Universidad Autónoma de Madrid

Facultad de Medicina

Departamento de
Anatomía, Histología y Neurociencia

El núcleo posterior medial del tálamo y su implicación en los procesos perceptivos y cognitivos

TESIS DOCTORAL

Carlos Castejón González

Dirigida por el Doctor
Ángel Núñez Molina

Madrid, 2020

Acércate a los que saben, pero huye de los sabios

Agradecimientos

Mi más cordial agradecimiento al Departamento de Anatomía, Histología y Neurociencia de la Facultad de Medicina de nuestra apreciada Universidad Autónoma de Madrid y a las personas que lo forman. Con muchas de ellas he tenido contacto durante la realización de esta Tesis y de algunas de dichas personas he recibido importante ayuda. Mucho he aprendido de ellas.

Así mismo, mi reconocimiento a los revisores de mis trabajos y publicaciones científicas, incluida esta Tesis, por su tiempo, dedicación y aportaciones.

Especial agradecimiento merece el director de esta Tesis, el Doctor Ángel Núñez, por su apoyo, compromiso y paciencia.

Fuera del ámbito académico, gracias de corazón a todas aquellas personas que me han ayudado prestándome apoyo y, especialmente, su tiempo. Creo que ellos mismos sabrán reconocerse.

Por último, es necesario recordar que toda esta investigación ha sido posible gracias a la utilización de animales de experimentación. Sin ellos, nuestro conocimiento actual sobre el cerebro sería considerablemente diferente.

Resumen

Se desconoce en gran medida cómo el sistema somestésico extrae información del flujo de señales sensoriales. La información procedente de las vibrisas es procesada principalmente por dos vías ascendentes paralelas hacia la corteza cerebral. Sin embargo, se desconoce la implicación funcional de las diferentes estructuras que componen dichas rutas. Mediante la combinación de técnicas electrofisiológicas y farmacológicas *in vivo* en ratas, encontramos diferencias significativas entre estas vías. Aunque está bien asumido que el POm y el VPM responden a la estimulación de las vibrisas contralaterales, encontramos que el primero es capaz de responder también a las ipsilaterales. Mediante la integración de señales simultaneas procedentes de vibrisas en ambos lados de la cara, está implicado en la representación de eventos táctiles bilaterales. Esto demuestra la implicación de los núcleos talámicos sensoriales de tipo 'higher-order' en la percepción bilateral. Encontramos que los núcleos POm están mutuamente conectados a través de la corteza formando un bucle o 'loop' funcional. Revelamos la naturaleza y el contenido de los mensajes transmitidos a través de este circuito mostrando que dichos mensajes son 'patrones estructurados de actividad sostenida'. Estos mensajes son transmitidos preservando su estructura integrada. La implicación de diferentes áreas fue investigada descubriendo que S1 juega un papel protagonista en dicho 'loop' POm-POm. También encontramos diferente implicación laminar en esta área en el procesamiento de actividad sostenida y en su transmisión entre hemisferios. Proponemos un modelo teórico en el que dichos 'patrones estructurados de actividad sostenida' generados por el POm pueden jugar un papel relevante en las funciones perceptivas, motoras y cognitivas. Además, demostramos que el POm está involucrado en la representación de patrones sensoriales complejos. Este núcleo es muy sensible a la activación simultanea de las vibrisas y a las complejas interacciones espaciotemporales que se producen entre ellas. La estructura espaciotemporal de dichos patrones y la complejidad de sus partes son reflejados en precisos cambios de actividad en el POm. Nuestros resultados sugieren que este núcleo podría ser un codificador general de patrones. La naturaleza (estructurada versus discreta), el tipo (sostenido versus transitorio) y el contenido (integrado versus segregado) de la actividad neural procesada y transmitida por estos núcleos determina su implicación funcional y puede permitir clasificarlos. Proponemos la hipótesis de los Componentes Complementarios para explicar estas diferencias. Además, revelamos la capacidad del POm para ajustar el procesamiento en las cortezas S1 y S2 mediante la inducción de una precisa inhibición en determinadas capas corticales. Esta modulación está mediada por neuronas GABAérgicas de la capa 1. La hipótesis de Computación Cortical por Resultados Discretos propuesta aquí puede explicar la implicación funcional de dicho ajuste.

Summary

How the somatosensory system extracts information from the flow of raw sensory signals is still unknown. Tactile information from whiskers is processed by two main parallel ascending pathways towards the cortex. However, the functional implication of the neural structures forming these routes remains unclear. Using a combination of electrophysiology and pharmacology *in vivo* in rats, we find profound differences between these pathways. Although it is well assumed that POm and VPM encode stimulations of the contralateral whisker pad, we show that POm is also able to respond to tactile stimulation of ipsilateral whiskers. POm is implicated in the representation of bilateral tactile events by integrating simultaneous signals arising from both whisker pads. This evidence demonstrates the implication of the higher-order sensory thalamus in bilateral perception. We find that POm nuclei are mutually connected through the cortex forming a functional POm-POm loop. We unravel the nature and content of the messages travelling through this loop showing that they are 'structured patterns of sustained activity'. These structured messages are transmitted preserving their integrated structure. The implication of different cortical areas was investigated revealing that S1 plays a protagonist role in this functional loop. Our results also demonstrate different laminar implication in the processing of sustained activity in this cortical area and its transmission between hemispheres. We propose a theoretical model in which these 'structured patterns of sustained activity' generated by POm may play important roles in perceptual, motor and cognitive functions. From a functional perspective, this proposal, supported by the results described here, provides a novel theoretical framework to understand the implication of the thalamus in cognition. In addition, we demonstrate that POm is implicated in the representation of complex sensory patterns. This nucleus is highly sensitive to multiwhisker stimuli involving complex spatiotemporal interactions. The dynamical spatiotemporal structure of sensory patterns and the different complexity of their parts are accurately reflected in precise POm activity changes. Our findings suggest that POm is a general encoder of patterns. The nature (structured versus discrete), type (sustained versus transient) and content (integrated versus segregated) of the neural activity processed and transmitted by these nuclei determine their functional implication and can be used to functionally classify them. The hypothesis of Complementary Components is proposed here to explain it. Moreover, we unravel the capacity of POm to adjust cortical processing in S1 and S2 by inducing precise inhibition in different layers. This modulation is mediated by GABAergic neurons in L1. The hypothesis of Cortical Computation by Discrete Results proposed here can explain the functional implication of this adjustment.

Lista de abreviaturas

Am: Amígdala
FS: Fast-spiking
GABA: Ácido gamma-amino butírico
INS: Corteza insular
LP: Núcleo lateral posterior del tálamo
M1: Corteza motora primaria
M2: Corteza motora secundaria
NMDA: Acido N-metil-D-aspártico
NRpT: Núcleo reticular pretalámico
POm: Núcleo postero-medial del tálamo
PrV: Núcleo principal del trigémino
PV: Parvalbúmina
Rt: Núcleo reticular talámico
S1: Corteza somatosensorial primaria
S1BF: Corteza somatosensorial primaria, zona de barriles
S1FL: Corteza somatosensorial primaria, región de la extremidad anterior
S2: Corteza somatosensorial secundaria
SC: Colículo superior
STR: Estriado
SEM: Error estándar de la media
SpV: Núcleo espinal del trigémino
SpVc: Núcleo espinal del trigémino, porción caudal
SpVi: Núcleo espinal del trigémino, porción interpolar
SpVic: Núcleo espinal del trigémino, porción interpolar caudal
SpVir: Núcleo espinal del trigémino, porción interpolar rostral
SpVo: Núcleo espinal del trigémino, porción oral
VPM: Núcleo ventral posterior medial del tálamo
VPMvl: Parte ventrolateral del VPM
VPMmv: Parte medioventral del VPM
ZI: Zona incerta

Índice

1. INTRODUCCIÓN

1. Sistemas sensoriales

Los sistemas sensoriales son vitales para el funcionamiento y supervivencia de los diferentes animales. Son la puerta de entrada de información al cerebro. Mediante ellos, éste adquiere dicha información y la utiliza para aprender y tomar decisiones.

Nuestro conocimiento sobre los sistemas sensoriales ha aumentado considerablemente en las últimas décadas, especialmente a nivel anatómico y fisiológico. Sin embargo, a nivel funcional, carecemos de un marco teórico general que permita explicar cómo dichos sistemas extraen información de su entorno y cómo hacen posible que pueda ser utilizada por el cerebro.

a. Complejidad de la estimulación sensorial

Los sistemas sensoriales han sido mayoritariamente estudiados usando estímulos simples. Sin embargo, en circunstancias naturales, la actividad sensorial a la que están expuestos dichos sistemas es compleja y constantemente variable. Esto requiere que este procesamiento pueda adaptarse a dicha variabilidad. Por otro lado, el procesamiento sensorial está, también, sujeto a los requerimientos que a nivel cognitivo o conductual exijan las tareas en curso. Parece, por lo tanto, necesario que dicho procesamiento tenga un carácter flexible. Sin embargo, desconocemos, actualmente, qué mecanismos soportan dicha flexibilidad de procesamiento.

b. Vías ascendentes paralelas en los sistemas sensoriales

Una característica común en la mayoría de los sistemas sensoriales es la existencia de vías ascendentes paralelas desde los receptores sensoriales hasta la corteza cerebral. Se desconoce actualmente el porqué de esta arquitectura anatómica. Dicha organización supone grandes costes para el organismo (por ejemplo, metabólicos) por lo que es de suponer que aportan un valor añadido a nivel funcional. De hecho, está presente en los diferentes sistemas sensoriales en la mayoría de las especies animales (Sherman and Guillery 2006).

c. El tálamo y sus proyecciones a la corteza cerebral

Otra característica común de la mayoría de los sistemas sensoriales es la implicación del tálamo. Esta estructura es, en realidad, un conjunto agrupado y heterogéneo de núcleos y subnúcleos. Están formado mayoritariamente por neuronas glutamatérgicas de proyección que extienden sus axones hacia diferentes destinos cerebrales y cuyas proyecciones están caracterizadas por tener una amplia variabilidad. Anatómicamente, estos núcleos reciben y proyectan sus axones de forma diferenciada dando lugar a un complejo entramado de conexiones corticales y subcorticales. La investigación sobre los diferentes núcleos y sus conexiones ha sido intensa y mucho hemos avanzado en su conocimiento. Sin embargo, nuevamente, dicho conocimiento es muy significativo a nivel anatómico y fisiológico pero escaso a nivel funcional. De hecho, actualmente desconocemos, en gran medida, qué función o funciones juega esta estructura en el funcionamiento del cerebro.

d. Clasificación de los núcleos talámicos

Las primeras clasificaciones se basaron, fundamentalmente, en criterios histológicos generando divisiones entre diferentes zonas talámicas (para una revisión detallada ver Jones, 2007). Esta segregación permitió asignar cierta funcionalidad a los diferentes núcleos.

Otras clasificaciones, utilizando criterios anatómicos, se han basado en las proyecciones que recibe y envía cada núcleo. Una primera distinción clasificó como ‘específicas’ aquellas proyecciones que terminaban focalmente en un área concreta de la corteza y como ‘inespecíficas’ aquellas que lo hacían de forma extensa en varias áreas corticales (Lorente de Nó, 1922; 1938). Más recientemente, una distinción ampliamente usada fue la clasificación ‘core’ y ‘matrix’ (Jones 2001). Las neuronas talámicas tipo ‘core’ solo proyectan hacia la corteza y lo hacen de forma espacialmente restringida y en capas corticales intermedias. Las neuronas tipo ‘matrix’ proyectan también a otras estructuras subcorticales como los ganglios basales, y en la corteza lo hacen de forma espacialmente extensa y en diferentes capas, incluida la capa 1. Sin embargo, nuevas técnicas han demostrado que las neuronas talámicas tienen una variabilidad en sus proyecciones mucho más compleja y rica (Rubio-Garrido y cols., 2009; Clascá et al., 2012; Ohno y cols. 2012; Kuramoto et al., 2017) y que la dicotomía anterior no vale para explicar dicha heterogeneidad. Nuevas

clasificaciones están siendo propuestas a medida que conocemos más detalladamente la variabilidad anatómica de las proyecciones de estas neuronas (Clascá y cols. 2016).

No muchos han sido los intentos por clasificar funcionalmente a los diferentes núcleos que componen el tálamo. Uno de ellos, es la clasificación que divide las proyecciones tálamo-corticales en dos tipos llamados 'drivers' y 'modulators' (Sherman and Guillery 1998). Las primeras trasmitirían la información principal a la corteza, mientras que las segundas jugarían un papel modulador. Aunque esta clasificación suponía un intento por asignar una determinada funcionalidad diferencial a estas proyecciones, los conocimientos que han ido apareciendo más recientemente, demuestran que no es válida para reflejar la complejidad funcional de esta estructura.

Otra clasificación, actualmente usada, divide al tálamo en núcleos primarios y núcleos de alto nivel ('first' versus 'higher-order'; Sherman and Guillery 2013). Los primeros recibirían información sensorial directamente de la periferia mientras que los segundos serian activados desde la capa 5 de la corteza cerebral. Nuevamente, esta dicotomía no es válida en base a los descubrimientos que actualmente conocemos sobre estos núcleos y sus proyecciones (revisado recientemente en Halassa y Sherman 2019). Por ejemplo, sabemos que estos núcleos de alto nivel pueden recibir de forma muy contundente desde ambas entradas.

En definitiva, nuestro conocimiento de esta estructura cerebral ha progresado desde los aspectos anatómicos más accesibles hacia sus aspectos menos evidentes como son los funcionales. Sin embargo, actualmente, ninguna clasificación recoge de forma precisa la complejidad de esta estructura cerebral.

e. La implicación del tálamo en los sistemas sensoriales

Diferentes núcleos talámicos están implicados en las diversas vías de información dentro de una misma modalidad sensorial. En base a esto, dichos núcleos sensoriales se han estudiado, tradicionalmente, dividiéndolos de forma básica en 'primarios' y 'secundarios'.

Los núcleos primarios han sido mucho más estudiados. Está bien asumido que estos núcleos están implicados en el procesamiento y transmisión de información sensorial con una topografía muy precisa y de carácter específico a nivel de contenido. Sin embargo, los núcleos secundarios han

sido menos estudiados. Actualmente, se considera que transmiten información sensorial menos específica y están caracterizados por tener una topografía poco precisa. Aunque recientemente, estos núcleos están siendo objeto de investigación de manera muy contundente, se desconoce aún, en gran medida, cómo es su funcionamiento y qué implicación funcional juegan en los procesos perceptivos.

f. La corteza cerebral y el procesamiento de información sensorial

La información sensorial procedente de los receptores sensoriales y transmitida por las vías ascendentes, llega a la corteza cerebral. Dicha información es recibida y procesada en diferentes capas corticales. Esta distribución depende del tipo de vía ascendente y suele ser similar en los diferentes sistemas sensoriales. En general, una de las vías suele terminar fundamentalmente en la capa granular de las cortezas primarias y en menor medida, en su capa 6, mientras que la otra vía evita la capa granular y termina principalmente en las capas 1 y 5. Actualmente, desde el punto de vista funcional, se desconoce el porqué de dicha distribución laminar.

Dentro de una modalidad sensorial concreta, los núcleos talámicos secundarios proyectan de forma paralela a varias cortezas sensoriales de dicha modalidad. Además, dichos núcleos proyectan a otras áreas corticales, así como a estructuras subcorticales.

g. Procesamiento de información sensorial y computación cortical

Una vez recibida por la corteza, el procesamiento de la información sensorial no se limita a capas específicas, sino que se produce por una interacción compleja entre ellas. Entender cómo es la computación que soporta dicho procesamiento sensorial en la corteza es uno de los objetivos más perseguidos en el campo de la neurociencia. Sin embargo, aunque nuestro conocimiento sobre este procesamiento se ha incrementado considerablemente en los últimos años, actualmente se desconocen, en gran medida, los mecanismos neurales que lo soportan y cómo la interacción entre los diferentes tipos neuronales sostiene esta computación.

h. Procesamiento discreto, ritmos y oscilaciones neurales

En los últimos años, ha resurgido el controvertido debate sobre si la computación que tiene lugar en la corteza es de carácter continuo o si por el contrario se produce de forma discreta. La idea de que el procesamiento de la información sensorial no se produce de forma continua, sino que ocurre en tramos discretos de tiempo ha sido defendida tradicionalmente por muchos autores (Pitts y McCulloch 1947; Harter 1967; Allport 1968; Varela y cols. 1981). Esta propuesta se basa en que el flujo sensorial puede ser dividido en ciclos perceptivos discretos. Esto da lugar a eventos sensoriales concretos que pueden ser comparados entre ellos para extraer información (Fig. 1).

En el sistema visual, la entrada continua de actividad sensorial es constantemente interrumpida por los movimientos microsacádicos de los ojos, generando eventos sensoriales discretizados. Este tipo de computación ha sido ampliamente descrita en este y en otros sistemas sensoriales (VanRullen et al., 2005). Por ejemplo, se ha demostrado que el procesamiento de información somatosensorial que ocurre en la corteza se produce en base a este tipo de computación discreta (Baumgarten et al., 2015).

Además, la evidencia experimental reciente demuestra un procesamiento de naturaleza discreta en otros procesos cognitivos incluida la atención, la memoria y la consciencia (VanRullen y Koch, 2003; Buschman y Miller, 2010; Lundqvist et al., 2016).

Este tipo de computación neural se ha asociado al hecho de que el funcionamiento del cerebro está caracterizado por ritmos y oscilaciones neurales. Dicho funcionamiento está ampliamente conservado entre especies (Buzsáki y Draguhn, 2004) y está funcionalmente asociado a diferentes procesos cognitivos. Sabemos que el mecanismo que lo soporta es la acción coordinada de los diferentes tipos de neuronas que forman el cerebro y que, dentro de este mecanismo, las interneuronas juegan un papel clave. Conocemos cada vez más sobre ellas, sus diferentes tipos y cómo contribuyen a la generación de las oscilaciones neurales. Sin embargo, sigue sin estar claro, cuál es el valor funcional de los diferentes ritmos y qué aporta funcionalmente cada uno de los elementos neurales implicados.

En su conjunto, esta evidencia sugiere que al menos una parte del procesamiento de información que realizan los sistemas sensoriales, se produce por computación de naturaleza discreta y asociada a un funcionamiento rítmico de la actividad neural.

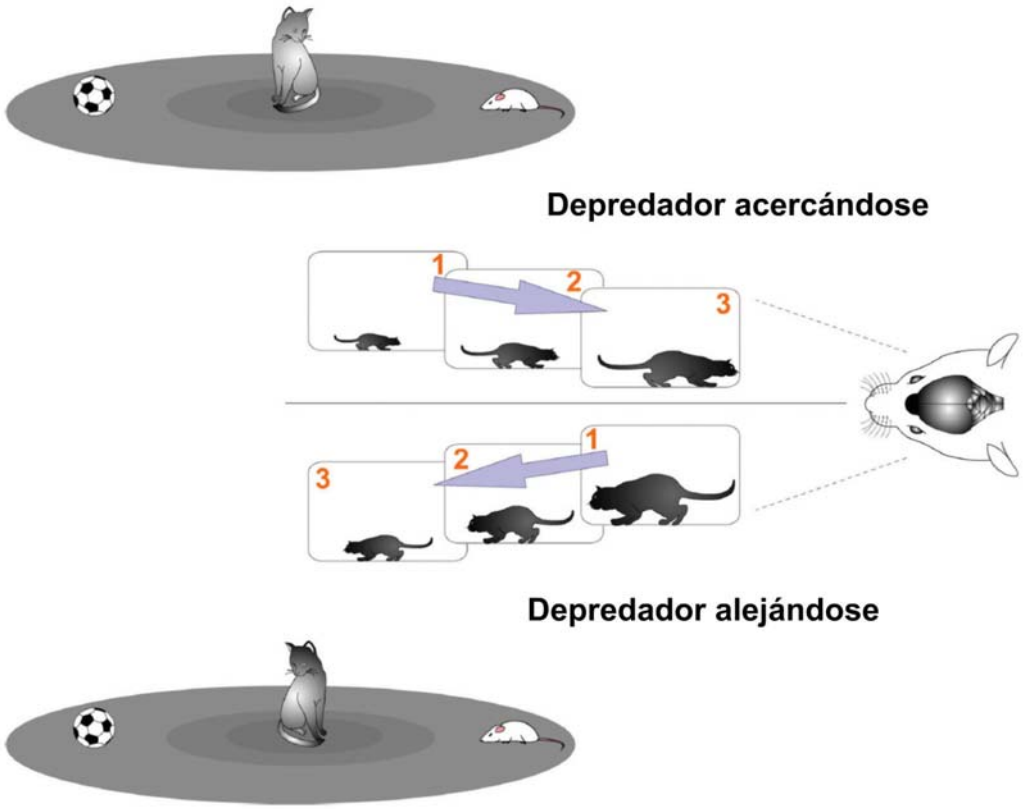


Fig. 1. La extracción de información relevante del entorno en el que se desenvuelve el animal es fundamental para su supervivencia. Para ello, se ha sugerido, que el cerebro muestrea el flujo de entrada sensorial para detectar cambios comparando sus contenidos. Esto da lugar a eventos sensoriales concretos (representados por las escenas 1-3 en esta ilustración) que pueden ser comparados entre ellos para extraer información y tomar la decisión adecuada en base a dicha información.

i. Computación cortical y asambleas funcionales de neuronas

Como ya hemos comentado, la computación que se realiza en la corteza sigue siendo uno de los desafíos más perseguidos en el campo de la neurociencia. La aparición de nuevas técnicas, y el desarrollo de las ya existentes, ha permitido la obtención de nuevos datos sobre el funcionamiento cortical tanto a nivel celular como a nivel de redes y conjuntos funcionales de neuronas. Nuevamente, este progreso no ha venido acompañado con un desarrollo teórico que permita

interpretar a nivel funcional los datos, cada vez más abundantes, obtenidos gracias al desarrollo de las técnicas y métodos de investigación.

Uno de los principales avances a nivel técnico ha sido la capacidad de estudiar conjuntos cada vez más amplios de neuronas durante su procesamiento de información. Esto ha permitido contrastar que las neuronas no funcionan de forma aislada, sino que lo hacen coordinadas en grupos y redes, de las que emerge la capacidad de computar (Hebb 1949; Pouget y cols. 2000; Yuste 2015). Como ya hemos comentado, dicha interacción coordinada entre grupos de neuronas juega un papel clave en la generación de los ritmos y oscilaciones características de la actividad neural.

En base a lo anterior, se ha propuesto que determinados conjuntos de neuronas forman unidades básicas de computación neural. El estudio de dichas asambleas funcionales de neuronas está acaparando un interés creciente en investigación. Sin embargo, se desconoce cómo estas asambleas están implicadas en la función computacional de la corteza. Sigue siendo un misterio cómo se forman y cómo afecta su dimensión espacial y temporal a su funcionalidad.

j. Extracción de información del flujo sensorial

La función de los sistemas sensoriales es la obtención de información del entorno. Esta función es vital para el animal puesto que determina su supervivencia. Es por lo tanto fundamental que la extracción de información relevante del flujo sensorial sea lo más optimizada posible. En base a esto, las regularidades y patrones juegan un papel básico en la extracción optimizada de información. De hecho, los sistemas sensoriales están caracterizados por su potente capacidad en la detección de dichas dinámicas sensoriales. A pesar de su relevancia, desconocemos cómo estos sistemas, codifican y extraen regularidades y patrones del entorno.

k. Uso de información sensorial en los procesos cognitivos

El cerebro utiliza el contenido relevante extraído por los sistemas sensoriales para organizar el comportamiento y funcionamiento general del animal. Dicho funcionamiento está soportado por los procesos cognitivos que tienen lugar en su cerebro. Por lo tanto, parece primordial entender cómo se produce la extracción de información sensorial y cómo se codifica para ser utilizada por

otras partes del cerebro durante dichos procesos cognitivos. Actualmente, desconocemos cómo se produce esta transformación de información relevante, extraída de su entorno, en actividad neural útil para ser utilizada en tareas actuales o futuras. Carecemos de teorías o explicaciones funcionales que permitan entender este aspecto tan vital del funcionamiento cerebral.

En parte, esto se debe a que desconocemos cómo se selecciona el contenido relevante, extrayéndolo del constante flujo de actividad sensorial a la que está expuesto el animal. De igual modo, desconocemos en base a qué criterios se produce este procedimiento y cómo son los mecanismos funcionales que lo soportan. Por otra parte, una vez seleccionado y extraído el contenido relevante, desconocemos cómo es codificado, qué estructuras están implicadas, que mecanismos utilizan y en qué formato se transforma para ser utilizado en los diferentes procesos cognitivos que exija las tareas en curso o que se utilizará en un futuro.

l. Implicación del tálamo en los procesos cognitivos

Es bien conocido que la expansión de la corteza a lo largo de la evolución ha dado lugar a nuevas capacidades. Esta expansión cortical no ha sido aislada, sino que ha venido acompañada, también, por un desarrollo a nivel talámico.

Hasta recientemente, el tálamo se ha estudiado mayoritariamente en el contexto de la función perceptiva, olvidando que su implicación es fundamental en otros procesos cognitivos. Esto se ha producido aun sabiendo que alteraciones en esta estructura han sido descritas en diferentes patologías neurológicas en las que diversos procesos cognitivos están gravemente afectados (por ejemplo, Scheibel 1997).

Esta situación ha ido progresivamente cambiando, y actualmente, está bien asumido, que el tálamo está implicado tanto en el procesamiento de información sensorial, como en los diferentes procesos cognitivos relacionados con la utilización de dicha información sensorial. Sin embargo, carecemos aún de una explicación funcional que nos permita entender cuál es su papel en dicha transformación y utilización del contenido extraído del flujo sensorial.

Afortunadamente, como hemos comentado, en los últimos años se está produciendo un interés creciente en conocer el papel que juega esta estructura en la función cognitiva.

2. El sistema de vibrisas en roedores

Los roedores utilizan fundamentalmente sus vibrisas para obtener información de su entorno cercano. Esta capacidad juega un papel vital para su supervivencia y es seguramente por esta razón, por la que ha evolucionado hasta estar exquisitamente perfeccionada. Sin embargo, aunque es un sistema ampliamente estudiado y conocemos en gran medida su arquitectura anatómica, a nivel funcional desconocemos cómo son las computaciones que realiza soportando esa extracción tan optimizada de información.

a. Exploración activa y extracción de información sensorial

Durante la exploración de su entorno, los roedores mueven de forma activa y rítmica las vibrisas a diferentes frecuencias (Carvell y Simons 1990). Este movimiento rítmico se denomina 'whisking' y juega un papel fundamental en el proceso perceptivo de este sistema. En base a estos movimientos, durante la exploración táctil, la entrada de actividad somatosensorial procedente de las vibrisas es constantemente dividida en eventos sensoriales discretizados. Sin embargo, este patrón de funcionamiento ha sido tradicionalmente obviado en el estudio de este sistema sensorial. Se desconocen actualmente los mecanismos funcionales que soportan dicho tipo de computación.

b. Vías de transmisión y procesamiento de la información somatosensorial en el sistema de vibrisas

La actividad sensorial recibida por los receptores sensitivos, situados en la base de las vibrisas en cada lado de la cara, es transmitida al complejo trigeminal en el tronco del encéfalo (Fig. 2). Dentro de este nivel juegan un papel fundamental el núcleo principal (PrV) y el núcleo espinal (SpV). Desde allí, una vez procesada, la actividad neural resultante es enviada al tálamo contralateral. Es entonces cuando varios núcleos talámicos reciben dicha información, la procesan y la transmiten, ipsilateralmente, a la corteza cerebral. Es importante resaltar que a pesar de ser esta, la ruta tradicionalmente asumida como fundamental en la transmisión de información sensorial procedente de las vibrisas, la arquitectura real que compone este sistema es bastante más compleja, incluyendo diferentes ramificaciones e involucrando a un gran número de estructuras cerebrales, entre las que podemos destacar el estriado y el tectum.

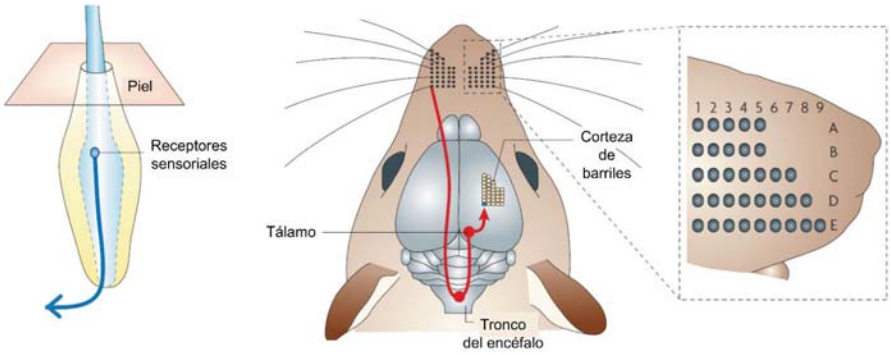


Fig. 2. Recepción y transmisión de información sensorial procedente de las vibrisas. La información sensorial es transmitida desde los receptores sensoriales en la base de cada vibrisa hacia el encéfalo. Dichas vibrisas están distribuidas espacialmente de forma precisa en ambos lados de la cara. Adaptado de Diamond y cols. (2008).

Como hemos descrito anteriormente, una característica común en la mayoría de los sistemas sensoriales, es la presencia de vías paralelas en el procesamiento y transmisión de información sensorial. Esta característica también la comparte el sistema de vibrisas y es especialmente evidente a nivel del tálamo y de sus proyecciones a la corteza. Dos de sus núcleos, el posterior medial (POm) y el ventral posterior medial (VPM) comparten su implicación en el procesamiento de información procedente de las vibrisas, pero tanto ellos como sus proyecciones presentan características diferenciadas. Esto da lugar a vías relativamente segregadas. La clasificación tradicionalmente usada para diferenciar dicha segregación, está descrita en la Fig. 3. Es importante destacar que, aunque esta clasificación, vista desde una perspectiva actual, no parece reflejar la verdadera complejidad de la arquitectura anatómica del sistema de vibrisas, ni la perfecta interacción o separación de los elementos tradicionalmente asignados como pertenecientes a cada una de ellas, si parece existir una segregación en cuanto al contenido que por ellas se transmite. La funcionalidad de este hecho y de cómo dichos contenidos diferenciados contribuyen durante el proceso perceptivo, es en gran medida desconocida. Dentro de estas vías, destacan la vía del sistema lemniscal y la vía del sistema paralemniscal. La primera incluye al núcleo principal (PrV) en el complejo del trigémino, al núcleo talámico VPM y termina en la capa 4 de la zona de barriles en la corteza somatosensorial primaria (S1; Woolsey and Van der Loos 1970; Wimmer et al. 2010). En esta vía, cada vibrisa está representada por un agregado especifico de neuronas en cada uno de estos niveles (denominados ‘barriletes’, ‘barriloides’ y ‘barriles’, respectivamente). Esto da lugar a mapas somatotópicos muy precisos en cada estación de la vía y que se corresponden con la distribución espacial de las vibrisas en el rostro del animal.

El sistema paralemniscal incluye al núcleo talámico POm que recibe la información sensorial relativa a las vibrisas de los núcleos principal (PrV) y espinal (SpV) en el complejo trigeminal. Desde el POm, el resultado de su procesamiento es transmitido a diferentes áreas corticales somestésicas y motoras, entre otras (Ohno y cols., 2012; Porrero., 2016). Este sistema es filogenéticamente más antiguo y no está caracterizado por poseer una somatotopía precisa.

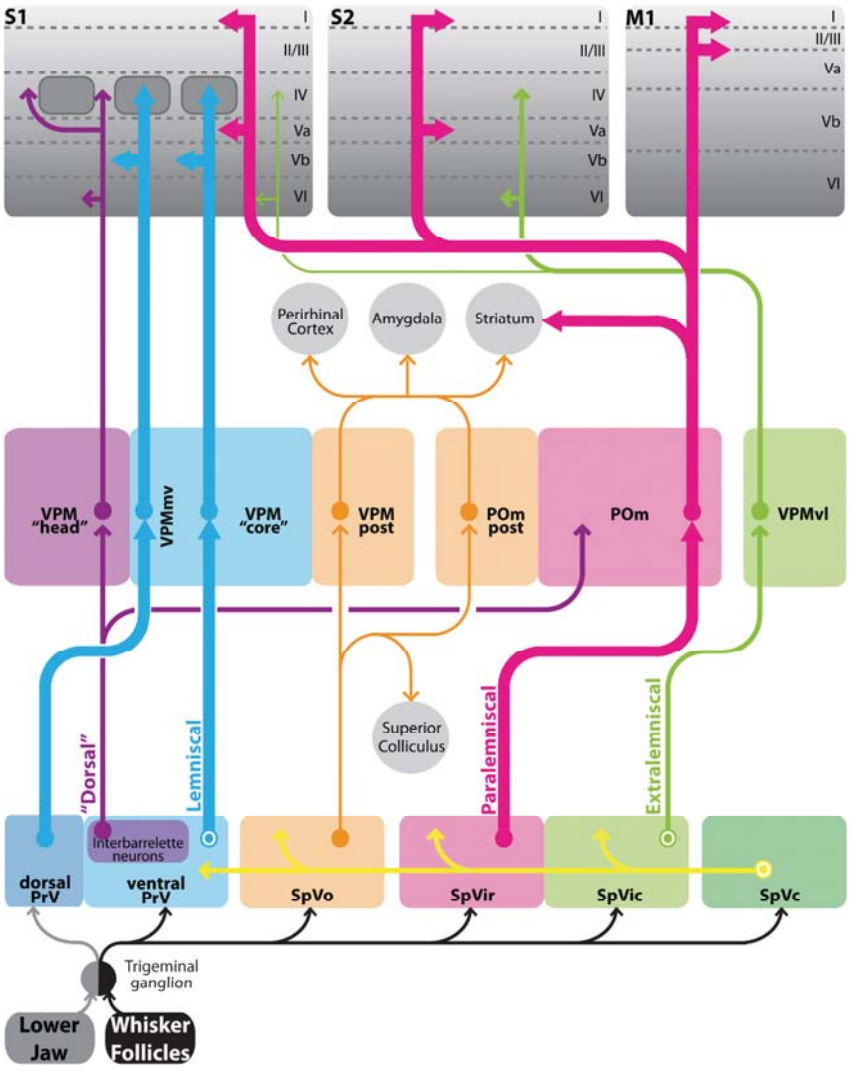


Fig. 3. Clasificación de las vías somatosensoriales implicadas en el procesamiento y transmisión de información sensorial de las vibrisas hacia la corteza cerebral. SpVo, núcleo espinal del trigémino, porción oral; SpVir, núcleo espinal del trigémino, porción interpolar rostral; SpVic, núcleo espinal del trigémino, porción interpolar caudal; SpVc, núcleo espinal del trigémino, porción caudal; VPMvl, parte ventrolateral del VPM; VPMmv, parte medioventral del VPM; S1, corteza somatosensorial primaria; S2, corteza somatosensorial secundaria; M1, corteza motora primaria. Tomado de Pouchelon y cols. (2012).

c. Núcleos talámicos POm y VPM

Aunque tanto el núcleo POm y como el VPM están implicados en el procesamiento de la información relativa a las vibrisas, la mayor parte de la investigación realizada sobre ellos, se ha focalizado en el segundo. Sin embargo, en los últimos años, se ha producido un interés creciente en conocer las características anatómicas, fisiológicas y funcionales también del primero.

Cuando comparamos estos núcleos, encontramos diferencias significativas entre ambos. Dos de ellas son, como ya hemos descrito, la diferencia en la precisión de su somatotopía en relación con las vibrisas y el contenido diferenciado que procesan y transmiten. Está actualmente asumido que el VPM procesa información sensorial muy específica, relacionada con cada una de las vibrisas y de aspectos concretos relacionados de forma individual con cada una de ellas. Sin embargo, el contenido de los mensajes que procesa y transmite el POm, se desconoce en gran medida. El conocimiento que tenemos sobre este contenido indica que, al contrario del VPM, no tiene dicho carácter específico (Diamond et al., 1992).

Además, la distribución regional y laminar de las proyecciones del VPM y POm a la corteza son profundamente distintas. El conjunto de dichas diferencias sugiere aspectos funcionales divergentes.

d. Proyecciones talamocorticales de los núcleos POm y VPM

Las proyecciones del POm hacia la corteza contactan principalmente a S1, pero también lo hacen a otras áreas como la corteza motora, somatosensorial secundaria (S2), corteza insular, área frontal asociativa, corteza ectorrinal y cortezas asociativas auditivas (Deschênes y cols., 1998; Ohno y cols., 2012; Porrero., 2016). En base a esta arquitectura anatómica, se las ha clasificado como de tipo ‘multi-específico’ (Fig. 4; Clascá y cols., 2016). Por el contrario, las proyecciones del VPM contactan de forma casi exclusiva a S1 y son tradicionalmente clasificadas como ‘específicas’. Como ya hemos comentado, esta profunda distinción anatómica sugiere diferencias funcionales evidentes. Sin embargo, desconocemos en gran medida dicha distinción a nivel funcional.

Además, estos dos tipos de proyecciones talámicas terminan en capas corticales diferentes. Las proyecciones del POm terminan en las capas 5A y 1 en S1, evitando la capa 4, mientras que en

otras áreas corticales lo hacen en varias capas incluyendo normalmente la 1 (Ohno y cols., 2012; Porrero., 2016). Sin embargo, las proyecciones del VPM a S1 terminan de forma focalizada en la capa 4 y en la capa 6A (Fig. 4). Actualmente, no está clara la razón por la que estas proyecciones contactan de forma paralela diferentes capas dentro de una misma área cortical. Nuevamente, desconocemos qué implicación funcional tiene dicha distribución anatómica.

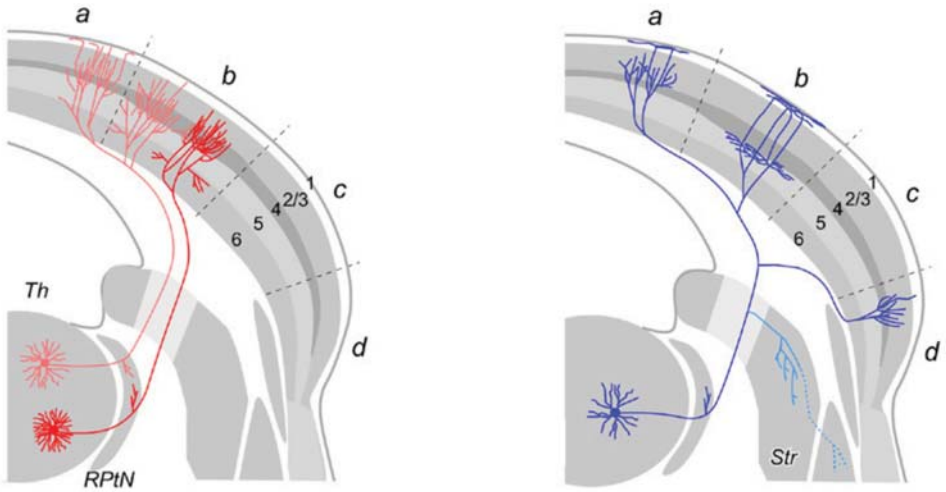


Fig. 4. Las neuronas de proyección del VPM y del POm contactan de forma diferente a la corteza cerebral y en base a eso, han sido diferenciadas en tipos distintos. Según la clasificación propuesta por Clascá cols. (2016), las primeras son consideradas del tipo 'específico' (en rojo) y las segundas, del tipo 'multispecífico' (en azul). En estas ilustraciones, tomadas de Clascá y cols. (2016), las capas corticales están definidas por los números y las letras a-d hacen referencia a cuatro posibles áreas corticales diferenciadas. Th, tálamo. RPtN, núcleo reticular. Str, estriado.

e. Procesamiento cortical de la información sensorial de las vibrisas

Varias áreas corticales están implicadas en el procesamiento de la información sensorial procedente de las vibrisas (Fig. 3). Dentro de cada área, las diversas capas que la componen contribuyen a dicho procesamiento interaccionando entre ellas. Las respuestas corticales evocadas por la estimulación sensorial han sido ampliamente estudiadas y nuestro conocimiento sobre ellas es

abundante. Sin embargo, cómo dicho procesamiento laminar contribuye a la función computacional que en la corteza se desarrolla, permanece poco claro. Esto es aplicable al resto de los sistemas sensoriales.

f. Extracción y utilización de información sensorial de las vibrisas por parte del cerebro

La información sensorial procedente de las vibrisas permite a los roedores desenvolverse con gran eficacia por su entorno cercano. Esto significa que dicha información táctil es utilizada para organizar su funcionamiento. Puesto que dicha organización funcional está soportada por los procesos cognitivos que tienen lugar en sus cerebros, parece primordial entender cómo se produce la extracción de información sensorial de las vibrisas y cómo se codifica para ser utilizada por parte del cerebro durante los procesos cognitivos que utilizan dicha información. Sin embargo, al igual que ocurre con el resto de los sistemas sensoriales, desconocemos cómo se produce dicha transformación.

Los roedores, y otros muchos animales, tienen la capacidad de detectar regularidades y patrones con mucha precisión. Desconocemos cómo el sistema somestésico extrae estas regularidades y patrones. Tampoco sabemos cómo contribuyen y qué aportan en este proceso cada uno de los elementos que componen este sistema.

2. HIPÓTESIS Y OBJETIVOS

Un aspecto común que aparece descrito en los apartados anteriores es la necesidad de aumentar nuestro conocimiento, especialmente a nivel funcional, de los diferentes procesos cerebrales implicados en el procesamiento de contenido sensorial, de los componentes que los soportan y de los mecanismos que utilizan para generar dicho procesamiento. Compartimos dicho objetivo, y de acuerdo a ello, hemos basado nuestras investigaciones. Partimos de la hipótesis de que el POm, en aquellos momentos, un núcleo mucho menos estudiado, podría jugar un papel más complejo e influyente en la función del sistema somestésico.

Utilizando el sistema de vibrisas de los roedores como modelo para realizar nuestros estudios, la intención de nuestra investigación se centró en conocer la implicación de las diferentes estructuras que componen las vías ascendentes de este sistema, así como la relación que mantienen entre ellas. Pare ello, se plantearon los siguientes objetivos específicos:

- Ampliar el conocimiento sobre el funcionamiento de los núcleos VPM y POm, prestando especial atención al segundo, por ser el más desconocido de los dos.

- Estudiar su implicación en el procesamiento sensorial.

- Conocer cómo codifican el contenido sensorial que reciben.

- Entender la naturaleza y contenido de los mensajes que envían a la corteza.

- Conocer su influencia sobre el procesamiento cortical de la información sensorial.

- Conocer cómo la corteza procesa estos mensajes.

Sin embargo, en el trascurso de nuestras investigaciones, cada resultado encontrado abría nuevas incógnitas y en base a ellas, nuevas cuestiones por resolver. Algunas de ellas fueron las siguientes:

- ¿Qué implicación funcional tiene la arquitectura anatómica paralela de los sistemas sensoriales?

- ¿Qué papel juega el tálamo en ellos y qué aportan funcionalmente los diferentes núcleos talámicos dentro de cada sistema?

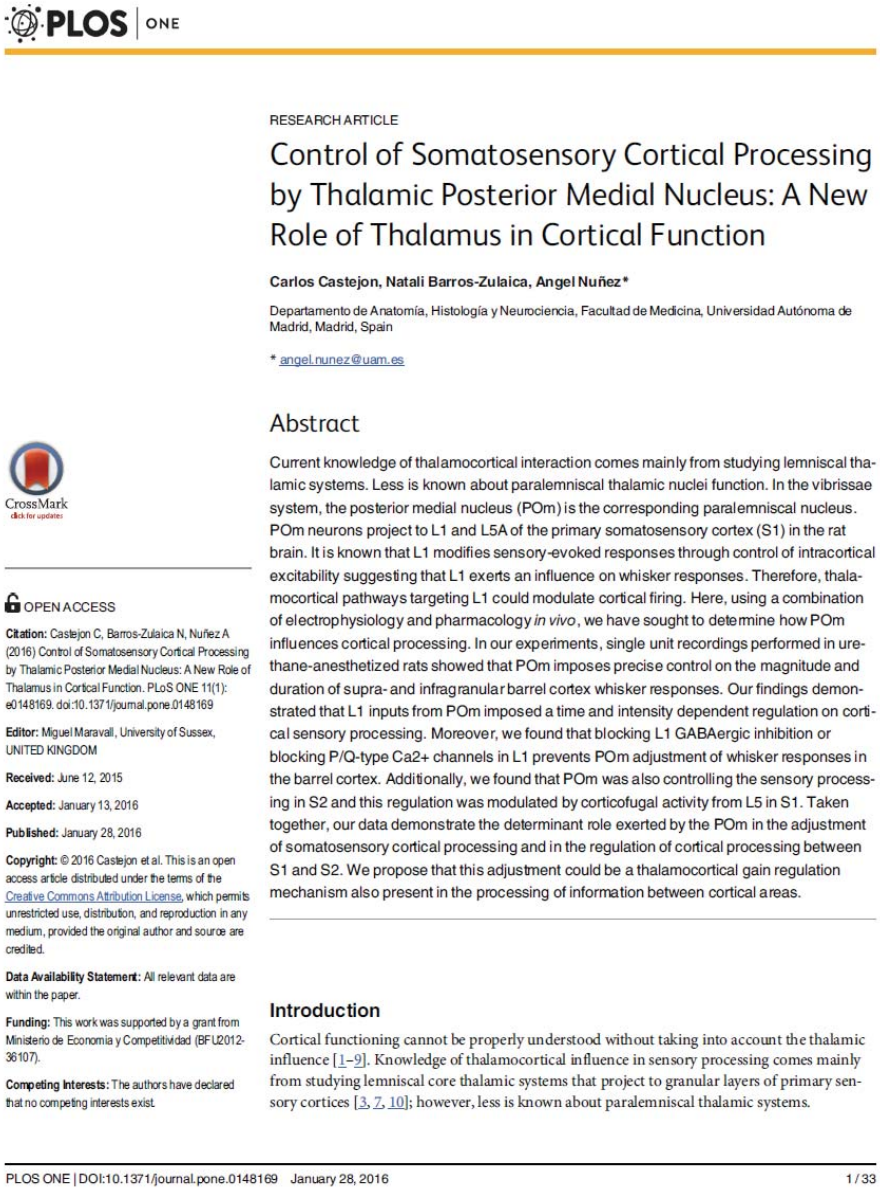
- ¿Cómo son codificados los contenidos relevantes extraídos del flujo sensorial para su posible utilización en los procesos cognitivos en los que sea requerida dicha información?

El planteamiento de estas cuestiones hizo que nuestros objetivos originales se ampliaran y orientaran hacia estos interrogantes. Algunas de estas últimas observaciones y propuestas derivadas de ellas están descritas en esta Tesis y pendientes de ser publicadas.

3. ARTÍCULOS CIENTÍFICOS PUBLICADOS

Esta Tesis contiene aquellos artículos científicos publicados en los que aparezco como autor principal. Son los siguientes:

ARTÍCULO CIENTÍFICO Nº 1: "Control of somatosensory cortical processing by thalamic posterior medial nucleus: A new role of thalamus in cortical function". C. Castejon, N. Barros-Zulaica, A. Nuñez. (2016) PLoS ONE 11(1):e0148169.



PLOS | ONE

RESEARCH ARTICLE

Control of Somatosensory Cortical Processing by Thalamic Posterior Medial Nucleus: A New Role of Thalamus in Cortical Function

Carlos Castejon, Natali Barros-Zulaica, Angel Nuñez*

Departamento de Anatomía, Histología y Neurociencia, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain

* angel.nunez@uam.es

Abstract

Current knowledge of thalamocortical interaction comes mainly from studying lemniscal thalamic systems. Less is known about paralemniscal thalamic nuclei function. In the vibrissae system, the posterior medial nucleus (POm) is the corresponding paralemniscal nucleus. POm neurons project to L1 and L5A of the primary somatosensory cortex (S1) in the rat brain. It is known that L1 modifies sensory-evoked responses through control of intracortical excitability suggesting that L1 exerts an influence on whisker responses. Therefore, thalamocortical pathways targeting L1 could modulate cortical firing. Here, using a combination of electrophysiology and pharmacology *in vivo*, we have sought to determine how POm influences cortical processing. In our experiments, single unit recordings performed in urethane-anesthetized rats showed that POm imposes precise control on the magnitude and duration of supra- and infragranular barrel cortex whisker responses. Our findings demonstrated that L1 inputs from POm imposed a time and intensity dependent regulation on cortical sensory processing. Moreover, we found that blocking L1 GABAergic inhibition or blocking P/Q-type Ca2+ channels in L1 prevents POm adjustment of whisker responses in the barrel cortex. Additionally, we found that POm was also controlling the sensory processing in S2 and this regulation was modulated by corticofugal activity from L5 in S1. Taken together, our data demonstrate the determinant role exerted by the POm in the adjustment of somatosensory cortical processing and in the regulation of cortical processing between S1 and S2. We propose that this adjustment could be a thalamocortical gain regulation mechanism also present in the processing of information between cortical areas.

CrossMark

OPEN ACCESS

Citation: Castejon C, Barros-Zulaica N, Nuñez A (2016) Control of Somatosensory Cortical Processing by Thalamic Posterior Medial Nucleus: A New Role of Thalamus in Cortical Function. PLoS ONE 11(1): e0148169. doi:10.1371/journal.pone.0148169

Editor: Miguel Maravall, University of Sussex, UNITED KINGDOM

Received: June 12, 2015

Accepted: January 13, 2016

Published: January 28, 2016

Data Availability Statement: All relevant data are within the paper.

Funding: This work was supported by a grant from Ministerio de Economía y Competitividad (BFU2012-36107).

Competing Interests: The authors have declared that no competing interests exist.

Introduction

Cortical functioning cannot be properly understood without taking into account the thalamic influence [1–9]. Knowledge of thalamocortical influence in sensory processing comes mainly from studying lemniscal core thalamic systems that project to granular layers of primary sensory cortices [3, 7, 10]; however, less is known about paralemniscal thalamic systems.

ARTÍCULO CIENTÍFICO Nº 2: Cortical neural computation by discrete results hypothesis". C. Castejon, A. Nuñez. (2016) Front. Neural Circuits 10:81.

frontiers in Neural Circuits

HYPOTHESIS AND THEORY
published: 19 October 2016
doi: 10.3389/fncir.2016.00081

Cortical Neural Computation by Discrete Results Hypothesis

Carlos Castejon and Angel Nuñez*

Department of Anatomy, Histology and Neuroscience, School of Medicine, Autonomous University of Madrid, Madrid, Spain

One of the most challenging problems we face in neuroscience is to understand how the cortex performs computations. There is increasing evidence that the power of the cortical processing is produced by populations of neurons forming dynamic neuronal ensembles. Theoretical proposals and multineuronal experimental studies have revealed that ensembles of neurons can form emergent functional units. However, how these ensembles are implicated in cortical computations is still a mystery. Although cell ensembles have been associated with brain rhythms, the functional interaction remains largely unclear. It is still unknown how spatially distributed neuronal activity can be temporally integrated to contribute to cortical computations. A theoretical explanation integrating spatial and temporal aspects of cortical processing is still lacking. In this Hypothesis and Theory article, we propose a new functional theoretical framework to explain the computational roles of these ensembles in cortical processing. We suggest that complex neural computations underlying cortical processing could be temporally discrete and that sensory information would need to be quantized to be computed by the cerebral cortex. Accordingly, we propose that cortical processing is produced by the computation of discrete spatio-temporal functional units that we have called "Discrete Results" (Discrete Results Hypothesis). This hypothesis represents a novel functional mechanism by which information processing is computed in the cortex. Furthermore, we propose that precise dynamic sequences of "Discrete Results" is the mechanism used by the cortex to extract, code, memorize and transmit neural information. The novel "Discrete Results" concept has the ability to match the spatial and temporal aspects of cortical processing. We discuss the possible neural underpinnings of these functional computational units and describe the empirical evidence supporting our hypothesis. We propose that fast-spiking (FS) interneuron may be a key element in our hypothesis providing the basis for this computation.

Keywords: cerebral cortex, sensory processing, cell ensembles, fast-spiking cells, brain oscillations, discrete computation, neural synchronization, processing resolution

OPEN ACCESS

Edited by:
Jessica Cardin,
Yale School of Medicine, USA

Reviewed by:
Amanda Casale,
University of California, San Diego, USA
Makoto Osanai,
Tohoku University, Japan

****Correspondence:***
Carlos Castejon
castejon.neuro@gmail.com

Received: *11 July 2016*
Accepted: *29 September 2016*
Published: *19 October 2016*

Citation:
Castejon C and Nuñez A (2016) Cortical Neural Computation by Discrete Results Hypothesis. Front. Neural Circuits 10:81. doi: 10.3389/fncir.2016.00081

INTRODUCTION

The cerebral cortex is possibly one of the most complex natural systems. Untangling its intricate functional microcircuit is one of the formidable challenges of neuroscience. However, despite its importance, how cortical computations are performed and the underlying neural mechanisms remain unclear.

ARTÍCULO CIENTÍFICO Nº 3: Higher-order thalamic implication in the codification of bilateral sensory events". Castejon C. and Nuñez A. (2020). bioRxiv. https://doi.org/10.1101/2020.05.01.073098.

Higher-order thalamic implication in the codification of bilateral sensory events

Carlos Castejon* and Angel Nuñez

Department of Anatomy, Histology and Neuroscience, Autónoma de Madrid University, Madrid, Spain

*For correspondence:
castejon.neuro@gmail.com

Abstract

In the rodent whisker system, it is well assumed that VPM and POm encode stimulations of the contralateral whisker pad. However, during tactile exploration usually whiskers are stimulated bilaterally. Accordingly, the integration of tactile information from the two sides of the body seems to be fundamental in the codification of these events. Here, to investigate whether POm could be able to codify these bilateral dynamics, whisker-evoked responses in this thalamic nucleus were examined by *in vivo* extracellular recordings in anesthetized rats using contralateral and ipsilateral stimuli. Strikingly, we found that POm is also able to respond to tactile stimulation of ipsilateral whiskers. Our findings reveal the implication of POm in the representation of bilateral tactile events by integrating simultaneous signals arising from both whisker pads and demonstrate the implication of the higher-order sensory thalamus in the codification of bilateral sensory events. This can have important implications in bilateral perceptual function.

ARTÍCULO CIENTÍFICO Nº 4: Thalamic codification of complex sensory patterns and its possible role in cognition". Castejon C, Martin-Cortecero J and Nuñez A. (2020). bioRxiv. https://doi.org/10.1101/2020.08.19.257667. Actualmente en proceso de revisión y pendiente de aceptación.

Thalamic codification of complex sensory patterns and its possible role in cognition

Carlos Castejon1*, Jesus Martin-Cortecero1,2 and Angel Nuñez1

1 Department of Anatomy, Histology and Neuroscience, Autónoma de Madrid University, Madrid, Spain

2 Institute of Physiology and Pathophysiology, Medical Biophysics, Heidelberg University, 69120 Heidelberg, Germany

*For correspondence:
castejon.neuro@gmail.com

Abstract

The function of the higher-order sensory thalamus remains unresolved. Here, POm nucleus was examined by *in vivo* extracellular recordings across a range of complex sensory patterns. We found that POm was highly sensitive to multiwhisker stimuli involving complex spatiotemporal interactions. The dynamical spatiotemporal structure of sensory patterns and the different complexity of their parts was accurately reflected in precise POm activity changes. Importantly, POm was also able to respond to ipsilateral stimulation and was implicated in the representation of bilateral tactile events by integrating simultaneous signals arising from both whisker pads. We found that POm nuclei are mutually connected through the cortex forming a functional POm-POm loop. We unravelled the nature and content of the messages travelling through this loop showing that they were 'structured patterns of sustained activity'. These structured messages were transmitted preserving their integrated structure. The implication of different cortical areas was investigated revealing that S1 plays a protagonist role in this functional loop. Our results also demonstrated different laminar implication in the processing of sustained activity in this cortical area and its transmission between hemispheres. We propose a theoretical model in which these 'structured patterns of sustained activity' generated by POm may play important roles in perceptual, motor and cognitive functions. From a functional perspective, this proposal, supported by the results described here, provides a novel theoretical framework to understand the implication of the thalamus in cognition. In addition, a profound difference was found between VPM and POm functioning. The hypothesis of Complementary Components is proposed here to explain it.

Los artículos anteriores se han adjuntado en su extensión completa en el apartado Anexos. Aparte de dichos artículos, he colaborado en otra publicación en la que no aparezco como autor principal, es la siguiente:

- ARTÍCULO CIENTÍFICO Nº 5: Frequency-specific response facilitation of supra and infragranular barrel cortical neurons depends on NMDA receptor activation in rats". Barros-Zulaica N, Castejon C, Nuñez A. Neuroscience. 2014; 281:178–94.

Este artículo no está incluido en esta Tesis y por lo tanto no va a ser descrito ni discutido aquí.

Neuroscience 281 (2014) 178–194

FREQUENCY-SPECIFIC RESPONSE FACILITATION OF SUPRA AND INFRAGRANULAR BARREL CORTICAL NEURONS DEPENDS ON NMDA RECEPTOR ACTIVATION IN RATS

N. BARROS-ZULAICA, C. CASTEJON AND A. NUÑEZ*

Departamento de Anatomía, Histología y Neurociencia, Facultad de Medicina, Universidad Autónoma de Madrid, 28029 Madrid, Spain

Abstract—Sensory experience has a profound effect on neocortical neurons. Passive stimulation of whiskers or sensory deprivation from whiskers can induce long-lasting changes in neuronal responses or modify the receptive field in adult animals. We recorded barrel cortical neurons in urethane-anesthetized rats in layers 2/3 or 5/6 to determine if repetitive stimulation would induce long-lasting response facilitation. Air-puff stimulation (20-ms duration, 40 pulses at 0.5–8 Hz) was applied to a single whisker. This repetitive stimulation increased tactile responses in layers 2/3 and 5/6 for 60 min. Moreover, the functional coupling (coherence) between the sensory stimulus and the neural response also increased after the repetitive stimulation in neurons showing response facilitation. The long-lasting response facilitation was due to activation of N-methyl-D-aspartate (NMDA) receptors because it was reduced by APV ((2*R*)-amino-5-phosphonovaleric acid, (2*R*)-amino-5-phosphonopentanoate) and MK801 application. Inactivation of layer 2/3 also blocked response facilitation in layer 5/6, suggesting that layer 2/3 may be fundamental in this synaptic plasticity processes. Moreover, i.p. injection of eserine augmented the number of layer 2/3 neurons expressing long-lasting response facilitation; this effect was blocked by atropine, suggesting that muscarinic receptor activation favors the induction of the response facilitation. Our data indicate that physiologically repetitive stimulation of a single whisker at the frequency at which rats move their whiskers during exploration of the environment induces long-lasting response facilitation improving sensory processing.

Key words: wavelet coherence, LTP, sensory plasticity, somatosensory system, thalamocortical network.

*Corresponding author. Address: Departamento de Anatomía, Histología y Neurociencia, Facultad de Medicina, Universidad Autónoma de Madrid, c/ Arzobispo Morcillo 4, 28029 Madrid, Spain. Tel: +34-91-207-3755; fax: +34-91-397-5338.
E-mail address: angel.nunez@uam.es (A. Nuñez).

Abbreviations: Ach, acetylcholine; APV, (2*R*)-amino-5-phosphonovaleric acid, (2*R*)-amino-5-phosphonopentanoate; LTP, long-term potentiation; MK801, dizocilpine; NMDA, N-methyl-D-aspartate receptor; PSTH, peristimulus time histogram; RF, receptive field; SEM, standard errors of the mean; VPM, ventral posteromedial thalamic.

http://dx.doi.org/10.1016/j.neuroscience.2014.09.057
0306-4522/

INTRODUCTION

The somatosensory barrel cortex is composed of local circuits heavily interconnected by vertical and horizontal projections (Feldmeyer, 2012; Feldmeyer et al., 2013). Sensory information from the whiskers passes via the brain stem and thalamus to layer 4 neurons in the barrel cortex. Sensory responses are relayed to layer 2/3 and then to layer 5 and layer 6, concomitant with feedback from layer 5 to layer 2/3 and layer 6 to layer 4. This vertical organization is linked horizontally by prominent projections within layer 2/3 and layer 5 (Douglas and Martin, 2004; Wester and Contreras, 2012). Distinct synaptic and intrinsic properties of these neurons may be involved in different sensory plasticity responses observed in the barrel cortex. Recently, it has been demonstrated that "N-methyl-D-aspartate (NMDA) spikes" and L-type voltage-gated Ca^{2+} channel activation increase the excitability of layer 5 neurons, thereby possibly mediating neuronal plasticity (Nuñez et al., 2012).

The barrel cortex of rodents is a remarkable structure that is capable of fine tactile discrimination based on whisker movements across objects or surfaces in repeated rhythmic sweeps at frequencies between 4 and 12 Hz (Carvell and Simons, 1990; Fanselow and Nicolelis, 1999), see for review (Moore, 2004). Sensory experience induces neuronal plasticity and has profound effects on synaptic responses in the neocortex. Long-term potentiation (LTP) of cortical synaptic potentials in response to repetitive stimulation is involved in sensory experience effects. For example, tetanic stimuli applied in layer 4 can induce LTP lasting several hours in layer 2/3 neurons (Glazewski et al., 1998). Repetitive whisker stimulation also induces a long-lasting increase in the amplitude of somatosensory-evoked potentials in layers 2/3 and 4 of the barrel cortex of neonatal rats or mice (Borgdorff et al., 2007; An et al., 2012), suggesting that it may participate in the activity-dependent wiring of the cortex during development. Moreover, multiwhisker stimulation at 2 or 8 Hz induces LTP in layers 2/3 and 4 of barrel cortical neurons of mature mice (Megevand et al., 2009), suggesting that sensory plasticity may contribute to information processing in adult animals.

Experiments on the possibility of inducing LTP in sensorially deprived barrel cortex provide further evidence on the role of LTP in cortical experience-dependent plasticity. In young adult rats with intact whiskers the incidence of LTP is relatively low,

4. RESULTADOS

ARTÍCULO CIENTÍFICO Nº 1: Control of somatosensory cortical processing by thalamic posterior medial nucleus: A new role of thalamus in cortical function". C. Castejon, N. Barros-Zulaica, A. Nuñez. (2016) PLoS ONE 11(1):e0148169.

Esta publicación describe una serie de experimentos diseñados para estudiar el funcionamiento del POm y la influencia que ejerce en el procesamiento cortical. Para ello, modificamos sus grados de actividad y estudiamos los efectos que esta manipulación produce en el procesamiento sensorial de la corteza. Son varios los resultados relevantes que encontramos. El primero de ellos, es la capacidad que tiene este núcleo de sostener su actividad para codificar la duración del estímulo somatosensorial (Fig. 5).

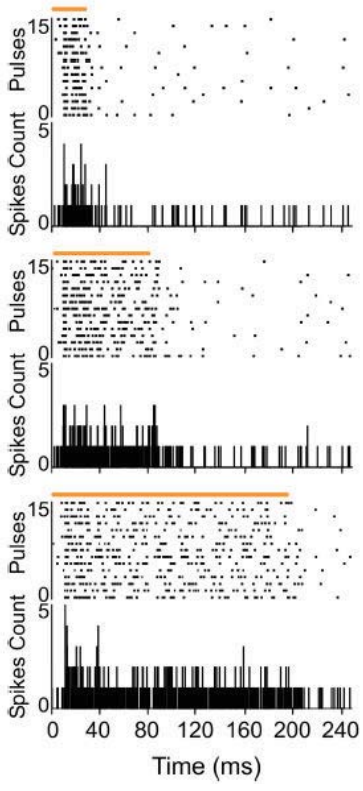


Fig. 5: Actividad sostenida en el POm codificando la duración del estimulo sensorial. Ejemplos representativos de las respuestas del POm a estímulos de diferente duración. Las líneas en color naranja indican la duración de la estimulación sensorial.

Demostramos que esta es una característica propia que lo diferencia del VPM. De acuerdo a estos resultados, proponemos que dichos núcleos juegan papeles funcionales distintos (ver Discusión General).

El segundo lugar, demostramos que la actividad del POm ejerce una gran influencia en el procesamiento de información que tiene lugar en la corteza S1.

Encontramos que sus respuestas sensoriales son controladas por este núcleo. Además, esta modulación no es igual en todas las capas corticales. Estos efectos son especialmente evidentes en la capa supragranular (capa 2/3).

La modulación de las respuestas sensoriales afecta significativamente a su magnitud y duración. Por un lado, la estimulación eléctrica del POm, aplicada en intervalos temporales precisos antes del estímulo sensorial, ocasiona una disminución en el número de disparos de la respuesta sensorial. Así mismo, produce una disminución de su duración.

Estos efectos fueron estudiados, en detalle, usando diferentes intensidades de estimulación eléctrica y diferentes intervalos entre la estimulación eléctrica del tálamo y el estímulo sensorial. Encontramos que la regulación que produce el POm en las respuestas corticales depende de su nivel de actividad. Cuanto mayor sea éste, mayor ajuste inducirá en ellas (Fig. 6).

Por otro lado, la desactivación farmacológica de este núcleo, produce los efectos contrarios. Es decir, ocasiona un aumento de la magnitud y duración de las respuestas. Además, produce un aumento de la actividad espontanea en la corteza.

Estos resultados, en su conjunto, mostraron que el POm tiene la capacidad de ejercer, también, una influencia supresora en la corteza. Este resultado es bastante novedoso puesto que tradicionalmente estaba asumido que este núcleo producía la activación de la corteza.

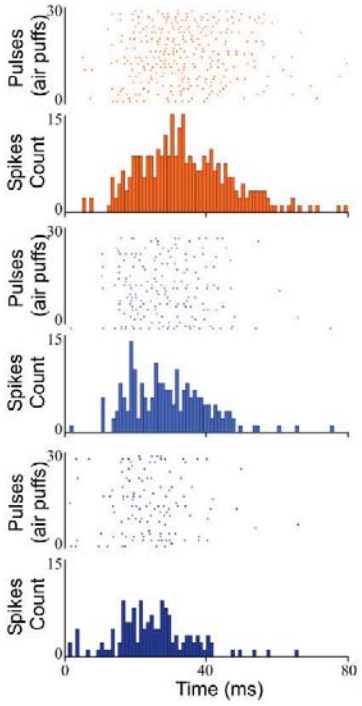


Fig. 6. El grado de actividad del POm determina el ajuste de las respuestas corticales. En estos rasters e histogramas se puede apreciar como la magnitud y duración de las respuestas sensoriales en la capa supragranular de la corteza S1 se reducen a medida que aumentamos el nivel de actividad en el POm (desde el panel superior hacia el inferior).

En tercer lugar, intentamos determinar a través de qué mecanismos ejercía el POm este efecto supresor. Estudios anteriores habían demostrado que la capa 1 de S1 produce una influencia supresora en las respuestas somatosensoriales (Shlosberg y cols., 2006). Puesto que una parte considerable de las proyecciones tálamocorticales de este núcleo terminan en esta capa, era razonable pensar que el POm podría estar produciendo los efectos descritos a través de capa 1. Varios de los experimentos que se describen en este artículo científico están relacionados con la comprobación de esta propuesta. Encontramos que la estimulación eléctrica de esta capa, aplicada antes del estímulo sensorial, producía los mismos efectos que la estimulación del POm. Por otro lado, la desactivación farmacológica del sistema GABAérgico en esta capa eliminaba dicha influencia. Además, esta desactivación ocasionaba el aumento de la magnitud y duración de las respuestas sensoriales. Es decir, los mismos efectos que producía la desactivación del POm. En su conjunto, estos resultados mostraban que el POm ejerce su influencia supresora sobre las respuestas corticales a través de la capa 1.

Además, efectos similares se encontraban cuando se desactivaban, farmacológicamente, los canales de Ca2+ tipo P/Q en la corteza. Esto sugería que los efectos inhibitorios producidos por el POm sobre la corteza eran debidos a la implicación de las interneuronas parvalbumina-positivas (PV).

En último lugar, en este estudio demostramos que el POm también está implicado en el control del procesamiento sensorial en S2. Además, encontramos que la transmisión de información desde capa 5 de S1 a S2 y la influencia que esta capa de S1 ejerce en S2, depende del POm. Esta evidencia confirma resultados descritos previamente *in vitro* (Theyel y cols. 2010).

ARTÍCULO CIENTÍFICO Nº 2: Cortical neural computation by discrete results hypothesis". C. Castejon, A. Nuñez. (2016) Front. Neural Circuits 10:81.

En este artículo proponemos un nuevo marco teórico para explicar cómo se produce el procesamiento optimizado de información en la corteza cerebral. Defendemos que dicho procesamiento optimizado es generado por la computación de unidades funcionales denominadas 'Resultados Discretos'. Describimos cómo son generadas estas unidades funcionales y cómo es la interacción entre los elementos neuronales que las forman. Explicamos el papel fundamental que juegan las interneuronas tipo 'fast-spiking' (FS) en la generación de estas unidades.

Así mismo discutimos las implicaciones funcionales que suponen nuestras propuestas teóricas y describimos la evidencia experimental existente apoyando dichas propuestas.

ARTÍCULO CIENTÍFICO Nº 3: Higher-order thalamic implication in the codification of bilateral sensory events". C. Castejon and A. Nuñez (2020). bioRxiv. https://doi.org/10.1101/2020.05.01.073098.

Este 'preprint' es un adelanto al artículo científico nº 4 en el que se describe la capacidad del POm de responder también a la estimulación de las vibrisas ipsilaterales. Este resultado es bastante novedoso puesto que estaba bien asumido que solo respondía a la estimulación de las

vibrisas contralaterales. Se describe también en este artículo que el VPM, a diferencia del POm, no responde la estimulación ipsilateral.

Además, nuestros resultados demostraron que el POm integra la información sensorial procedente de ambos lados del cuerpo (Fig. 7). Esto tenía especial relevancia en nuestro campo de estudio puesto que demostraba, por primera vez, la implicación de este núcleo talámico en la percepción bilateral.

Anatómicamente, cómo la información ipsilateral llega al POm y por qué circuitos lo hace no se muestran en este artículo.

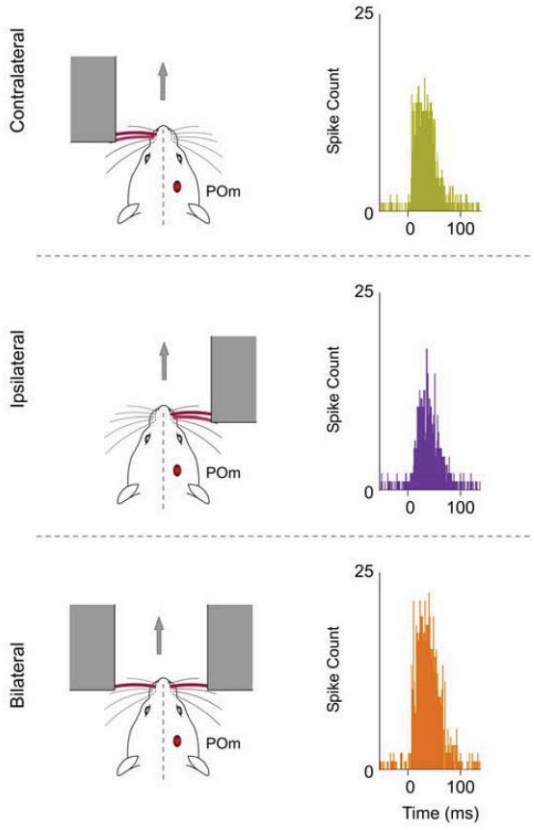


Fig. 7. Implicación del POm en la percepción bilateral. El POm no solo está implicado en el procesamiento de la actividad sensorial procedente de las vibrisas contralaterales (histograma en color verde) sino que también responde a la estimulación de las ipsilaterales (histograma en color violeta). Como puede apreciarse en el ejemplo del panel inferior, sus respuestas se ven incrementadas cuando la estimulación se produce de forma simultánea bilateralmente (histograma en color naranja).

ARTÍCULO CIENTÍFICO Nº 4: Thalamic codification of complex sensory patterns and its possible role in cognition". C. Castejon, J. Martin-Cortecero and A. Nuñez. (2020). bioRxiv. https://doi.org/10.1101/2020.08.19.257667.

En este articulo revelamos los siguientes aspectos importantes relacionados con el funcionamiento del POm en la función perceptiva y su posible implicación en los procesos cognitivos.

En primer lugar, confirmamos que el POm es capaz de producir actividad sostenida que permite codificar la duración de los eventos sensoriales y además mostramos que esta capacidad es muy precisa, incluso ante estímulos de muy breve duración. También demostramos que es capaz de mantener de forma sostenida su actividad, incluso ante eventos sensoriales de larga duración.

En segundo lugar, mostramos que este núcleo se activa más cuando los estímulos sensoriales son más complejos. Demostramos que esto se produce por la integración de señales de las diferentes vibrisas y describimos cómo son esos procesos de integración de señales solapadas que dan lugar a este incremento de actividad talámica. Se desconocían los procesos de integración que soportan esta característica funcional del POm.

En tercer lugar, describimos cómo la capacidad de producir actividad sostenida y la capacidad de integrar información a nivel espacial permiten a este núcleo generar variaciones precisas en su actividad reflejando cambios en la estructura espaciotemporal de los eventos sensoriales.

Es importante destacar que dichas capacidades del POm son propias y no son generadas por influencia de la corteza, sino que siguen presentes cuando ésta es eliminada (Fig. 8)

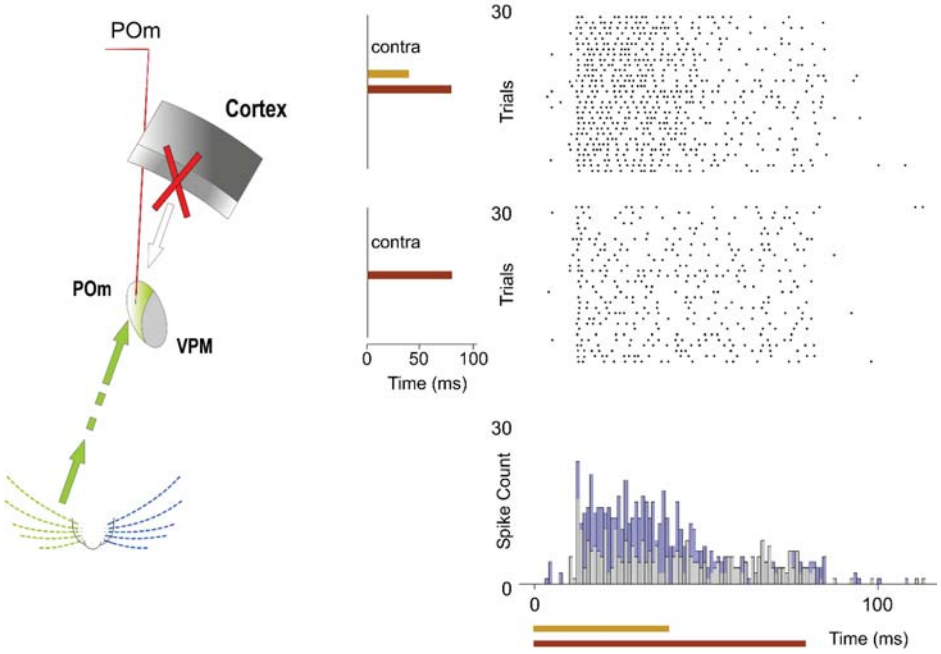


Fig. 8. La capacidad del POm de sostener su actividad codificando la duración del estímulo y su capacidad de integrar señales solapadas no se pierden al eliminar la corteza cerebral. Histogramas y rasters de unas respuestas del POm registradas en dicha condición.

En cuarto lugar, como ya describimos en el artículo anterior, confirmamos que el POm responde también a la estimulación de las vibrisas ipsilaterales. Así mismo, encontramos que la anestesia afecta profundamente al funcionamiento del POm y que sus respuestas ante estímulos ipsilaterales se pierden cuando aumenta el nivel de anestesia. Esto podría explicar por qué dicha capacidad no había sido descrita anteriormente.

En quinto lugar, describimos la implicación de este núcleo en la percepción bilateral. Tiene la capacidad de procesar e integrar las entradas sensoriales producidos por estímulos contralaterales e ipsilaterales. Describimos estos procesos de integración en detalle.

En sexto lugar, mostramos que el VPM no comparte las capacidades descritas en el POm demostrando profundas diferencias entre estos núcleos durante el procesamiento de los eventos sensoriales. Así mismo, sugerimos una propuesta funcional para explicar dicha diferencia (Hipótesis de los Componentes Complementarios, descrita en la Discusión General).

En séptimo lugar, describimos la ruta por la que el POm recibe la información sensorial producida por los estímulos ipsilaterales y los circuitos y estructuras implicadas. Encontramos que esta información es trasmitida desde el otro POm, en el hemisferio opuesto, a través de la corteza cerebral. Mediante el uso de farmacología y lesiones en diferentes áreas corticales, demostramos que en esta vía juega un papel protagonista la corteza S1. Su desactivación o lesión elimina casi en su totalidad la transmisión de información procedente del otro POm.

Así mismo, encontramos que la actividad generada por el POm es transmitida al otro POm manteniendo su estructura integrada. Proponemos el concepto de 'cogtainers' como modelo teórico para dar un posible sentido funcional a este hecho y su posible implicación en los procesos cognitivos (ver Discusión General). Además, demostramos que tanto el contenido como el tipo de actividad que procesa el POm es completamente diferente a la que procesa el VPM (ver la Hipótesis de los Componentes Complementarios en la Discusión General).

Por último, estudiando la implicación de las diferentes capas de S1, encontramos que es la capa infragranular la que está soportando la transmisión de actividad sostenida y su intercambio entre hemisferios.

5. DISCUSIÓN SOBRE LOS RESULTADOS PUBLICADOS

1. El control del POm sobre el procesamiento cortical

Tradicionalmente estaba bien asumido que el POm y los núcleos de tipo similar, producían un efecto facilitador en la corteza (Theyel y cols., 2010; Viaene y cols., 2011; Gambino y cols., 2014; Mease y cols. 2016). Esto hacía que las investigaciones sobre este tema y los resultados obtenidos de ellas tendieran a diseñarse e interpretarse desde esta perspectiva. Por otro lado, el desconocimiento acerca del tipo de neuronas corticales sobre las que contactaban las proyecciones procedentes del POm, hacía difícil que este aspecto anatómico fuera esclarecedor.

En Castejón y cols. (2016) describimos, por primera vez, que el POm tiene la capacidad de controlar de forma contundente y constante la excitabilidad de la corteza y que, en base a ello, impone un control preciso sobre el procesamiento cortical. Esta regulación da lugar a un ajuste en las respuestas sensoriales corticales afectando profundamente a su magnitud y estructura temporal.

Dichos resultados demostraban que la influencia que ejerce el POm sobre la corteza no se limita a inducir activación en ella, sino que es significativamente más compleja. Esto suponía un cambio en la forma de entender la influencia de este núcleo y aportaba una visión más rica de su implicación funcional. Por extensión, dicha perspectiva podría ser aplicada a otros núcleos talámicos similares en otras modalidades sensoriales o implicados en otros aspectos cognitivos.
Así ha ocurrido y los efectos supresores descritos por nosotros en S1 y S2 producidos por el POm, acaban de ser demostrados en el núcleo lateral posterior (LP) y su relación con la corteza en el sistema auditivo (Chou et al. 2020) y recientemente por parte del núcleo pulvinar en el sistema visual (Fang et al. 2020).

2. Ajuste del procesamiento por influencia 'top-down' y 'bottom-up'

Además, nuestros resultados demuestran que este ajuste del procesamiento cortical, basado en los cambios de actividad del POm, no solo se produce inducido por la actividad sensorial

entrante de la periferia ('bottom-up'), sino que dicho ajuste también es inducido por actividad procedente de la corteza. Demostramos que el POm también ajusta el procesamiento en S2 y lo hace en función de la actividad que recibe de S1 (Castejon y cols. 2016; Fig. 9).

Estos resultados tienen especial relevancia a la hora de entender la implicación de este núcleo en procesos cognitivos tales como la atención. Sugieren que la capacidad que tiene el POm de ajustar la forma de procesamiento que se produce en la corteza podría ser inducida tanto por influencia 'bottom-up' como 'top-down', Esto le implicaría como un elemento clave en los procesos atencionales.

De acuerdo con esta idea, dicha capacidad ya ha sido demostrada en otros núcleos similares al POm en otras modalidades sensoriales (Desimone y cols. 1990; Saalmann y cols. 2012).

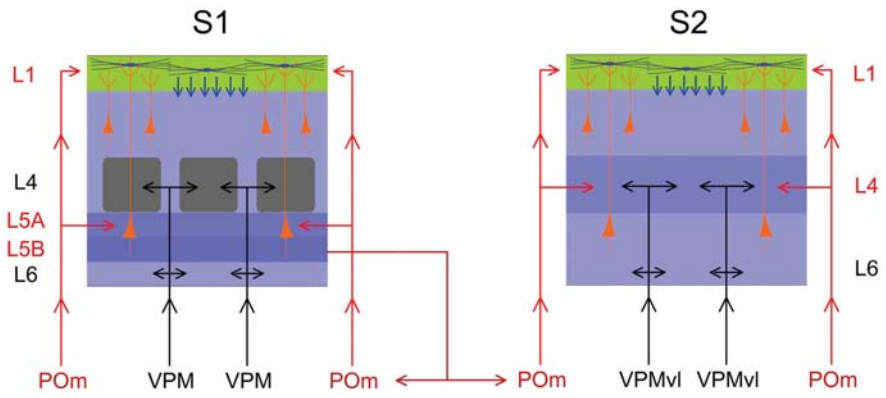


Fig. 9. El POm también está implicado en el control del procesamiento sensorial en S2. Esta regulación está influenciada por las aferencias procedentes de la capa 5 de S1 (Castejón y cols., 2016). Estos resultados confirman *in vivo* experimentos descritos anteriormente *in vitro* (Theyel y cols. 2010).

3. Mecanismos de control cortical

El hecho de que el POm tuviera la capacidad de controlar el procesamiento cortical y de poder ajustar las respuestas sensoriales allí procesadas, incitaba a investigar cuáles podrían ser los mecanismos que utilizaba para realizar dicha influencia. Como ya hemos comentado anteriormente, nuestros resultados demostraban que el POm ejerce su influencia reguladora principalmente a través

de la capa 1 y lo hacía usando al sistema GABAérgico como intermediario necesario en dicha regulación.

En apoyo a este hallazgo, la implicación de la capa 1 en los efectos supresores inducidos por otros núcleos de tipo similar al del POm, ha sido confirmada recientemente en el sistema auditivo y visual (Chou et al. 2020; Fang et al. 2020).

Más concretamente, nuestros resultados sugerían la implicación de las interneuronas PV+ como elementos clave en este proceso. Recientemente nuestras observaciones se han confirmado, puesto que se ha demostrado, que el POm ejerce una influencia directa en la activación de estas interneuronas en S1 (Audette y cols., 2017).

4. La actividad talámica determina el ajuste cortical

Una vez descrita la capacidad del POm de ajustar el procesamiento cortical y el mecanismo que utiliza para realizarlo, estudiamos en base a qué criterio se producía dicho control. Así, en Castejon y cols. (2016), describimos que el ajuste del procesamiento lo determina el grado de actividad de este núcleo. Cuanto mayor era este grado de actividad, con mayor intensidad se producía dicho ajuste.

Nuestros resultados sugerían que el POm transmitiría a la corteza la actividad global del conjunto de vibrisas en tiempo real, de manera que esta información contextual sería la que modularía el procesamiento sensorial en la corteza. Desconocíamos, en aquel momento, cómo este núcleo era capaz de determinar la actividad global que los eventos sensoriales producían en el conjunto de vibrisas. Además, tampoco era conocida la naturaleza de esa actividad resultante y en qué formato era transmitida a la corteza para producir el ajuste anteriormente descrito. Estos interrogantes fueron investigados y han sido descritos recientemente en Castejon y cols. (2020). En dicho artículo científico demostramos que la actividad del POm es la resultante de su integración, en tiempo real, de las diferentes señales procedentes del conjunto de las vibrisas. Así mismo, sabemos que, como resultado de dicha computación, el contenido de los mensajes que transmite este núcleo a la corteza es soportado por actividad neural de naturaleza sostenida e integrada.

5. Complejidad de la estimulación sensorial y flexibilidad de procesamiento

Debido a que la actividad sensorial a la que están expuestos los sistemas sensoriales es compleja y constantemente variable, el procesamiento sensorial debe adaptarse a dicha variabilidad. Además, debe adaptarse a los requerimientos cognitivos o conductuales que exijan las acciones o tareas en curso.

Como ya hemos comentado, se desconocen los mecanismos funcionales que soportan dicha flexibilidad de procesamiento. Los resultados derivados de nuestros experimentos demuestran que el tálamo juega un papel fundamental en dicha flexibilidad.

6. La capacidad de integrar para representar la variabilidad y complejidad sensorial

Primero hay que codificar dicha variabilidad y adaptar el procesamiento en base a dicho resultado. Las observaciones descritas en Castejon y cols. (2020) son consistentes con esta idea. En dicho artículo, mostramos que dicha variabilidad es reflejada en cambios precisos de actividad en el POm. Esta representación está soportada por su capacidad de sostener su actividad y de producir cambios en dicha actividad en función de la integración que realiza de las diferentes señales a lo largo del desarrollo del evento sensorial.

7. Actividad integrada como factor determinante de la flexibilidad del procesamiento cortical

Como hemos comentado, el grado preciso de actividad presente en el POm en un determinado momento determina el ajuste, en tiempo real, del procesamiento cortical (Castejon y cols. 2016). Los cambios de actividad talámica generadas para cada evento sensorial como resultado de los procesos de integración de señales que realiza este núcleo (Castejon y cols. 2020) permiten que dicho evento pueda ser procesado de forma óptima por la corteza. Por lo tanto, este mecanismo talámico permite que se produzca un ajuste del procesamiento sensorial en función de cómo es el estímulo sensorial. Es decir, permite optimizar la flexibilidad de procesamiento. Dichos

cambios precisos en la actividad del POm permiten ajustar, en tiempo real, el procesamiento cortical a este rango tan amplio de posibles estímulos.

8. Implicación de las interneuronas PV+

El ajuste producido por el POm sobre el procesamiento cortical se perdía cuando desactivábamos farmacológicamente los canales de Ca2+ tipo P/Q en la corteza (Castejon y cols. 2016). Este tipo de canales se encuentran ampliamente expresados en los terminales axónicos de las interneuronas PV+ y juegan un papel fundamental en la inhibición que ejercen estas células sobre las neuronas piramidales (Toledo-Rodriguez y cols. 2005; Hefft y Jonas 2005; Zaitsev y cols. 2007). Además, es bien sabido que la eliminación de dichos canales de forma específica en estas interneuronas impide su efecto inhibidor sobre la excitabilidad de las neuronas piramidales (Rossignol y cols. 2013).

El ajuste producido por el POm sobre el procesamiento cortical también se perdía, de forma similar, cuando desactivábamos farmacológicamente el sistema GABAérgico en la corteza (Castejon y cols. 2016). En conjunto, esta evidencia sugería que los efectos inhibitorios producidos por el POm sobre la corteza eran debidos a la implicación de estas interneuronas PV+. Recientemente se ha confirmado que el POm ejerce una influencia directa en la activación de estas interneuronas en S1 (Audette y cols., 2017). Por lo tanto, este tipo de células juegan un rol fundamental en el mecanismo de ajuste que utiliza el POm para regular el procesamiento cortical.

Hemos sugerido (Castejon y Nunez 2016) que estas interneuronas tienen un papel esencial en la generación de unidades funcionales básicas en la computación cortical. En base a ello, el POm tendría una influencia determinante en la generación de dichas unidades y, por lo tanto, en la discretización del procesamiento cortical (ver Discusión General).

Puesto que defendemos que el flujo de información sensorial tiene que ser discretizado para poder ser computado de forma óptima por la corteza (Castejon y Nunez 2016), proponemos que son las fluctuaciones de actividad del POm las que determinan dicha discretización. Esto asigna una nueva función al tálamo y, concretamente al POm y núcleos del mismo tipo, implicándolos en el control de la computación de tipo discreto que ocurre en la corteza.

A esta propuesta la hemos denominado 'efecto discretizador basado en fluctuaciones de actividad talámica' (ver la Hipótesis de los Resultados Discretos en la Discusión General).

9. El POm es muy sensible a las interacciones espaciotemporales en la activación de las vibrisas y a la complejidad del evento sensorial

Estudiamos el funcionamiento de este núcleo usando patrones sensoriales de diferente complejidad (Castejon y cols. 2020). Encontramos que el POm es muy sensible a las interacciones espaciotemporales producidas durante la activación de múltiples vibrisas. Diferentes patrones sensoriales y la diferente complejidad de sus partes constituyentes son reflejados en precisos cambios de actividad en este núcleo. Demostramos que el POm codifica dicha complejidad aumentando su actividad. Por lo tanto, cuanto más simple sea el estímulo, menor será la actividad que produce en él.

Puesto que, mayoritariamente, los estímulos sensoriales usados para estudiar el procesamiento sensorial del sistema de vibrisas han sido estímulos poco complejos, esto puede explicar por qué estaba tradicionalmente asumido que el POm respondía débilmente a la estimulación de las vibrisas (Diamond y col. 1992).

En definitiva, nuestros resultados demuestran que el POm juega un papel relevante en la codificación de estímulos complejos y que, por lo tanto, se requiere suficiente complejidad sensorial para capturar la verdadera dinámica funcional de este núcleo.

10. Producción de solapamientos para extraer información

Acabamos de comentar que el POm es muy sensible a las interacciones y solapamientos espaciotemporales producidas durante la activación de múltiples vibrisas. Desde esta perspectiva, los movimientos intencionados de 'whisking' pueden optimizar el número, la frecuencia y la variación de los solapamientos entre vibrisas activadas para maximizar la extracción de información (por ejemplo, regularidades) de objetos, superficies y texturas durante su exploración.

Se sabe que los roedores utilizan diferentes estrategias de 'whisking' ajustando los movimientos de las vibrisas. Este movimiento está caracterizado por ser de gran amplitud durante la exploración más grosera y por movimientos de pequeña amplitud y frecuencias más altas cuando necesitan aumentar la resolución en la extracción de información detallada.
De acuerdo con esto, encontramos que el POm tiene la capacidad de integrar señales simultaneas de diferentes vibrisas con mucha precisión incluso cuando se utilizaron estímulos de muy corta duración (20 ms). Este hallazgo es compatible con el rango de frecuencias descrito en estos animales: entre 4-12 Hz (movimientos grandes) y 12-25 Hz (movimientos pequeños; Carvell y Simons 1990).

En consecuencia, la generación intencionada de solapamientos precisos de subconjuntos específicos de vibrisas y el ajuste de su frecuencia permitiría a estos animales obtener la resolución óptima necesaria para resolver diferentes requisitos perceptivos.

11. Implicación del POm en la percepción bilateral

Está bien asumido que el POm procesa información sensorial de las vibrisas contralaterales. Nuestros resultados demuestran que este núcleo también recibe y procesa información de las vibrisas ipsilaterales (Castejon y Nunez 2020; Castejon y cols. 2020; Fig. 10). Esto indica que su función perceptiva se basa en la información recibida de ambos lados del cuerpo y supone un cambio en la forma de entender el papel de este núcleo, y del tálamo en general, en percepción.

En su conjunto, nuestras observaciones demuestran la implicación del tálamo en el procesamiento somatosensorial bilateral y sugiere una visión más amplia de su implicación funcional.

Desde un punto de vista neurológico, dichos resultados pueden explicar por qué lesiones unilaterales en el tálamo pueden ocasionar déficits perceptivos bilaterales. Esto es clínicamente relevante para el campo de la neurología. Además, estos hallazgos también pueden explicar por qué la presencia de la estimulación táctil de un lado del cuerpo puede afectar al procesamiento de la estimulación del otro lado (por ejemplo, en paradigmas de interferencia sensorial). Por lo tanto, puede tener cierta relevancia también en el campo de la psicología experimental.

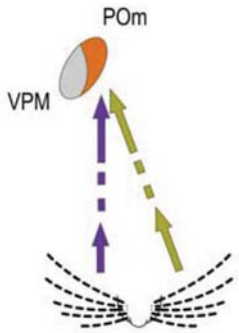


Fig. 10. El POm responde también a la activación de las vibrisas ipsilaterales. El VPM no responde a la estimulación ipsilateral. Esto demuestra importantes diferencias entre estos dos núcleos y sugiere que juegan una función diferente en la percepción bilateral.

12. Comunicación e interacción interhemisférica entre tálamos

En Castejon y Nunez (2020) describíamos, por primera vez, que el POm responde también a la estimulación de las vibrisas ipsilaterales. Inmediatamente surgió la cuestión de por dónde está llegando esa información al POm. De tal forma que las diferentes posibles rutas fueron estudiadas, y posteriormente, descritas en Castejon y cols. (2020). Encontramos que los núcleos POm, en ambos hemisferios, están mutualmente conectados a través de la corteza cerebral formado un 'loop' funcional (Fig. 11).

Estos resultados tienen una serie de implicaciones importantes. La primera es el hecho de que, tradicionalmente, el estudio del tálamo se ha basado fundamentalmente en su relación con la corteza. Sin embargo, la interacción de un tálamo con su opuesto, en el otro hemisferio, ha recibido poca o ninguna atención. Mayoritariamente, han sido estudiados de forma aislada, obviando su posible interacción. Así mismo, el intercambio de información entre ellos seguía siendo un misterio.

Nuestros resultados sugieren una perspectiva más rica, en cuanto a su funcionamiento, al demostrar la comunicación e intercambio de información entre núcleos talámicos a través de la corteza.

También, supone un cambio de perspectiva a la hora de comprender cuál es el destino último de la información sensorial. Por tradición, se considera que la corteza cerebral es

principalmente el destino final de la información sensorial y es allí donde se da uso ha dicha información. Nuestros resultados desafían esta visión jerárquica y sugieren que dicha perspectiva no refleja la verdadera complejidad de los sistemas sensoriales. Demostramos que el POm también recibe información sensorial a través de la corteza, la procesa y la envía codificada a sus destinatarios. Algunos de ellos son áreas corticales, pero otros son subcorticales tales como los ganglios basales (Ohno y cols., 2012; Alloway y cols., 2014; Porrero, 2016). Por lo tanto, estructuras como el estriado, pueden utilizar dicho contenido procesado en el POm para realizar sus funciones sin que dicha información final haya sido generada o refinada en la corteza. Esto revindica un protagonismo mayor al tálamo y revela que la corteza también es una estructura de paso y no siempre un destino final.

En apoyo a esta idea, nuestros resultados muestran que la información sensorial relacionada con las vibrisas contralaterales procede de la periferia, ascendiendo directamente desde ella. Sin embargo, la información sensorial de las vibrisas ipsilaterales procede también de los receptores periféricos, pero llega al POm por una ruta más larga, formada por una parte considerada tradicionalmente como 'ascendente' (desde fuera hacia la corteza) y otra parte considerada 'descendente' (desde la corteza hacia el exterior). Esto supone un cambio a la hora de entender las vías de transmisión en los sistemas sensoriales y sugiere que el funcionamiento de estos sistemas no debe interpretarse, exclusivamente, desde el punto de vista jerárquico tradicionalmente asumido.

13. El bucle POm-POm: los núcleos POm están conectados entre sí a través de la corteza

Nuestros resultados muestran que la actividad sensorial producida por la estimulación ipsilateral llega al POm desde el otro POm. Encontramos que esta información es transmitida por un 'loop' formado por proyecciones talamocorticales, interhemisféricas y corticotalámicas. Confirmamos esta vía interhemisférica inactivando diferentes áreas de la corteza en ambos hemisferios y demostrando que la actividad ipsilateral llega principalmente al POm a través de S1, pero que otras áreas corticales también están implicadas. Esto demostraba que ambos núcleos están conectados entre sí formando una red compleja de proyecciones talamocorticales, interhemisféricas y corticotalámicas paralelas (Fig. 11).

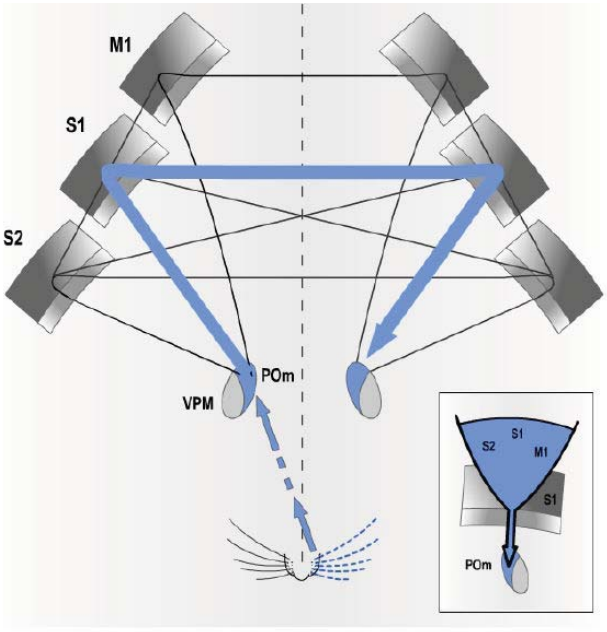


Fig. 11. El bucle POm-POm está formado por una red funcional de proyecciones talamocorticales, interhemisféricas y corticotalámicas paralelas. Nuestros resultados revelaron que S1 juega un papel protagonista en este bucle funcional. El recuadro ilustra la idea de que S1 puede actuar como un 'embudo' que recolecta la actividad de diferentes áreas y envía esta información a través de proyecciones corticotalámicas al POm del mismo hemisferio. Este complejo bucle interhemisférico permite la integración bilateral en el tálamo y está implicado en la transmisión bidireccional de actividad sostenida entre el tálamo y la corteza. Esta transmisión de actividad sostenida puede estar funcionalmente implicada en procesos cognitivos (ver Discusión General).

De acuerdo con este hallazgo, está anatómicamente descrito que MI y S2 también reciben proyecciones talamocorticales del POm (Ohno et al. 2012), que están respectivamente conectadas interhemisféricamente (Carvell y Simons, 1987, Kinnischtzke et al. 2014) y que tienen proyecciones corticotalámicas al POm (Alloway et al. 2008; Liao et al. 2010). Además, sabemos que el POm trasmite actividad sensorial a S2 y a M1 (Theyel et al., 2010; Castejon et al.2016; Casas-Torremocha et al.2019) y también se han descrito respuestas sensoriales bilaterales en S2 (Debowska et al. , 2011).

Sin embargo, es importante tener en cuenta que la actividad ipsilateral remanente que encontramos después de la desactivación de S1 no permite la codificación y representación de la

duración del evento táctil ipsilateral o su estructura espacio-temporal con alta precisión. Esto indica que, aunque diferentes áreas corticales están paralelamente implicadas en el bucle POm-POm, S1 juega el papel protagonista.

Es relevante destacar que este 'loop' interhemisférico entre núcleos talámicos similares al POm puede ser una característica común del tálamo y, por lo tanto, estar presente en otras modalidades sensoriales, así como en otros núcleos implicados en funciones cognitivas.

14. 'Embudo funcional' en S1

Nuestros resultados demostraban que la información sensorial procedente de las vibrisas ipsilaterales era recibida por el POm a través de las proyecciones corticofugales procedentes principalmente de S1 (Castejon y cols. 2020). Esto es coherente con el hecho de que la gran mayoría de las proyecciones corticales desde la capa 5 hacia el POm provienen de S1 (Veinante et al. 2000b; Casas-Torremocha, 2017; Fig. 12).

En base a esta evidencia, propusimos que S1 parece estar actuando como un 'embudo' que recolecta información de diferentes áreas corticales en ambos hemisferios y la transmite al POm (Fig. 11). En apoyo a esta idea, es sabido que, S1 recibe proyecciones intra e interhemisféricas de diferentes áreas corticales entre las que destacan las regiones corticales motora y S2 (Porter y White 1983; Carvell y Simons, 1987; Kinnischtzke et al. 2014).

Además, estas áreas producen una fuerte entrada directa a L6b y L5 en S1 (Mao et al. 2011; Zolnik et al., 2020). También se han confirmado las proyecciones corticofugales de L6b a POm (Bourassa et al.1995; Hoerder-Suabedissen et al.2018). L6b recibe una inervación sustancial de las áreas corticales motoras y sensitivas del hemisferio opuesto (Zolnik et al. 2020). A diferencia del POm, VPM no recibe información cortical de las proyecciones corticofugales de L5 o L6b en S1 (Hoogland et al.1991; Hoerder-Suabedissen et al.2018). En línea con esto, VPM no responde a estímulos ipsilaterales.

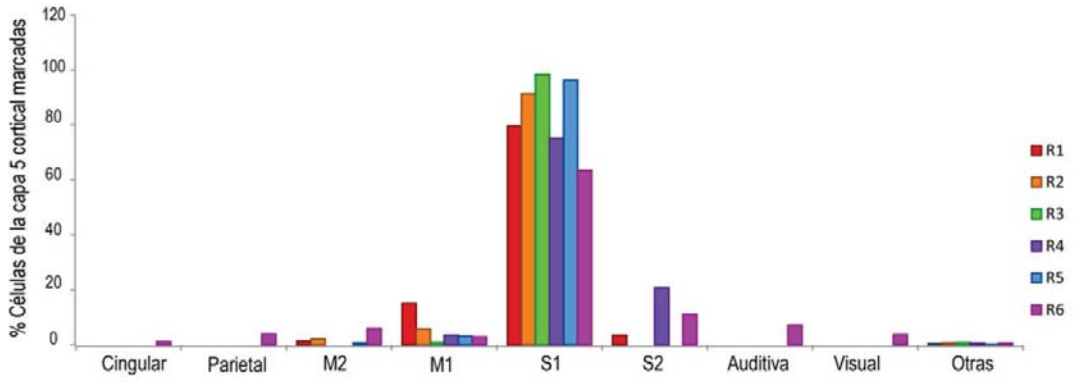


Fig. 12. Casi la totalidad de las proyecciones corticales desde L5 hacia POm provienen de S1. En este grafico se muestran el porcentaje de células marcadas en la capa 5 en cada área de la corteza procedente de seis casos diferentes (R1-R6). Las áreas que mostraban menos del 2% de todas las células marcadas están agrupadas en "Otras". Imagen tomada de Casas-Torremocha, 2017.

15. Integración de señales bilaterales en el tálamo

En Castejon y cols. (2020) además de revelar la implicación del POm en el procesamiento de información sensorial procedente de las vibrisas de ambos lados de la cara, también describimos cómo se produce la integración de ambas señales en este núcleo. Dichos fenómenos de integración bilateral habían sido estudiados en la corteza cerebral, sin embargo, el papel del tálamo era desconocido. Nuevamente, nuestras observaciones aportan un perspectiva más amplia y rica de esta estructura cerebral.

Encontramos que el POm integra constantemente la información sensorial bilateral y que la actividad ipsilateral llega a este núcleo a través del 'loop' POm-POm en el que están implicadas las proyecciones corticofugales de S1.

Nuestras observaciones son coherentes con la evidencia experimental existente. Es bien sabido, que el POm recibe aferencias corticales y subcorticales (Hoogland y cols., 1991; Lavallée y cols., 2005) y que incluso pueden converger en la misma célula talámica (Groh y cols. 2014). Se había descrito que la activación de las proyecciones corticofugales de la capa 5 en S1, por estimulación optogenética, aumenta las respuestas sensoriales ascendentes dentro de una ventana de tiempo concreta (Groh y cols. 2014). Esto sugería una integración de ambas señales en el POm. Sin

embargo, se desconocía qué papel jugaban dichas proyecciones cortifugales, qué contenido trasmitían y los detalles de cómo era procesado dicho contenido en el POm.

En Castejon y cols. (2020) aprovechamos nuestro descubrimiento de que la estimulación sensorial ipsilateral produce la activación de estas fibras corticotalámicas para investigar, en condiciones más fisiológicas, la interacción funcional de las entradas subcortical y cortical usando eventos bilaterales y la implicación de estas proyecciones en el funcionamiento del POm.

En dicho artículo científico, demostramos, por primera vez, la transmisión de actividad sensorial procedente de las vibrisas ipsilaterales a través de las proyecciones corticotalámicas. Además, demostramos que el proceso de integración de la entrada subcortical y cortical es más complejo de lo que se había descrito anteriormente. Describimos en detalle cómo ocurre dicho proceso y demostramos que dicha computación está implicada en la codificación de los estímulos bilaterales. Mostramos, además, que el intervalo temporal entre los estímulos contra e ipsilaterales es crítico para la integración bilateral en el POm de la información sensorial. El retardo (~ 10 ms) que observamos para la información ipsilateral determinó la interacción entre los estímulos bilaterales y el incremento de la actividad del POm por la integración de estos (Fig. 13).

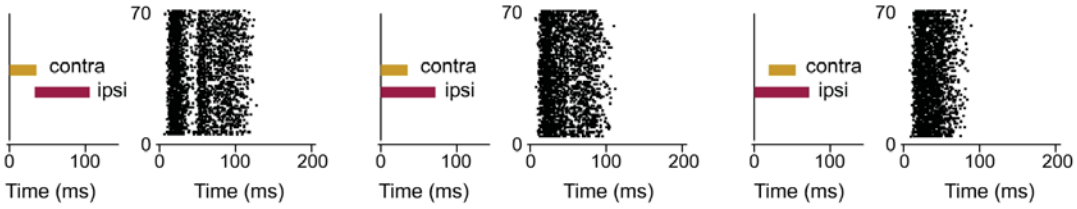


Fig. 13. La diferencia en las latencias de las respuestas evocadas por estímulos contralaterales e ipsilaterales determina la integración bilateral en el POm.

Por otro lado, nuestros trabajos demuestran que la desactivación de la corteza impide la llegada al POm de información procedente de las vibrisas ipsilaterales, pero no de las vibrisas contralaterales (Fig. 14; Castejon y cols. 2020). Dicha eliminación de la corteza impide la integración de señales bilaterales, pero no altera las capacidades de sostener e integrar actividad, presentes en este núcleo (Castejon y cols. 2020).

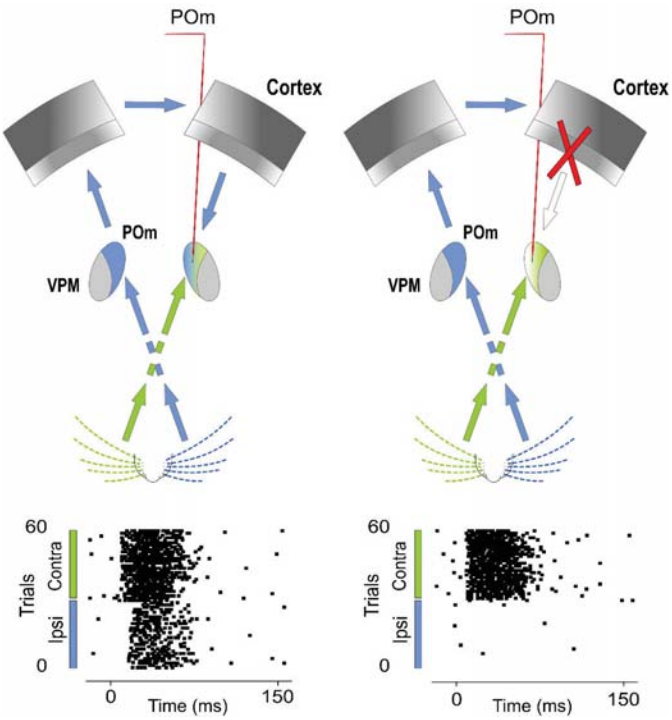


Fig. 14. La eliminación de la corteza impide la llegada al POm de información procedente de las vibrisas ipsilaterales, pero no de las vibrisas contralaterales.

En definitiva, nuestros resultados aportan una visión más global del funcionamiento de estas proyecciones que contactan al POm, del contenido que por ellas se transmite y de cómo dichos contenidos son procesados e integrados en este núcleo. Permiten entender la implicación de dichas proyecciones como pertenecientes a un sistema más global y no como elementos aislados.

16. La actividad sostenida del POm y su transmisión entre estructuras cerebrales

En Castejon y cols. (2016) demostramos que el POm está implicado en el procesamiento y transmisión de actividad sostenida. Este hecho no es compartido por el VPM. En Castejon y cols. (2020) describimos cómo es transmitida dicha actividad a través de las proyecciones talamocorticales, interhemisféricas y corticotalámicas en las que está comprometido este núcleo.

Puesto que está bien asumida la implicación de este tipo de actividad en diferentes procesos cognitivos que tienen lugar en el cerebro (Fuster, 1982; Goldman-Rakic y col. 1987), nuestros resultados implicando al tálamo en el procesamiento y transmisión de este tipo de actividad puede tener repercusiones importantes a la hora de entender dichos procesos (ver Discusión General).

17. Diferente implicación laminar en el procesamiento de actividad sostenida

A pesar de haber sido ampliamente estudiadas, se desconoce, en gran medida, la implicación de las diferentes capas corticales en el procesamiento sensorial. Se conocen profundas diferencias entre las respuestas de las diferentes capas, pero dichas peculiaridades no han sido bien definidas ni clasificadas.

En nuestros trabajos hemos tratado de estudiar la implicación de las diferentes capas de S1 en el procesamiento de información procedente de las vibrisas, así como su relación con los núcleos talámicos de los que reciben proyecciones.

Dicho análisis laminar reveló que las respuestas sensoriales en las distintas capas de S1 tenían diferente estructura temporal (Castejon y cols. 2020; Fig. 15). Solo encontramos respuestas sostenidas a lo largo de la duración del estímulo en la capa infragranular. Sin embargo, no todas las respuestas en esta capa fueron sostenidas. Esto está de acuerdo con la complejidad de esta capa, formada por diferentes subcapas. Las respuestas sostenidas se observaron principalmente en la parte superficial de la capa infragranular. Dicha parte se corresponde con la capa 5.

Además, de manera similar a las respuestas del VPM, las respuestas supra y granulares solo se activaron de manera transitoria al inicio de los estímulos contralaterales, pero no encontramos respuestas sostenidas a lo largo de la presencia del estímulo. Esto está de acuerdo con hallazgos anteriores que muestran respuestas sensoriales con ratios de disparo altos en las neuronas de la capa 5 (de Kock et al., 2007; Castejon et al. 2016) y disparos escasos en capas supragranulares de S1 (de Kock et al., 2007; Petersen y Crochet 2013; Clancy et al.2015; Peron et al.2015).

Nuestros resultados sugieren una implicación funcional diferenciada entre capas (ver apartado Componentes Complementarios en Discusión General).

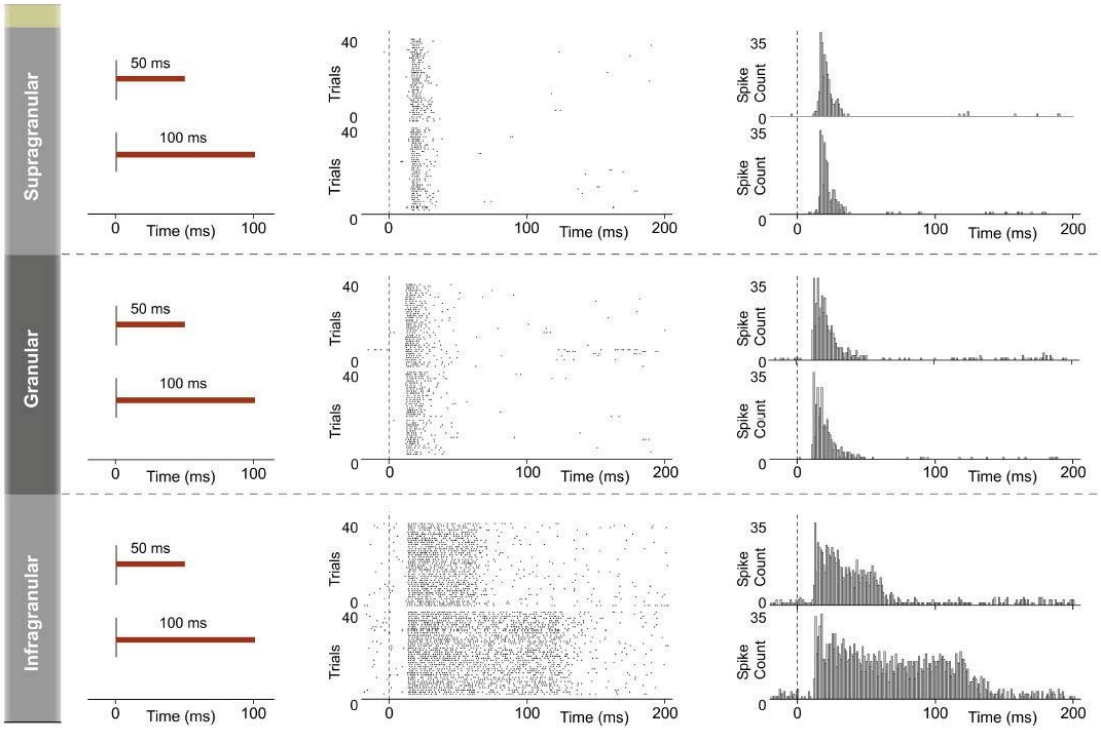


Fig. 15. Las respuestas sensoriales en las distintas capas de S1 tienen diferente estructura temporal. Respuestas sostenidas a lo largo de la duración del estímulo solo se encontraron en la capa infragranular. Las respuestas en las capas supra y granulares solo se activaron de manera transitoria al inicio de los estímulos contralaterales, pero no de manera sostenida a lo largo de la presencia del estímulo (Castejon y cols. 2020).

18. Diferente implicación laminar en la transmisión de actividad sostenida entre hemisferios

Nuestros resultados también demostraron, por primera vez, diferente implicación laminar en la transmisión de actividad sostenida entre hemisferios (Castejon y cols. 2020). Usando estímulos ipsilaterales con diferentes duraciones para examinar la implicación de estas capas en la transferencia interhemisférica de actividad sostenida, encontramos que solo la capa infragranular mostró respuestas sostenidas evocadas por la estimulación ipsilateral. Estos resultados demuestran que la capa infragranular soporta la transmisión de actividad sostenida en el bucle POm-POm (Fig. 16).

El hecho de que, en nuestros experimentos, las respuestas sostenidas se observaran de forma mayoritaria en la parte superficial de la capa infragranular correspondiendo con la capa 5, es coherente con varias observaciones. La primera, con el hecho de que la gran mayoría de las proyecciones corticales desde la capa 5 hacia el POm provienen de S1 (Veinante et al. 2000b; Casas-Torremocha, 2017). La segunda, con el hecho de que es S1 desde donde se transmite al POm, casi en su totalidad, la actividad sostenida procedente del POm opuesto. Esto sugiere un protagonismo especial de esta capa en el procesamiento y transmisión de este tipo de actividad.

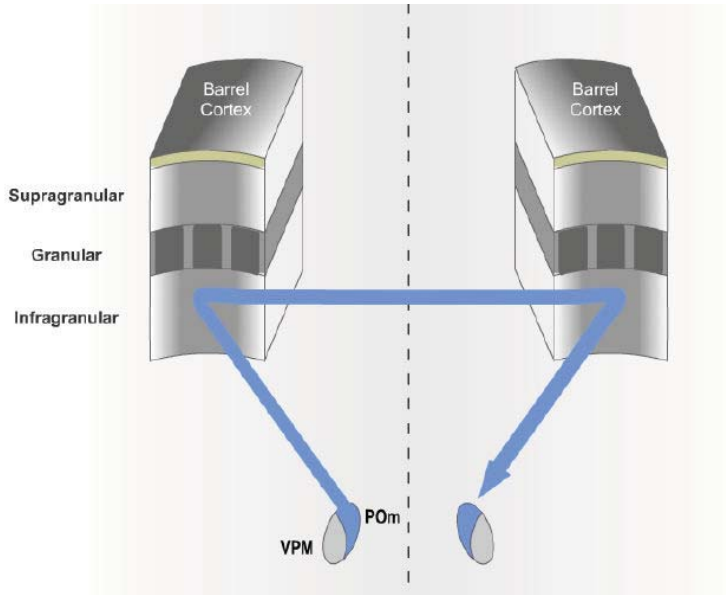


Fig. 16. Implicación de la capa infragranular en la transmisión de actividad sostenida entre hemisferios. Nuestros resultados indican que la capa infragranular de S1 soporta la transmisión de actividad sostenida a través del bucle POm-POm. Esto puede ser una característica básica del funcionamiento cerebral aplicable a otros núcleos talámicos (por ejemplo, el pulvinar en primates) y áreas corticales (por ejemplo, visual primaria).

En resumen, nuestros hallazgos revelan diferentes perfiles laminares de las respuestas corticales y demuestran diferente implicación laminar en el procesamiento de la actividad sostenida y en su transmisión entre hemisferios. Esto podría ser una característica común de las cortezas sensoriales.

19. Transmisión de 'patrones estructurados' a través del 'loop' POm-POm

Nuestros resultados demuestran que la actividad codificada por el POm es transmitida al otro POm manteniendo fielmente su estructura integrada (Fig. 17).

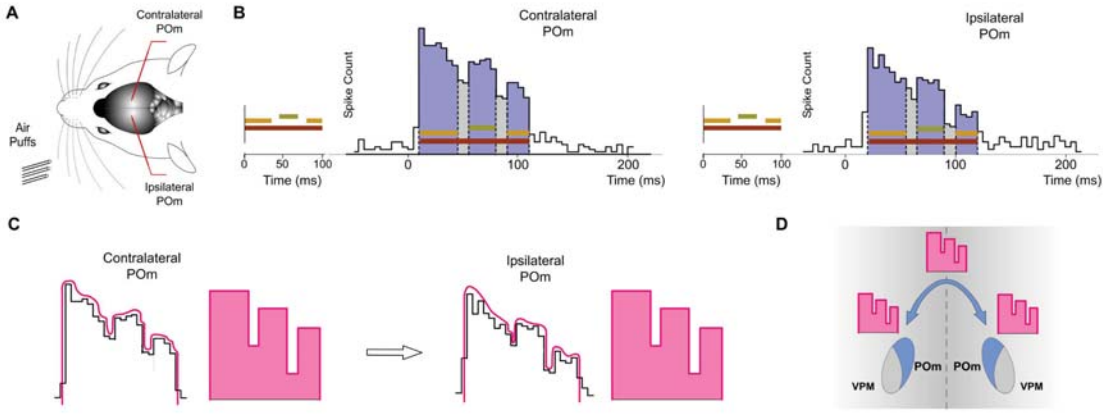


Fig. 17. Transmisión de 'patrones estructurados' a través del 'loop' POm-POm. (A, B) Registrando simultáneamente en ambos tálamos encontramos que los patrones de actividad generada por integración de señales sensoriales en un POm fueron transmitidos al otro POm manteniendo fielmente su estructura integrada (Castejon y cols. 2020). (C) Estos 'patrones estructurados' de actividad talámica (representados en color rosa) pueden jugar un papel esencial en la extracción de información sensorial por parte del tálamo y en la transmisión de contenido con otras estructuras cerebrales.

Las precisas secuencias de fluctuaciones generadas por integración en el POm reflejando la estructura de los patrones sensoriales, fueron detectados en el POm opuesto conservado dicha precisión (Castejon y cols. 2020). Esto indicaba que el contenido de dicho mensaje, transferido entre ellos, podría ser codificado con dicho objetivo.

En base a ello, propusimos el concepto de 'cogtainers' como modelo teórico para dar un posible sentido funcional a este hecho y su posible implicación en los procesos cognitivos (ver Discusión General).

20. Contenido de los mensajes que envía el POm, su influencia y utilización en la corteza

En su conjunto, hemos descrito el contenido de los mensajes que envía el POm a la corteza y el formato en el que lo hace (Castejón y cols. 2020). Además, hemos mostrado que dicho contenido es utilizado para funciones distintas.

Las proyecciones del POm terminan en las capas 5a y 1 en S1 (Ohno y cols., 2012; Porrero., 2016). Nuestros resultados demuestran que la actividad presente en el POm y transmitida a través de sus proyecciones a capa 1, determina el ajuste del procesamiento cortical (Castejón y cols. 2016) y por lo tanto permite su flexibilidad de procesamiento. La transmitida a través de sus proyecciones a capa 5a, parece jugar otras funciones, entre ellas, la de formar parte del circuito por el que se transmite dicho contenido al otro hemisferio y, por lo tanto, sostener la percepción bilateral (Castejon y cols 2020). Estos resultados y las implicaciones derivadas de ellos (ver Discusión General), eran desconocidos antes de nuestros trabajos.

21. Implicación del POm y de su ajuste del procesamiento cortical en los fenómenos de interferencia sensorial en la corteza

Ha sido tradicionalmente bien descrito que cuando varias vibrisas eran activadas de forma simultánea, se producía mayoritariamente una reducción en sus correspondientes respuestas corticales. Este efecto de tipo supresor producido por dichas interacciones ha sido ampliamente confirmado (Simons 1985; Higley y Contreras 2003; Hirata y Castro-Alamancos 2008). Sin embargo, a pesar de que este fenómeno de interferencia sensorial se ha estudiado a lo largo de varias décadas y ha acaparado la atención de muchos investigadores en nuestro campo, el mecanismo que soporta dicho efecto ha permanecido desconocido. Los resultados descritos en Castejon y cols. (2016) y Castejon y cols. (2020) permiten ahora explicar cómo se produce dicho efecto y también definir el mecanismo neural implicado.

La integración por parte del POm de las diferentes señales solapadas producidas por la activación simultanea de varias vibrisas, ajusta el procesamiento cortical produciendo cambios en la estructura temporal de las respuestas sensoriales, afectando también a su magnitud y duración. Este

efecto hace que cuando las vibrisas son activadas de forma conjunta, sus correspondientes respuestas corticales se vean afectadas y atenuadas en su magnitud y duración por dicho ajuste inducido por el POm.

Apoyando esta explicación, el efecto supresor producido por la activación conjunta de las vibrisas ocurre fundamentalmente y de forma más intensa en la capa supragranular (capa 2/3) y, sin embargo, es poco significativa en la capa granular (Hirata y Castro-Alamancos 2008). Como hemos comentado antes, el ajuste inducido por el POm no es igual en todas las capas corticales. Sus efectos son especialmente evidentes en la capa supragranular (capa 2/3). La magnitud y duración de las respuestas sensoriales en esta capa se ven significativamente reducidas por dicho ajuste (Castejon y cols. 2016). Además, hemos demostrado que estos efectos son producidos a través de la capa 1. Las respuestas de la capa granular no se ven significativamente afectadas por dicho efecto al no tener sus neuronas dendritas apicales alcanzando la capa 1.

Dicha evidencia, en su conjunto, apoya la implicación del POm y su influencia en la corteza como explicación de estos fenómenos de interferencia sensorial en la corteza. Esto tiene especial relevancia a la hora de entender los procesos atencionales que tradicionalmente han sido estudiados usando dichos fenómenos de interferencia.

22. Diferencias entre VPM y POm

A lo largo de nuestros artículos científicos describimos las profundas diferencias encontradas entre los núcleos talámicos VPM y POm (Castejón y cols., 2016; Castejón y Nunez 2020; Castejón y cols., 2020). Algunas de estas diferencias ya eran conocidas como, por ejemplo, la diferencia en cuanto a la precisión de su somatotopía. Otras han sido descritas, por primera vez, en nuestros artículos.

Entre ellas cabe destacar la capacidad del POm de mantener su actividad de forma sostenida durante la presencia de los eventos sensoriales (Castejón y cols., 2016) y la capacidad de responder a la estimulación de las vibrisas ipsilaterales (Castejón y Nunez 2020). Dichas capacidades no están presentes en el VPM. Además, describimos la capacidad del POm de integrar las señales procedentes del conjunto de las vibrisas (Castejón y cols., 2020).

Tomadas juntas, dichas diferencias, ahora conocidas en mayor medida, sugieren implicaciones funcionales distintas. En base a ellas, hemos sugerido varias propuestas para explicar su posible implicación funcional (ver Discusión General).

23. Contenido 'segregado' versus 'integrado'

Ya hemos comentado, que al contrario de la del VPM, la somatotopía del POm es difusa y escasamente precisa. Poco era conocido, hasta ahora, de la posible implicación funcional de dicha característica. Nuestros resultados aportan nuevo conocimiento sobre este tema, demostrando que el contenido que surge del procesamiento realizado por el POm, hace referencia al conjunto de las vibrisas y a la interacción entre ellas (Castejón y cols., 2020). Demostramos que este núcleo genera este resultado por integración de las diferentes señales procedentes de todo el conjunto de las vibrisas. Por lo tanto, el hecho de que sus neuronas respondan a la activación de un gran número de estas, es esencial para la realización de su papel funcional. Además, demostramos que esta integración incluye información sensorial procedente de ambos lados del cuerpo (Castejón y cols. 2020).

Estas observaciones evidencian la profunda diferencia en cuanto al contenido que procesan y transmiten estos núcleos talámicos. En el VPM, el contenido relacionado con cada vibrisa se mantiene segregado y asilado del resto. Además, el contenido que procesa y transmite solo procede de las vibrisas contralaterales. Por el contrario, el contenido del POm, es integrado, conteniendo información de todas las vibrisas en ambos lados de la cara. En base a dicha discrepancia, hemos diferenciado y denominado a estos tipos distintos de contenido como 'segregado' versus 'integrado' (ver la Hipótesis de los Componentes Complementarios en la Discusión General).

24. Nueva clasificación talámica

Aparte de la diferenciación en cuanto al contenido, nuestros resultados también demuestran que el tipo de la actividad neuronal procesada y transmitida por el POm y el VPM es diferente (sostenida versus transitoria). Dichas diferencias determinan su implicación funcional y pueden usarse para clasificarlos.

Nuestra propuesta de los Componentes Complementarios puede contribuir a explicar funcionalmente esta dicotomía (ver la Hipótesis de los Componentes Complementarios en la Discusión General). Uno de los Componentes es 'estructurado' (POm), soportado por actividad neural sostenida e integrada y de contenido, también, integrado. Mientras que otro Componente es 'discreto' (VPM), soportado por actividad transitoria y segregada y de contenido, asimismo, segregado.

Dicha distinción puede estar presente en otros sistemas sensoriales. En base a ello, aquellos núcleos con características similares al POm, formaría parte del tipo 'estructurado'. Aquellos similares al VPM, lo serían del tipo 'discreto'.

Además, dicha distinción podría aplicarse al tálamo en su conjunto. La distinción entre 'estructurado' y 'discreto' puede aplicarse a los diferentes núcleos que componen esta estructura independiente de su implicación funcional (perceptiva, motora, cognitiva, etc.).

25. Anestesia y funcionamiento del POm

Se ha demostrado experimentalmente que durante la vigilia o bajo una sedación leve, la actividad del POm es significativamente mayor que durante los estados de anestesia profunda (Masri y cols. 2008; Sobolewski y cols. 2015; Zhang y Bruno 2019). Esto sugiere que el funcionamiento normal de este núcleo está significativamente alterado por la anestesia.

Nuestros hallazgos están de acuerdo con esta idea. Por un lado, encontramos que la capacidad del POm de sostener su actividad durante la presencia del estímulo sensorial está afectada por la anestesia. Dosis suplementarias de uretano redujeron fuertemente o incluso eliminaron la actividad sostenida en el POm. Además, encontramos que su capacidad de integrar señales procedentes de las diferentes vibrisas también se encuentra disminuida.
Por otro lado, las respuestas del POm a la estimulación ipsilateral solo se encontraron a partir de las 5 o 6 horas después de la aplicación del uretano (1.3 - 1.5 g / kg i.p.). Además, observamos que dosis suplementarias eliminaron estas respuestas una vez encontradas. Esto indica que la transmisión de la actividad sensorial entre hemisferios a través del 'loop' POm-POm también es afectada por la anestesia. De acuerdo con esto, se ha demostrado previamente que el aumento del nivel de sedación produce la eliminación de las respuestas a la estimulación ipsilateral en S1

(Armstrong-James y George, 1988). Este hecho podría explicar por qué las respuestas del POm a la estimulación ipsilateral no se habían descrito previamente.

En conjunto, nuestras observaciones muestran que la anestesia reduce la actividad sostenida y su transmisión interhemisférica entre estructuras cerebrales. Esto puede tener implicaciones importantes en los procesos cognitivos superiores como la consciencia.

En cuanto al POm, esta evidencia indica que altos niveles de anestesia deterioran la dinámica real de funcionamiento de este núcleo.

26. Limitaciones de nuestros trabajos y resultados

Se ha descrito la existencia de diversas zonas dentro del POm diferenciadas por las proyecciones que reciben (Groh y cols. 2014). Actualmente, el asunto de la complejidad de este núcleo, especialmente a nivel funcional, sigue siendo controvertido y nuevas investigaciones parecen necesarias a la hora de aclararlo.

Nuestros registros se han centrado principalmente en la parte dorsolateral del POm donde mayoritariamente aparecen representadas las vibrisas. No formaba parte de nuestros objetivos estudiar la heterogeneidad de este núcleo ni delimitar detalladamente sus diferentes posibles subáreas. Sin embargo, cierta información relevante puede extraerse de nuestra investigación. Las respuestas sostenidas fueron encontradas a lo largo de su distribución rostro-caudal. Lo mismo ocurrió con su capacidad de integrar señales de diferentes vibrisas, incluidas las ipsilaterales.

Sería interesante estudiar estos fenómenos en otras partes del cuerpo como, por ejemplo, la representación de las extremidades, y conocer si esas zonas comparten estas habilidades. Es posible que la capacidad de procesar y transmitir actividad sostenida sea una característica general de este núcleo. Otras capacidades puede que no. Por ejemplo, funcionalmente, parece razonable que los procesos de integración bilateral estén optimizados para determinadas partes del cuerpo y reducidos o inexistentes para otras partes. Pendiente queda investigarlo.

Además, es necesario conocer la anatomía detallada de las proyecciones de las diferentes neuronas que forman el circuito interhemisférico del 'loop' POm-POm. Este conocimiento podría ser aplicable a otros posibles 'loops' similares en otras modalidades sensoriales. Pendientes quedan también estas tareas.

En nuestros trabajos demostramos una distinción en cuanto al tipo de actividad neural que procesan y transmiten las vías lemniscal y paralemniscal. Sería interesante investigar los mecanismos que soportan dicha diferenciación.

Por otra parte, está bien asumida la influencia de los diferentes neuromoduladores sobre el funcionamiento del tálamo. Concretamente, se ha descrito previamente que las respuestas del POm son afectadas significativamente por dicha influencia (Masri et al., 2006). Es necesario estudiar esta modulación más ampliamente.

En línea con lo anterior, la zona incerta (ZI) proyecta al POm y ejerce cierta regulación sobre él (Barthó y cols., 2002). Pendiente queda, por tanto, examinar los efectos que estas influencias pueden ejercer sobre las capacidades talámicas aquí descritas.

Así mismo, el POm recibe proyecciones de otras estructuras cerebrales. Entre ellas, podemos destacar al colículo superior (SC; LeDoux y cols., 1987). Siguiendo la línea de nuestros resultados (Castejon y cols. 2016), recientemente se ha descrito que el SC puede ejercer regulación sobre la corteza utilizando al POm como intermediario (Gharaei y cols. 2020). Sería interesante estudiar esta interacción en más detalle. Además, en nuestros trabajos sugerimos la posibilidad de que una parte de la actividad que recibe el POm proveniente de las vibrisas ipsilaterales puede estar recibiéndola del SC a través de la comisura colicular (Castejon y cols. 2020), dicha posibilidad queda por ser confirmada.

Por otro lado, dado que el POm también proyecta a otras estructuras cerebrales como los ganglios basales o la amígdala (Ohno et al. 2012; Alloway y cols., 2014; Porrero, 2016), sería interesante estudiar cómo es la influencia de este núcleo sobre ellas. Puesto que estas estructuras son inervadas por las mismas neuronas que proyectan a la corteza (Fig. 18) y teniendo en cuenta nuestros resultados sobre la transmisión de información desde el POm hacia ella (Castejón y cols. 2016; Castejón y cols. 2020), es razonable pensar que el contenido, su formato y el tipo de la actividad que lo soporta sería el mismo. Esto queda pendiente de ser confirmado.

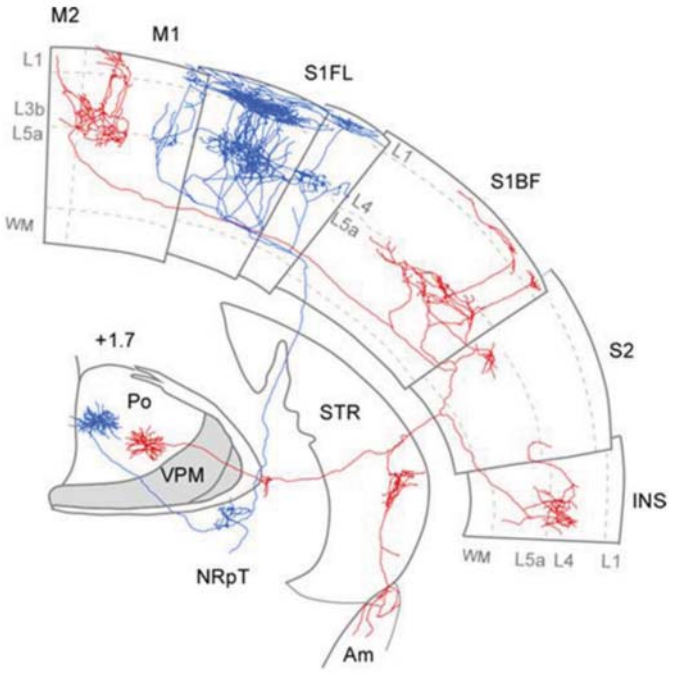


Fig. 18. El estriado y la amígdala son inervados por colaterales de los axones de las mismas mismas neuronas que proyectan a la corteza. Am: Amígdala; INS: corteza insular; NRpT: Núcleo reticular pretalámico; S1BF: Corteza somatosensorial primaria, zona de barriles; S1FL: Corteza somatosensorial primaria, región de la extremidad anterior; STR: Estriado. Imagen tomada de Porrero, 2016.

Otro aspecto funcionalmente muy relevante pendiente de investigar es la influencia del POm sobre las otras áreas corticales a las que proyecta, así como la interacción funcional e intercambio de contenido con ellas. Puesto que nuestros resultados y propuestas sugieren una nueva interpretación de la implicación del POm más allá de dicha función perceptiva, especial relevancia podría tener en estos fenómenos la implicación del POm en la codificación y generación de contenido con fines cognitivos y ejecutivos como, por ejemplo, aquellos relacionados con procesos atencionales, de reconocimiento y familiaridad o toma de decisiones. Nuestro concepto de 'cogtainers' podría tener relevancia a la hora de entender su implicación en dichos procesos. Por ejemplo, las proyecciones de este núcleo con la corteza ectorhinal (Ohno et al. 2012; Porrero, 2016), podrían estar implicadas en la transmisión de estos 'cogtainers' con fines cognitivos.

Desde esta nueva perspectiva, el estudio de la implicación del POm más allá de su función perceptiva supone un cierto reto a la hora de su diseño experimental. Este camino a recorrer pasa por estudiar su implicación funcional durante la realización de tareas que pongan en evidencia su aportación a dichos procesos. Esto requiere inevitablemente, su estudio en animales despiertos y expuestos a dichas exigencias funcionales.

En nuestros trabajos, la aplicación de patrones sensoriales tan precisos como los utilizados en Castejon y cols. (2020) son difícilmente realizables en animales despiertos. El control y reproducibilidad de este tipo de estimulación estarían enormemente afectados. Puesto que, como demuestran nuestros trabajos, el POm juega un papel fundamental en la codificación de patrones complejos y en la transmisión del contenido resultante de dicha codificación, esto supone un gran reto a la hora de estudiar la implicación funcional de dichos contenidos en animales no anestesiados.

6. DISCUSIÓN SOBRE LAS IMPLICACIONES FUNCIONALES

Tal y como argumentamos en la Introducción, un reto fundamental para la neurociencia actual, es la necesidad de aumentar nuestro conocimiento a nivel funcional de los diferentes procesos cerebrales, de los componentes que los soportan y de los mecanismos que utilizan para generar dichas funciones. En general, carecemos de explicaciones y marcos teóricos que nos permitan interpretar, dentro de una perspectiva funcional, la implicación de las diferentes vías, circuitos, estructuras y núcleos, áreas y capas corticales, así como de las neuronas y demás células que los forman. Mucho se ha incrementado, en los últimos años, el conocimiento científico sobre ellos, fundamentalmente sobre su anatomía y fisiológica y especialmente a nivel celular y molecular, donde el grado de detalle alcanzado es espectacular. Sin embargo, esto no ha ido acompañado con un desarrollo teórico que permitiera asociar, interpretar y dar sentido a la cantidad de datos experimentales que este campo de la ciencia genera constantemente. Esta escasez de teorías y propuestas funcionales es común en todos los niveles de interpretación en el estudio del cerebro y sus funciones mentales.

A lo largo de nuestra investigación hemos tratado de proponer explicaciones que permitieran una mejor interpretación de las observaciones que íbamos encontrando. Como puede apreciarse en nuestros artículos científicos, los resultados experimentales descritos en ellos han ido, siempre, acompañados de propuestas funcionales basadas en dicha evidencia experimental.

En esta Discusión General, vamos a comentar brevemente algunas de estas propuestas y modelos teóricos relacionándolas con nuestras observaciones experimentales.

1. Extracción de información y codificación de patrones sensoriales

Tal y como hemos descrito en apartados anteriores, los resultados derivados de nuestras investigaciones contribuyen a poder entender mejor cómo el cerebro procesa el flujo de actividad sensorial que recibe y cómo extrae y codifica dicho contenido informativo. Estos resultados destacan la implicación del tálamo en dicho proceso y aportan detalles relevantes sobre la computación que allí tiene lugar.

a. Fluctuaciones de actividad en el POm para codificar patrones

Usando sus vibrisas, los roedores tienen la capacidad de localizar, identificar y discriminar formas, objetos y otras dinámicas sensoriales en su entorno con una precisión muy alta. Nuestros resultados sugieren que el tálamo puede jugar un papel fundamental en dicha capacidad. En Castejon y cols. (2020) demostramos que el POm está implicado en el procesamiento y codificación de patrones sensoriales complejos. Mostramos que este núcleo es muy sensible a la activación simultanea de las vibrisas y a las complejas interacciones que se producen entre ellas. Demostramos su implicación en la codificación de dichas dinámicas y describimos cómo se produce dicha codificación. Mostramos que es su capacidad de sostener su actividad a lo largo de la duración del evento sensorial, junto con su capacidad de integrar las señales sensoriales procedentes del conjunto de las vibrisas, lo que le permite generar una representación de los eventos sensoriales basada en su estructura espacio-temporal. Dicha estructura y la variante complejidad de sus partes son reflejados en cambios precisos de actividad. En base a esto, dichas fluctuaciones o secuencias de actividad generados por el POm puede ser considerados 'patrones estructurados de actividad talámica integrada'.

A nivel funcional, las fluctuaciones de actividad integrada pueden ser usadas para detectar referencias espacio-temporales en el flujo continuo de actividad sensorial. Podrían, por tanto, usarse para detectar los límites de una secuencia, permitir la extracción de regularidades y patrones de dicho flujo. Además, estas fluctuaciones, también pueden servir como guías en el ajuste sensoriomotor, el reconocimiento de patrones, la discriminación perceptiva y la toma de decisiones, entre otras funciones.

b. POm como codificador general de patrones

A lo largo de nuestros protocolos de estimulación sensorial, encontramos que el POm genera las mismas secuencias de fluctuaciones en su actividad cuando se aplica un mismo patrón sensorial, pero activando diferentes vibrisas en cada ocasión. Este hallazgo está de acuerdo con la somatotopía poco precisa de este núcleo y tiene especial relevancia, puesto que sugiere que la función de la integración producida por el POm, no es la representación combinada de vibrisas específicas, sino la codificación de patrones genéricos del conjunto de ellas. Esto sugiere que el POm es un codificador general de patrones.

c. El papel de los núcleos sensoriales secundarios en la codificación de patrones sensoriales

Nuestros resultados sugieren que la codificación de patrones sensoriales complejos puede ser también una función típica de los núcleos similares al POm en otras modalidades sensoriales. Su implicación funcional puede permitir, a dichos sistemas, la extracción de patrones y regularidades del flujo constante de actividad sensorial entrante. Esta propuesta queda pendiente de ser confirmada también en otros sistemas sensoriales.

2. 'Patrones estructurados de actividad integrada'

a. Transmisión de 'patrones estructurados de actividad integrada'

La actividad sostenida se ha relacionado tradicionalmente con procesos cognitivos como la memoria de trabajo (Fuster, 1982; Goldman-Rakic y col. 1987). Sin embargo, aún se desconoce cómo se genera, mantiene y transfiere esta actividad. Además, aunque la comunicación intra- e interhemisférica durante los procesos perceptivos y cognitivos está bien asumida, el intercambio de actividad sostenida entre estructuras cerebrales es actualmente poco entendido.

Hasta ahora, no se habían realizado estudios que definieran la comunicación de actividad sostenida entre núcleos talámicos en ambos hemisferios. Teniendo en cuenta la relevancia del tálamo y de la actividad sostenida en los procesos cognitivos, dicha investigación toma especial interés.

En nuestros trabajos hemos estudiado dicha comunicación a través del `loop` POm-POm, investigando la naturaleza y el contenido de la actividad transmitida por las proyecciones talamocorticales, cortico-corticales y corticotalámicas que lo forman (Castejon y cols. 2020). Mostramos que se produce un constante intercambio de actividad sostenida entre estos núcleos y además demostramos que dicha actividad sostenida tiene un carácter estructurado como resultado de los procesos de integración que tienen lugar en el tálamo. Mostramos que las fluctuaciones en la actividad sostenida generadas en este núcleo por la integración de señales sensoriales, se transmiten,

a través de estos circuitos intra- e interhemisféricos, manteniendo la estructura resultante de dicha integración (Fig. 17). Esta actividad 'estructurada' representa contenido sensorial codificado por el POm.

Proponemos que este mecanismo de codificación y transmisión de contenido puede ser una característica funcional básica, no solo del POm, sino de otros núcleos y estructuras cerebrales.

En su conjunto, nuestro trabajo aporta nuevo conocimiento sobre cómo se codifica la información sensorial y cómo se transmite entre estructuras cerebrales. Además, sugiere una visión más compleja a la hora de entender el formato de la actividad neural implicada en los procesos perceptivos y cognitivos (ver la Hipótesis de los Componentes Complementarios descrita más adelante).

b. Uso de 'patrones estructurados de actividad integrada' como 'plantillas funcionales'

Tradicionalmente, el estudio de los sistemas sensoriales se ha centrado mayoritariamente en estudiar cómo éstos procesan la información sensorial entrante y prestando poca atención a cómo luego puede ser utilizada y en qué formato se hace. Por otro lado, el estudio de la función cognitiva se ha producido, mayoritariamente, de forma focalizada en procesos cognitivos concretos y normalmente asilados del resto del funcionamiento cerebral. Esta falta de visión conjunta ha limitado enormemente nuestro conocimiento sobre cómo es la interacción entre los diferentes procesos perceptivos y cognitivos, cómo comparten información y en qué formato se transmite y utiliza.

Nuestros resultados muestran que el POm juega un papel fundamental en la codificación de información sensorial del conjunto de las vibrisas mediante la generación de cambios precisos de actividad talámica. Ya hemos descrito que estas fluctuaciones de actividad constituyen 'patrones estructurados de actividad integrada' (Fig. 19). Además, hemos propuesto que estos patrones de actividad talámica permiten la extracción de información relevante para que pueda ser utilizada en los procesos cognitivos que requieran dicha información. En base a ello, en Castejon y cols. (2020) propusimos que dichos 'patrones de actividad estructurada' podrían transmitirse y usarse como

'plantillas' funcionales. Es decir, que los patrones de actividad estructurada codificados por el POm pueden ser unidades funcionales básicas para la percepción y extracción de información (Fig. 20).

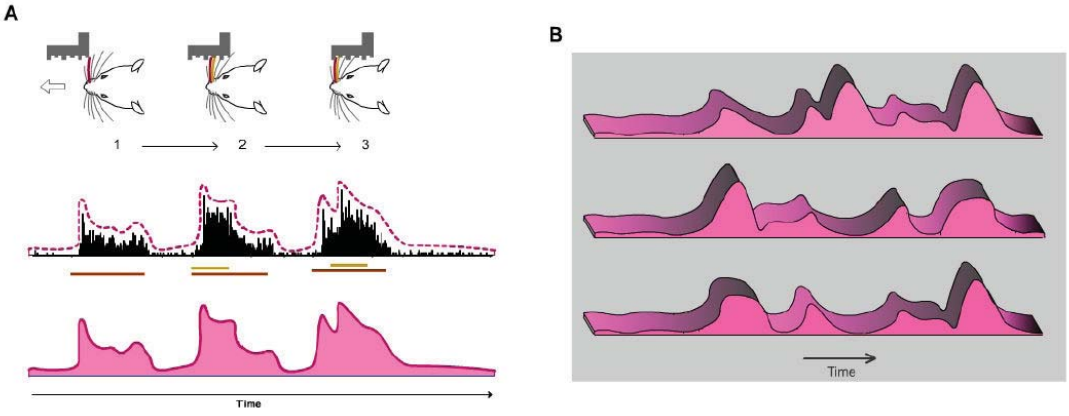


Fig. 19. Implicación talámica en el proceso de extracción de patrones de información sensorial en bruto y su posible relevancia en diferentes funciones cerebrales. (A) Durante la exploración táctil de objetos o superficies, se producen secuencialmente diferentes superposiciones o solapamientos espacio-temporales entre vibrisas. La codificación de estas interacciones por el POm genera secuencias o patrones de fluctuaciones de actividad talámica (1→2→3 en el ejemplo ilustrado aquí). En base a ello, funcionalmente, los diferentes patrones sensoriales son representados con precisión por sus correspondientes 'patrones estructurados de actividad integrada' en el POm. Algunos ejemplos simulados se muestran en (B).

Estos patrones integrados pueden jugar otros roles funcionales más allá de su implicación en percepción. Entre ellos, podrían permitir la conversión de información sensorial en representaciones funcionales relevantes para tareas cognitivas actuales o futuras. Es decir, podrían seleccionarse, adquirirse y usarse como 'plantillas' también para funciones cognitivas. En base a ello, hemos propuesto que esos patrones sean llamados 'cogtainers' cuando sean utilizados para dichas funciones (Castejon y cols. 2020). Esta propuesta funcional proporciona un modelo teórico novedoso para comprender la implicación del tálamo en la percepción y la cognición.

Desde esta perspectiva, como ya hemos comentado, una función importante de los núcleos talámicos sensoriales secundarios podría ser la generación de dichas unidades perceptivas extraídas

del flujo sensorial y convertidas en unidades funcionales que podrían usarse para los requisitos cognitivos actuales o futuros.

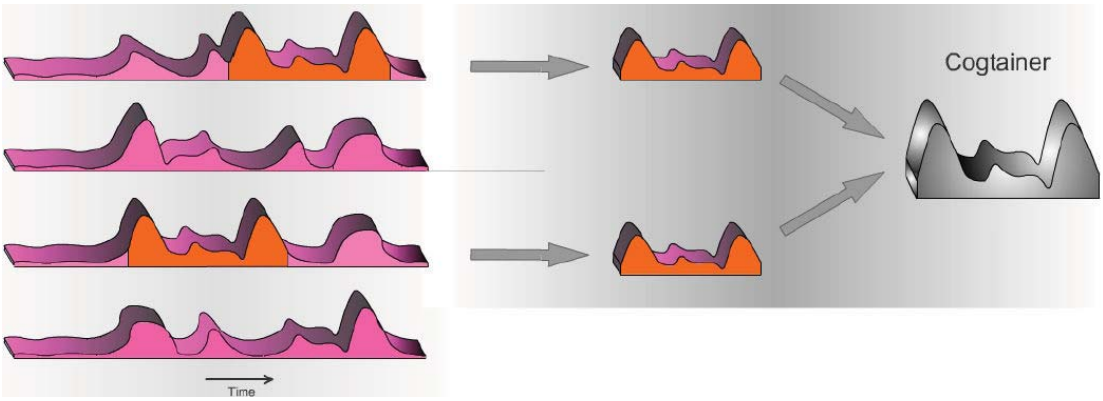


Fig. 20. La codificación de patrones sensoriales por parte del POm puede proporcionar un mecanismo para detectar señales espacio-temporales, puntos de referencia o límites que permitan la extracción de regularidades y patrones funcionalmente relevantes del flujo continuo de actividad sensorial. Desde una perspectiva funcional, los patrones seleccionados de actividad estructurada (representados en naranja) podrían extraerse y usarse como 'plantillas' para diferentes funciones cerebrales. Aquí, proponemos que cuando estas unidades funcionales se utilizan para requisitos cognitivos, podrían denominarse 'cogtainers'.

c. Un mismo contenido para diferentes funciones

Varias implicaciones funcionales surgen de la propuesta descrita en el apartado anterior. La primera hace referencia al hecho de que las neuronas del POm tienen axones talamocorticales 'multiespecíficos' (Clasca et al.2016) que inervan varias áreas corticales, incluidas S1, S2, M1 y M2, con diferentes perfiles laminares. En base a esto, nuestros resultados sugieren que los mismos 'patrones estructurados de actividad integrada' generados por el POm pueden ser enviados en paralelo a diferentes destinos corticales. Por lo tanto, estas áreas y capas corticales pueden usar el mismo mensaje para diferentes funciones (por ejemplo, perceptiva en S1-S2 y motora en M1-M2).

En segundo lugar, dado que el POm también proyecta a otras estructuras cerebrales, incluida la amígdala, los ganglios basales, la corteza insular o ectorhinal (Ohno et al. 2012), nuestros resultados sugieren que estas estructuras pueden usar las mismas 'plantillas' generadas por

el POm para diversas funciones, tales como la discriminación perceptiva, su relevancia para la toma de decisiones, su significado motivacional o su reconocimiento y familiaridad.
Esto indica que el POm, no solo codifica estas 'plantillas' funcionales, sino que puede distribuirlas directamente a un conjunto amplio de destinatarios.

La existencia de neuronas con características anatómicas y funcionales similares a las del POm en otras partes del tálamo puede permitir esta capacidad funcional en otros núcleos.

3. Representación de contenido durante los procesos cognitivos

a. 'Cogtainers' para funciones cognitivas

Se desconoce actualmente cómo el contenido de los procesos cognitivos es representado y en qué formato está codificado para poder ser procesado. En Castejon y cols. (2020) propusimos que la transmisión de 'cogtainers' a través de los diferentes circuitos cerebrales (ej. circuitos talamocorticales, corticocorticales y corticotalámicos) soporta el acceso y uso de información durante los procesos cognitivos. Estas unidades permitirían la representación y el mantenimiento de información en las diferentes estructuras que los reciben. Desde una perspectiva funcional, esta propuesta proporciona un modelo teórico nuevo para entender la implicación del tálamo, incluyendo el POm, en la cognición.

La interacción entre la corteza (ej. la corteza prefrontal) y el tálamo juega un papel fundamental en la función cognitiva. Defectos en los núcleos secundarios en el tálamo producen graves déficits cognitivos. Proponemos, como hemos comentado antes, que otros núcleos talámicos con características anatómicas y funcionales similares a las del POm pueden jugar un papel esencial en los procesos cognitivos y ejecutivos mediante la generación y transmisión de cogtainers. De hecho, se han descrito respuestas talámicas que sostienen representaciones prefrontales durante diferentes procesos cognitivos (Bolkan et al., 2017; Guo et al., 2017; Schmitt et al., 2017; Rikhye et al. 2018). Proponemos que estos efectos podrían ser la consecuencia de la transmisión de patrones de actividad 'estructurada' por los núcleos talámicos a sus destinatarios. El nuevo concepto de cogtainers podría usarse para explicar funcionalmente estos hallazgos.

4. Hipótesis de los Resultados Discretos

Como hemos comentado anteriormente, las neuronas no funcionan de forma aislada, sino que lo hacen coordinadas en grupos y redes de las que emerge la capacidad de computar. El estudio de dichas asambleas funcionales de neuronas está acaparando, especialmente a nivel cortical, un interés creciente en la investigación. Sin embargo, se desconoce cómo estas asambleas de neuronas están implicadas en la función computacional de la corteza. Sigue siendo un misterio cómo se forman y cómo afecta su dimensión espacial y temporal a su funcionalidad.

Por otro lado, el dilema sobre si el procesamiento cortical es continuo o discreto ha vuelto a tomar mucha relevancia en los últimos años (VanRullen y Koch, 2003; Buschman y Miller, 2010).

Ambos temas han sido abordados por nuestra parte, como queda patente en nuestros trabajos. Hemos propuesto la hipótesis de los Resultados Discretos que surge como un intento de aportar una nueva interpretación de dichos fenómenos. Así mismo, los resultados experimentales obtenidos a lo largo de nuestra investigación también pueden ser interpretados desde esta propuesta teórica. Dicha interpretación es descrita a continuación.

5. Modelo de discretización del procesamiento cortical en base a fluctuaciones de actividad talámica

a. Fluctuaciones de actividad en el POm para discretizar el procesamiento cortical

En nuestra hipótesis de los 'Resultados Discretos' hemos sugerido que el procesamiento avanzado de información en la corteza se produce por computación discreta. En base a ello, esta hipótesis sugiere que la información sensorial necesita ser discretizada para poder ser procesada de forma óptima por la corteza cerebral (Castejon y Nunez. 2016). Esto da lugar a secuencias de Resultados Discretos. Dichas secuencias son el mecanismo que utiliza la corteza para realizar su procesamiento de información y es el mecanismo utilizado para extraer, codificar y transmitir contenido.

Proponemos que las fluctuaciones en la actividad del POm pueden jugar un papel determinante en este proceso. Nuestros resultados experimentales demuestran que los eventos sensoriales son representados por secuencias de fluctuaciones en la actividad del POm (Castejon et al. 2020). Por lo tanto, estos patrones de actividad talámica parecen ser la base sobre las que se generan las secuencias de Resultados Discretos en la corteza.

En apoyo a esta propuesta, hemos demostrado que los cambios de actividad del POm determinan de forma muy precisa el procesamiento cortical afectando principalmente a su dimensión temporal (Castejon et al. 2016).

Proponemos, en base a ello, que las fluctuaciones de actividad permiten la óptima discretización del input sensorial. Cambios precisos en la actividad del POm, producidos por la integración de señales, determinan de forma precisa cuándo se debe discretizar el flujo de procesamiento cortical.

Este mecanismo permite ajustar, en tiempo real, el procesamiento discreto en función de las características de la actividad sensorial entrante. Diferentes patrones de actividad en el POm generarán diferentes secuencias de Resultados Discretos y por lo tanto diferentes contenidos.

Desde una perspectiva funcional, este modelo de discretización del procesamiento cortical en base a fluctuaciones de actividad talámica, aporta un nuevo rol a este núcleo. Además, dicho mecanismo funcional sería aplicable a otros núcleos talámicos de similares características. Cambios precisos en la actividad de estos núcleos determinarían el procesamiento discreto en sus correspondientes cortezas. A nivel general, este modelo funcional podría ser denominado ‘efecto discretizador basado en fluctuaciones de actividad talámica’ (Fig. 21).

En resumen, nuestra propuesta de discretización del procesamiento cortical aporta una nueva implicación funcional a este tipo de núcleos talámicos, a su relación con la corteza y con la computación que en ella tiene lugar.

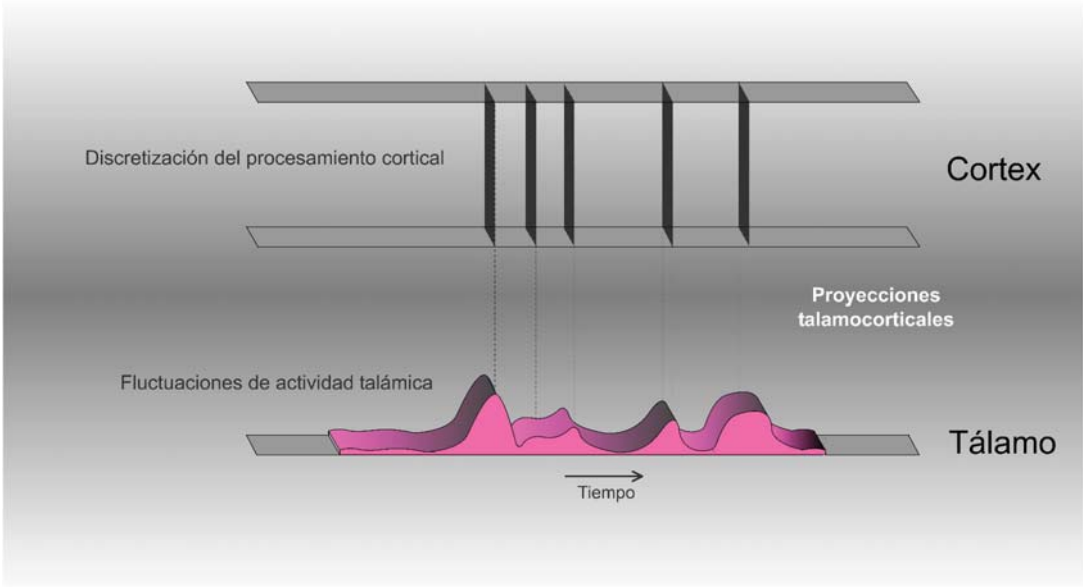


Fig. 21. Fluctuaciones de actividad talámica para discretizar el procesamiento cortical. Patrones de actividad talámica producidos por integración de señales determinan de forma precisa cuándo se debe discretizar el flujo de procesamiento cortical.

b. Fluctuaciones de actividad en el POm y oscilaciones corticales

Se ha demostrado a nivel experimental, la implicación de los núcleos de orden superior en la regulación de los ritmos en la corteza cerebral. Nuestra propuesta de discretización del procesamiento cortical en base a fluctuaciones de actividad en determinados núcleos del tálamo permite explicar dicha observación. La estructura temporal del procesamiento cortical se ve afectada por este proceso y así puede observarse en las oscilaciones neurales que caracterizan la computación cortical.

c. Implicación de las interneuronas PV+ en la discretización

Las interneuronas PV+ también conocidas como 'fast-spiking' por su tipo de funcionamiento, son uno de los elementos comunes que aparecen implicados en nuestros resultados y propuestas teóricas. Por un lado, nuestra hipótesis de los 'Resultados Discretos' propone que son ellas las que soportan la computación discreta y las que forman las unidades funcionales de ese tipo

de computación en la corteza (Castejon y Nunez 2016). Por otro lado, son los elementos clave que utiliza el POm para controlar el procesamiento cortical (Castejon y col. 2016). Por lo tanto, jugarían un papel fundamental en el proceso de discretización del procesamiento cortical basado en las fluctuaciones de actividad talámica.

En apoyo a esta propuesta, es bien sabido que dichas neuronas juegan un papel clave en el control de la estructura temporal del procesamiento cortical y en la generación de los ritmos asociados a dicho procesamiento en la corteza (Traub y cols. 1996; Sohal y cols. 2009; Cardin y cols. 2009).

6. Hipótesis de los Componentes Complementarios

Son conocidas, cada vez en mayor medida, las conexiones anatómicas de los núcleos talámicos con sus correspondientes áreas corticales. Sin embargo, es también fundamental, conocer cómo es su relación a nivel funcional. Por otro lado, es necesario tener conocimiento de cuál es la naturaleza y el formato de los mensajes que entre ellos se intercambian y de cómo son codificados para ser transmitidos y recibidos de forma adecuada. Estos aspectos eran poco conocidos antes de nuestros trabajos.

La investigación que hemos desarrollado en el sistema de vibrisas y los resultados derivados de ella, aportan un nuevo conocimiento acerca del formato de dichos mensajes y de la actividad neural que los soporta. En base a dichos resultados, propusimos la hipótesis de los Componentes Complementarios. Dicha propuesta puede ser también aplicada a otros sistemas sensoriales.

a. Componentes Complementarios

Tal y como se describe en el conjunto de artículos publicados, nuestros resultados muestran profundas diferencias en el funcionamiento de los dos diferentes núcleos que componen las vías ascendentes implicadas en el procesamiento y transmisión de información sensorial de las vibrisas en los roedores. En base a estas observaciones, ahora sabemos que el contenido y el formato de los

mensajes que se procesan y transmiten, en cada uno de estos núcleos y sus correspondientes proyecciones, es diferente.

A lo largo de nuestros resultados, descritos en los diferentes artículos científicos, se observaron diferencias importantes entre los modos de respuesta del VPM y del POm. Describimos, por primera vez, que el POm permanece activo de forma sostenida durante la presencia del evento sensorial (Castejon y cols. 2016). Sin embargo, el VPM solo lo hace, de forma transitoria. Esto indicaba que el efecto de la duración del estímulo en la respuesta era totalmente diferente para los dos núcleos. El tipo de actividad que procesan, por lo tanto, era distinta. El VPM se caracteriza por una actividad transitoria, mientras que la del POm es sostenida (Castejon y cols. 2016). Esta diferenciación afecta a su implicación funcional.

Además, no encontramos cambios significativos en las respuestas del VPM por la aplicación de estímulos complejos, activando múltiples vibrisas, respecto a la estimulación de dichas vibrisas de forma aislada. Esto, está de acuerdo con hallazgos anteriores que muestran que la respuesta del VPM a la activación simultánea de varias vibrisas es muy similar a la activación individual de esas vibrisas (Aguilar y Castro-Alamancos 2005). Esto indica que la información relacionada con cada vibrisa tiende a permanecer segregada con respecto a la de las demás. Sin embargo, nuestros resultados muestran que el POm se activa más cuando los estímulos aumentan su complejidad y que juega un papel fundamental en la representación de estos eventos táctiles complejos. Ahora sabemos que dichos efectos son generados gracias a su capacidad de integrar información de diferentes vibrisas (Castejon y cols. 2020). Ya hemos comentado anteriormente que, en base a esta diferencia fundamental, el contenido que procesa y transmite el VPM puede ser denominado como ‘segregado’, mientras que el del POm puede ser definido como ‘integrado’. Esta diferenciación afectaría también a su implicación funcional.

Además, a diferencia del POm, el VPM no responde a estímulos ipsilaterales (Castejon y Nunez 2020). Dado que la integración de la información táctil de los dos lados del cuerpo es fundamental en la percepción bilateral, nuestros resultados sugieren una implicación diferente de estos núcleos en esta función.

Como ya hemos comentado, se desconocía el formato en el que está codificado el contenido que computan estos núcleos. Así mismo, las peculiaridades de la actividad neural que soporta dicho contenido tampoco estaban bien caracterizadas.

En nuestros artículos definimos el tipo (‘sostenida’ versus ‘transitoria’) y el contenido (‘integrado’ versus ‘segregado’) de la actividad neuronal procesada y transmitida por estos núcleos (Castejon y cols. 2016, 2020). En base a dicho conocimiento, desconocido antes de nuestros trabajos, propusimos la hipótesis de los Componentes Complementarios (Castejon y cols. 2020) para interpretar funcionalmente dicha diferenciación. Uno de los Componentes es ‘estructurado’, soportado por actividad neural sostenida e integrada y de contenido, también, integrado. Otro Componente es ‘discreto’, caracterizado por actividad transitoria y segregada. Su contenido es, asimismo, segregado.

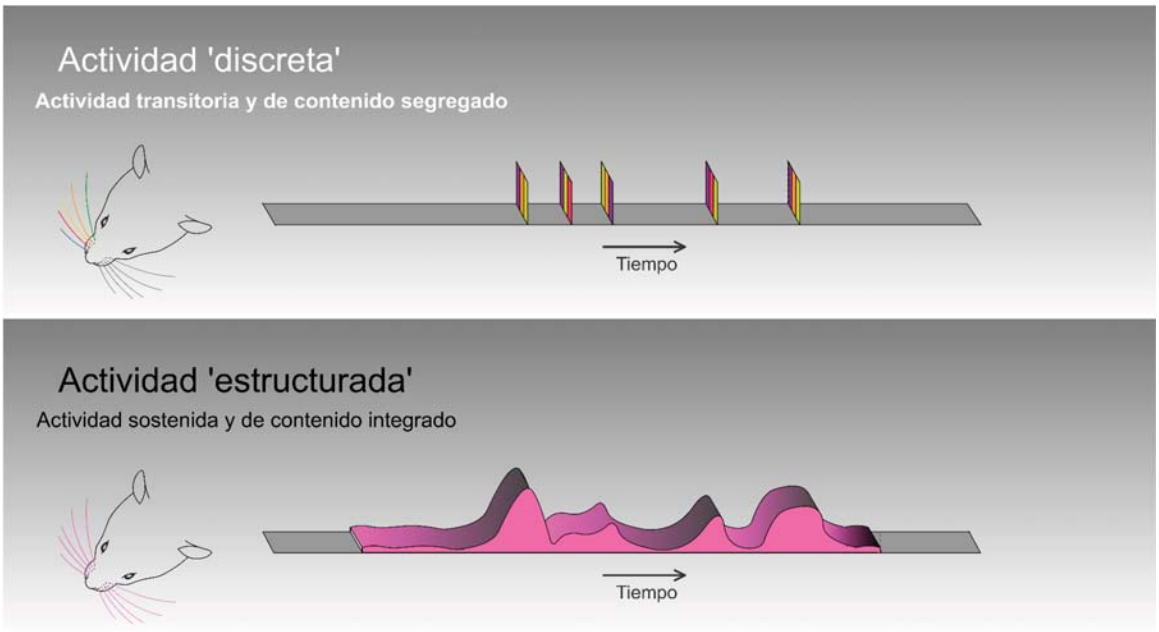


Fig. 22. Actividad ‘estructurada’ versus actividad ‘discreta’. La actividad ‘estructurada’ del POm está formada por fluctuaciones de actividad sostenida e integrada relacionada con el conjunto de las vibrisas. La actividad ‘discreta’ en el VPM está formada por elementos transitorios y segregados relacionados con vibrisas concretas.

b. Roles funcionales diferentes pero complementarios

Como hemos descrito anteriormente, la información sensorial de las vibrisas es procesada y transmitida a la corteza a través de varias vías ascendentes paralelas (Diamond et al., 1992; Veinante et al., 2000a). En su conjunto, nuestros resultados muestran que las respuestas del VPM son diferentes de las respuestas del POm y sugieren diferencias funcionales significativas entre estos dos núcleos talámicos. Además, encontramos que dichas diferencias también están presentes, a nivel del trigémino, entre diferentes subnúcleos (Castejón y cols. 2016) y a nivel cortical, entre diferentes capas (Castejón y cols. 2020). Esto demostraba una dicotomía clara entre las vías lemniscal y paralemniscal y sugería funcionalidades distintas en cada una de ellas.

Puesto que más vías han sido diferenciadas dentro del sistema de vibrisas (Fig. 3), es razonable pensar que más 'componentes' pueden estar 'complementariamente' implicados en la extracción optimizada de información sensorial en este sistema.

c. Componentes Complementarios en los sistemas sensoriales

Los resultados, descritos en nuestros artículos y en esta Tesis, demostrando diferencias importantes, pero funcionalmente complementarias entre el POm y el VPM, están de acuerdo con la hipotésis funcional de los Componentes Complementarios.

Nuestros resultados muestran que patrones sensoriales similares evocan secuencias de fluctuaciones de actividad en el POm también similares. Dicho efecto ocurre incluso cuando diferentes vibrisas son activadas usando el mismo patrón sensorial. Esto sugiere que la función de este núcleo no se basa en una precisa somatotopía sino en la extracción de patrones generales. Por lo tanto, proponemos que para optimizar la extracción de información del flujo sensorial y complementar el papel del POm como un codificar general de patrones, este Componente debe ser complementado con otro Componente adicional que aporte la precisa somatotopía de la que carece. Según nuestra propuesta, este puede ser el papel funcional del sistema lemniscal. Dicho sistema es filogenéticamente más reciente que el paralemniscal y está caracterizado por tener una somatotopía muy precisa (Diamond 1995; Simons 1995).

Este funcionamiento por Componentes Complementarios puede ser una característica común en la mayoría de los sistemas sensoriales.

d. Optimización en la extracción de información por Componentes Complementarios

La propuesta funcional de los Componentes Complementarios puede explicar por qué la información táctil de las vibrisas se procesa por vías ascendentes paralelas hacia la corteza. Esta arquitectura paralela también está presente en la mayoría de los sistemas sensoriales en el cerebro y se conserva en todos los animales (Sherman y Guillery 2006). En base a ello, proponemos que los sistemas sensoriales han evolucionado para optimizar la extracción de información del entorno y que la aparición de vías 'complementarias' (como la vía lemniscal somatosensorial) durante la evolución fue esencial en esa optimización funcional.

A nivel general en los diferentes sistemas sensoriales, la aparición, a lo largo de la evolución, de nuevos Componentes aportaba un valor funcional añadido, basado en la optimización y mejora del proceso de obtención de información del entorno. Puesto que dicho proceso es vital para la supervivencia, su optimización supone una ventaja fundamental.

e. Componentes Complementarios en la corteza cerebral

Además, nuestros resultados demuestran un procesamiento laminar distinto del mismo estímulo por parte de la corteza. Muestran que el contenido, el tipo y la naturaleza de los mensajes que reciben, procesan y transfieren estas capas es diferente (Castejon y cols. 2020). Por lo tanto, los diferentes Componentes también están asociados con distintos perfiles laminares. Allí, también pueden jugar roles funcionales diferentes pero complementarios. Esto podría explicar los diferentes perfiles de actividad que describimos en las capas corticales de S1 (Castejon y cols. 2016, 2020).

f. Componentes Complementarios en el funcionamiento cerebral

Nuestros resultados indican que estos Componentes son transmitidos de forma independiente a lo largo de las diferentes proyecciones y conexiones cerebrales. Esto sugiere que el

funcionamiento por Componentes Complementarios podría ser un principio básico del cerebro y que, en base a ello, la separación de Componentes en rutas diferenciadas puede ser una característica esencial en los circuitos cerebrales. Por lo tanto, dicha segregación de Componentes tiene que tenerse en cuenta a la hora de estudiar las diferentes estructuras cerebrales, las áreas y capas corticales, los circuitos y redes que las conectan y los mensajes que se intercambian entre estos elementos.

En apoyo a esta propuesta, en Castejon y cols. (2020) demostramos que estos Componentes son transmitidos de forma independiente entre los hemisferios cerebrales. Mostramos que el Componente de actividad 'estructurada' es transmitido entre los tálamos a través de un circuito interhemisférico complejo pero diferenciado (Fig. 11). Demostramos, además, que dentro de S1, el área cortical protagonista en dicho circuito, solo su capa infragranular está implicada en transmisión dicho Componente (Fig. 16).

En general, lo anterior indica que los Componentes Complementarios pueden ser una característica general del funcionamiento cerebral. Por lo tanto, a la hora de entender el procesamiento de información que tiene lugar en el cerebro, debemos tener en cuenta la separación de Componentes.

7. EVALUACIÓN DE LOS OBJETIVOS PLANTEADOS

Formaba parte de nuestros objetivos aumentar el conocimiento sobre la implicación de las diferentes estructuras que componen las vías ascendentes del sistema de vibrisas, así como sobre la relación e influencia que mantienen entre ellas. En base a ello, hemos estudiado tanto los núcleos principal (PrV) y espinal (SpV) en el complejo trigeminal, como los núcleos VPM y POm en el tálamo, como las cortezas somatosensoriales S1 y S2. Por lo tanto, se ha estudiado y obtenido datos a lo largo de diferentes vías y de sus diferentes niveles. Esto ha dado lugar a que dichos datos pudieran ser comparados e interpretados de forma conjunta, permitiendo entenderlos desde una perspectiva de sistema global. Esto no es lo habitual en el campo de la neurociencia en el que existe la tendencia a investigar y describir de forma aislada aspectos concretos respecto a los diferentes elementos que componen un sistema.

Principal protagonismo ha recibido el POm, al que hemos prestando especial atención, por ser, en aquellos momentos, el más desconocido. Hemos estudiado su funcionamiento e implicación en el procesamiento sensorial, así como su forma de codificar el contenido que procesa y que intercambia con la corteza. La influencia que ejerce este núcleo sobre el procesamiento cortical también ha sido ampliamente estudiada.

Nuestra investigación del POm siempre ha ido acompañada a la del VPM. Esto ha permitido contrastarlos, diferenciarlos y entenderlos mejor. De esta comparativa hemos obtenido observaciones relevantes y han surgido propuestas funcionales como las que en esta Tesis hemos descrito.

La corteza cerebral también ha sido considerablemente estudiada y algunas de las observaciones obtenidas aparecen descritas en los artículos científicos que acompañan esta Tesis.

En general, consideramos que hemos alcanzado los objetivos específicos que nos propusimos. Parte del conocimiento que hemos obtenido a lo largo de este proceso de investigación, ha sido descrito aquí y en las publicaciones que están adjuntadas en el apartado Anexos.

Tal y como suele ocurrir en ciencia, a medida que comprendemos mejor un determinado fenómeno, se abren nuevas cuestiones aún más interesantes. Algunas de ellas, las más inmediatas,

también recibieron nuestra atención y fueron estudiadas, otras muchas quedan pendientes de hacerlo.

Hemos tratado de no limitarnos a describir nuestras observaciones y resultados, sino a proponer explicaciones que permitieran interpretarlos desde una perspectiva funcional. Varias de esas explicaciones han sido desarrolladas y han dado lugar a propuestas y modelos teóricos sobre los temas tratados. En nuestros artículos científicos se han descrito algunos de ellos.

Los resultados que hemos aportado y las propuestas que hemos generado a partir de ellos, contribuyen en su conjunto, a entender mejor el procesamiento y uso de información por parte del cerebro, así como los detalles de los mecanismos neurales que soportan dicha computación.

Esperamos que dicha evidencia experimental y teórica sirvan para estimular el interés por el estudio del cerebro y que contribuyan al enorme reto de poder generar, algún día, una teoría general del funcionamiento de este preciado órgano.

8. CONCLUSIONES

1. El POm tiene la capacidad de sostener su actividad a lo largo de la presencia del evento sensorial. En base a ello, está implicado en el procesamiento y transmisión de actividad neural sostenida. Esta característica es compartida por el SpVi pero no por el VPM ni el PrV. Las respuestas de estos últimos núcleos son de carácter transitorio. Esta dicotomía funcional diferencia al sistema paralemniscal del lemniscal.

2. El POm es muy sensible a las interacciones espaciotemporales en la activación de las vibrisas y a la complejidad del evento sensorial.

3. El POm tiene la capacidad de integrar de forma precisa las señales sensoriales procedentes del conjunto de las vibrisas.

4. El POm está implicado en el procesamiento y codificación de patrones sensoriales complejos.

5. Es su capacidad de sostener su actividad y de producir cambios en dicha actividad en función de la integración que realiza de las diferentes señales a lo largo del desarrollo del evento sensorial lo que le permite generar una representación de los eventos sensoriales basada en su estructura espacio-temporal. Dicha estructura y la variante complejidad de sus partes son reflejados en cambios precisos de actividad talámica.

6. Estas capacidades del POm son propias y no son generadas por influencia de la corteza, sino que siguen presentes cuando ésta es eliminada.

7. El POm impone un control preciso sobre el procesamiento cortical. Dicha influencia no se limita a inducir activación en ella, sino que es significativamente más compleja.

8. El POm controla de forma contundente y constante la excitabilidad de la corteza S1. La desactivación farmacológica de este núcleo produce un aumento de la magnitud y duración de las respuestas corticales. Además, produce un aumento de la actividad espontanea en la corteza.

9. En contra de lo asumido hasta la fecha, el POm tiene la capacidad de ejercer, también, una influencia supresora en la corteza. Esta regulación da lugar a un ajuste en las respuestas sensoriales corticales afectando profundamente a su magnitud y estructura temporal.

10. El grado preciso de actividad presente en el POm en un determinado momento determina el ajuste, en tiempo real, del procesamiento cortical. Dicha actividad es la resultante de la integración, en tiempo real, de las diferentes señales procedentes del conjunto de las vibrisas. Cuanto mayor sea, mayor intensidad en dicho ajuste.

11. Esta modulación no es igual en todas las capas corticales. Sus efectos son especialmente evidentes en la capa supragranular (capa 2/3).

12. El POm ejerce su influencia reguladora a través de la capa 1 y lo hace usando al sistema GABAérgico como intermediario necesario en dicha regulación.

13. Los efectos inhibitorios producidos por el POm sobre la corteza parecen ser debidos a la implicación de las interneuronas parvalbumina-positivas (PV).

14. El POm también está implicado en el control del procesamiento sensorial en S2.

15. La transmisión de información desde la capa 5 de S1 a S2 y la influencia que esta capa de S1 ejerce en S2, depende del POm.

16. El contenido de los mensajes que transmite este núcleo a la corteza es soportado por actividad neural de naturaleza sostenida e integrada.

17. El POm está implicado en la percepción bilateral.

18. Los núcleos POm en ambos tálamos están mutuamente conectados formando un bucle interhemisférico a través de la corteza cerebral.

19. La actividad codificada por el POm es transmitida al otro POm manteniendo su estructura integrada.

20. Esta información es intercambiada constantemente entre ambos núcleos transmitida por una red paralela de proyecciones talamocorticales, cortico-corticales y corticotalámicas en la que juega un papel protagonista la corteza S1.

21. Existe una diferente implicación de las diferentes capas de S1 en la transmisión de actividad sostenida. Es la capa infragranular la que está soportando la transmisión de actividad sostenida y su intercambio entre hemisferios. Esto sugiere que las proyecciones del POm a capa 5a en S1 están implicadas en la transmisión de dicha actividad permitiendo la integración bilateral en el tálamo opuesto.

22. El POm responde también a la estimulación de las vibrisas ipsilaterales. El VPM, a diferencia del POm, no responde la estimulación ipsilateral. Estas respuestas tampoco se encuentran a nivel del complejo trigeminal.

23. El POm integra la información sensorial procedente de las vibrisas en ambos lados del cuerpo. Esta integración, no ocurre a nivel del complejo trigeminal.

24. El VPM no comparte las capacidades descritas en el POm demostrando profundas diferencias entre estos núcleos durante el procesamiento de los eventos sensoriales.

25. En el VPM, el contenido relacionado con cada vibrisa se mantiene segregado y asilado del resto. Además, el contenido que procesa y transmite solo procede de las vibrisas contralaterales. Por el contrario, el contenido del POm, es integrado, conteniendo información de todas las vibrisas en ambos lados de la cara. En base a dicha discrepancia, proponemos que estos tipos distintos de contenido sean denominados como ‘segregado’ versus ‘integrado’.

26. La anestesia afecta profundamente al funcionamiento del POm. Sus respuestas ante estímulos ipsilaterales se pierden cuando aumenta el nivel de anestesia. También elimina la actividad sostenida en este núcleo.

9. Bibliografía

Aguilar JR and Castro-Alamancos MA (2005). Spatiotemporal Gating of Sensory Inputs in Thalamus during Quiescent and Activated States. J. Neurosci. 25, 10990–11002.

Alloway KD, Olson ML, Smith JB (2008). Contralateral corticothalamic projections from MI whisker cortex: Potential route for modulating hemispheric interactions. Journal of Comparative Neurology 510, 100–116.

Alloway KD, Smith JB, Watson GD (2014). Thalamostriatal projections from the medial posterior and parafascicular nuclei have distinct topographic and physiologic properties. J Neurophysiol. 111, 36-50.

Allport DA (1968). Phenomenal simultaneity and the perceptual moment hypothesis. Br. J.Psychol. 59, 395–406.

Armstrong-James M and George MJ (1988). Bilateral receptive fields of cells in rat Sm1 cortex. Exp Brain Res 70, 155–165.

Audette NJ, Urban-Ciecko J, Matsushita M, Barth AL (2017). POm thalamocortical input drives layer-specific microcircuits in somatosensory cortex. Cereb. Cortex 10, 1-17.

Barthó P, Freund TF, Acsády L (2002). Selective GABAergic innervation of thalamic nuclei from zona incerta. Eur. J. Neurosci. 16, 999-1014.

Baumgarten TJ, Schnitzler A, Lange J (2015). Beta oscillations define discrete perceptual cycles in the somatosensory domain. Proc. Natl. Acad. Sci. USA 112, 12187–12192.

Bolkan SS, Stujenske JM, Parnaudeau S, Spellman TJ, Rauffenbart C, Abbas AI et al. (2017). Thalamic projections sustain prefrontal activity during working memory maintenance. Nat. Neurosci. 20, 987–996.

Bourassa J, Pinault D, Deschênes M (1995). Corticothalamic Projections from the Cortical Barrel Field to the Somatosensory Thalamus in Rats: A Single-fibre Study Using Biocytin as an Anterograde Tracer. Eur. J. Neurosci. 7, 9-30.

Buschman TJ and Miller EK (2010) Shifting the spotlight of attention: evidence for discrete computations in cognition. Front. Hum.Neurosci. 4:194.

Buzsáki G and Draguhn A (2004). Neuronal oscillations in cortical networks. Science 304, 1926–1929.

Cardin JA, Carlén M, Meletis K, Knoblich U, Zhang F, Deisseroth K, et al. (2009). Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature 459, 663–667.

Carvell GE and Simons DJ (1987). Thalamic and corticocortical connections of the second somatic sensory area of the mouse. J. Comp. Neurol. 265, 409–427.

Carvell GE and Simons D J (1990). Biometric analyses of vibrissal tactile discrimination in the rat. J. Neurosci. 10, 2638–2648.

Casas-Torremocha D (2017). Análisis anatómico-funcional del flujo de información en el núcleo posterior del tálamo de roedores. Tesis Doctoral. Universidad Autónoma de Madrid.

Casas-Torremocha D, Porrero C, Rodriguez-Moreno J, García-Amado M, Lübke JHR, Núñez Á, et al. (2019). Posterior thalamic nucleus axon terminals have different structure and functional impact in the motor and somatosensory vibrissal cortices. Brain Struct Funct 224, 1627–1645.

Castejon C, Barros-Zulaica N, Nunez A (2016). Control of somatosensory cortical processing by thalamic Posterior Medial Nucleus: A new role of thalamus in cortical function. PLoS ONE 11(1):e0148169.

Castejon C and Nuñez A (2016). Cortical neural computation by discrete results hypothesis. Front. Neural Circuits 10:81.

Castejon C and Nuñez A (2020). Higher-order thalamic implication in the codification of bilateral sensory events. bioRxiv. https://doi.org/10.1101/2020.05.01.073098.

Chou X, Fang Q, Yan L, Zhong W, Peng B, Li H, et al. (2020). Contextual and cross-modality modulation of auditory cortical processing through pulvinar mediated suppression eLife 9:e54157.

Clancy KB, Schnepel P, Rao AT, Feldman DE (2015). Structure of a single whisker representation in layer 2 of mouse somatosensory cortex. J. Neurosci. 35, 3946–3958.

Clascá F, Rubio-Garrido P, Jabaudon D (2012). Unveiling the diversity of thalamocortical neuron subtypes. Eur. J. Neurosci. 35, 1524–1532.

Clascá F, Porrero C, Galazo MJ, Rubio-Garrido P, Evangelio M. (2016). Anatomy and development of multi-specific thalamocortical axons: implications for cortical dynamics and evolution. In: Rockland KS (ed) Axons and brain architecture. Elsevier, Amsterdam, pp 69–92.

de Kock CPJ, Bruno RM, Spors H, Sakmann B (2007). Layer- and cell-type-specific suprathreshold stimulus representation in rat primary somatosensory cortex. J. Physiol. (Lond.) 581, 139–154.

Debowska W, Liguz-Lecznar M, Kossut M (2011). Bilateral Plasticity of Vibrissae SII Representation Induced by Classical Conditioning in Mice. J Neurosci 31, 5447–5453.

Deschênes M, Bourassa J, Parent A (1995). Two different types of thalamic fibers innervate the rat striatum. Brain Res. 701, 288-292.

Deschênes M, Bourassa J, Doan VD, Parent A (1996). A single-cell study of the axonal projections arising from the posterior intralaminar thalamic nuclei in the rat. Eur. J. Neurosci.8, 329-43.

Deschênes M, Veinante P, Zhang ZW (1998). The organization of corticothalamic projections: reciprocity versus parity. Brain Res. Rev. 28, 286-308.

Desimone R, Wessinger M, Thomas L, Schneider W (1990). Attentional control of visual perception: cortical and subcortical mechanisms. Cold Spring Harb Symp Quant Biol 55, 963-971.

Diamond ME, Armstrong-James M, Ebner FF (1992). Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus. J. Comp. Neurol. 318, 462-476.

Diamond ME (1995). Somatosensory thalamus of the rat. In Cerebral Cortex vol. 11. Jones EG y Diamond IT eds New York: Plenum Press; 189-219.

Diamond ME, von Heimendahl M, Knutsen PM, Kleinfeld D, Ahissar E (2008). 'Where' and 'what' in the whisker sensorimotor system. Nat. Rev. Neurosci. 9, 601–612.

Fang Q, Chou XL, Peng B, Zhong W, Zhang LI, Tao HW (2020). A differential circuit via Retino-Colliculo-Pulvinar pathway enhances feature selectivity in visual cortex through surround suppression. Neuron 105, 355–369.

Furuta T, Timofeeva E, Nakamura K, Okamoto-Furuta K, Togo M, Kaneko T, Deschenes M (2008). Inhibitory gating of vibrissal inputs in the brainstem. J Neurosci. 28, 1789–1797.
Fuster JM, Bauer RH, Jervey J P (1982). Cellular discharge in the dorsolateral prefrontal cortex of the monkey in cognitive tasks. Exp. Neurol. 77, 679–694.

Gambino F, Pagès S, Kehayas V, Baptista D, Tatti R, Carleton A, Holtmaat A (2014). Sensory-evoked LTP driven by dendritic plateau potentials in vivo. Nature 515, 116-119.

Gharaei S, Honnuraiah S, Arabzadeh E, Stuart GJ (2020). Superior colliculus modulates cortical coding of somatosensory information. Nat. commun. 11:1693.

Goldman-Rakic PS (1987). Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. In Handbook of physiology, the nervous system, higher functions of the brain (ed. F. Plum), sect. I, vol. V, pp. 373-417. Bethesda, MD: American Physiological Society.

Groh A, Bokor H, Mease RA, Plattner V M, Hangya B, Stroh A, et al. (2014). Convergence of cortical and sensory driver inputs on single thalamocortical cells. Cereb. Cortex 24, 3167–3179.

Guo ZV, Inagaki HK, Daie K, Druckmann S, Gerfen CR, Svoboda K (2017). Maintenance of persistent activity in a frontal thalamocortical loop. Nature 545, 181–186.

Halassa M and Sherman SM (2019). Thalamocortical Circuit Motifs: A General Framework. Neuron 103, 762-770.

Harter MR (1967). Excitability cycles and cortical scanning: a review of two hypotheses of central intermittency in perception. Psychol. Bull. 68, 47–58.

Hebb D (1949). The Organization of Behavior: A Neuropsychological Theory. New York, NY: Wiley-Interscience.

Hefft S and Jonas P (2005). Asynchronous GABA release generates long-lasting inhibition at a hippocampal inter-neuron-principal neuron synapse. Nat. Neurosci. 8, 1319–28.

Higley MJ and Contreras D (2003). Nonlinear integration of sensory responses in the rat barrel cortex: an intracellular study in vivo. J Neurosci. 23, 10190-10200.

Hirata A and Castro-Alamancos MA (2008). Cortical transformation of wide-field (multiwhisker) sensory responses. J. Neurophysiol. 100, 358–370.

Hoerder-Suabedissen A, Hayashi S, Upton L, Nolan Z, Casas-Torremocha D, Grant E et al. (2018). Subset of cortical layer 6b neurons selectively innervates higher order thalamic nuclei in mice. Cereb. Cortex 28, 1882–1897.

Hoogland PV, Wouterlood FG, Welker E, Van der Loos H (1991). Ultrastructure of giant and small thalamic terminals of cortical origin: a study of the projections from the barrel cortex in mice using Phaseolus vulgaris leuco-agglutinin (PHA-L). Exp. Brain Res. 87, 159-172.

Jones EG (2007). The Thalamus, Second Edition. Cambridge, Mass: Cambridge University Press.

Kinnischtzke AK, Simons DJ, Fanselow EE (2014). Motor cortex broadly engages excitatory and inhibitory neurons in somatosensory barrel cortex. Cereb. Cortex 24, 2237–2248.

Kuramoto E, Pan S, Furuta T, Tanaka YR, Iwai H, Yamanaka A et al. (2017). Individual mediodorsal thalamic neurons project to multiple areas of the rat prefrontal cortex: A single neurontracing study using virus vectors. J. Comp. Neurol. 525, 166–185.

Lavallée P, Urbain N, Dufresne C, Bokor H, Acsády L, Deschênes M (2005). Feedforward inhibitory control of sensory information in higher-order thalamic nuclei. J Neurosci. 25, 7489-7498

LeDoux JE, Ruggiero DA, Forest R, Stornetta R, Reis DJ (1987). Topographic organization of convergent projections to the thalamus from the inferior colliculus and spinal cord in the rat. J. Comp. Neurol. 264, 123-146.

Liao CC, Chen RF, Lai WS, Lin RCS, Yen CT (2010). Distribution of large terminal inputs from the primary and secondary somatosensory cortices to the dorsal thalamus in the rodent. J. Comp. Neurol. 518, 2592–2611

Lorente de Nó R (1992). La corteza cerebral del ratón (primera contribución – la corteza "acústica".) Trab Lab Inv Biol Univ Mad 20: 41-78. Reimpresión traducida al inglés en Somatosens Mot Res 9, 3-36.

Lorente de Nó R (1938). The cerebral cortex: architecture, intracortical connections and motor projections. En: The Physiology of the Nervous System, Fulton JF (Ed). Oxford University Press: Oxford, London pp. 291-340.

Lundqvist M, Rose J, Herman P, Brincat SL, Buschman TJ, Miller EK (2016). Gamma and beta bursts underlie working memory. Neuron 90, 152–164.

Mao T, Kusefoglu D, Hooks BM, Huber D, Petreanu L, Svoboda K (2011). Long-range neuronal circuits underlying the interaction between sensory and motor cortex. Neuron 72, 111–123

Martín-Cortecero J and Núñez A (2014). Tactile response adaptation to whisker stimulation in the lemniscal somatosensory pathway of rats. Brain Res. 1591, 27-37.

Masri R, Trageser JC, Bezdudnaya T, Li Y, Keller A (2006). Cholinergic regulation of the posterior medial thalamic nucleus. J. Neurophysiol. 96, 2265–2273.

Mease RA, Metz M, Groh A (2016). Cortical sensory responses are enhanced by the higher-order thalamus. Cell Reports 14, 208-15.

Ohno S, Kuramoto E, Furuta T, Hioki H, Tanaka YR, Fujiyama F, et al. (2012). A morphological analysis of thalamocortical axon fibers of rat posterior thalamic nuclei: a single neuron tracing study with viral vectors. Cereb. Cortex 22, 2840–2857.

Peron SP, Freeman J, Iyer V, Guo C, Svoboda K (2015). A cellular resolution map of barrel cortex activity during tactile behavior. Neuron 86, 783–799.

Petersen CCH, and Crochet S (2013). Synaptic computation and sensory processing in neocortical layer 2/3. Neuron 78, 28–48.

Pitts W and McCulloch WS (1947). How we know universals: the perception of auditory and visual forms. Bull. Math. Biophys. 9, 127–147.

Porrero C (2016). Arquitecturas axónicas y organización de las neuronas de proyección multiespecífica del tálamo: estudio en el núcleo posterior del ratón. Tesis Doctoral. Universidad Autónoma de Madrid.

Porter LL and White EL (1983). Afferent and efferent pathways of the vibrissal region of primary motor cortex in the mouse. J. Comp. Neurol. 214, 279–289.

Pouchelon G, Frangeul L, Rijli FM, Jabaudon D (2012) Patterning of pre-thalamic somatosensory pathways. Eur. J. Neurosci. 35, 1533–1539.

Pouget A, Dayan P, Zemel R (2000). Information processing with population codes. Nat. Rev. Neurosci. 1, 125–132.

Rikhye RV, Gilra A, Halassa MM (2018). Thalamic regulation of switching between cortical representations enables cognitive flexibility. Nat. Neurosci. 21, 1753–1763.

Rossignol E, Kruglikov I, van den Maagdenberg AM, Rudy B, Fishell G (2013). CaV 2.1 ablation in cortical interneurons selectively impairs fast-spiking basket cells and causes generalized seizures. Ann Neurol. 74, 209–22.

Rubio-Garrido P, Pérez de Manzo F, Porrero C, Galazo MJ, Clasca F (2009). Thalamic input to apical dendrites in neocortical layer 1 is massive and highly convergent. Cereb. Cortex 19, 2380–2395.

Saalmann YB, Pinsk MA, Wang L, Li X, Kastner S (2012). The pulvinar regulates information transmission between cortical areas based on attention demands. Science 337, 753–756

Scheibel AB (1997). The thalamus and neuropsychiatric illness. J. Neuropsychiatry Clin. Neurosci. 9, 342–353.

Schmitt LI, Wimmer RD, Nakajima M, Happ M, Mofakham S, Halassa MM (2017). Thalamic amplification of cortical connectivity sustains attentional control. Nature 545, 219–223.

Sherman SM and Guillery RW (1998). On the actions that one nerve cell can have on another: distinguishing ‘‘drivers’’ from ‘‘modulators’’. Proc. Natl. Acad. Sci. USA 95, 7121–7126.

Sherman SM and Guillery RW (2006). Exploring the thalamus and its role in cortical function. 2nd ed. Cambridge, Mass: MIT Press.

Sherman SM and Guillery RW (2011). Distinct functions for direct and transthalamic corticocortical connections. J. Neurophysiol. 106, 1068–1077.

Sherman SM and Guillery RW (2013). Functional Connections of Cortical Areas: A New View from the Thalamus. Cambridge, Mass: MIT Press.

Sherman SM and Guillery RW (2014). The lateral geniculate nucleus and pulvinar. Cambridge, Mass: MIT Press.

Shlosberg D, Amitai Y, Azouz R (2006). Time-dependent, layer-specific modulation of sensory responses mediated by neocortical layer 1. J Neurophysiol. 96, 3170–3182.

Simons DJ (1985). Temporal and spatial integration in the rat SI vibrissa cortex. J. Neurophysiol. 54, 615–635.

Sobolewski A, Kublik E, Swiejkowski DA, Kamiński, J, Wróbel A (2015). Alertness opens the effective flow of sensory information through rat thalamic posterior nucleus. Eur. J. Neurosci. 41, 1321–1331.

Sohal VS, Zhang F, Yizhar O, Deisseroth K (2009) Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 459, 698–702.

Theyel B, Llano D, Sherman SM (2010). The corticothalamocortical circuit drives higher-order cortex in the mouse. Nat. Neurosci. 13, 84-88.

Toledo-Rodriguez M, Blumenfeld B, Wu C, Luo J, Attali B, Goodman P, Markram H (2004). Correlation maps allow neuronal electrical properties to be predicted from single-cell gene expression profiles in rat neocortex. Cereb. Cortex 14, 1310–1327.

Traub RD, Whittington MA, Stanford IM, Jefferys JG (1996) A mechanism for generation of long-range synchronous fast oscillations in the cortex. Nature 383, 621–624.

VanRullen R and Koch C (2003). Is perception discrete or continuous? Trends Cogn. Sci. 7, 207–213.

VanRullen R, Reddy L, Koch C (2005). Attention-driven discrete sampling of motion perception. Proc. Natl. Acad. Sci. USA 102, 5291–5296.

Varela FJ, Toro A, John ER, Schwartz EL (1981). Perceptual framing and cortical alpha rhythm. Neuropsychologia 19, 675–686.

Veinante P, Jacquin MF, Deschênes M (2000a). Thalamic projections from the whisker-sensitive regions of the spinal trigeminal complex in the rat. J. Comp. Neurol. 420, 233–243.

Veinante P, Lavallée P, Deschênes M (2000b). Corticothalamic projections from layer 5 of the vibrissal barrel cortex in the rat. J. Comp. Neurol. 424, 197–204.

Viaene AN, Petrof I, Sherman SM (2011). Properties of the thalamic projection from the posterior medial nucleus to primary and secondary somatosensory cortices in the mouse. Proc. Natl. Acad. Sci. USA 108, 18156-18161.

Wimmer VC, Bruno RM, de Kock CP, Kuner T, Sakmann B. (2010). Dimensions of a projection column and architecture of VPM and POm axons in rat vibrissal cortex. Cereb Cortex. 20, 2265–76.

Woolsey TA, Van der Loos H (1970). The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res. 17, 205–42.

Yuste R (2015). From the neuron doctrine to neural networks. Nat. Rev. Neurosci. 16, 487–497.

Zaitsev AV, Povysheva NV, Lewis DA, Krimer LS (2007). P/Q-type, but not N-type, calcium channels mediate GABA release from fast-spiking interneurons to pyramidal cells in rat prefrontal cortex. J Neurophysiol. 97, 3567–3573.

Zhang W and Bruno RM (2019). High-order thalamic inputs to primary somatosensory cortex are stronger and longer lasting than cortical inputs. eLife 8, e44158.

Zolnik TA, Ledderose J, Toumazou M, Trimbuch T, Oram T, Rosenmund C, et al. (2020). Layer 6b Is Driven by Intracortical Long-Range Projection Neurons. Cell Reports 30, 3492-3505.e5.

10. Anexos

Artículo científico nº 1

PLOS | ONE

RESEARCH ARTICLE

Control of Somatosensory Cortical Processing by Thalamic Posterior Medial Nucleus: A New Role of Thalamus in Cortical Function

Carlos Castejon, Natali Barros-Zulaica, Angel Nuñez*

Departamento de Anatomía, Histología y Neurociencia, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain

* angel.nunez@uam.es

OPEN ACCESS

Citation: Castejon C, Barros-Zulaica N, Nuñez A (2016) Control of Somatosensory Cortical Processing by Thalamic Posterior Medial Nucleus: A New Role of Thalamus in Cortical Function. PLoS ONE 11(1): e0148169. doi:10.1371/journal.pone.0148169

Editor: Miguel Maravall, University of Sussex, UNITED KINGDOM

Received: June 12, 2015

Accepted: January 13, 2016

Published: January 28, 2016

Data Availability Statement: All relevant data are within the paper.

Funding: This work was supported by a grant from Ministerio de Economia y Competitividad (BFU2012-36107).

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Current knowledge of thalamocortical interaction comes mainly from studying lemniscal thalamic systems. Less is known about paralemniscal thalamic nuclei function. In the vibrissae system, the posterior medial nucleus (POm) is the corresponding paralemniscal nucleus. POm neurons project to L1 and L5A of the primary somatosensory cortex (S1) in the rat brain. It is known that L1 modifies sensory-evoked responses through control of intracortical excitability suggesting that L1 exerts an influence on whisker responses. Therefore, thalamocortical pathways targeting L1 could modulate cortical firing. Here, using a combination of electrophysiology and pharmacology in vivo, we have sought to determine how POm influences cortical processing. In our experiments, single unit recordings performed in urethane-anesthetized rats showed that POm imposes precise control on the magnitude and duration of supra- and infragranular barrel cortex whisker responses. Our findings demonstrated that L1 inputs from POm imposed a time and intensity dependent regulation on cortical sensory processing. Moreover, we found that blocking L1 GABAergic inhibition or blocking P/Q-type Ca2+ channels in L1 prevents POm adjustment of whisker responses in the barrel cortex. Additionally, we found that POm was also controlling the sensory processing in S2 and this regulation was modulated by corticofugal activity from L5 in S1. Taken together, our data demonstrate the determinant role exerted by the POm in the adjustment of somatosensory cortical processing and in the regulation of cortical processing between S1 and S2. We propose that this adjustment could be a thalamocortical gain regulation mechanism also present in the processing of information between cortical areas.

Introduction

Cortical functioning cannot be properly understood without taking into account the thalamic influence [1–9]. Knowledge of thalamocortical influence in sensory processing comes mainly from studying lemniscal core thalamic systems that project to granular layers of primary sensory cortices [3, 7, 10]; however, less is known about paralemniscal thalamic systems.

In the rodents, vibrissal information is conveyed to the somatosensory cortex via several parallel pathways [11–19]. In the lemniscal pathway, the ventral posterior medial nucleus of the thalamus (VPM) projects to L4, L5B and L6A in the primary somatosensory cortex (S1). In the extralemniscal pathway, the ventral tier of VPM projects mainly to L4 and L6 [67] in the secondary somatosensory cortex (S2). And in the paralemniscal pathway, the posterior medial nucleus (POm) projects to L1 and L5A in S1 and also to S2 [18–24]. It has been proposed that, whereas these ascending pathways appear to be parallel anatomically, they may not be functionally equivalent [39].

Thalamic VPM nucleus can be considered a "First order" relay station [5, 9], receiving sensory information from the principal trigeminal nucleus (PrV). POm nucleus is largely more complex to classify since it receives sensory information from the interpolar division of the spinal trigeminal nucleus (SpVi) and also from L5 of the somatosensory cortical areas [5, 15, 21, 25, 26]. There are several important differences between both nuclei: VPM is topographically well organized [19, 27–30]. In contrast, POm neuronal responses show poor spatial resolution [1, 15, 27, 31] with receptive fields composed of multiple vibrissae [12]. Recordings from both nuclei revealed different adaptation process to repetitive stimuli [1, 32, 33]. Offset latencies remained constant in POm neurons across the different stimulation frequencies [1]. In agreement with those findings, other studies found that onset and offset latencies of SPVi paralemniscal neuronal responses were not affected by deflecting the vibrissae at different frequencies [32, 34]. These properties of paralemniscal neurons render them poorly suited for coding specific stimulus content features. It has been proposed that signals conveyed by the lemniscal pathway involve high-resolution encoding of contact and texture information relayed from the vibrissae [17, 35]. The role of POm and the paralemniscal system in sensory processing is less clear. It is known that paralemniscal system processes temporal features of tactile stimuli [1, 34], and is involved in nociceptive transmission [32, 36–38]. Also, it has been suggested that POm neurons represent (temporal- to rate-code transformation by thalamocortical loops) the temporal frequency of whisker movements by latency and spike count [1, 34] and that the POm is involved in temporal processing related to sensory-motor control of whisker movement [17, 34, 35]. Other authors have reported that whisking in air, without vibrissae contacts, fails to evoke significant activity in POm neurons [32]. Actually, the nature and function of the messages that POm thalamic nucleus transfers to the cortex are still under debate.

It has been proposed that the role of the paralemniscal projection is to provide modulatory inputs to barrel cortex [39]. However, the possible mechanisms by which these projections could regulate the cortex are unknown.

Here, we have sought to determine POm influences in cortical processing by single-unit recordings in somatosensory cortex of urethane-anesthetized rats. Our findings demonstrate that POm modulates magnitude and duration of S1 cortical responses to sensory input. We found that GABAergic inhibitory transmission in L1 is implicated in the regulation of cortical excitability and sensory response magnitude and duration. Our results are consistent with a previous work that described L1 inhibitory influence on whisker responses [40]. Accordingly, we demonstrate that POm exerts its control of cortical sensory responses mainly through L1.

Additionally, it has been suggested that 'Higher order' thalamic nuclei play a key role in corticocortical communication [41, 42]. In S1, L5 corticofugal neurons send the processed information to the POm and to various subcortical regions [9, 15, 25, 26, 43]. Recently, both anatomical and physiological findings have shown that ascending inputs from the brainstem and descending inputs from L5 converge on single thalamocortical neurons in POm [25]. Both individual pathways interact functionally in a time-dependent manner [25]. From here, POm neuron projections also target other cortical areas including the primary motor cortex (M1) and higher-order somatosensory cortical regions [18, 21]. Furthermore, it is well described the

reciprocal connections between these areas. These connections are likely to play a crucial role in sensory-motor integration and sensory learning. However, both the function of that trans-thalamic pathway and the nature of the messages that are relayed through the POm from one cortical area to another remain unclear.

In this study, we propose that cortical sensory response modulation by POm could be also present in the processing of information between somatosensory cortical areas. We performed a complementary set of experiments to test this hypothesis and found that POm is also controlling the sensory processing in S2 and this regulation is modulated by corticofugal activity from L5 in S1 [25]. In sum, our findings demonstrate the determinant role exerted by the POm in the adjustment of barrel cortex sensory processing and in the regulation of cortical processing between somatosensory cortical areas.

Materials and Methods

Animal procedures and electrophysiology

All animal procedures were approved by the Ethical Committee of the Universidad Autonoma de Madrid, in accordance with European Community Council Directive 2010/63/UE. Rats were group housed with a 12-h light/dark cycle and had free access to food and water. Every effort was made to minimize the number and suffering of the animals used. Experiments were performed on 98 (36 males and 62 females) urethane-anesthetized (1.6 g/kg i.p.) adult Sprague Dawley rats weighing 200–250 g. Animals were placed in a Kopf stereotaxic frame in which surgical procedures and recordings were performed. The animals breathed freely. The body temperature was maintained at 37°C; the end-tidal CO_2 and heart rate were monitorized. Local anaesthetic (Lidocaine 1%) was applied to all skin incisions. The level of anesthesia was monitored and kept constant (absence of whisker movements and pinch withdrawal reflex) using supplemental doses of urethane. The skull was exposed and then openings were made to allow electrode penetrations to different neuronal stations in the cortex, thalamus and brainstem. Tungsten microelectrodes (2–5 MΩ) were driven using a microdrive system. Extracellular recordings were made of putative excitatory neurons in the interpolar division of the ipsilateral spinal trigeminal complex (SpVi; AP 11.5–14; L 2.5–3.5, D 8.5–9.5; in mm from Bregma; [44], contralateral posterior medial nucleus (POm; AP 2.5–4.5, L 2–2.5, D 5–6.5) of the thalamus and contralateral vibrissal region of the primary (S1; AP 0.5–4, L 5–7) and secondary (S2; AP 0–3.7; L 7–7.5) somatosensory cortices. In S1, barrel cortical neurons were recorded in supragranular (D: 200–600 μm) or infragranular (D: 900–1500 μm) layers. In S2, neurons were recorded along the cortical depth (D: 400–1300 μm). To estimate the depths of recorded neurons, we used the micromanipulator axial depth readings.

Sensory stimulation

Controlled whisker deflections were performed by brief air puffs (20–200 ms) applied to one whisker (deflected in caudal direction) at 0.5 Hz using a pneumatic pressure pump (Picospritzer) that delivers an air pulse through a 1 mm inner diameter polyethylene tube (1.2–2 kg/cm^2) avoiding skin stimulation. We choose this precise stimulus to assure the effect of our protocols and to avoid complex, likely nonphysiological responses. Vibrissae were cut 9 mm from the skin in order to allow a controlled mechanical stimulation of single vibrissae and to evoke reproducible responses. Details on train duration, pulse duration and number of consecutive deflections applied are provided in figures. We determined receptive field size of single units by deflecting individual vibrissae with a hand-held probe and monitoring the audio conversion of the amplified activity signal.

Electrical stimulation

Electrical microstimulation was carried out with single square pulses (0.5 ms, 5–80 μA; S88 Grass Stimulator). We applied these pulses at 0.5 Hz to avoid possible adaptation phenomena. Electrical stimulation (E-stimulation) was applied in POm, VPM, L5 or L1 in S1 cortex, using 120 μm diameter stainless steel bipolar electrodes. The E-stimulation parameters were digitally controlled by Spike2 software (Cambridge Electronic Design, Cambridge, UK) and transmitted to the current source via a digital-to-analog converter built in to the CED Power 1401 data acquisition unit (Cambridge Electronic Design). We tried to establish the minimal, but effective, stimulation parameters for detecting changes in cortical neural responses and to avoid possible antidromic activity [45] in order to study only orthodromic effects. Stimulation within the current range used in our study (<80 μA) is estimated to activate cells within a maximal radius of 0.5 mm [46]. At the end of each E-stimulation experiment we applied a train of 20 pulses (0.5 ms; same intensity) at high frequency (100 Hz) to check for antidromic activity. We did not find evoked spikes having the ability to follow this high frequency E-stimulation. Thus, none of the cortical recorded neurons were antidromically activated by thalamic E-stimulation at the intensities used. None of the E-stimulation parameters used here induced subtle motor effects, whisking or facial twitching.

We identify the placement of the electrodes on histological sections or according to their response pattern. Only the data from cases in which the electrode tip was unambiguously well localized inside the corresponding thalamic nucleus or cortical layer were quantitatively analyzed.

Pharmacological study

The following drugs were used: Muscimol (5-(aminomethyl)-isoxazol-3-ol; selective agonist for γ-aminobutyric acid receptor-A ($GABA_A$) receptors; 1 mM), Picrotoxin (PTX; prototypic antagonist of $GABA_A$ receptors; 1mM) and Cav2.1 (P/Q- type) voltage-gated calcium channels blocker ω-agatoxin-IVa (AGA; 0.1 μM). Drugs were injected through a cannula connected to a Hamilton syringe (1 μl). The syringe was driven using a microdrive system to inject the drug solution into the cortical surface or into the thalamic nucleus (AP 3.3 mm, L 2.5 mm to the Bregma for POm, or L 3.2 mm for VPM and D 4.8–6.8 mm from the surface of the brain; [44]). The piston of the syringe was moved manually at a slow speed (infusion speed 0.3 μl/min). A volume of 0.1 to 0.3 μl of muscimol was infused unilaterally into the corresponding thalamic nuclei. PTX or AGA was applied to the surface of the cortex (1 μl). Given the potential of $GABA_A$ receptors antagonists, PTX in our experiments, to induce seizures (e.g. [47–49]), all rats were carefully monitored for indicators of seizures after infusions. None of the PTX injections elicited tremor, motor convulsions or more subtle seizure effects such as jaw or facial twitching.

Histology

Upon completion of the experiments, animals were deeply anesthetized with sodium-pentobarbital (50 mg/kg) and then perfused transcardially with saline followed by formalin (4% in saline). Subsequently, 50 μm thick sections were prepared for Nissl staining for verification of cannula placement and to locate the stimulation and recording electrode tracks. Placements of the lesions were determined using a light microscope and mapped onto coronal sections of a rat brain stereotaxic atlas [44].

Data acquisition and analysis

Raw signal was filtered (0.3–3 kHz band pass), amplified via an AC preamplifier (DAM80; World Precision Instruments, Sarasota, USA), and fed into a computer (sampled at 10 kHz)

with the temporal references of the stimuli for off-line analysis. Single-unit activity was extracted with the aid of commercial software Spike2 (Cambridge Electronic Design, Cambridge, UK) for spike waveform identification and analysis. Furthermore, we also supervise waveforms to confirm that units were well isolated. The sorted spikes were stored at a 1-ms resolution and isolated single-units were analyzed and quantified. We defined response magnitude as the total number of spikes per stimulus occurring between response onset and offset from the peristimulus time histogram (PSTH, bin width 1 ms). Response onset was defined as the first of three consecutive bins displaying significant activity (three times higher than the mean spontaneous activity) after stimulus and response offset as the last bin of the last three consecutive bins displaying significant activity. Response duration was defined as the time elapsed from the onset to offset responses. The baseline firing rate was calculated from mean firing within a 10 s window before the first stimulus (air puff). In all figures, raster plots represent each spike as a dot for sample neuron. Spikes were aligned on stimulus presentation (Time 0 ms). In some figures, PSTHs and rasters are shown from multi-units recordings just to clarify the effects.

Statistical analysis was performed using GraphPad Prism 5 software (San Diego, CA, USA). For all experiments, data analysis was based on single unit responses. For normally distributed data (Shapiro-Wilk normality test), comparisons of activities of single units in different conditions were performed by using paired two-tailed t test, where $P<0.05$ was considered significant. Data are presented as means ± SEM. Non-normally distributed data were compared with Wilcoxon-matched pairs test (as indicated in the text).

Results

Our experiments were designed to study thalamic POm influence in somatosensory cortical processing. First, we studied and characterized the firing pattern of POm responses to whisker deflections. After that, to test whether POm activity modulates cortical tactile processing, we investigated whisker response changes in barrel cortex by electrically stimulating the POm immediately before whisker stimulus or by muscimol-induced inactivation of the POm. Finally, we pharmacologically blocked GABAergic inhibitory transmission in L1 to understand the contribution of this layer in POm regulation of cortical processing.

Additionally, to determine the possible role exerted by the POm in the adjustment of somatosensory cortical processing between S1 and S2, we performed a complementary set of experiments investigating whisker response changes in S2 by electrically stimulating S1 and by muscimol-induced inactivation of the POm.

POm responses lasted the duration of the stimulus

Performing experiments in 10 rats, we firstly characterized the firing pattern of POm neurons delivering air-puffs of different durations (20–200 ms) to one whisker, avoiding skin stimulation. We found multivibrissae receptive fields (mean receptive field size: 6.1±2.5 vibrissae; range: 3–12; n = 118) at all POm recording sites. Our recordings from POm revealed a sustained response along stimulus presence, as shown by the raster of spikes in response to 0.5 Hz periodic vibrissae deflections (Fig 1). Specifically, 72% of the recorded neurons exhibited this response pattern (85 of 118). We also found that 83% of the recorded neurons in SpVi exhibited the same pattern (85 of 102; data not shown). These findings demonstrated the presence of this sustained response pattern along the paralemniscal pathway.

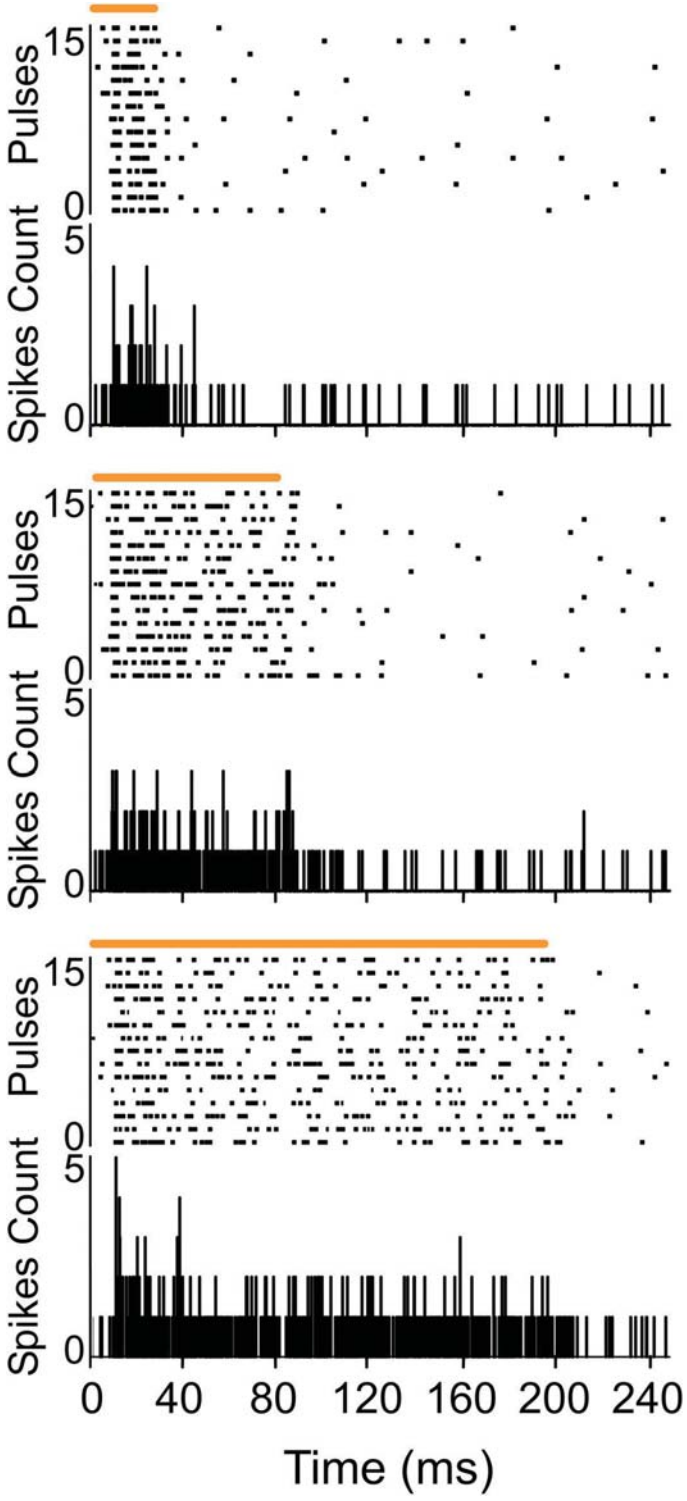


Fig 1. POm responses lasted the duration of the whisker stimulus. Raster plots and PSTHs showing sustained multiunit POm responses evoked by different stimulus duration (top: 20 ms, middle: 80 ms and bottom: 200 ms). Air puff duration is indicated by horizontal orange lines.

doi:10.1371/journal.pone.0148169.g001

POm activity modulates sensory cortical processing

To examine the influence of POm nucleus on infra- and supragranular neurons in barrel cortex, we compared whisker responses in several experimental conditions increasing or decreasing POm activity.

POm E-stimulation evokes orthodromic spikes in infra- and supragranular layers of barrel cortex. We investigated whisker response changes in barrel cortex by POm electrical stimulation (E-stimulation) immediately before whisker stimulus (air puff) application in 15 rats (Fig 2). We restricted our recordings to infra- and supragranular layers of barrel cortex (see Discussion). Cortical neurons were silent or displayed a low firing rate in spontaneous conditions (0.89±0.1 spikes/s in infragranular layers, n = 69; 0.69±0.1 in supragranular layer, n = 51). Whisker deflections caused short-latency spikes in infra- and supragranular layers of barrel cortex. All neurons displayed a contralateral response to whisker displacements. Spike shape and firing pattern (low spontaneous firing rate and reduced tactile response to the deflection) provide strong support to the notion that recordings were obtained from pyramidal cells, as has been previously reported [50–54].

First, we stimulated electrically the POm (single pulse of 0.5 ms; 15–80 μA) alone. POm E-stimulation elicited spikes (for example see Fig 2C) in infra- and supragranular layers of barrel cortex. In infragranular layers the latencies of these spikes varied in the range of 5–50 ms (mean latency: 23.67±0.9 ms; n = 69). In supragranular layers in the range of 5–50 ms (mean latency: 16.30±0.5 ms; n = 51). These findings are in agreement with recent studies suggesting that POm projections make excitatory synapses with barrel cortex pyramidal cells [20, 39, 43].

Also, in all cases, we checked for potential rebound excitation (potential delayed spikes >150 ms in infra- or >50 ms in supragranular layers after E-stimulation offset). However, after POm E-stimulation we did not find rebound excitation even at maximal intensity used in our experiments (80 μA;).

Anatomically POm receives corticothalamic inputs from infragranular layers, thus, infragranular activity elicited by thalamic POm E-stimulation could also result from antidromic activation of corticothalamic axons. This would induce cortical responses characterized by minimal response variability and failure to show neural response fatigue [55, 56]. In contrast, orthodromic stimulation would activate cortical sites through neural pathways, characterized by substantial response timing variability and decremental cortical responses with repeated electrical stimulation pulses. At the end of each E-stimulation experiment we applied a train of 20 pulses (0.5 ms; same intensity) at high frequency (100 Hz) to check for antidromic activity. We did not find evoked spikes having the ability to follow this high frequency E-stimulation. None of the cortical recorded neurons were antidromically activated by thalamic E-stimulation at the intensities used. Thus, the results obtained in our experiments were due to orthodromic cortical activation from thalamic inputs.

Increasing POm activity by E-stimulation modulates sensory response in barrel cor- tex. To examine the effects of POm E-stimulation on infra- and supragranular neurons, we compared cortical sensory responses before and during POm E-stimulation (500 ms before each vibrissae stimulus; Fig 2). We applied the E-stimulation protocol defined by two blocks of 30 pulses (air puff 20 ms duration) delivered to one whisker at 0.5 Hz. In the second block, we stimulated electrically the POm just before each sensory stimulus (Fig 2D). Quantitative measures of neural responses were examined to determine how paralemniscal thalamic E-stimulation affected cortical responses to vibrissae deflections. We found that POm E-stimulation was accompanied by a marked change in cortical sensory responses in a layer specific manner. Following POm E-stimulation just before whisker stimulus, cortical sensory response magnitude and duration significantly decreased. These effects were demonstrated both by the rasters and

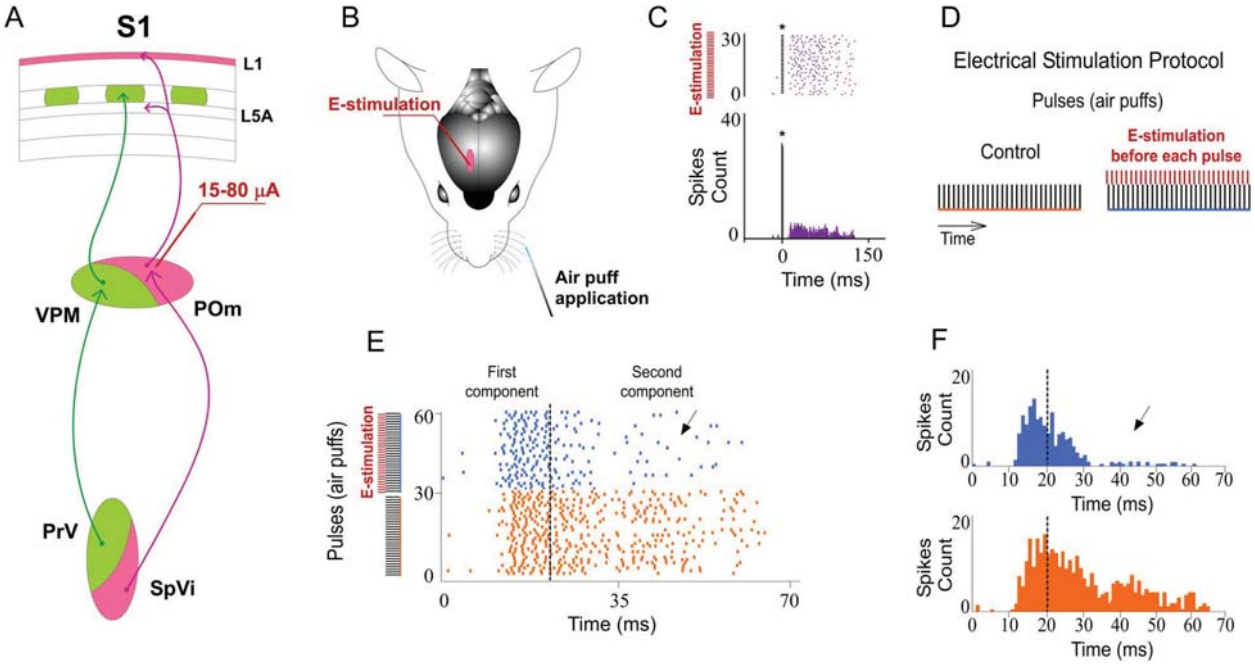


Fig 2. Increasing POm activity by POm E-stimulation just before sensory stimulus modulates whisker cortical responses. (A) Schematic diagram summarizing the main components of the lemniscal (green) and paralemniscal (pink) thalamocortical circuitry to barrel cortex. (B) Schematic diagram indicating the experimental protocol used in our study. (C) In agreement with recent studies suggesting that POm projections make excitatory synapses with barrel cortex pyramidal cells [20, 39, 43, 73], POm E-stimulation alone (single pulse of 0.5 ms; 15–80 μA) elicited orthodromic spikes in infra- and supragranular layers of barrel cortex. An example of evoked orthodromic spikes in the barrel cortex infragranular layer by POm E-stimulation is shown. * indicates stimulation artifacts. (D) Experimental protocol. The 'Electrical Stimulation Protocol' consisted of two blocks of 30 pulses (air puff 20 ms) delivered to the principal whisker at 0.5 Hz. We stimulated electrically the POm, VPM, L1 or L5 in S1 50–1000 ms before each pulse in the second block (blue) applied 60 s after the first block (orange). (E, F) POm E-stimulation 500 ms before whisker stimulus reduced sensory responses in infra- and supragranular layers of S1. Raster plots (E) and PSTHs (F) are shown for a sample multiunit infragranular response. Vertical dashed lines separate response components. POm E-stimulation shortened responses and reduced spikes mainly in the second response component (arrows). Spikes are aligned on sensory stimulus (air puff) presentation (Time 0 ms). POm E-stimulation was applied 500 ms before air puffs (31 to 60 pulses; red bars).

doi:10.1371/journal.pone.0148169.g002

by the peristimulus time histograms (PSTHs; Fig 2F). Results were consistent across all animals (n = 15).

Taken into account that POm projections target specifically L5A, we performed a preliminary analysis of single-units from different deeps. POm E-stimulation induced similar response decrease in both superficial (900–1200 μm) and deep (1200–1500 μm) infragranular units (-27%; n = 27; P<0.001 and -33%; n = 31; P<0.001, respectively). A total of 82% of superficial infragranular neurons (27 of 33) and 86% of deep infragranular neurons (31 of 36) decreased their sensory responses correlated with POm E-stimulation. Therefore, we combined all these single units across different depths into a single neuronal population termed infragranular layer. In this cortical layer, POm E-stimulation before each vibrissae stimulus induced a mean response decrease from 2.08±0.1 spikes/stimulus in control condition (before the application of the POm E-stimulation) to 1.48±0.1 spikes/stimulus during POm E-stimulation condition (-29%; n = 80; P< 0.001). A total of 89% of infragranular neurons (80 of 90) displayed changes in responses correlated with POm E-stimulation. The latency of the vibrissae response onset did not change while offset latencies significantly decreased during POm E-stimulation. Onset tactile responses had on average 13.20±0.12 ms latency in control and 13.09±0.10 ms after POm E-stimulation (-1%; n = 80; P = 0.41). Offset tactile responses decreased on average from

59.64±0.55 in control condition to 46.14±0.32 ms during POm E-stimulation (-23%; n = 80; P< 0.001).

In supragranular layers, POm E-stimulation applied before each whisker stimulus induced a mean response decrease from 1.95±0.1 spikes/stimulus in control condition to 1.33±0.1 spikes/stimulus in POm E-stimulation condition (-32%; n = 67; P < 0.001). A total of 90% neurons (67 of 74) displayed changes correlated with POm E-stimulation. Onset tactile responses had on average 14.92±0.22 ms latency and 14.14±0.18 ms after POm E-stimulation (-4%; n = 67; P = 0.06). Offset tactile responses had on average 41.64±0.6 ms latency and was reduced to 32.81±0.71 ms after POm E-stimulation (-21%; n = 67; P<0.001).

In both layers, POm E-stimulation before whisker stimulus resulted in decreased spike count.

However, this reduction was not homogeneous along the sensory response (Fig 2E and 2F). Previous reports from our laboratory have described two different components of tactile responses and the relevant implication of N-methyl-D-aspartate (NMDA) receptors manly in the late component of the response [57, 58]. Accordingly, here we divided each PSTH in two components: the first (from onset to 20 ms) and the second (from 20 ms to offset) components. We found important differences between these components. In infragranular layers, the first component of the PSTH did not decrease (from 1.02±0.1 to 0.97±0.1 spikes/stimulus; -5%; n = 80; P = 0.44). However, spikes were suppressed abruptly in the second component of the response by POm E-stimulation (from 1.06±0.1 to 0.50±0.1 spikes/stimulus; -52%; n = 80; P<0.001). In supragranular layers, the first component decreased from 1.15±0.1 to 0.94±0.1 (-19%; n = 67; P< 0.001) and from 0.80±0.1 to 0.40±0.1 (-50%; n = 67; P<0.001) in the second component. These findings demonstrate the important differences between both components.

As a control for specificity of the POm E-stimulation site, we also stimulated electrically (single pulse of 15–80 μA, 0.5 ms) the VPM in 9 rats. We found that VPM E-stimulation alone elicited short latencies spikes in infra- and supragranular layers of barrel cortex. In infragranular layers the latencies of these spikes varied in the range of 4–38 ms (mean latency: 13.42±0.5 ms; n = 38) and in supragranular layers in the range of 4–30 ms (mean latency: 12.37±0.4 ms; n = 50). We also applied high frequency VPM E-stimulation (a train of 20 pulses at 100 Hz). Cortical spikes decreased with increasing pulse number consistent with orthodromic stimulation. Also, we test the possibility of rebound excitation. We did not find delayed rebound excitation occurring 38 ms after VPM E-stimulation (for example see Fig 3A) within the current range used in our study (<80 μA). However, applying VPM E-stimulation with a higher intensity (>130 μA) we found rebound activity in same tested cases (data not shown).

After that, we compared cortical sensory responses before and after VPM E-stimulation (500 ms before each stimulus). Cortical responses increased their magnitude in both layers (quantified in Fig 3B; P<0.001in both layers). This effect was more prevalent in the second component of the response. In infragranular layers, we found an increased number of spikes from 0.96±0.1 to 1.01±0.1 spikes/stimulus (5%; n = 38; P = 0.031) in the first component. Spikes in the second component of the response were also increased by VPM E-stimulation (from 1.09±0.2 to 1.36±0.2 spikes/stimulus; 25%; n = 38; P<0.001; Fig 3). Similarly, the number of spikes in the first component increased from 1.13±0.1 to 1.21±0.1 (7%; n = 50; P<0.001) and from 0.69±0.1 to 0.89±0.1 (29%; n = 50; P<0.001; Fig 3) in the second component of supragranular layer neurons. A total of 73% of infragranular layer neurons (38 of 52) and 76% of supragranular neurons (50 of 66) displayed increments in responses correlated with VPM E-stimulation.

These findings suggest significant differences between POm and VPM thalamic nuclei. Our results showed that VPM E-stimulation alone elicited shorter latencies orthodromic spikes in infra- and supragranular layers of barrel cortex than POm E-stimulation. VPM orthodromic spikes varied in the range of 4–38 ms in infra- and of 4–30 ms in supragranular layers.

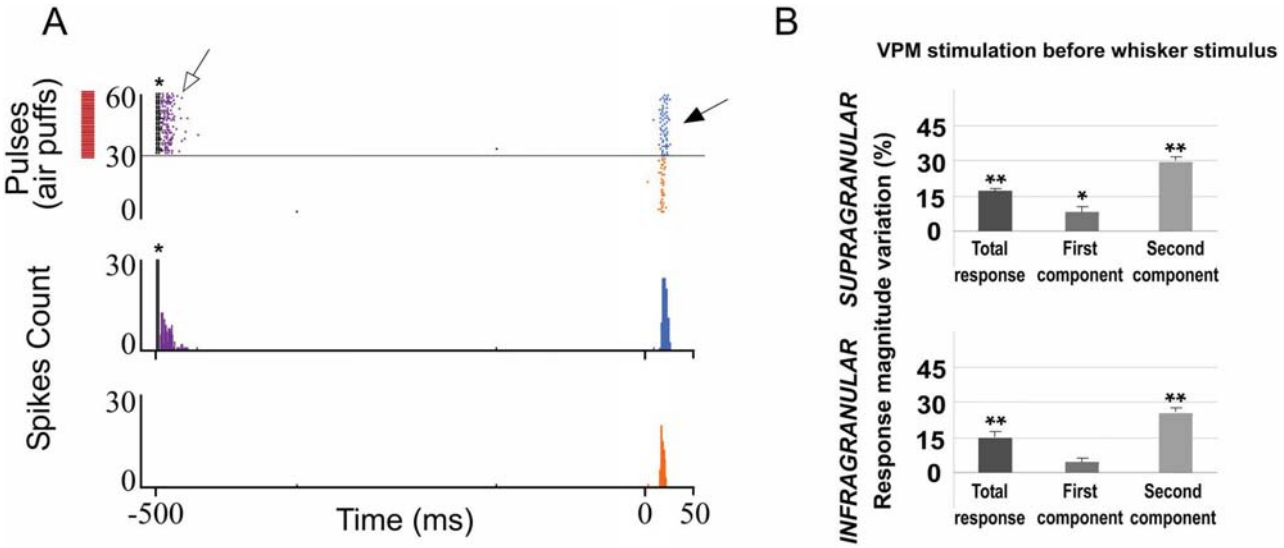


Fig 3. VPM E-stimulation just before whisker stimulus enhances sensory responses in barrel cortex. (A) Raster plots and PSTHs are shown for a sample supragranular neuron. VPM E-stimulation was applied 500 ms before pulses 31 to 60 (red bars). In contrast to POm E-stimulation, spikes mainly in the second component of the response were strongly increased by VPM E-stimulation (filled arrow). We did not find delayed rebound excitation occurring 30 ms after VPM E-stimulation within the current range used in our study (<80 μA). VPM E-stimulation evoked cortical spikes (open arrow). * indicates E-stimulation artifacts. (B) Change (%) in mean sensory response magnitude by VPM E-stimulation 500 ms before stimulus. Total response was increased in both layers by VPM E-stimulation. First component of infragranular responses was not significantly affected. Spikes in the second component were strongly increased in both layers.

doi:10.1371/journal.pone.0148169.g003

However, POm E-stimulation (same intensity) elicited evoked-spikes lasting up to 150 ms in infra- and up to 50 ms in supragranular layers. These results are in agreement with other studies showing that evoked bursts of EPSCs in neocortical neurons triggered by VPM neurons had faster decay times than those from POm neurons [20].

Moreover, in contrast to VPM E-stimulation, following POm E-stimulation just before whisker stimulus, cortical responses magnitude and duration significantly decreased. These opposite results from VPM or POm E-stimulation on whisker cortical responses suggested a different functional role of these thalamic nuclei in somatosensory processing.

POm inactivation enhances whisker response magnitude and duration in barrel cor- tex. To further understand the POm implication in cortical sensory processing, we pharmacologically inactivated POm neurons by infusing a small volume (0.1–0.3 μl; 1 mM) of the $GABA_A$ receptor agonist muscimol in 16 rats. Surprisingly, inactivating POm enhanced sensory responses in infra- and supragranular layers within a few minutes (<5 min) of the injection (Fig 4). We found enhanced tactile responses in 37 out of 51 neurons (67%) and 51 of 59 neurons (86%) in infra- and supragranular layer, respectively (measured at 15 min after injection). The evoked spikes in response to whisker stimulation were enhanced from 1.96±0.3 spikes/stimulus to 2.26±0.3 spikes/stimulus (15%; n = 37; P<0.001) in infragranular layers and from 1.86±0.2 spikes/stimulus to 2.16±0.3 spikes/stimulus (16%; n = 51; P<0.001) in supragranular layers (Fig 4C). The response facilitation was evident in the second response component (Fig 4C). In infragranular layers, the first component was not affected (from 1.04±0.2 to 1.03 ±0.2 spikes/stimulus; -2%; n = 37; P = 0.4). In contrast, spikes in the second component of the response were increased abruptly by POm inactivation (from 0.92 ± 0.1 to 1.23 ± 0.2 spikes/stimulus; 34%; n = 37; P<0.001). In supragranular layers, the first component was also not affected (from 1.13±0.2 to 1.17±0.2 spikes/stimulus; 3%; n = 51; P = 0.61) while the second component increased from 0.73±0.1 to 1.01±0.1 spikes/stimulus (37%; n = 51; P<0.001). The

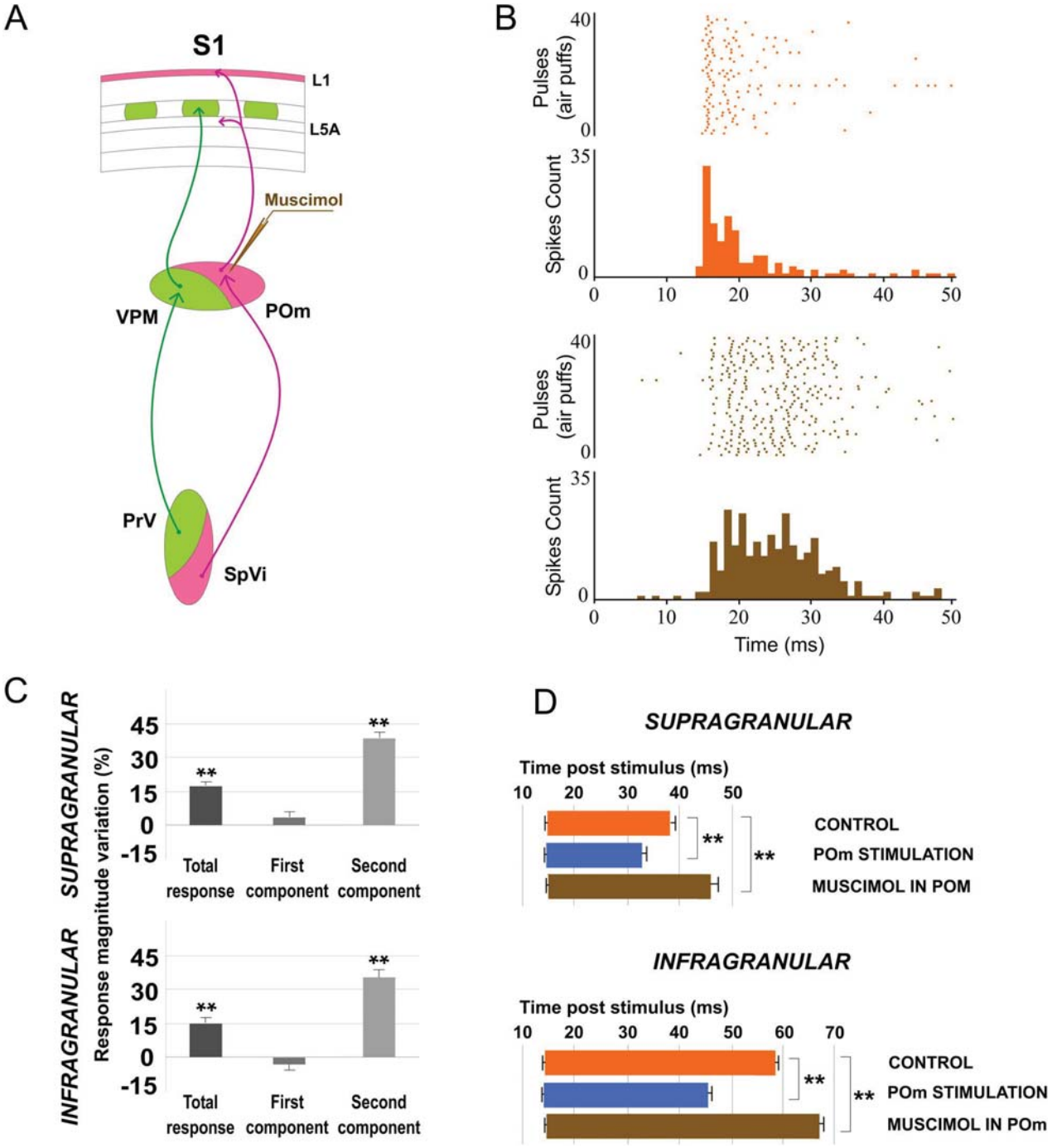


Fig 4. Muscimol-induced inactivation of the POm. (A) Schematic diagram indicating the experimental manipulation of the paralemniscal (pink) thalamocortical circuitry to barrel cortex. (B) POm inactivation enhanced responses in S1 mainly in the second component. Raster plots and PSTHs are shown for a sample supragranular neuron before (top) and after (bottom) POm inactivation. Also the pattern of spikes in the response was changed after POm inactivation suggesting that POm imposes a precise control of cortical responses. (C) Percentage change in mean response magnitude when POm was inactivated with muscimol. Spikes were strongly enhanced in the second component of the response. (D) Mean onset and offset latencies and response duration in Control (orange), in POm E-stimulation (blue) and in POm inactivation condition (brown). Response duration decreased with POm E-stimulation and increased in POm inactivation condition. We did not find differences in onset latencies but offset latencies changed significantly. Horizontal bars represent response duration.

doi:10.1371/journal.pone.0148169.g004

response onset latency was not significantly modified under muscimol application in POm (Fig 4D). However, the response offset latency was increased in infra- (12%; n = 37; P<0.001) and supragranular layers (22%; n = 51; P<0.001; Fig 4D).

Also, spontaneous activity was increased from 0.94±0.2 to 1.23±0.3 spikes/s (31%; n = 51; P<0.001) in infragranular neurons and from 0.70±0.2 to 0.95±0.2 spikes (35%; n = 37; P<0.001) in supragranular neurons. These results suggest that POm activity modulates cortical excitability of the barrel cortex.

To determine if this effect was specific of POm nucleus, we pharmacologically inactivated VPM neurons with muscimol (0.1–0.3 μl; 1 mM) in 9 rats. The magnitude of cortical responses diminished within a few minutes (<5 min) after the injection (Fig 5). A total of 82% of infragranular layer neurons (27 of 33) and 94% of supragranular layer neurons (29 of 31) displayed significant reduction in responses correlated with VPM inactivation. The evoked spikes in response to whisker sensory stimulation were reduced from 1.97±0.3 to 1.29±0.2 spikes/stimulus (-34%, P<0.001; n = 27) in infragranular layers and from 1.72±0.3 to 0.97±0.2 spikes/stimulus (-44%, P<0.001; n = 29) in supragranular layers (Fig 5B). Our results are in agreement with other studies showing that VPM lesions abolish cortical responses evoked by whisker stimulation [59].

These findings suggest more differences between POm and VPM thalamic nuclei. In contrast to VPM inactivation, following POm inactivation cortical response magnitude significantly increased. In both layers, the first component was not affected by POm inactivation. However, spikes in both components of cortical sensory responses were strongly abolished after VPM inactivation. Again, these opposite results from VPM or POm inactivation on whisker cortical responses suggest a different functional role of these thalamic nuclei in somatosensory processing.

POm regulation on cortical sensory processing is time and intensity-dependent

To further understand these effects we investigated sensory response changes according to the interval between POm E-stimulation and sensory stimulus. We found that response magnitude and duration of cortical neurons changed by POm E-stimulation intervals before sensory stimulus. The results are summarized and quantified in Fig 6. This figure also demonstrates the important differences between both cortical layers. In infragranular layers, the first component was not significantly affected at any time interval (50–1000 ms). However, spikes in the first component were strongly reduced at all intervals in supragranular layers. In addition, we found a significant reduction of spikes in the second response component in both infra- and supragranular layers. Moreover, in supragranular layers, we did not find significant response changes at longer intervals than 700 ms. In contrast, we found a significant reduction of spikes even at 1000 ms in infragranular layer. These findings implicate different dynamics between both layers, especially on the first response component.

We also found that response duration and magnitude of cortical neurons decreased with increasing E-stimulation intensity (Fig 7), indicating that POm E-stimulation effects are also intensity-dependent. Reduction in whisker response magnitude and duration by POm E-stimulation at two current intensity ranges (15–45μA and 50–80 μA) are quantified in Fig 8. We found that increasing POm E-stimulation intensity mainly reduced second component spikes, shortening the duration of sensory responses.

POm exerts its control of cortical sensory responses mainly through L1

Recent studies suggest that POm projections make excitatory synapses with barrel cortex pyramidal cells [20, 39, 43]. Accordingly, we have showed above that POm E-stimulation alone

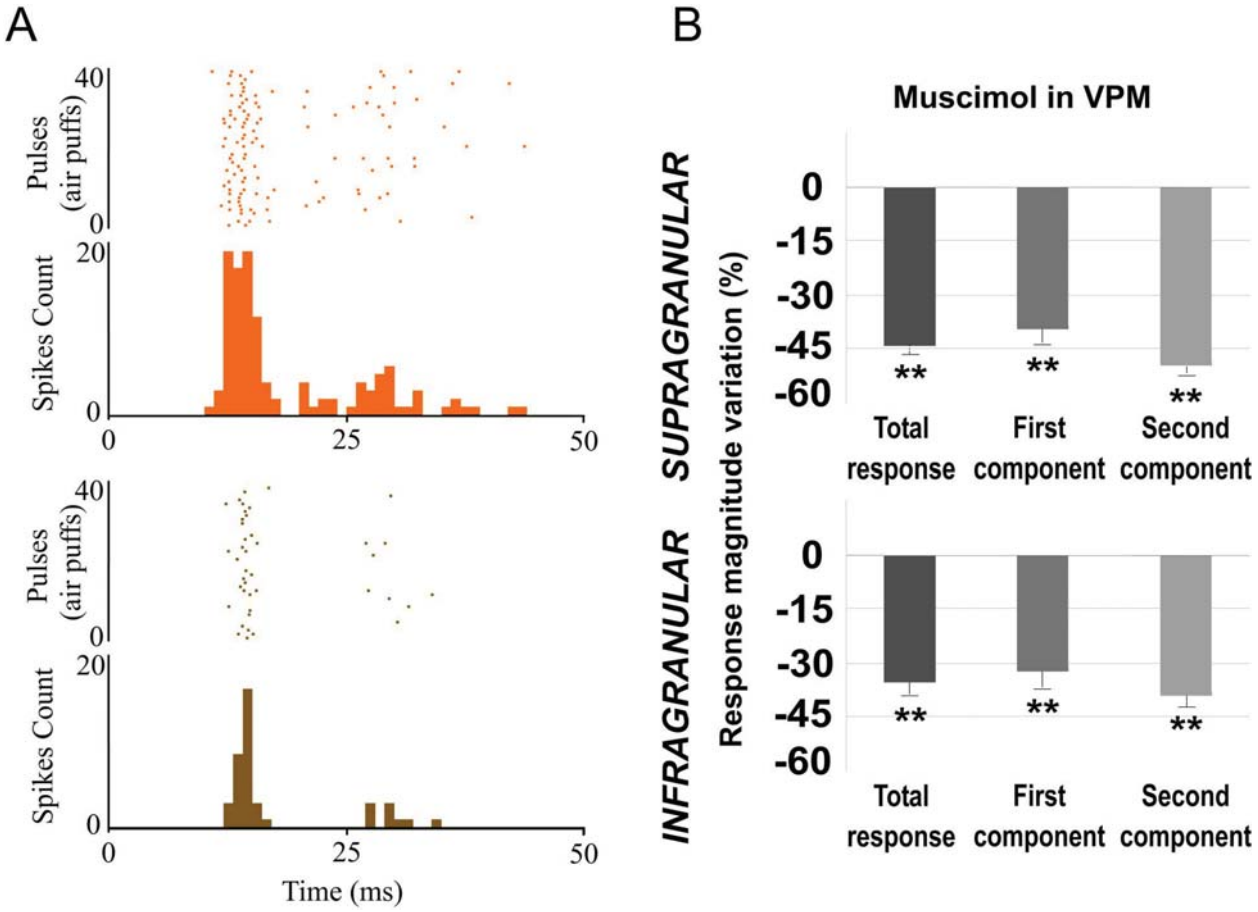


Fig 5. Muscimol-induced inactivation of the VPM. Inactivating VPM decreased responses in infra- and supragranular layers of S1. (A) Raster plots and PSTHs are shown for a supragranular sample neuron before (orange) and after (brown) VPM inactivation. (B) Mean response magnitude change (%) evoked by VPM inactivation. In both layers, spikes of sensory responses were strongly abolished after VPM inactivation by muscimol.

doi:10.1371/journal.pone.0148169.g005

elicited excitatory orthodromic spikes in infra- and supragranular layers of barrel cortex. However, our results also showed that POm E-stimulation just before sensory stimulus reduced magnitude and duration of cortical whisker responses. Moreover, POm inactivation by muscimol caused an enhancement of both sensory cortical responses and spontaneous cortical activity in the barrel cortex. How can these intriguing effects be explained? It is well described that blocking activity in L1 increases whisker-evoked responses [40], suggesting that L1 exerts an inhibitory influence on whisker responses. Since L1 receives strong inputs from POm [18, 19, 21, 23, 28], it is then possible that POm exerts its control of cortical sensory responses through L1. To test this hypothesis, we perform the following experiments.

Blocking inhibitory transmission in L1 enhances whisker response in barrel cortex. It is known that L1 inputs generate direct, rapid excitatory postsynaptic potentials in L1 interneurons [60, 85]. Accordingly, in the barrel cortex, whisker-evoked sensory information is rapidly relayed to L1 neurons, which, in turn, act to powerfully inhibit whisker-evoked responses [40, 60]. Since L1 is composed of more than 90% of GABAergic neurons [61, 62], to further understand the contribution of L1 in POm regulation of cortical sensory processing, we pharmacologically blocked GABAergic inhibitory transmission in L1 in 12 rats. Picrotoxin (PTX; antagonist of $GABA_A$ receptors; 1 mM) application (1 μl) to the cortical surface was accompanied by a

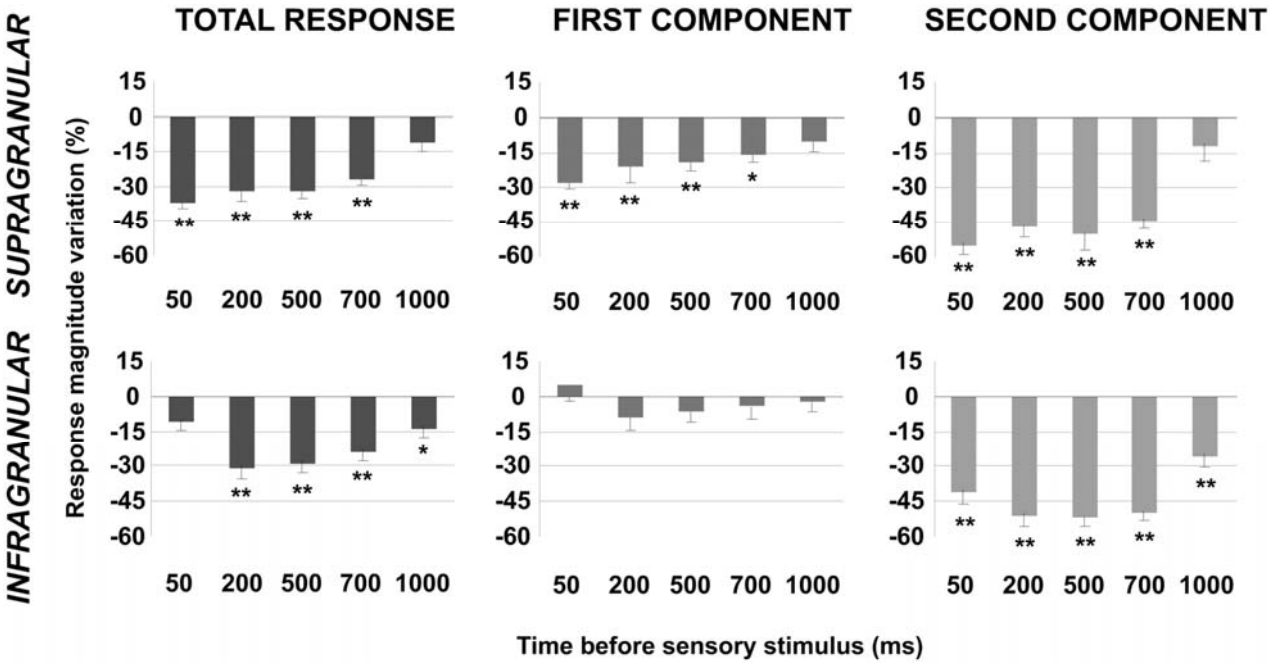


Fig 6. The effect of POm E-stimulation is time-dependent. This figure shows the change in mean sensory response magnitude by different POm E-stimulation intervals (50–1000 ms) before whisker stimulus. Supragranular total response was significantly reduced by POm E-stimulation at intervals ranged from 50 to 700 ms but not at 1000 ms. Total response of infragranular neurons was reduced at intervals from 200 to 1000 ms but not at 50 ms. In infragranular layers, the first response component (from onset to 20 ms) was not significantly affected at any time interval. In contrast, in supragranular layers, spikes in the first component were reduced at intervals <1000 ms. Spike reduction by POm E-stimulation was more prevalent in the second component of the responses in both layers (from 20 ms to offset). In supragranular neurons, spikes in the second component were decreased significantly at several time intervals from 50 to 700 ms before stimulus. The most powerful effect was found at 50 ms. Spikes in the second component of infragranular neurons were reduced significantly at time intervals from 50 to 1000 ms before sensory stimulus. In infragranular layer, the numbers of single units analyzed in each interval are: n = 38 (50 ms), n = 40 (200 ms), n = 80 (500 ms), n = 55 (700 ms) and n = 40 (1000 ms). In supragranular layer, n = 35 (50 ms), n = 33 (200 ms), n = 67 (500 ms), n = 45 (700 ms) and n = 32 (1000 ms). In all figures: * P<0.05; ** P<0.01.

doi:10.1371/journal.pone.0148169.g006

marked change in cortical sensory responses in infra- and supragranular layers. Spontaneous activity rates were significantly affected, as is depicted in Fig 9. The baseline firing rate was increased from 0.88±0.3 to 1.20±0.3 spikes/s (36%; n = 22; P<0.001; Wilcoxon-matched pairs test) in infragranular layer and from 0.59±0.2 to 0.79±0.2 spikes/s (38%; n = 21; P<0.001; Wilcoxon-matched pairs test) in supragranular layer.

It is known that Cav2.1 (P/Q- type) voltage-gated calcium channels are expressed on parvalbumin (PV) interneuron axon terminals and mediate GABA release from fast spiking interneurons to pyramidal cells [63–65]. To study in more detail the inhibitory implication in POm control of cortical processing, we applied P/Q- type voltage-gated calcium channels blocker ω-agatoxin-IVa (0.1 μM) to the cortical surface (1 μl) in 10 rats. We found that cortical sensory response magnitude and duration significantly increased 15 min after injection. A total of 88% of infragranular layer neurons (23 of 26) and 91% of supragranular neurons (21 of 23) displayed increments in sensory responses after blocking P/Q-type calcium channels in superficial cortex. Spontaneous activity rates were also significantly affected (Fig 9). The baseline firing rate was increased from 0.97±0.3 to 1.18±0.3 spikes/s (22%; n = 23; P<0.001; Wilcoxon-matched pairs test) in infragranular layer and from 0.66±0.2 to 0.86 ± 0.2 spikes/s (31%; n = 22; P<0.001; Wilcoxon-matched pairs test) in supragranular layer.

A total of 92% of infragranular layer neurons (22 of 24) and 81% of supragranular neurons (21 of 26) displayed increments in sensory responses after blocking GABAergic inhibitory

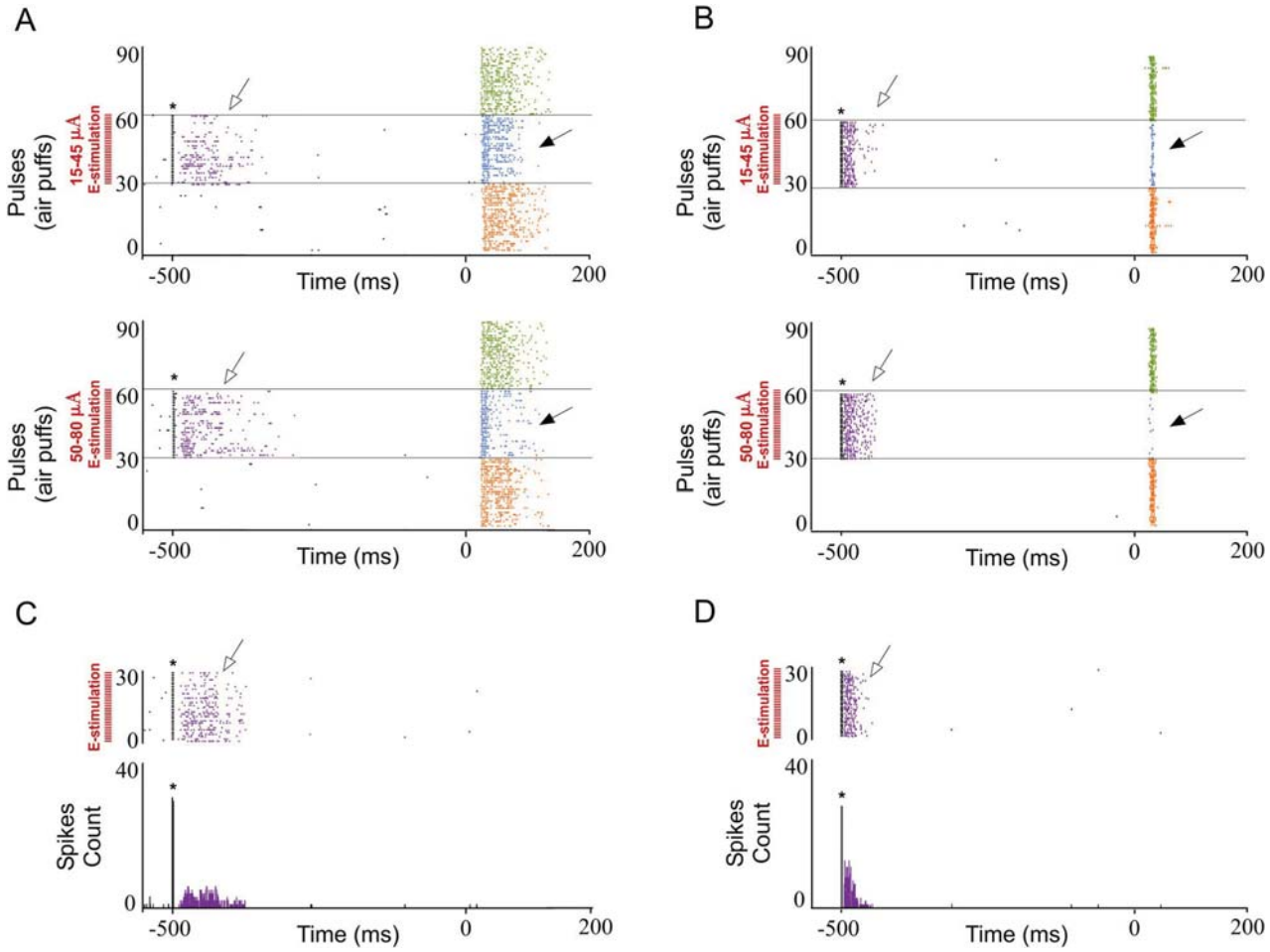


Fig 7. The effect of POm E-stimulation is intensity-dependent. Response duration and magnitude of cortical neurons decreased with increasing E-stimulation intensity. Raster plots and PSTHs are shown for a sample infragranular (A) and supragranular (B) responses after 15–45 μA (top) and 50–80 μA (bottom). Increasing POm E-stimulation intensity shortened responses more strongly, reducing spikes in the second response component (filled arrows). Control condition before (orange) and after (green) POm E-stimulation condition (blue) are shown. POm E-stimulation was applied 500 ms before pulses 31 to 60. Whisker stimulus presentation was applied at Time 0 ms. POm E-stimulation applied alone elicited orthodromic (open arrows) but not rebound activity in infra- (C) and supragranular layers (D). * indicates E-stimulation artifacts.

doi:10.1371/journal.pone.0148169.g007

transmission in L1. Cortical response magnitude (Fig 10A) and duration were significantly increased 15 min after PTX application. Again, this effect was more prevalent in the second component of the response. In infragranular layers, we found an increased number of spikes from 0.84±0.2 to 0.98±0.2 spikes/stimulus (16%; n = 22; P<0.001; Wilcoxon-matched pairs test) in the first component. Spikes in the second component of the response were increased from 1.12±0.2 to 1.55±0.3 spikes/stimulus (38%; n = 22; P<0.001; Wilcoxon-matched pairs test; Fig 10A). In supragranular layers, we found an increased number of spikes from 0.97±0.2 to 1.19±0.2 (22%; n = 21; P<0.001; Wilcoxon-matched pairs test) in the first component and from 0.91±0.1 to 1.20±0.2 (32%; n = 21; P<0.001; Wilcoxon-matched pairs test; Fig 10A) in the second component.

In infragranular layers the latency of the response onset did not change (from 14.17±0.33 to 14.38±0.32 ms; 1%; n = 22; P = 0.37; Wilcoxon-matched pairs test) while offset latency increased from 55.46±0.67 to 69.29±1.54 ms (25%; n = 22; P<0.001; Wilcoxon-matched pairs

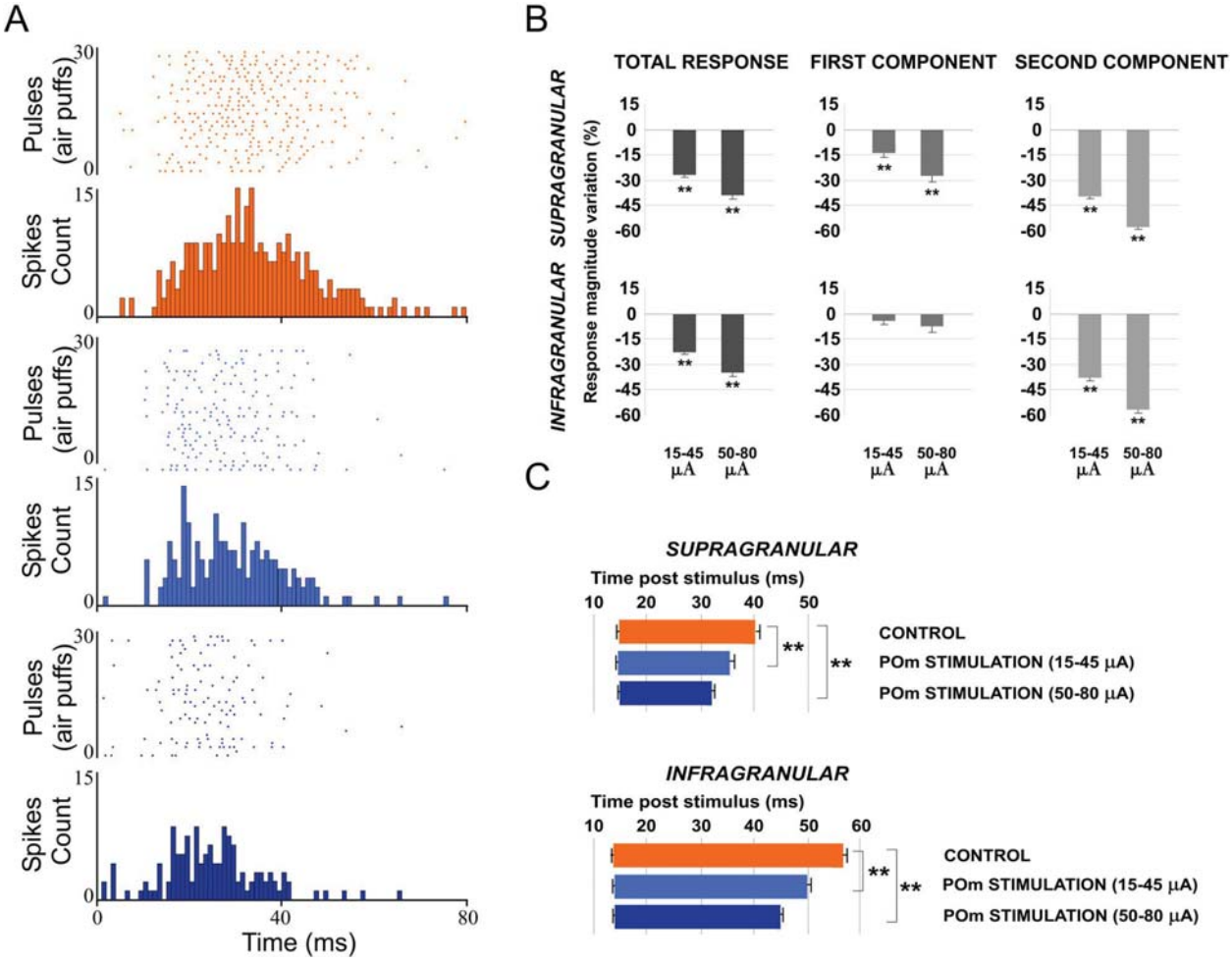


Fig 8. Increasing POm E-stimulation intensity enhances the reduction of second component spikes, shortening the duration of the sensory response. (A) Raster plots and PSTHs are shown for a supragranular whisker response change after increasing POm E-stimulation intensity before sensory stimulus. Control response (orange), 15–45μA POm E-stimulation (blue) and 50–80 μA POm E-stimulation (dark blue). (B) Response magnitude variation (%) with different POm E-stimulation intensities before sensory stimulus. (C) Increasing POm E-stimulation intensity before stimulus shortened the responses offset latencies. Horizontal bars represent response duration. In infragranular layers, the numbers of single units analyzed in B and C are: n = 57. In supragranular, n = 53.

doi:10.1371/journal.pone.0148169.g008

test;). In supragranular layers the onset latency was not modified (from 14.35±0.36 to 14.64 ±0.37 ms; 2%; n = 21; P = 0.23; Wilcoxon-matched pairs test). In contrast, offset latency increased from 38.81±1.57 to 46.96±1.02 ms (21%; n = 21; P<0.001; Wilcoxon-matched pairs test).

Whisker response magnitude and duration increased in infra- and supragranular layers (Fig 10B). In infragranular layers, the first component did not increase significantly (from 0.78±0.2 to 0.83±0.2 spikes/stimulus; 6%; n = 23; P = 0.07; Wilcoxon-matched pairs test). Spikes in the second component of the response were increased from 1.06±0.3 to 1.22±0.3 spikes/stimulus (15%; n = 23; P<0.001; Wilcoxon-matched pairs test; Fig 10B). In supragranular layers, the first component was not affected (from 0.73±0.1 to 0.79±0.1 spikes/stimulus; 8%; n = 22; P = 0.062; Wilcoxon-matched pairs test) while the second component was increased (0.68±0.1 to 0.81±0.1 spikes/stimulus; 19%; n = 22; P<0.001; Wilcoxon-matched pairs test; Fig 10B).

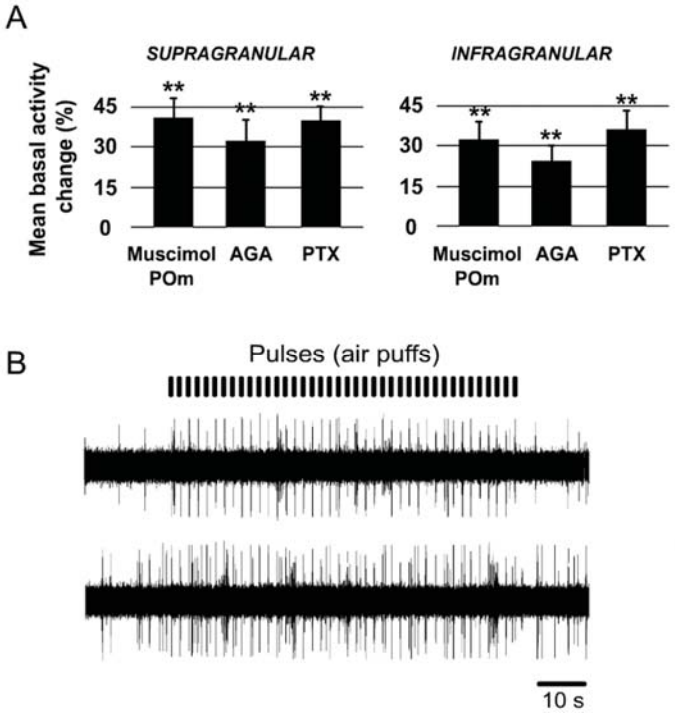


Fig 9. Cortical spontaneous activity changes in different tested conditions. (A) Mean basal activity change (%) in different conditions (muscimol in POm; AGA and PTX in cortical surface). In all conditions cortical basal activity in S1 was significantly increased after drugs applications. The baseline firing rate was calculated from mean firing within a 10 s window before the first pulse (air puff). (B) Muscimol-induced inactivation of the POm enhanced sensory responses in infra- and supragranular layers and increased cortical spontaneous activity. Raw cortical extracelular recordings are shown before (top) and after (bottom) muscimol application. These recordings show the enhancement of cortical sensory responses to whisker deflections (pulses). Cortical spontaneous activity was also increased after muscimol-induced inactivation of the POm.

doi:10.1371/journal.pone.0148169.g009

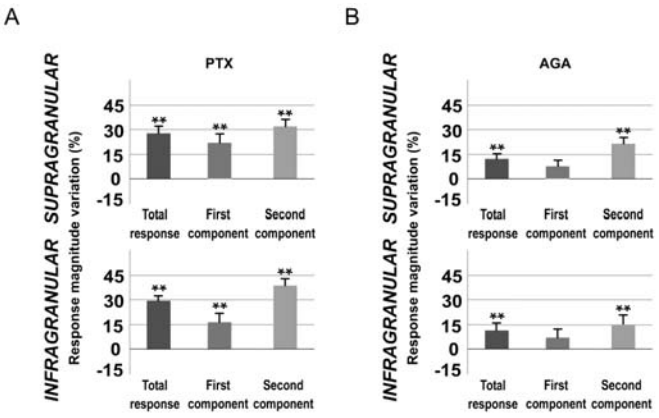


Fig 10. Blocking GABAergic inhibitory transmission in L1 enhances whisker responses. Change (%) in mean sensory response magnitude by PTX (A) or AGA (B) application in L1. Blocking GABAergic inhibitory transmission in L1 by PTX increased significantly whisker response magnitude in infra- (bottom row) and supragranular layers (top row) more strongly in the second component of the response. AGA application enhanced sensory responses in infra- and supragranular neurons. However, in both layers, AGA did not induce significant effects in the first component. * P<0.005; ** P<0.001.

doi:10.1371/journal.pone.0148169.g010

In accordance with previous studies [40], we confirm that L1 exerts an inhibitory influence on whisker responses. Our results demonstrate that GABAergic inhibitory transmission in L1 is implicated in the regulation of cortical excitability and sensory response magnitude and duration.

L1 GABAergic system is crucial in sensory cortical regulation by POm

Next, in that condition of L1 inhibitory transmission inactivation by PTX, we applied E-stimulation to the POm before (500 ms) each whisker stimulus (Fig 11). We found that response magnitude did not significantly decrease by POm E-stimulation (infragranular layers: 1%; n = 22; P = 0.67; Wilcoxon-matched pairs test; in supragranular layers: -4%; n = 21; P = 0.18; Wilcoxon-matched pairs test; Fig 12A, Total response). Response duration was also not affected by POm E-stimulation in this condition. Offset latencies were not reduced (in infragranular layer: -5%; n = 22; P = 0.098; Wilcoxon-matched pairs test; and in supragranular layer: -3%; n = 21; P = 0.9; Wilcoxon-matched pairs test; Fig 12B). Onset latencies in both layers were not significantly affected.

Blocking P/Q-type Ca2+ channels in L1 prevents POm electrical stimulation effect. When we applied POm E-stimulation before (500 ms) whisker stimulus in P/Q-type voltage-gated calcium channels blocked condition we found that cortical sensory responses did not significantly decrease. Response magnitude did not significantly decrease by POm E-stimulation in infragranular layers (-3%; n = 23; P = 0.15) and in supragranular layers (-6%; n = 22; P = 0.09; Wilcoxon-matched pairs test; Fig 13A Total response). Response duration was also not affected by POm E-stimulation in this condition. In infragranular layers, offset latencies were not reduced (-3%; n = 23; P = 0.39; Wilcoxon-matched pairs test) and the same was found in supragranular layers (-6%; n = 22; P = 0.08; Wilcoxon-matched pairs test; Fig 13B). Onset latencies in both layers were not affected.

L1 E-stimulation just before whisker stimulus modulates sensory response magnitude and duration in barrel cortex. To further confirm whether the observed POm modulation of cortical responses was mediated by L1, we investigated cortical response changes by applying L1 E-stimulation in S1 before sensory stimulus in 6 rats. Similar to POm E-stimulation, L1 E-stimulation (single pulse of 5–10 μA, 0.5 ms) before (150 ms) each whisker stimulus was accompanied by a marked change in cortical sensory responses. Magnitude and duration of cortical responses significantly decreased. Again, magnitude reduction was more prevalent in the second component of the response (Fig 14). In infragranular layers, the first component was not affected (from 1.11±0.1 to 1.08±0.1 spikes/stimulus; -3%; n = 33; P = 0.67). Evoked spikes were decreased in the second component of the response by L1 E-stimulation from 0.96 ±0.2 to 0.67±0.1 spikes/stimulus (-30%; n = 33; P< 0.001). In infragranular layers, 72% of neurons (33 of 46) displayed significant changes in responses correlated with L1 E-stimulation. In supragranular layers both response components were affected. The first component decreased from 1.02±0.1 to 0.83±0.1 spikes/stimulus (-19%; n = 39; P< 0.001) and from 0.78±0.1 to 0.48 ±0.1 spikes/stimulus (-38%; n = 39; P<0.001) in the second component. A total of 85% of supragranular layer neurons (39 of 46) displayed changes correlated with L1 E-stimulation.

The latency of the response onset did not change in infragranular neurons (13.82±0.13 ms in control and 13.68±0.1 ms after L1 E-stimulation; -1%; n = 33; P = 0.62). However, as occurred in POm E-stimulation condition, the main effect was found in offset latency (from 55.18±1.04 to 51.21±1.1 ms; -7%; n = 33; P<0.001), decreasing the duration of the response. The latency of the response onset was reduced in supragranular neurons from 14.67±0.3 to 13.93±0.25 ms (-5%; n = 39; P = 0.002) and offset latencies decreased from 40.58±1.54 to 34.33 ±1.4 ms (-15%; n = 39; P<0.001), as well.

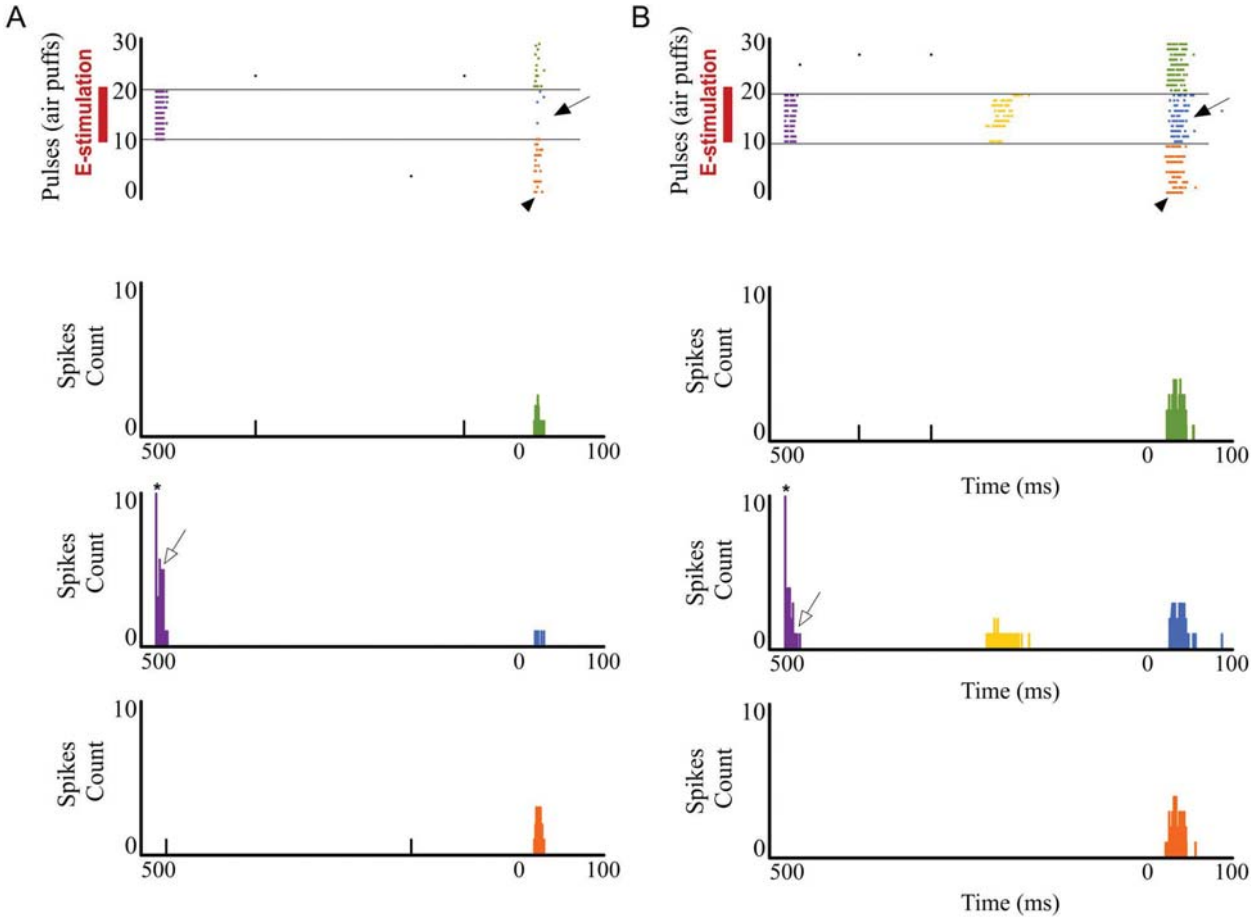


Fig 11. POm E-stimulation before whisker stimulus does not decrease cortical response magnitude and duration when PTX is applied in L1. Raster plots and PSTHs are shown for a supragranular neuron before (A) and after (B) GABAergic inhibitory inactivation in L1. Before PTX application, sensory response (filled arrows) of this example neuron was abolished by POm E-stimulation before whisker stimulus. However, POm E-stimulation did not reduce sensory response when GABAergic inhibitory transmission in L1 was inactivated (B). GABAergic inactivation in L1 allowed POm E-stimulation to cause rebound spikes (in yellow). PTX effect is also shown in the sensory response (arrowheads) enlargement after PTX application. Control condition before (orange) and after (green) POm E-stimulation condition (blue) are shown. POm E-stimulation was applied 500 ms before pulses (air puffs) 11 to 20. Open arrows indicate orthodromic spikes elicited by POm E-stimulation. * indicates stimulation artifacts.

doi:10.1371/journal.pone.0148169.g011

These results were similar to POm E-stimulation suggesting that the observed effects produced by POm E-stimulation in sensory cortical responses were mainly mediated by L1.

POm controls the sensory processing in S2 and this regulation is modulated by corticofugal activity from L5 in S1

POm neuron projections also target other cortical areas including S2 [18, 21]. It is well described the reciprocal connections between these areas. The above results demonstrate that POm modulates magnitude and duration of S1 cortical responses to sensory input. This sensory response adjustment could be also present in the processing of information between somatosensory cortical areas. Then, to determine the possible role exerted by the POm in the adjustment of somatosensory cortical processing between S1 and S2, we performed a complementary set of experiments investigating whisker response changes in S2 by electrically stimulating S1 and by muscimol-induced inactivation of the POm. The following results describe

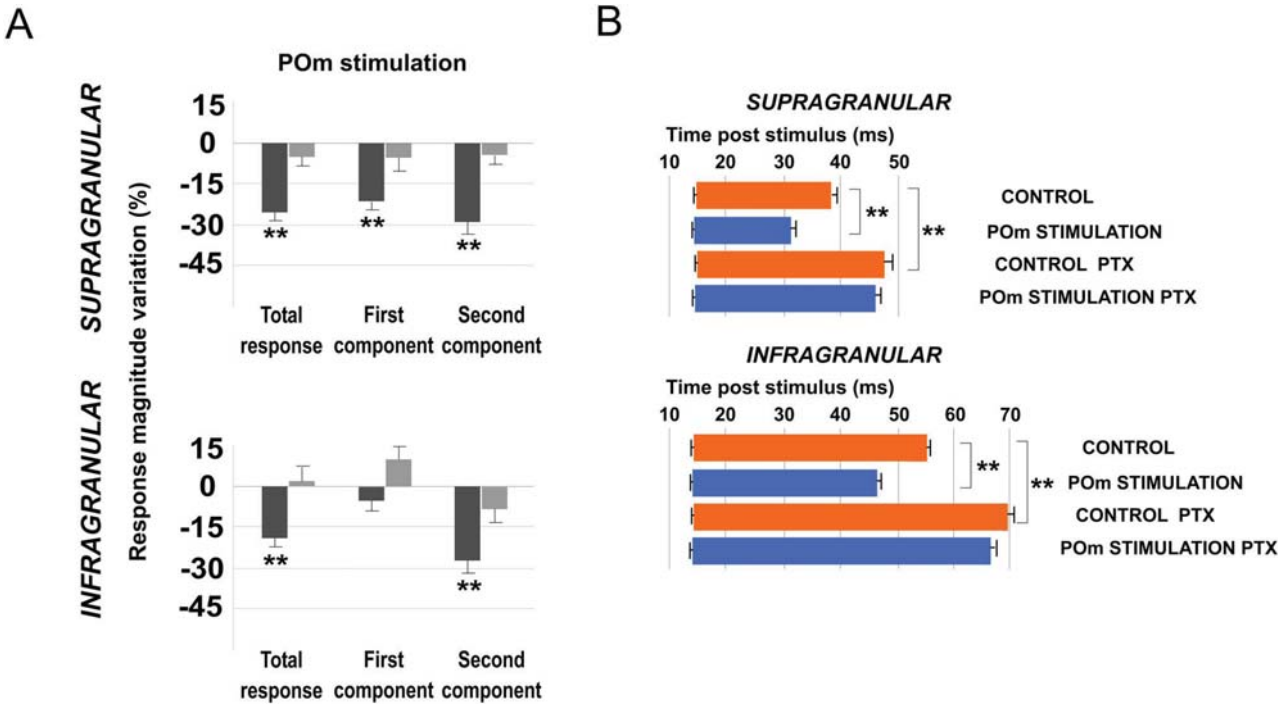


Fig 12. POm E-stimulation before whisker stimulus does not decrease cortical response magnitude and duration when PTX is applied in L1. (A) Percentage change in mean response magnitude by POm E-stimulation before (black) and after PTX application (grey). POm E-stimulation did not decrease cortical response magnitude when GABAergic inhibitory transmission in L1 was blocked. (B) PTX application in L1 increased whisker offset response latency in infra- and supragranular layers. POm E-stimulation before whisker stimulus did not decrease cortical response duration when PTX was applied in L1. Control (orange) and POm E-stimulation (blue) conditions are shown.

doi:10.1371/journal.pone.0148169.g012

below demonstrate that POm activity is also controlling the sensory processing in S2 and this regulation is modulated by corticofugal activity from L5 in S1.

L5 E-stimulation in S1 before sensory stimulus modulates whisker response in S2. It is known that L5 corticofugal neurons in S1 project to the POm (see Introduction). From here, POm neuron projections also target other cortical areas including higher-order somatosensory cortical regions.

We recorded vibrissal responses in the whisker representation area of S2 in 11 rats. We found that S2 neurons displayed a low firing rate in spontaneous conditions (0.87±0.6 spikes/s; n = 40) and displayed a contralateral response to whisker displacements. Then, we investigated sensory response changes in S2 neurons by L5 E-stimulation in S1 before whisker stimulus (150 ms). S1 L5 E-stimulation alone (single pulse of 0.5 ms; 5–30 μA) elicited strong activity in S2 (Fig 15B). The latencies of these evoked spikes varied in the range of 8–40 ms (mean latency: 21.61±0.7 ms; n = 40). When we stimulated electrically the L5 of S1 before each sensory stimulus, response magnitude decreased from 1.95± 0.3 to 1.35± 0.2 spikes/stimulus (-30%; n = 36; $P<0.001$). A total of 90% of S2 recorded units (36 of 40) displayed reduction in responses correlated with L5 E-stimulation in S1. First and second component results are described and quantified in Fig 16B. The latency of the response onset did not change (from 14.45±0.1 to 14.25±0.13 ms; -1%; n = 36; P = 0.31) but offset latency decreased by L5 E-stimulation (from 44.33±0.21 to 35.63±0.46 ms; -20%; n = 36; $P<0.001$).

Cortico-cortical sensory processing adjustment is abolished when POm is inactivated with muscimol. Next, to demonstrate that POm was implicated in the effects described

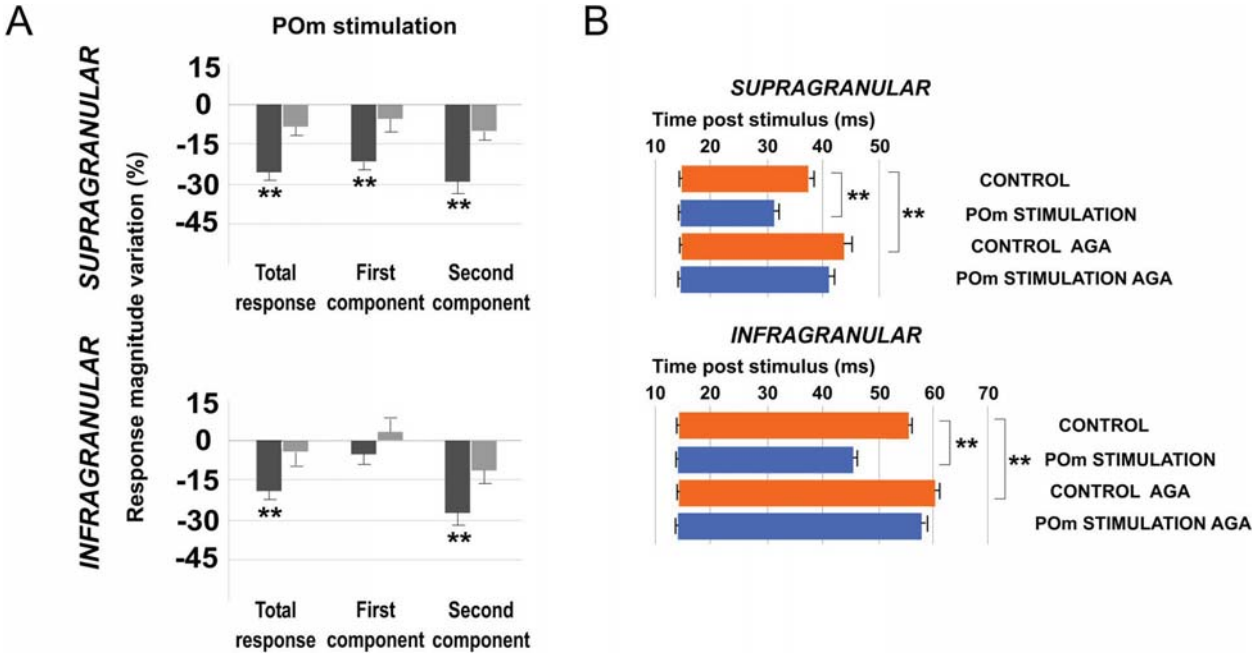


Fig 13. POm E-stimulation before whisker stimulus does not decrease cortical response magnitude and duration when AGA is applied in L1. (A) Percentage change in mean response magnitude by POm E-stimulation before (black) and after AGA application (grey). POm E-stimulation before each stimulus (500 ms) did not decrease cortical responses when P/Q-type voltage-gated calcium channels were blocked. (B) AGA application in L1 increased whisker offset response latency in infra- and supragranular layers. POm E-stimulation did not decrease cortical response duration when P/Q-type voltage-gated calcium channels were blocked. Control (orange) and POm E-stimulation (blue) conditions are shown.

doi:10.1371/journal.pone.0148169.g013

above, we inactivated the POm by infusing a small volume (0.1–0.3 μl; 1 mM) of muscimol. We found that 15 min after muscimol application, L5 E-stimulation in S1 could not reduce sensory response spikes in S2 (Fig 16). Change in mean sensory response magnitude by stimulating L5 of S1 before and after POm muscimol inactivation is quantified in Fig 16B. These findings indicate that POm activity is also controlling the sensory processing in S2 and this regulation is modulated by corticofugal activity from L5 in S1.

Furthermore, we found that S2 robust activity in response to L5 E-stimulation in S1 alone was eliminated after POm inactivation (Fig 16C and 16D white arrows) with a subsequent return after washout (data not shown). This finding is in agreement with other studies on corticothalamocortical communication implicating the POm in information transfer to higher-order cortical areas [25, 41, 42].

In sum, our results demonstrate that POm is implicated in the adjustment of information processing between somatosensory cortical areas.

Discussion

Here, using a combination of electrophysiology and pharmacology in vivo, we show that POm modulates magnitude and duration of supra- and infragranular barrel cortex whisker responses. Our findings demonstrate that L1 inputs from POm impose a time and intensity dependent regulation on cortical sensory processing. Moreover, we found that L1 GABAergic system mediates this process and that blocking P/Q-type Ca2+ channels in L1 prevents POm adjustment of whisker responses in the barrel cortex. Additionally, we found that POm is also controlling the sensory processing in S2 and this regulation is modulated by corticofugal activity from L5 in S1. Taken together, our data demonstrate the determinant role exerted by the

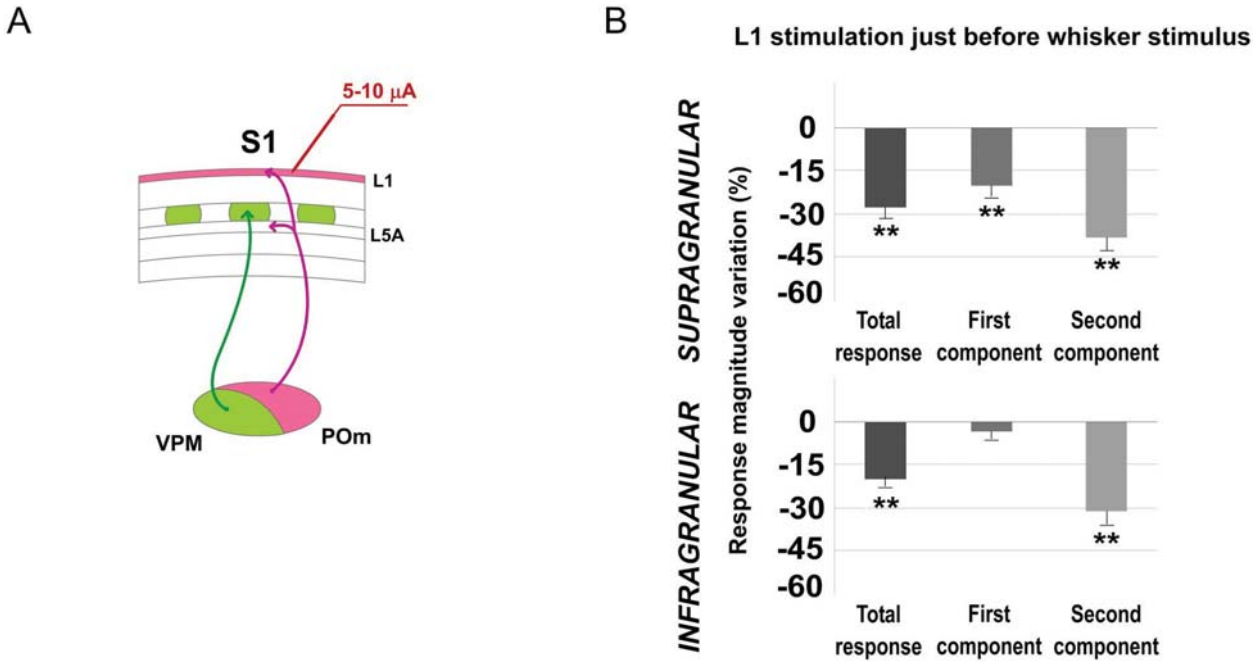


Fig 14. L1 E-stimulation before sensory stimulus modulates cortical responses. (A) Schematic diagram indicating the experimental manipulation of the barrel cortex L1. (B) Mean response magnitude variation (%) by L1 E-stimulation is quantified in this Fig Magnitude reduction was more prevalent in the second component of the response in both layers. In infragranular layers, in the first component we did not find a significant decrease of spikes. However, spikes in the second component were decreased strongly by L1 E-stimulation. In supragranular layers, in both components we found a significant reduction of spikes.

doi:10.1371/journal.pone.0148169.g014

POm in the adjustment of somatosensory cortical processing and in the regulation of cortical processing between S1 and S2. We propose that this adjustment could be a thalamocortical gain regulation mechanism also present in the processing of information between cortical areas.

Antidromic or rebound activities are not implicated in our thalamic E-stimulation effects

It is known that low intensity thalamic E-stimulation strongly activates thalamocortical neurons [45]. Yang and collaborators demonstrated that thalamic E-stimulation was capable of eliciting a cortical response that resembles the cortical activity pattern evoked by a whisker stimulus [59]. Their E-stimulation protocol (single current pulse; 10–150 μA, 100 μs duration) activated only a small region of thalamic tissue. Intensity used in our experiments (<80 μA) was estimated to activate neurons within a maximal radius that would not exceed 0.5 mm [46], suggesting that the effect induced by the E-stimulation was likely concentrated around the stimulation site. In our experiments, no cortical evoked responses were elicited when the thalamic E-stimulation was performed outside the POm or VPM. In these cases, we did not observe any detectable changes in cortical sensory responses by thalamic E-stimulation (data not shown). We assume that thalamic E-stimulation minimally affects neighbouring structures, however because POm and VPM are immediately adjacent to each other, we can not rule out possible mixed effects between VPM and POm E-stimulation. To clarify this issue we performed a set of complementary studies. Muscimol inactivation of these nuclei in separate experiments demonstrated different thalamic influence in cortical processing. VPM

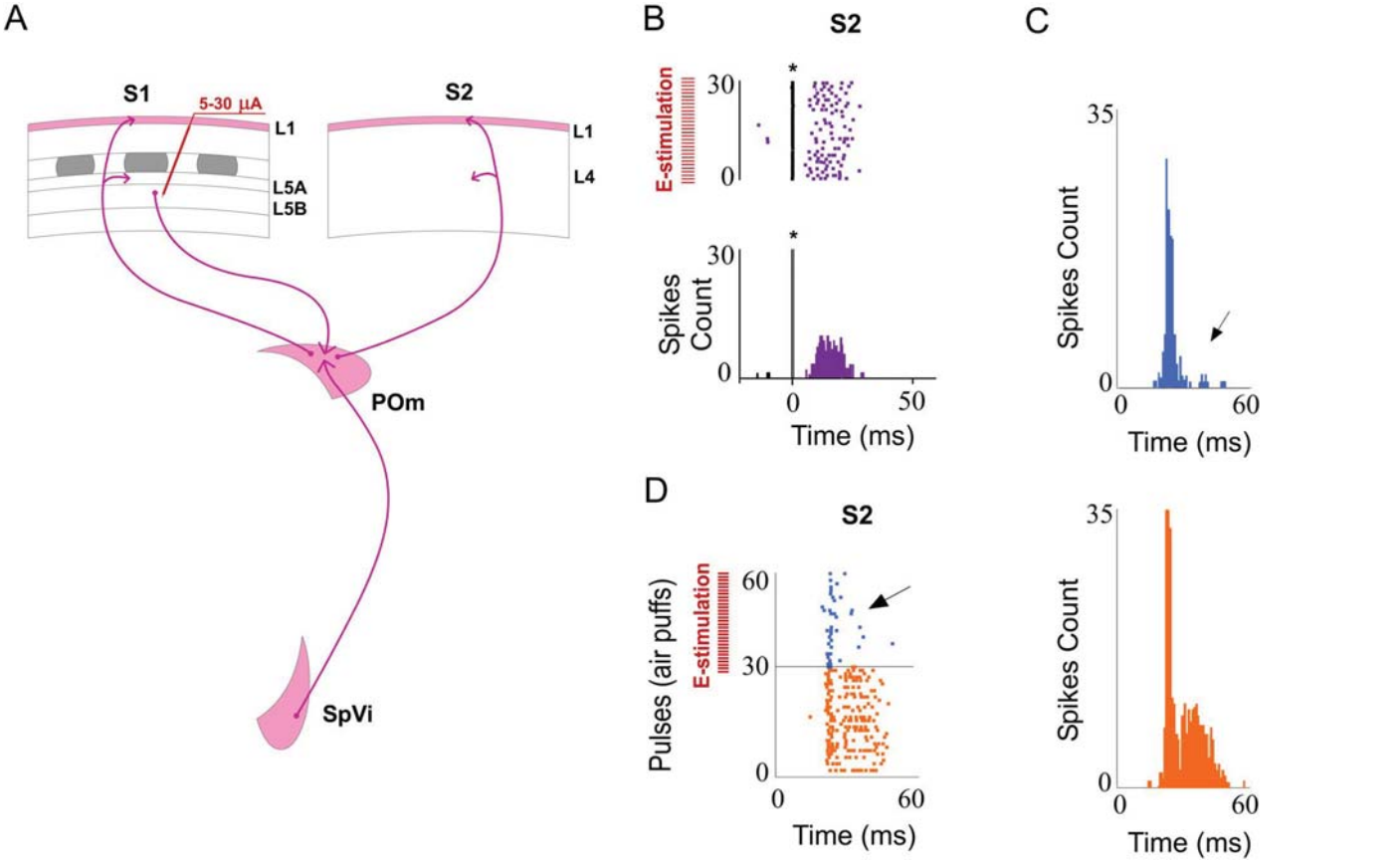


Fig 15. L5 E-stimulation in S1 before sensory stimulus modulates S2 whisker responses. (A) Schematic diagram summarizing the corticothalamocortical circuitry from S1 to S2 through the POm. The experimental manipulation of the barrel cortex is also shown. (B) An example of S2 evoked orthodromic spikes by L5 E-stimulation in barrel cortex is shown. * indicates stimulation artifacts. (C, D) L5 E-stimulation in barrel cortex just before whisker stimulus reduced responses in S2. Raster plots (D) and PSTHs (C) are shown for a sample infragranular response. L5 E-stimulation in S1 shortened responses and reduced spikes mainly in the second response component (arrows). Control (orange) and POm E-stimulation (blue) conditions are shown. Spikes are aligned on sensory stimulus (air puff) presentation (Time 0 ms). L5 E-stimulation in S1 was applied 150 ms before air puffs (31 to 60 pulses; red bars).

doi:10.1371/journal.pone.0148169.g015

inactivation by muscimol abolished whisker responses. However, POm inactivation enhanced spontaneous activity and whisker responses. Moreover, it is known that L1 receives synaptic inputs from POm but not from VPM or L4. We found that E-stimulation of L1 or POm caused similar effects in cortical sensory responses. These findings together with electrode tip position on histological sections allow us to discriminate E-stimulation effects and to understand the different function of these nuclei on cortical processing. Moreover, it is known that VPM lesions abolished the cortical responses evoked by whisker stimulation [59]. Therefore, in our POm inactivation experiments a further indication that the muscimol did not affect the VPM was the increase of cortical whisker responses.

We did not find rebound activity induced by POm or VPM E-stimulation within the current range used (<80 µA). However, we found excitatory rebound activity in some cases applying VPM E-stimulation with a higher intensity (>150 µA) (data not shown). POm E-stimulation did not elicit excitatory rebound activity even at 150 µA (data not shown). We only found excitatory rebound activity at the intensities used in our studies (<80 µA) when GABAergic inhibitory transmission in L1 was blocked by PTX. GABAergic inactivation in L1 increased

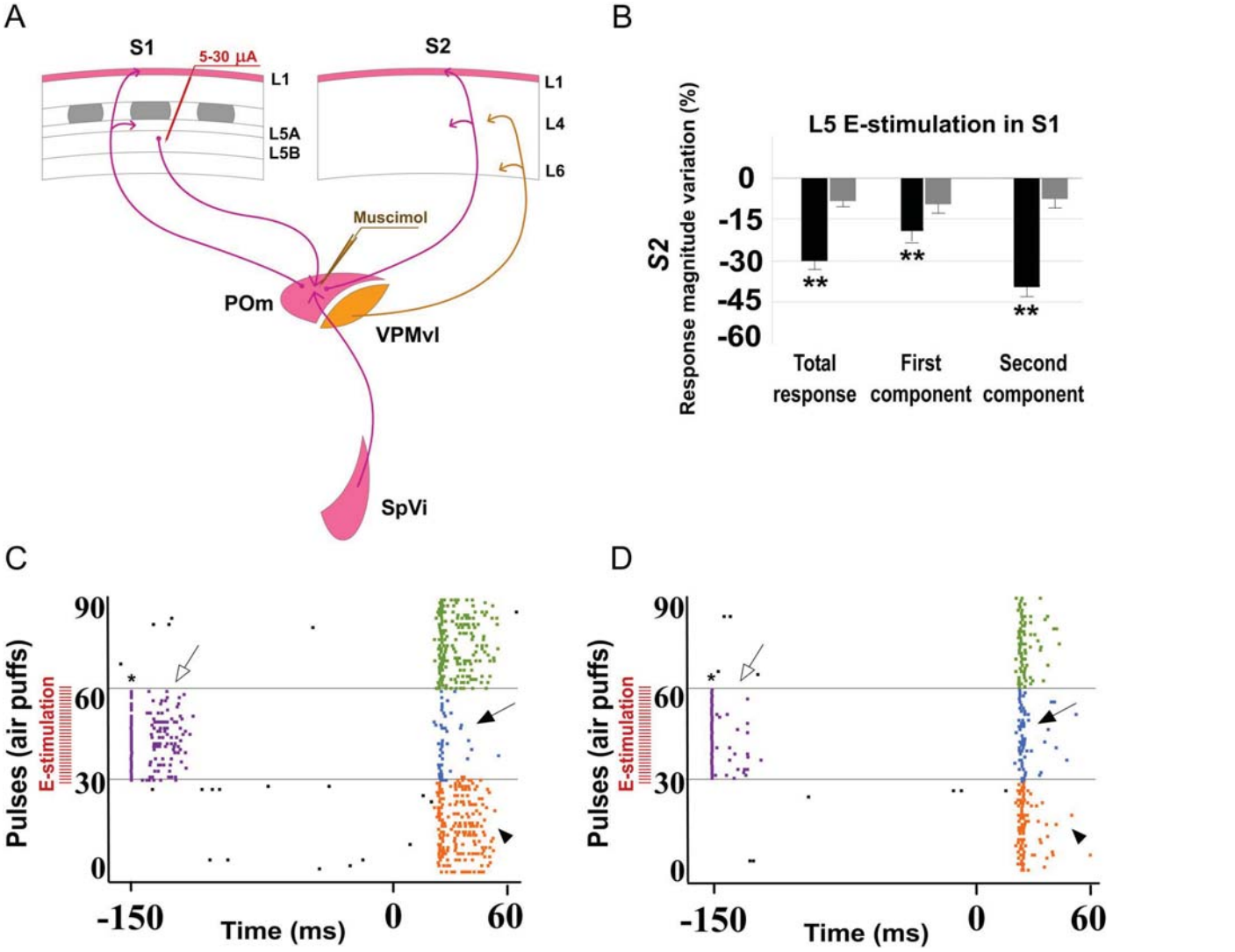


Fig 16. L5 E-stimulation in S1 does not modulate sensory responses in S2 when POm is inactivated. (A) Schematic diagram summarizing the corticothalamocortical circuitry from S1 to S2 through the POm and the extralemniscal pathway to S2 from the VPMvl thalamic nucleus. The experimental manipulation of the barrel cortex and POm is also shown. (B) Change (%) in mean S2 sensory response magnitude by stimulating L5 of S1 before (black) and after POm muscimol inactivation (gray). L5 E-stimulation in S1 did not reduce significantly sensory response spikes in S2 when POm was inactivated. ** $P<0.001$. (C) Sensory responses in S2 were reduced when we applied E-stimulation in L5 of S1 before each stimulus (filled arrows). This effect was abolished when the POm was inactivated with muscimol (D). Control condition before (orange) and after (green) POm E-stimulation condition (blue) are shown. E-stimulation was applied 150 ms before pulses 31 to 60. Spikes evoked in S2 by E-stimulation of L5 in S1 were eliminated by POm inactivation (open arrows). At the stimulation intensities used in this experiment, we have not observed antidromic activation in S2. Sensory responses were significantly decreased after muscimol application but not totally eliminated. Only spikes in the second component of the response were abolished (arrowheads). Spikes in the first component were not reduced by POm inactivation. * indicates stimulation artifacts.

doi:10.1371/journal.pone.0148169.g016

whisker response magnitude, increased basal activity and allowed POm E-stimulation to cause rebound spikes in some cases as shown in Fig 11B. Moreover, in our experiments we used different time intervals between POm E-stimulation and sensory stimulus ranged from 50 to 1000 ms. We consider that the time of these intervals is both, variant and long enough to allow rebound activity to be detected. However, we did not find it, ruling out rebound activity implication in our thalamic E-stimulation effects. We consider that our results support an absence

of implication of POm adaptation in our E-stimulation results. For example, sensory cortical whisker responses were strongly reduced at all intervals (1–20 Hz) in supragranular layers (Fig 6). Yet infragranular responses were not significantly reduced by POm E-stimulation at 20 Hz, a frequency high enough to cause adaptation. Furthermore, L1 E-stimulation induced similar cortical effects. In agreement with that, POm E-stimulation before whisker stimulus did not reduce cortical sensory response when GABAergic inhibitory transmission in L1 was inactivated.

Anatomically, POm receives corticothalamic inputs from L5, thus, infragranular layers activity elicited by POm E-stimulation could also result from antidromic activation of corticothalamic neurons and their axon collaterals. Thus, E-stimulation of the thalamus that is intended to activate thalamocortical afferents may also produce antidromic activation of corticothalamic neurons that subsequently contributes, via axon collaterals, to the synaptic response in the infragranular layers. Cortical studies have demonstrated that orthodromic stimulation effects are stronger than antidromic effects even between areas with strong direct projections [68–70]. Previous thalamocortical studies demonstrated that the threshold for antidromic activation was significantly higher than for orthodromic activation [45, 71]. Rose and Metherate found that mean orthodromic cortical response threshold from stimulating thalamic afferents was 28 μA. Antidromic stimulation of corticothalamic projections resulted in a mean threshold of 214 μA. This implies that low-current thalamic stimulation activates relatively few corticothalamic neurons and that it can strongly activate thalamocortical neurons. Furthermore, the threshold for evoking an antidromic spike in pyramidal neurons by L1 E-stimulation is higher than the threshold required to elicit synaptic responses in the same neuron [40]. In our experiments, we did not observe antidromic activation in infra- or supragranular recordings at stimulation intensities used in these experiments. Antidromic contribution to our findings was therefore ruled out.

L1 implication in POm control of cortical sensory responses

L1 is an important site of integration as it contains feedback corticocortical inputs from other cortical areas and TC inputs mainly from high order nuclei. In our experiments, we found that L1 inputs from POm impose precise regulation on cortical processing. In some of our experiments, we used PTX to block GABAergic transmission in L1, as was also used in other recent cortical studies in vitro [74] and in vivo [75, 76]. We also use AGA to block P/Q-type Ca2 + channels [63–65]; see below). Our results showed that POm E-stimulation before whisker stimulus did not reduce cortical sensory responses when PTX or AGA was applied over cortical surface. We did not try to determine whether these drugs reached other cortical layers, which could have directly inactivated inhibitory influence in those layers. However, we found in our experiments that POm E-stimulation did not reduce sensory responses in infra- and supragranular layers within a few minutes (<5 min) of the PTX or AGA application over the cortical surface. Taking into account that this effect was produced rapidly at the same time in both layers and since the diffusion of the drug into the infragranular layers requires more time, we consider these effects to be induced mainly by L1.

These results were similar to those resulting from POm inactivation. Furthermore, similar to POm E-stimulation, L1 E-stimulation before sensory stimulus also reduced responses in infra- and supragranular layers. It is known that L1 E-stimulation evokes two types of laminar activity in barrel cortex depending on intensity [40]. At lower intensities (<10 μA) the synaptic activation evoked by this E-stimulation was restricted to L1 and upper L2. In contrast, at higher intensities (>10 μA) L1 E-stimulation activated the entire cortical column. In our L1 E-stimulation protocol, we applied low intensities (<10 μA) to examine the effect of L1 activation on

whisker responses. We can not rule out the possibility that in our experiments L1 E-stimulation activated other cortical layers. Even L1 E-stimulation can antidromically activate vertically projecting axons of Martinotti interneurons inducing effects in other layers [40, 77]. Since, we found in our experiments similar cortical effects induced by POm E-stimulation and by L1 E-stimulation, we consider ruling out these possibilities.

In the rat barrel cortex, the border between L5 and L6 has been described at depths of 1400–1600 μm [24, 78]. In our experiments, neurons were recorded in depths from 200 to 600 μm and from 900 to 1500 μm. According to this anatomical data, we must consider that infragranular neurons recorded in our experiments were mainly from L5. Since POm strongly innervates L5A [18, 21], we considered to separate our infragranular recordings in two groups according to the depths of the recordings. A preliminary analysis of single-units from both groups (superficial and deep recordings) showed similar quantitative modulation by POm manipulations. L5A and L5B pyramidal neurons have an apical dendrite reaching L1. In accordance with that, our findings show that POm may exert its control of cortical sensory responses mainly through L1. This layer also contains a dense plexus of apical dendrites of supragranular pyramidal neurons but not of granular neurons [60, 79]. One remaining unknown is the function of L5A inputs from POm.

POm modulates the temporal integration window of cortical sensory responses

Recent studies suggest that POm projections make excitatory synapses with barrel cortex pyramidal cells [20, 39, 43, 73]. According to them, in our experiments, POm E-stimulation alone elicited orthodromic spikes in infra- and supragranular layers of barrel cortex. However, our results also showed that POm E-stimulation just before sensory stimulus reduced magnitude and duration of cortical whisker responses. Moreover, unexpectedly, we found that POm inactivation by muscimol caused an enhancement of both sensory cortical responses and spontaneous cortical activity in the barrel cortex suggesting that POm is tonically regulating cortical excitability in this region. How can these intriguing effects be explained? Our findings show that POm exerts its control of cortical sensory response magnitude and duration using the GABAergic inhibitory system in L1. Therefore, L1 inhibitory interneurons are other potential targets of POm projections. In the mouse prefrontal cortex, a recent study described that matrix thalamocortical projections terminate in outer L1, and their activation drives robust synaptic responses in L1 interneurons [80]. They found that L1 thalamocortical projections preferentially drove inhibitory interneurons of L1 and were much more effective at exciting L1 interneurons than L2/3 pyramidal cells. Accordingly, it is known that L1 inputs generate direct, rapid excitatory postsynaptic potentials in L1 interneurons [60, 85]. These interneurons could rapidly truncate afferent excitation of infra- and supragranular pyramidal neurons, limiting the temporal window during which action potentials can be generated. Our results are also in agreement with that idea. We found that POm E-stimulation or L1 E-stimulation reduced spikes mainly in the second response component. Therefore, this interplay between excitation and inhibition at the level of the barrel cortex could provide a "window of opportunity" for generating cortical responses. Our findings are consistent with that hypothesis. As our results show, the duration of the responses is regulated by POm activity. L1 inputs from POm could activate L1 GABAergic interneurons strengthening cortical inhibition, which shortens the response window. We found that increasing POm E-stimulation intensity reduced more strongly the duration of cortical responses (see Results; Fig 8). A relevant assumption supported by our data is that prolonged response duration (prolonged window) was observed when GABAergic inhibitory transmission in L1 was blocked (Fig 12B).

Accordingly, we found that response magnitude and duration of cortical neurons changed by POm E-stimulation intervals before sensory stimulus (described in Fig 6). Therefore, that interval determines the outcome of the interaction. Recently, both anatomical and physiological findings have shown that ascending inputs from the brainstem and descending inputs from L5 converge on single thalamocortical neurons in POm [25]. Both individual pathways interact functionally in a time-dependent manner and when co-activated, increase the output of thalamus supralinearly [25]. Moreover, Shlosberg et al. found that when pairing L1 E-stimulation with whisker deflection, the interval between the stimuli determined the outcome of the interaction, with facilitation of sensory responses dominating the short (<10 ms) intervals and suppression prevailing at longer (>10 ms) intervals [40]. Then, same effects could be induced by POm E-stimulation using those intervals.

We propose this mechanism could allow the temporal cortical integration of inputs from distinct pathways and could act to "reset" the network to generate the next cortical response avoiding the somatosensory cortex be captured by a single stimulus.

Since it is well described that POm is involved in temporal processing related to sensory-motor control of whisker movement [17, 34, 35], it is then possible that this mechanism could play a crucial role in sensory-motor interaction allowing the POm to control the temporal integration of the incoming tactile information during whisking exploration. The accuracy of whisking could be controlled by POm activity to optimize sensory processing. Accordingly, it has been suggested that the whisker sensory-motor system is involved in closed-loop computations [94, 95]. In particular, single unit responses from whisker sensory and motor areas show generic signatures of phase-sensitive detection and control at the level of thalamocortical and corticocortical loops [94, 95]. These loops are likely to be components within a greater closed-loop vibrissa sensory-motor system, which optimizes sensory processing. Our results are in agreement with that proposal.

Possible implication of parvalbumin interneurons in POm control of cortical responses

L1 inhibitory interneurons provide a direct source of apical dendritic inhibition to supra- and infragranular layer pyramidal neurons [80–82]; and also form inhibitory synapses onto other L1 interneurons and L2/3 interneurons [83–86]. Interneurons of L1 are heterogeneous [60, 79, 87–90]. To study in more detail the L1 inhibitory implication in POm control of cortical processing, we applied Cav2.1 (P/Q- type) voltage-gated calcium channels blocker and found that blocking P/Q-type Ca2+ channels avoided POm E-stimulation effects. It is known that these channels are expressed on parvalbumin (PV) interneuron axon terminals and mediate GABA release from fast spiking interneurons to pyramidal cells [63–65]. Consequently, it is possible that presumed PV+ interneurons were implicated in a dynamic control of sensory cortical processing by POm. Other studies have demonstrated that PV+ interneurons participate in control gain of sensory responses [86, 91, 92]. Furthermore, recent findings demonstrate that the conditional ablation of Cav 2.1 channel function from cortical PV+ interneurons alters GABA release from these cells, impairs their ability to constrain cortical pyramidal cell excitability [93].

The main effect of POm manipulation occurs in the second component of cortical response: possible NMDA receptors implication and cortical plasticity

It is known that short-latency spikes evoked by whisker stimulation in the barrel cortex are mainly mediated through non-NMDA receptors while NMDA receptors are implicated mainly

in spikes generated later after them [72]. Studies from our laboratory confirmed the implication of NMDA receptors in the late component of cortical tactile responses [57, 58]. A recent study suggest that POm associated synaptic pathways in barrel cortex are responsible for these mediating whisker-evoked NMDA receptor dependent spikes [73], in agreement with our results. Since these receptors have been directly implicated in cortical synaptic plasticity, our findings have important consequences in sensory processing implicating the POm in the control of cortical synaptic plasticity by reducing the time-window of activation in cortical neurons.

POm implication in the regulation of cortical processing between S1 and S2

Our results demonstrate the determinant role exerted by the POm in the adjustment of somatosensory cortical processing in S1 and S2. We found that vibrissal stimulus responses recorded in S1 and S2 were modulated in magnitude and duration by POm activity. These effects were abolished when we inactivated the POm with muscimol. Since our results show that POm exerts its control of barrel cortex sensory responses mainly using L1 GABAergic system, it is then possible that the same mechanism could be used by the POm to regulate sensory responses in S2 (Fig 17). Accordingly, strong POm connections to L1 in S2 have been described [18, 96].

However, in contrast to S1, it is known that L4 of S2 receives a strong projection from the POm [39, 43]. In agreement with that, in our experiment, whisker sensory responses in S2 were reduced after POm inactivation (for example see Fig 16D, arrowheads). Spikes in the second component of the response were abolished. However, spikes in the first component were not reduced by POm inactivation suggesting they come from a different pathway. Ascending whisker signals reach S2 not only through the POm. It is known that S2 receives (focally in L4 and L6; extralemniscal pathway) from thalamocortical neurons located in the ventrolateral part of the VPM [66, 67]. Since this pathway should not be affected by POm inactivation in our experiments, it is then possible that spikes in the first component of S2 whisker responses were caused by extralemniscal inputs. The short latencies of these spikes rule out the possible VPM-S1-S2 route.

We show in our experiments that vibrissal stimulus responses recorded in S2 were reduced in magnitude and duration when we applied E-stimulation in L5 of S1 before the whisker stimulus. It is possible that L6 neurons, which send feedback inputs to thalamus, were also affected by L5 E-stimulation. However, a recent study demonstrates that stimulation of L6 does not activate S2 via this circuit [43]. In our experiments, this cortical sensory processing adjustment between S1 and S2 was abolished when POm was inactivated with muscimol. L5 E-stimulation in S1 could not reduce sensory response spikes in S2 after POm inactivation (Fig 16). Furthermore, we found that S2 robust activity in response to L5 E-stimulation in S1 alone was eliminated after POm inactivation. This finding is in agreement with other studies on corticothalamocortical communication implicating the POm in information transfer to higher-order cortical areas [25, 41, 42].

POm activity modulation of cortical processing. Functional implication

There is a huge range of stimuli that reach the cortex, each with different intensities and durations. To process them the system must have the capacity to regulate itself to detect the weakest ones and not be saturated by the strongest ones. This allows the system to process a wider range of stimuli improving the ability to detect and identify tactile features. Based on our findings, we propose that control of cortical sensory processing exerted by POm could be part of a

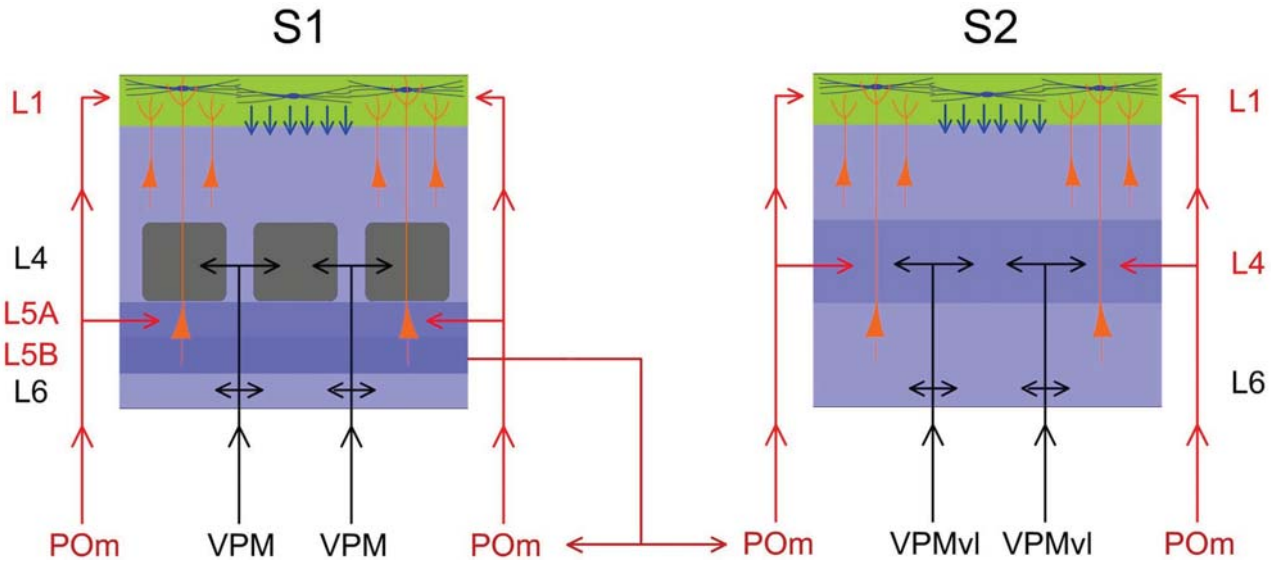


Fig 17. POm influence on somatosensory cortical response modulation. Schematic diagram summarizing the ascending thalamocortical pathways to S1 and S2. Corticothalamocortical circuitry from S1 to S2 through the POm is also shown. L5B corticofugal neurons in S1 project to the POm [9, 15, 25, 26, 43]. Ascending inputs from the brainstem and descending inputs from L5 converge on single thalamocortical neurons in POm [25]. Both individual pathways interact functionally in a time-dependent manner [25]. From here, POm neuron projections also target S2 [18, 21]. POm is also controlling the sensory processing in S2 and this regulation is modulated by corticofugal activity from L5 in S1 [25]. Whisker-evoked sensory information is rapidly relayed to L1 neurons, which, in turn, act to powerfully inhibit whisker-evoked responses [40, 60]. In accordance with previous studies [40], we confirm that L1 exerts an inhibitory influence on whisker responses.

doi:10.1371/journal.pone.0148169.g017

mechanism that has the ability to regulate the processing gain, depending on the relative intensities of stimuli across the entirety of vibrissae space. This integration of multi-whisker activity could be achieved by the POm and transmitted to the cortex to adjust the sensory processing.

Sensory activity carried by this pathway could allow the adjustment of the specific sensory content processing in the cortex. Our results show that there is a fundamental difference between the lemniscal and paralemniscal thalamic nuclei in terms of cortical influence. We must consider that these parallel pathways have a complementary function in sensory processing. Lemniscal and paralemniscal parallel ascending projection systems from the thalamus could convey specific sensory content and stimuli global sensory activity, respectively. Global activity carried by the paralemniscal pathway could allow the POm to instruct the cortex how to handle the incoming lemniscal information, which, overall, produces a precise qualitative assessment of the perceived stimulus in its specific context. Therefore, the level of activity in the POm could determine the cortical sensory processing regulation. POm could detect the changes in sensory activity (stimulus intensity and duration) and could adjust the gain and timing of cortical processing accordingly.

Our results unmask a new role of POm (and maybe other "higher-order nuclei") in cortical processing and suggest a novel framework to understand thalamocortical interaction according to which POm modulates the temporal integration window of cortical sensory responses in a POm activity-dependent manner. This could be a common feature in other sensory systems.

Acknowledgments

We thank Drs. F. Clasca and C. Porrero for their constructive comments.
We thank M. Callejo for technical assistance.

Author Contributions

Conceived and designed the experiments: CC AN. Performed the experiments: CC NBZ. Analyzed the data: CC NBZ AN. Wrote the paper: CC NBZ AN.

References

1. Ahissar E, Sosnik R, Haidarliu S. Transformation from temporal to rate coding in a somatosensory thalamocortical pathway. Nature. 2000; 406(6793):302–6. PMID: 10917531
2. Castro-Alamancos MA. Dynamics of sensory thalamocortical synaptic networks during information processing states. Prog Neurobiol. 2004; 74(4):213–47. PMID: 15556288
3. Jones EG. The thalamic matrix and thalamocortical synchrony. Trends Neurosci. 2001; 24(10):595–601. PMID: 11576674
4. McCormick DA, Bal T. Sensory gating mechanisms of the thalamus. Curr Opin Neurobiol. 1994; 4 (4):550–6. PMID: 7812144
5. Sherman SM, Guillery RW. Distinct functions for direct and transthalamic corticocortical connections. J Neurophysiol. 2011; 106(3):1068–77. doi: 10.1152/jn.00429.2011 PMID: 21676936
6. Steriade M. Synchronized activities of coupled oscillators in the cerebral cortex and thalamus at different levels of vigilance. Cereb Cortex. 1997; 7(6):583–604. PMID: 9276182
7. Jones EG. A new view of specific and nonspecific thalamocortical connections. Adv Neurol. 1998; 77:49–71; discussion 2–3. PMID: 9709817
8. Poulet JF, Fernandez LM, Crochet S, Petersen CC. Thalamic control of cortical states. Nature neuroscience. 2012; 15(3):370–2. doi: 10.1038/nn.3035 PMID: 22267163
9. Sherman SM. Thalamocortical interactions. Curr Opin Neurobiol. 2012; 22(4):575–9. doi: 10.1016/j.conb.2012.03.005 PMID: 22498715
10. Castro-Alamancos MA, Connors BW. Thalamocortical synapses. Prog Neurobiol. 1997; 51(6):581–606. PMID: 9175158
11. Castro-Alamancos MA. Properties of primary sensory (lemniscal) synapses in the ventrobasal thalamus and the relay of high-frequency sensory inputs. J Neurophysiol. 2002; 87(2):946–53. PMID: 11826059
12. Diamond ME, Armstrong-James M, Ebner FF. Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus. J Comp Neurol. 1992; 318(4):462–76. PMID: 1578013
13. Feldmeyer D, Brecht M, Helmchen F, Petersen CC, Poulet JF, Staiger JF, et al. Barrel cortex function. Prog Neurobiol. 2013; 103:3–27. doi: 10.1016/j.pneurobio.2012.11.002 PMID: 23195880
14. Nicolelis MAL, Fanselow EE. Thalamcortical optimization of tactile processing according to behavioral state. Nat Neurosci. 2002; 5(6):517–23. PMID: 12037519
15. Veinante P, Jacquin MF, Deschenes M. Thalamic projections from the whisker-sensitive regions of the spinal trigeminal complex in the rat. J Comp Neurol. 2000; 420(2):233–43. PMID: 10753309
16. Woolsey TA, Van der Loos H. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res. 1970; 17(2):205–42. PMID: 4904874
17. Ahissar E, Zacksenhouse M. Temporal and spatial coding in the rat vibrissal system. Prog Brain Res. 2001; 130:75–87. PMID: 11480290
18. Ohno S, Kuramoto E, Furuta T, Hioki H, Tanaka YR, Fujiyama F, et al. A morphological analysis of thalamocortical axon fibers of rat posterior thalamic nuclei: a single neuron tracing study with viral vectors. Cereb Cortex. 2012; 22(12):2840–57. doi: 10.1093/cercor/bhr356 PMID: 22190433
19. Waite P. Trigeminal sensory system. In: Paxinos G, editor. The rat nervous system. 3 ed. San Diego: Academic; 2004. p. 817–51.
20. Bureau I, von Saint Paul F, Svoboda K. Interdigitated paralemniscal and lemniscal pathways in the mouse barrel cortex. PLoS Biol. 2006; 4(12):e382. PMID: 17121453
21. Clasca F, Rubio-Garrido P, Jabaudon D. Unveiling the diversity of thalamocortical neuron subtypes. Eur J Neurosci. 2012; 35(10):1524–32. doi: 10.1111/j.1460-9568.2012.08033.x PMID: 22606998
22. Herkenham M. Laminar organization of thalamic projections to the rat neocortex. Science. 1980; 207 (4430):532–5. PMID: 7352263
23. Rubio-Garrido P, Perez-de-Manzo F, Porrero C, Galazo MJ, Clasca F. Thalamic input to distal apical dendrites in neocortical layer 1 is massive and highly convergent. Cereb Cortex. 2009; 19(10):2380–95. doi: 10.1093/cercor/bhn259 PMID: 19188274

24. Wimmer VC, Bruno RM, de Kock CP, Kuner T, Sakmann B. Dimensions of a projection column and architecture of VPM and POm axons in rat vibrissal cortex. Cereb Cortex. 2010; 20(10):2265–76. doi: 10.1093/cercor/bhq068 PMID: 20453248

25. Groh A, Bokor H, Mease RA, Plattner VM, Hangya B, Stroh A, et al. Convergence of cortical and sensory driver inputs on single thalamocortical cells. Cereb Cortex. 2014; 24(12):3167–79. doi: 10.1093/cercor/bht173 PMID: 23825316

26. Killackey HP, Sherman SM. Corticothalamic projections from the rat primary somatosensory cortex. J Neurosci. 2003; 23(19):7381–4. PMID: 12917373

27. Haidarliu S, Yu C, Rubin N, Ahissar E. Lemniscal and Extralemniscal Compartments in the VPM of the Rat. Front Neuroanat. 2008; 2:4. doi: 10.3389/neuro.05.004.2008 PMID: 18958201

28. Petersen CC. The functional organization of the barrel cortex. Neuron. 2007; 56(2):339–55. PMID: 17964250

29. Simons DJ, Carvell GE. Thalamocortical response transformation in the rat vibrissa/barrel system. J Neurophysiol. 1989; 61:311–30. PMID: 2918357

30. Veinante P, Deschenes M. Single- and multi-whisker channels in the ascending projections from the principal trigeminal nucleus in the rat. J Neurosci. 1999; 19(12):5085–95. PMID: 10366641

31. Jacquin MF, Golden J, Rhoades RW. Structure-function relationships in rat brainstem subnucleus interpolaris: III. Local circuit neurons. J Comp Neurol. 1989; 282(1):24–44. PMID: 2708592

32. Masri R, Bezdudnaya T, Trageser JC, Keller A. Encoding of stimulus frequency and sensor motion in the posterior medial thalamic nucleus. J Neurophysiol. 2008; 100(2):681–9. doi: 10.1152/jn.01322.2007 PMID: 18234976

33. Sitnikova EY, Raevskii VV. The lemniscal and paralemniscal pathways of the trigeminal system in rodents are integrated at the level of the somatosensory cortex. Neurosci Behav Physiol. 2010; 40(3):325–31. doi: 10.1007/s11055-010-9259-7 PMID: 20148310

34. Sosnik R, Haidarliu S, Ahissar E. Temporal frequency of whisker movement. I. Representations in brain stem and thalamus. J Neurophysiol. 2001; 86(1):339–53. PMID: 11431515

35. Yu C, Derdikman D, Haidarliu S, Ahissar E. Parallel thalamic pathways for whisking and touch signals in the rat. PLoS Biol. 2006; 4(5):e124. PMID: 16605304

36. Frangeul L, Porrero C, Garcia-Amado M, Maimone B, Maniglier M, Clasca F, et al. Specific activation of the paralemniscal pathway during nociception. Eur J Neurosci. 2014; 39(9):1455–64. doi: 10.1111/ejn.12524 PMID: 24580836

37. Masri R, Keller A. Chronic pain following spinal cord injury. Adv Exp Med Biol. 2012; 760:74–88. PMID: 23281514

38. Sewards TV, Sewards M. Separate, parallel sensory and hedonic pathways in the mammalian somatosensory system. Brain Res Bull. 2002; 58(3):243–60. PMID: 12128150

39. Viaene AN, Petrof I, Sherman SM. Properties of the thalamic projection from the posterior medial nucleus to primary and secondary somatosensory cortices in the mouse. Proc Natl Acad Sci USA. 2011; (108):18156–18161. doi: 10.1073/pnas.1114828108 PMID: 22025694

40. Shlosberg D, Amitai Y, Azouz R. Time-dependent, layer-specific modulation of sensory responses mediated by neocortical layer 1. J Neurophysiol. 2006; 96(6):3170–82. PMID: 17110738

41. Guillery RW. Anatomical pathways that link perception and action. Prog Brain Res. 2005; 149:235–56. PMID: 16226588

42. Guillery RW, Sherman SM. Branched thalamic afferents: what are the messages that they relay to the cortex? Brain Res Rev. 2011; 66(1–2):205–19. doi: 10.1016/j.brainresrev.2010.08.001 PMID: 20696186

43. Theyel BB, Llano DA, Sherman SM. The corticothalamocortical circuit drives higher-order cortex in the mouse. Nature Neurosci. 2010; 13(1):84–8. doi: 10.1038/nn.2449 PMID: 19966840

44. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 2007.

45. Rose HJ, Metherate R. Thalamic stimulation largely elicits orthodromic, rather than antidromic, cortical activation in an auditory thalamocortical slice. Neuroscience. 2001; 106(2):331–40. PMID: 11566504

46. Ranck JB Jr. Which elements are excited in electrical stimulation of mammalian central nervous system: a review. Brain Res. 1975; 98(3):417–40. PMID: 1102064

47. Bragin A, Penttonen M, Buzsáki G. Termination of epileptic afterdischarge in the hippocampus. J Neurosci. 1997; 17(7):2567–79. PMID: 9065516

48. Castro-Alamancos MA. Neocortical synchronized oscillations induced by thalamic disinhibition in vivo. J Neurosci. 1999; 19(18).

49. Steriade M, Contreras D. Spike-wave complexes and fast components of cortically generated seizures. I. Role of neocortex and thalamus. J Neurophysiol. 1998; 80(3):1439–55. PMID: 9744951

50. Chakrabarti S, Zhang M, Alloway KD. MI neuronal responses to peripheral whisker stimulation: relationship to neuronal activity in si barrels and septa. J Neurophysiol. 2008; 100(1):50–63. doi: 10.1152/jn.90327.2008 PMID: 18450580

51. de Kock CP, Bruno RM, Spors H, Sakmann B. Layer- and cell-type-specific suprathreshold stimulus representation in rat primary somatosensory cortex. J Physiol (London). 2007; 581(Pt 1):139–54.

52. de Kock CP, Sakmann B. Spiking in primary somatosensory cortex during natural whisking in awake head-restrained rats is cell-type specific. Proc Natl Acad Sci U S A. 2009; 106(38):16446–50. doi: 10.1073/pnas.0904143106 PMID: 19805318

53. Manns ID, Sakmann B, Brecht M. Sub- and Suprathreshold Receptive Field Properties of Pyramidal Neurons in Layers 5A and 5B of Rat Somatosensory Barrel Cortex. J Physiol (London). 2004; 556 (2):601–22.

54. Wright N, Fox K. Origins of cortical layer V surround receptive fields in the rat barrel cortex. J Neurophysiol. 2010; 103(2):709–24. doi: 10.1152/jn.00560.2009 PMID: 19939962

55. Atencio CA, Shih JY, Schreiner CE, Cheung SW. Primary auditory cortical responses to electrical stimulation of the thalamus. J Neurophysiol. 2014; 111(5):1077–87. doi: 10.1152/jn.00749.2012 PMID: 24335216

56. Swadlow HA. Neocortical efferent neurons with very slowly conducting axons: strategies for reliable antidromic identification. J Neurosci Methods. 1998; 79(2):131–41. PMID: 9543479

57. Barros-Zulaica N, Castejon C, Nuñez A. Frequency-specific response facilitation of supra and infragranular barrel cortical neurons depends on NMDA receptor activation in rats. Neuroscience. 2014; 281:178–94.

58. Nuñez A, Dominguez S, Buño W, Fernandez de Sevilla D. Cholinergic-mediated response enhancement in barrel cortex layer V pyramidal neurons. J Neurophysiol. 2012; 108(6):1656–68. doi: 10.1152/jn.00156.2012 PMID: 22723675

59. Yang JW, An S, Sun JJ, Reyes-Puerta V, Kindler J, Berger T, et al. Thalamic network oscillations synchronize ontogenetic columns in the newborn rat barrel cortex. Cereb Cortex. 2013; 23(6):1299–316. doi: 10.1093/cercor/bhs103 PMID: 22593243

60. Zhu Y, Zhu JJ. Rapid arrival and integration of ascending sensory information in layer 1 nonpyramidal neurons and tuft dendrites of layer 5 pyramidal neurons of the neocortex. J Neurosci. 2004; 24 (6):1272–9. PMID: 14960597

61. Prieto JJ, Peterson BA, Winer JA. Morphology and spatial distribution of GABAergic neurons in cat primary auditory cortex (AI). J Comp Neurol. 1994; 344(3):349–82. PMID: 7914896

62. Winer JA, Larue DT. Populations of GABAergic neurons and axons in layer I of rat auditory cortex. Neuroscience. 1989; 33(3):499–515. PMID: 2636704

63. Hefft S, Jonas P. Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse. Nature Neurosci. 2005; 8(10):1319–28. PMID: 16158066

64. Toledo-Rodriguez M, Blumenfeld B, Wu C, Luo J, Attali B, Goodman P, et al. Correlation maps allow neuronal electrical properties to be predicted from single-cell gene expression profiles in rat neocortex. Cereb Cortex. 2004; 14(12):1310–27. PMID: 15192011

65. Zaitsev AV, Povysheva NV, Lewis DA, Krimer LS. P/Q-type, but not N-type, calcium channels mediate GABA release from fast-spiking interneurons to pyramidal cells in rat prefrontal cortex. J Neurophysiol. 2007; 97(5):3567–73. PMID: 17329622

66. Bokor H, Acsady L, Deschenes M. Vibrissal responses of thalamic cells that project to the septal columns of the barrel cortex and to the second somatosensory area. J Neurosci. 2008; 28(20):5169–77. doi: 10.1523/JNEUROSCI.0490-08.2008 PMID: 18480273

67. Pierret T, Lavallee P, Deschenes M. Parallel streams for the relay of vibrissal information through thalamic barreloids. J Neurosci. 2000; 20(19):7455–62. PMID: 11007905

68. Bullier J, McCourt ME, Henry GH. Physiological studies on the feedback connection to the striate cortex from cortical areas 18 and 19 of the cat. Exp Brain Res. 1988; 70(1):90–8. PMID: 3402571

69. Girard P, Hupe JM, Bullier J. Feedforward and feedback connections between areas V1 and V2 of the monkey have similar rapid conduction velocities. J Neurophysiol. 2001; 85(3):1328–31. PMID: 11248002

70. Movshon JA, Newsome WT. Visual response properties of striate cortical neurons projecting to area MT in macaque monkeys. J Neurosci. 1996; 16(23):7733–41. PMID: 8922429

71. Beierlein M, Connors BW. Short-term dynamics of thalamocortical and intracortical synapses onto layer 6 neurons in neocortex. J Neurophysiol. 2002; 88(4):1924–32. PMID: 12364518

72. Armstrong-James M, Welker E, Callahan CA. The contribution of NMDA and Non-NMDA receptors to fast and slow transmission of sensory information in the rat Sl barrel cortex. J Neurosci. 1993; 13 (5):2149–60. PMID: 8097531

73. Gambino F, Pages S, Kehayas V, Baptista D, Tatti R, Carleton A, et al. Sensory-evoked LTP driven by dendritic plateau potentials in vivo. Nature. 2014; 515(7525):116–9. doi: 10.1038/nature13664 PMID: 25174710

74. Salling MC, Harrison NL. Strychnine-sensitive glycine receptors on pyramidal neurons in layers II/III of the mouse prefrontal cortex are tonically activated. J Neurophysiol. 2014; 112(5):1169–78. doi: 10.1152/jn.00714.2013 PMID: 24872538

75. Dilgen J, Tejeda HA, O'Donnell P. Amygdala inputs drive feedforward inhibition in the medial prefrontal cortex. J Neurophysiol. 2013; 110(1):221–9. doi: 10.1152/jn.00531.2012 PMID: 23657281

76. Pezze M, McGarrity S, Mason R, Fone KC, Bast T. Too little and too much: hypoactivation and disinhibition of medial prefrontal cortex cause attentional deficits. J Neurosci. 2014; 34(23):7931–46. doi: 10.1523/JNEUROSCI.3450-13.2014 PMID: 24899715

77. Cottam JC. Identifying the functional role of Martinotti cells in cortical sensory processing. J Neurophysiol. 2009; 102(1):9–11. doi: 10.1152/jn.00290.2009 PMID: 19420125

78. Oberlaender M, de Kock CP, Bruno RM, Ramirez A, Meyer HS, Dercksen VJ, et al. Cell type-specific three-dimensional structure of thalamocortical circuits in a column of rat vibrissal cortex. Cereb Cortex. 2012; 22(10):2375–91. doi: 10.1093/cercor/bhr317 PMID: 22089425

79. Chu Z, Galarreta M, Hestrin S. Synaptic interactions of late-spiking neocortical neurons in layer 1. J Neurosci. 2003; 23(1):96–102. PMID: 12514205

80. Cruikshank SJ, Ahmed OJ, Stevens TR, Patrick SL, Gonzalez AN, Elmaleh M, et al. Thalamic control of layer 1 circuits in prefrontal cortex. J Neurosci. 2012; 32(49):17813–23. doi: 10.1523/JNEUROSCI.3231-12.2012 PMID: 23223300

81. Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J. Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. Science. 2009; 325(5941):756–60. doi: 10.1126/science.1171958 PMID: 19661433

82. Williams SR, Stuart GJ. Dependence of EPSP efficacy on synapse location in neocortical pyramidal neurons. Science. 2002; 295:1907–10. PMID: 11884759

83. Christophe E, Roebuck A, Staiger JF, Lavery DJ, Charpak S, Audinat E. Two types of nicotinic receptors mediate an excitation of neocortical layer I interneurons. J Neurophysiol. 2002; 88(3):1318–27. PMID: 12205153

84. Letzkus JJ, Kampa BM, Stuart GJ. Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. J Neurosci. 2006; 26(41):10420–9. PMID: 17035526

85. Jiang X, Wang G, Lee AJ, Stornetta RL, Zhu JJ. The organization of two new cortical interneuronal circuits. Nature Neurosci. 2013; 16(2):210–8. doi: 10.1038/nn.3305 PMID: 23313910

86. Lee AJ, Wang G, Jiang X, Johnson SM, Hoang ET, Lante F, et al. Canonical Organization of Layer 1 Neuron-Led Cortical Inhibitory and Disinhibitory Interneuronal Circuits. Cereb Cortex. 2014.

87. Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. Interneurons of the neocortical inhibitory system. Nat Rev Neurosci. 2004; 5(10):793–807. PMID: 15378039

88. Zhou FM, Hablitz JJ. Layer I neurons of rat neocortex .1. Action potential and repetitive firing properties. J Neurophysiol. 1996; 76(2):651–67. PMID: 8871189

89. Kubota Y. Untangling GABAergic wiring in the cortical microcircuit. Curr Opin Neurobiol. 2014; 26:7–14. PMID: 24650498

90. Wozny C, Williams SR. Specificity of synaptic connectivity between layer 1 inhibitory interneurons and layer 2/3 pyramidal neurons in the rat neocortex. Cereb Cortex. 2011; 21(8):1818–26. doi: 10.1093/cercor/bhq257 PMID: 21220765

91. Atallah BV, Bruns W, Carandini M, Scanziani M. Parvalbumin-expressing interneurons linearly transform cortical responses to visual stimuli. Neuron. 2012; 73(1):159–70. doi: 10.1016/j.neuron.2011.12.013 PMID: 22243754

92. Wilson NR, Runyan CA, Wang FL, Sur M. Division and subtraction by distinct cortical inhibitory networks in vivo. Nature. 2012; 488(7411):343–8. doi: 10.1038/nature11347 PMID: 22878717

93. Rossignol E, Kruglikov I, van den Maagdenberg AM, Rudy B, Fishell G. CaV 2.1 ablation in cortical interneurons selectively impairs fast-spiking basket cells and causes generalized seizures. Ann Neurol. 2013; 74(2):209–22. doi: 10.1002/ana.23913 PMID: 23595603

94. Ahissar E, Kleinfeld D. Closed-loop neuronal computations: focus on vibrissa somatosensation in rat. Cereb Cortex. 2003; 13:53–62. PMID: 12466215

95. Ahissar E, Oram T. Thalamic Relay or Cortico-Thalamic Processing? Old Question, New Answers Cereb. Cortex. 2015; 25(4):845–848.

96. Herkenham M (1980) Laminar organization of thalamic projections of the rat neocortex. Science 207:532–534. PMID: 7352263

Artículo científico nº 2

frontiers in Neural Circuits

HYPOTHESIS AND THEORY
published: 19 October 2016
doi: 10.3389/fncir.2016.00081

Cortical Neural Computation by Discrete Results Hypothesis

Carlos Castejon* and Angel Nuñez

Department of Anatomy, Histology and Neuroscience, School of Medicine, Autonomous University of Madrid, Madrid, Spain

One of the most challenging problems we face in neuroscience is to understand how the cortex performs computations. There is increasing evidence that the power of the cortical processing is produced by populations of neurons forming dynamic neuronal ensembles. Theoretical proposals and multineuronal experimental studies have revealed that ensembles of neurons can form emergent functional units. However, how these ensembles are implicated in cortical computations is still a mystery. Although cell ensembles have been associated with brain rhythms, the functional interaction remains largely unclear. It is still unknown how spatially distributed neuronal activity can be temporally integrated to contribute to cortical computations. A theoretical explanation integrating spatial and temporal aspects of cortical processing is still lacking. In this Hypothesis and Theory article, we propose a new functional theoretical framework to explain the computational roles of these ensembles in cortical processing. We suggest that complex neural computations underlying cortical processing could be temporally discrete and that sensory information would need to be quantized to be computed by the cerebral cortex. Accordingly, we propose that cortical processing is produced by the computation of discrete spatio-temporal functional units that we have called "Discrete Results" (Discrete Results Hypothesis). This hypothesis represents a novel functional mechanism by which information processing is computed in the cortex. Furthermore, we propose that precise dynamic sequences of "Discrete Results" is the mechanism used by the cortex to extract, code, memorize and transmit neural information. The novel "Discrete Results" concept has the ability to match the spatial and temporal aspects of cortical processing. We discuss the possible neural underpinnings of these functional computational units and describe the empirical evidence supporting our hypothesis. We propose that fast-spiking (FS) interneuron may be a key element in our hypothesis providing the basis for this computation.

OPEN ACCESS

Edited by:
Jessica Cardin,
Yale School of Medicine, USA

Reviewed by:
Amanda Casale,
University of California, San Diego, USA
Makoto Osanai,
Tohoku University, Japan

*Correspondence:
Carlos Castejon
castejon.neuro@gmail.com

Received: 11 July 2016
Accepted: 29 September 2016
Published: 19 October 2016

Citation:
Castejon C and Nuñez A (2016) Cortical Neural Computation by Discrete Results Hypothesis. Front. Neural Circuits 10:81. doi: 10.3389/fncir.2016.00081

Keywords: cerebral cortex, sensory processing, cell ensembles, fast-spiking cells, brain oscillations, discrete computation, neural synchronization, processing resolution

INTRODUCTION

The cerebral cortex is possibly one of the most complex natural systems. Untangling its intricate functional microcircuit is one of the formidable challenges of neuroscience. However, despite its importance, how cortical computations are performed and the underlying neural mechanisms remain unclear.

There is increasing evidence that the power of the cortical processing is produced by populations of neurons forming dynamic neuronal ensembles. Theoretical proposals (Lorente de Nó, 1938; Hebb, 1949; Hopfield, 1982; Engel et al., 2001; Buzsáki, 2010; Yuste, 2015) and multineuronal experimental studies (Fujisawa et al., 2008; Miller et al., 2014) have revealed that ensembles of neurons can form emergent functional units. However, it is still unknown how distributed neuronal activity can be functionally integrated to contribute to cortical computations. Moreover, no one knows what these functional units look like, or how they emerge. In sum, how these ensembles are implicated in cortical computations is still a mystery.

Although cell ensembles have been associated with brain rhythms, the functional interaction remains largely unclear. It is still unknown how spatially distributed neuronal activity can be temporally integrated to contribute to cortical computations. A theoretical explanation integrating spatial and temporal aspects of cortical processing is still lacking.

In this Hypothesis and Theory article, we propose a new functional theoretical framework to explain the computational roles of these ensembles in cortical processing. We suggest that complex neural computations underlying cortical processing could be temporally discrete. Accordingly, we propose that cortical processing is produced by the computation of discrete spatio-temporal functional units that we have called ''Discrete Results'' (Discrete Results Hypothesis). Furthermore, we propose that precise dynamic sequences of Discrete Results is the mechanism used by the cortex to extract, code, memorize and transmit neural information.

As we describe in the next sections, this proposal has the ability to match the spatial and temporal aspects of cortical processing. Moreover, our hypothesis represents a novel functional mechanism by which information processing is computed in the cortex. We discuss the possible neural underpinnings of this proposal and describe the empirical evidence supporting our hypothesis.

NEURONAL PROCESSING BY DISTRIBUTED NEURONAL ACTIVITY

The power of the cortex lies in the dynamic coordination of neurons (Hebb, 1949; Pouget et al., 2000; Yuste, 2015). Coordinated activity of large ensembles of spatially distributed cells across the cortex provides the source for the processing and encoding of sensory information. Experimental work supports this idea. Highly distributed representations of tactile information have been described in the cortex (Nicolelis et al., 1997). In the visual cortex, sensory stimuli recruit intrinsically generated cortical ensembles (Miller et al., 2014; Okun et al., 2015). Representation of motor programs via cell ensembles has been described (Hommel, 2004). Moreover, the auditory cortex is dominated by broad scale dynamics in which a complete representation of sounds emerges only at a global scale (Bathellier et al., 2012).

Accordingly, multineuronal recording studies (Fujisawa et al., 2008; Miller et al., 2014) have revealed that ensembles of neurons can form emergent functional units. Therefore, they may be the building blocks used in cortical processing. However, important questions concerning integration of cortical activity remain unresolved. It is still unknown how these ensembles are present in the cortex and how spatially distributed cells can functionally contribute to unified stimulus codification. Moreover, although cell ensembles have long been thought to be associated with brain rhythms (Harris et al., 2003), a theoretical explanation integrating spatial and temporal aspects of cortical processing has yet to be proposed.

RHYTHMIC NEURONAL ACTIVITY IN THE BRAIN

Mammalian brain activity is rich in rhythms. The preservation of that rhythmic activity during the course of evolution demonstrates its relevance and appears to reflect a common functional mechanism for neural processing (Buzsáki and Draguhn, 2004). These rhythms are involved in perception, attention, memory, consciousness and movement execution (Gray et al., 1989; Engel et al., 2001; Fries et al., 2001; Brown, 2007; Buschman and Miller, 2010; Baldauf and Desimone, 2014). They play a key role in neural communication (Fries, 2005; Schroeder and Lakatos, 2009; Siegel et al., 2012). Moreover, it has been suggested that oscillatory activity contributes to spike synchronization of distributed neurons (Gray et al., 1989; Nuñez et al., 1992; Engel et al., 2001) and that synchrony underlies binding of separate features enabling perceptual unity (Singer and Gray, 1995).

Excitation and inhibition play a key role in the generation of rhythmic activity (Steriade et al., 1993; Whittington et al., 1995; Traub et al., 1996; Cardin et al., 2009). However, the mechanisms underlying oscillations and synchrony are still not well understood. Furthermore, current theories about their computational role are incomplete (Thiele and Stoner, 2003; Roelfsema et al., 2004; Hermes et al., 2015). It remains unclear what function they play in neural processing. They may contribute to discretize the computation.

DISCRETE NEURAL COMPUTATION

The brain receives a constant flow of analog sensory information from the environment. Successful interaction with the world depends on accurate processing of that information. Therefore, the challenging task that the brain faces is to rapidly extract changing relevant features from the environment in order to respond adequately. It requires a precise detection of changes sampling the flow of sensory information to compare its contents. Consequently, a primary function of the brain could be to discretize the continuous flow of information, compare those sampled units and extract relevant information from that computation (**Figure 1**). In sum, although the brain receives a constant flow of analog sensory information from the environment, sensory stimuli changes will be sampled in a discrete manner.

Scientists have long theorized that our cognition operates discontinuously within a framework of discrete cycles

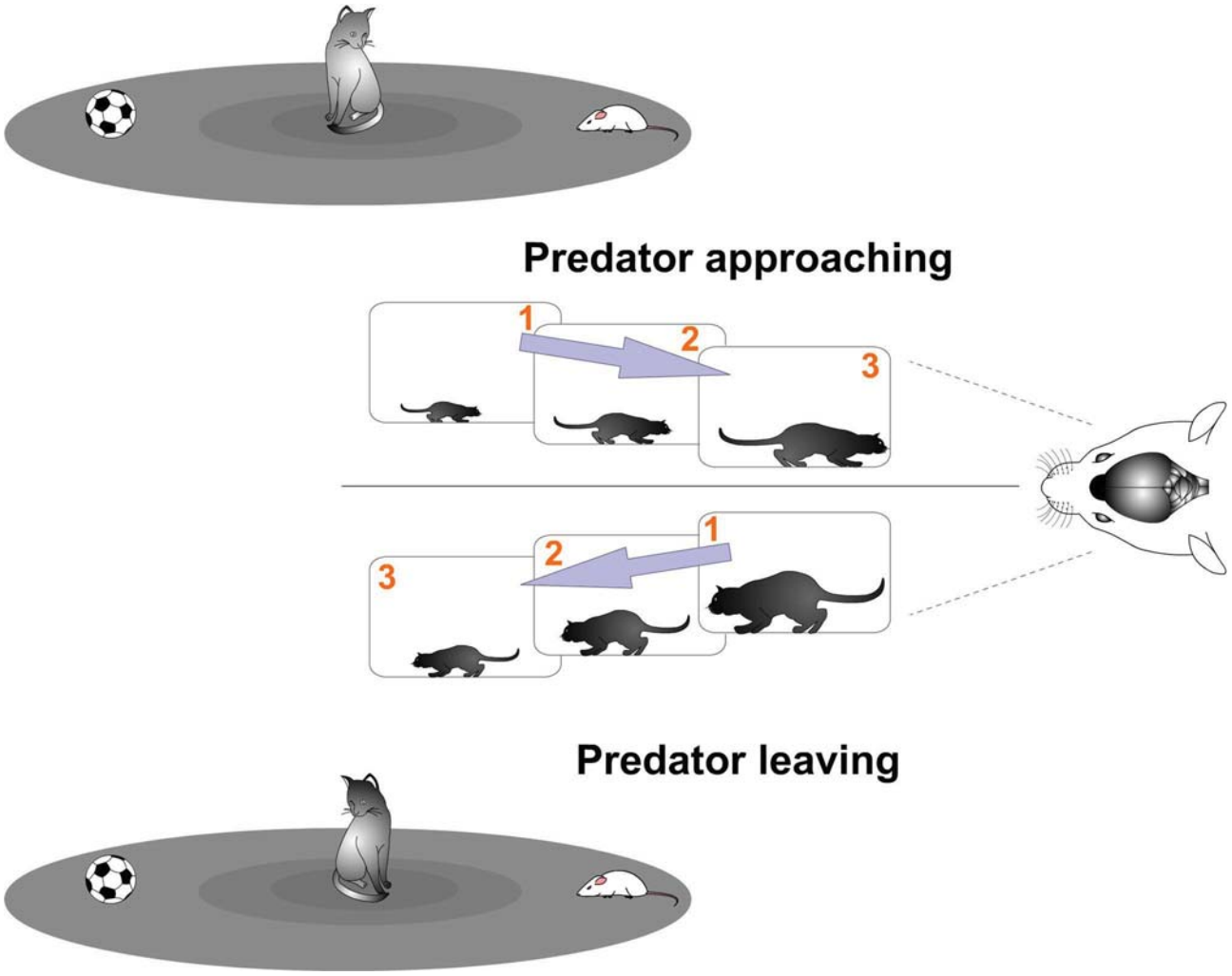


FIGURE 1 | Discrete neural processing. Successful interaction with the world depends on accurate processing of rapidly changing stimuli. It requires a precise detection of changes sampling the flow of sensory information to compare its contents. Of particular interest are relevant events, such as the appearance of a predator. A challenge faced by the prey's brain is to rapidly extract predator changing features from the scene in order to react satisfactorily. In this hypothetical example, the prey's visual system actively samples the visual environment. Discrete samples are taken separately by brief temporal periods. Predator movements (choosing between the prey and the ball) can be detected by computing differences between the subsamples (see "Cortical Neural Computation by Discrete Results Hypothesis: Functional Spatio-Temporal Units of Computation" Section). That computation allows the prey to know if the predator is approaching or leaving and to take appropriate action.

(Pitts and McCulloch, 1947; Harter, 1967; Allport, 1968; Varela et al., 1981; VanRullen and Koch, 2003; Buschman and Miller, 2010). Accordingly, neural systems could undergo oscillatory activity patterns. These oscillations could divide the neural processing into a series of discrete computational events.

There are relevant experimental data demonstrating this discrete processing. Discrete computations are well described in visual perception (VanRullen et al., 2005). One example is microsaccadic eye movements by which the visual system acquires fine spatial detail (Ko et al., 2010). Accordingly, vision is interrupted and sensory processing is discretized, separating it into distinct epochs. Recent evidence has been shown for discrete perceptual sampling in the somatosensory domain (Baumgarten et al., 2015). They demonstrated that somatosensory perception operates in a discrete mode, with sensory input being sampled by discrete perceptual cycles. Memory (Lundqvist et al., 2016) and attention are other examples of this computational nature (Buschman and Miller, 2009; Busch and VanRullen, 2010). It is known that oscillatory neuronal activity in the frontal eye field reflects the successive cycles of a sequential attentional exploration process during visual search (Buschman and Miller, 2009). Furthermore, discretized processing and encoding in the hippocampus is well described (Buzsáki, 2005). However, how the brain, especially the cerebral cortex, performs this computation is unknown.

CORTICAL NEURAL COMPUTATION BY DISCRETE RESULTS HYPOTHESIS: FUNCTIONAL SPATIO-TEMPORAL UNITS OF COMPUTATION

One of the most challenging problems we face in neuroscience is to understand how the cortex performs computations. Here we suggest that complex neural computations underlying cortical processing could be temporally discrete. But how does the cortex perform this computation? We propose that cortical processing is produced by the computation of discrete emergent functional units that we have called Discrete Results (Discrete Results Hypothesis). As we describe in the next sections, this novel concept has the ability to match the spatial and temporal aspects of cortical processing. We discuss the possible neural underpinnings of these spatio-temporal computational units and describe the empirical evidence supporting our hypothesis.

Our Discrete Results Hypothesis suggests that the computational principle of the cortex lies in the precise temporal coordination of spikes of spatially distributed neurons. It is necessary to divide the temporal and spatial dimension of that proposal for a better clarification.

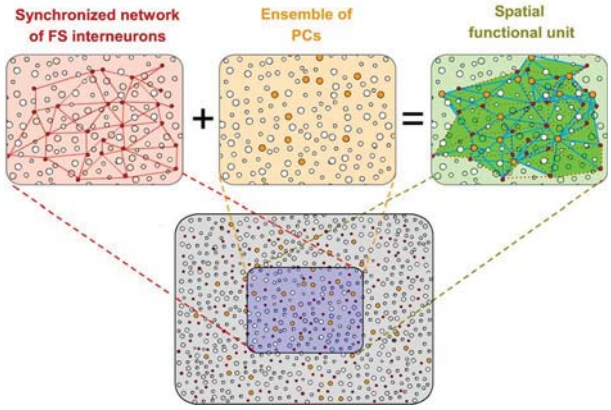


FIGURE 2 | Spatial functional units of cortical processing. A specific spatially distributed set of pyramidal cells (PCs; in orange) that are controlled by a specific coupled network of fast-spiking (FS) inhibitory cells (in red) form functional spatial units ("Ensembles") of cortical computation (in green). All PCs organized by that particular inhibitory network form part of that Ensemble. Individual PCs could participate in different Ensembles. The members and spatial extension of the Ensemble is determined by the inhibitory network. Red lines represent mutual connections between FS inhibitory cells in the network. These neurons innervate strategically the PCs (blue lines) extending a blanket of precise inhibition onto them.

Spatial Dimension of Cortical Processing: The "Ensemble" as a Functional Spatial Unit

In the cortex, most neuronal activity occurs in the form of coactive groups of cells defining neuronal ensembles (Miller et al., 2014). However, it is unclear how they emerge, with which neurons, what and how the relation is between the members, what spatial and temporal extension they have and what exactly an ensemble functionally means.

In our hypothesis, we define ''Ensemble'' as a specific spatially distributed set of excitatory neurons (referred to as pyramidal cells hereafter, PCs) that are controlled by a definite synchronized network of fast-spiking (FS) inhibitory cells (see ''Neural Underpinnings of Discrete Results Hypothesis : Spatio-Temporal Integration By Fast-Spiking Cells Synchronized Network.'' Section). All PCs organized by that particular synchronized inhibitory network form part of that Ensemble. It means that the Ensemble is formed by all the PCs whose firing could be transiently constrained by that specific synchronized inhibitory network (**Figure 2**). The members and spatial extension of the Ensemble is determined by that inhibitory network. Moreover, individual PCs could participate in different emergent Ensembles.

These emergent clusters of PCs form functional spatial units of cortical computation. However, that spatial aspect must be complemented with a temporal one.

Temporal Dimension of Cortical Processing: "Temporal Structure of Spikes"

Neurons are temporally precise on very fine timescales (Mainen and Sejnowski, 1995; Shmiel et al., 2005; Butts et al., 2007). Experimental evidence indicates that the exact time point at which a spike occurs plays an important role in information processing (Markram et al., 1997). Moreover, the precise timing of neuronal spiking is vital for coding of information (Singer and Gray, 1995). Therefore, this temporal precision is likely to be crucial for cortical computation. However, the functional significance remains unclear.

The Discrete Results hypothesis suggests that cortical processing is produced by a highly ordered temporal organization. Spike timing of PCs in a particular Ensemble is constrained by an inhibitory network generating a precise structured firing. We propose that this network constrains PCs spikes in temporal precise manner creating what we have called ''Temporal Structure of Spikes'' (**Figure 3**). We defined it as the accurate spike timing organization resulting from the precise temporal suppression of PCs spikes in the Ensemble. Spikes from PCs occur independently but organized inside the temporal structure. We propose that this Temporal Structure of Spikes is very important in the processing, coding and transfer of information in the cerebral cortex. The temporally structured firing activity enables information to be processed and coded in a way that downstream networks can compute. Accordingly, the existence of this specific temporal structure implies that failures in that precision of spikes will result in processing dysfunctions. An example of the importance of the temporal structure is the spike-timing-dependent plasticity (Caporale and Dan, 2008). This temporal structure is not fixed. It can be dynamically adjusted (for example by sensory input or by top-down influence) to meet the finest processing resolution depending on perceptual, task or attentional demands. Furthermore, the structure could be adjusted by neuromodulators. Accordingly, variations in the temporal structure will produce changes in the rate and temporal

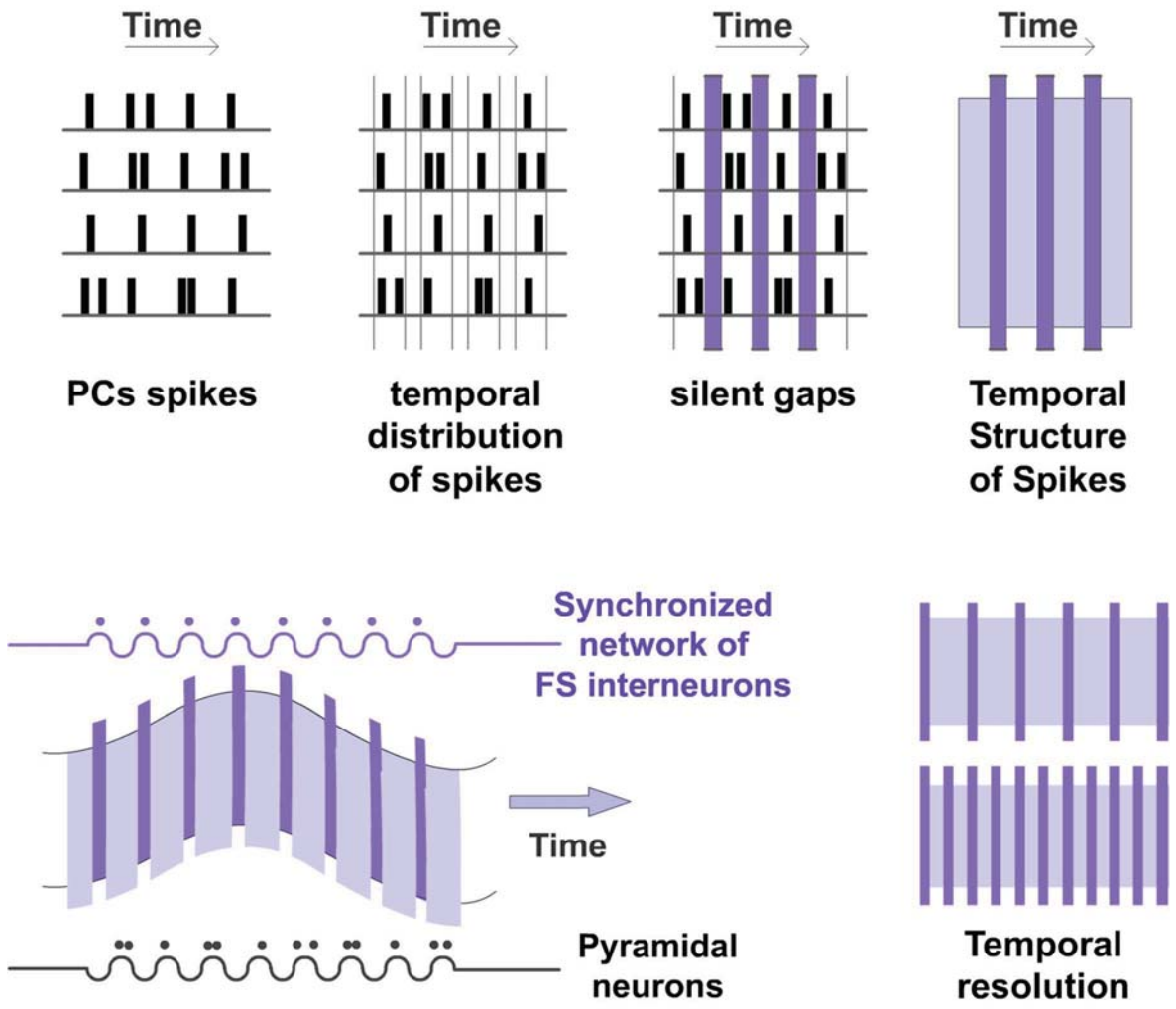


FIGURE 3 | Temporal structure of spikes. Spike timing of PCs is constrained by FS inhibitory interneurons. These cells set the timing and rate of action potentials produced by PCs limiting the temporal window during which they can be generated (Pouille and Scanziani, 2001). Moreover, PCs cannot discharge when they are shunted by strong inhibition. Consequently, the synchronized spiking of the inhibitory network creates temporal order among the PCs firing, generating precise silent periods that we have called "Silent Gaps" during which action potentials cannot be generated (vertical columns in purple). Rhythmic inhibition of the interneuron network provides alternating periods of spiking and no spiking in the Ensemble of PCs constraining these spikes into discrete periods generating a scaffold that we have called "Temporal Structure of Spikes". Rows represent hypothetical spike rasters for an Ensemble of four PCs. Spikes from these PCs occur independently but organized inside that temporal structure (top). These cycles would result in the oscillations observed in the brain (bottom left). The temporal structure can be dynamically adjusted to meet the finest processing resolution depending on perceptual, task or attentional demands (bottom right).

precision of PCs firing in the Ensemble. PCs spikes latencies and synchronization between them will vary accordingly. Consequently, changes in that temporal precision will code different content.

Experimental data provide support for this proposal. It is known that spike timing of PCs is constrained by the inhibitory cells. FS interneurons quickly limit the temporal window during which action potentials can be generated (Pouille and Scanziani, 2001; Li et al., 2015). Consequently, PCs are more likely to fire at precise points in time (Cardin et al., 2009). More importantly, PCs cannot discharge when they are shunted by strong inhibition. We propose that these precise inhibitory inputs to PCs generate strict periods of no spiking (''Silent Gaps'') in the Ensemble. Our hypothesis suggests that these precise Silent Gaps are very important for cortical computation. They divide the neural processing in the Ensemble into a series of

discrete computational events that we have called ''Discrete Results''.

It is also known that FS interneurons generate synchronized networks by mutual chemical and electrical connections in the neocortex (Whittington et al., 1995; Traub et al., 1996; Galarreta and Hestrin, 1999; Gibson et al., 1999). Electrical synapses generate highly precise transmission between interneurons of these networks. We propose that this coupling promotes the harmonized firing of connected neurons (Jones et al., 2000; Deans et al., 2001; Bartos et al., 2007) forming a synchronized network imposing a time-dependent spike restriction in the Ensemble of PCs. Different synchronized networks of FS interneurons create different sets of possible Ensembles. The simultaneous firing of the FS interneurons in the inhibitory network generates a synchronized inhibitory activity at their postsynaptic PCs in the Ensemble. The synchronized spiking of the inhibitory network could be fast enough to adjust the onset spiking of PCs (Woodruff et al., 2011; Li et al., 2015) and to create a temporal structure or scaffold providing alternating windows of no spiking in the emergent Ensemble of PCs. The rhythmic functioning of this network creates a sequence of temporal discrete events. This network rhythmically concentrates PCs discharges to particular discrete moments providing observable oscillation cycles at population level.

In sum, the inhibitory network forms a spatial structure of synchronized FS inhibitory cells and then this synchronized

DISCRETE RESULT: A FUNCTIONAL SPATIO-TEMPORAL UNIT OF COMPUTATION

Spatio-temporal activity patterns play an important role in cortical mechanisms of information processing (Ayzenshtat et al., 2010). Consequently, we propose that PCs compute and communicate information by using specific spatio-temporal patterns of spiking. Our hypothesis suggests that the cortex generates and employs these precise patterns to perform its computations. Thus cortical processing depends on the precise temporally structured relations among the respective spikes of PCs of the Ensemble. Information is encoded in the precise relations between temporal structured discharges. Individual spikes of PCs in the Ensemble take functional relevance when inserted into that temporal structure, forming a Discrete Result. Precise silent periods (Silent Gaps) inside the structure discretize the processing and allow for the formation of these discrete spatio-temporal functional units. All PCs belonging to the same Ensemble participate in the Discrete Result. Therefore, all PCs in the Ensemble have the opportunity to fire. Spikes from these PCs occur independently but organized inside the temporal structure. Each Discrete Result emerges transiently formed by the combination of the firing and silent responses (no firing) of all PCs in the Ensemble (**Figure 4**). Thus, PCs silent responses are also important in cortical computation and codification. The same Ensemble can form multiple Discrete Results. This

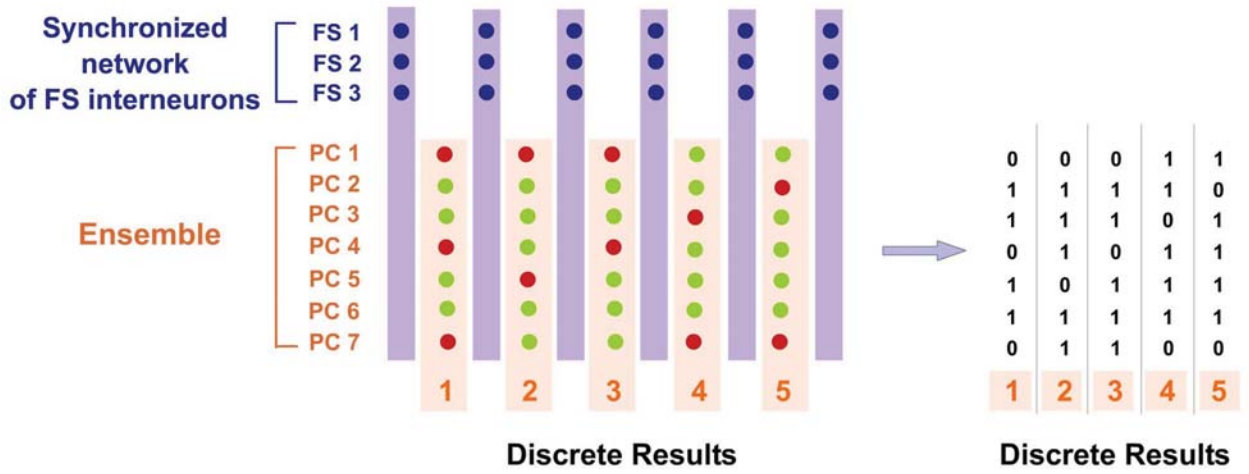


FIGURE 4 | Discrete Results: neural computational units. PCs belonging to the same Ensemble participate in the Discrete Result. Therefore, all PCs in the Ensemble have the opportunity to fire. Spikes from these PCs occur independently but organized inside the temporal structure. Each potential Discrete Result emerges transiently formed by the combination of the firing and silent responses (no firing) of all PCs in the Ensemble. Therefore, PCs silent responses are also important in cortical computation and codification. Moreover, the same Ensemble can form multiple Discrete Results. This means that the same PCs of the Ensemble process and encode multitude of contents. Consequently, there are great potential neural possibilities that could form a Discrete Result and also enormous possibilities that could form different sequences of Discrete Results. In this hypothetical example, the Ensemble is formed by seven PCs and the synchronized network of FS interneurons by three cells. Five possible Discrete Results are shown. PCs spikes are displayed as green dots and silent responses (no spikes) as red dots. On the right, a digital (binary) representation of these hypothetical Discrete Results is shown.

means that the same PCs of the Ensemble, process and encode multitude of contents. Consequently, there are great potential neural possibilities that could form a Discrete Result and also enormous possibilities that could form different sequences of Discrete Results. Therefore, the number of possible representations that can be formed is titanic. This mechanism could explain why the cortex is so robust to damage. Moreover, the content coded by a Discrete Result depends also on the resolution of the Temporal Structure of Spikes.

Individual PCs could participate in different Ensembles and be potentially implicated in multiple representations. Furthermore, different synchronized networks of FS interneurons create different sets of possible Ensembles. Accordingly, the cortex performs computations using multiple Ensembles in parallel creating a multitude of Discrete Results simultaneously. Moreover, different sets of possible Ensembles are created by different synchronized networks of FS interneurons along the cortical processing hierarchy. Discrete Results at higher levels integrate computational results from previous stages. Therefore, each Discrete Result constitutes a functional unit that has the ability to process, integrate and represent specific content (Discrete Results) from previous computations (**Figure 5**). Consequently, in sensory

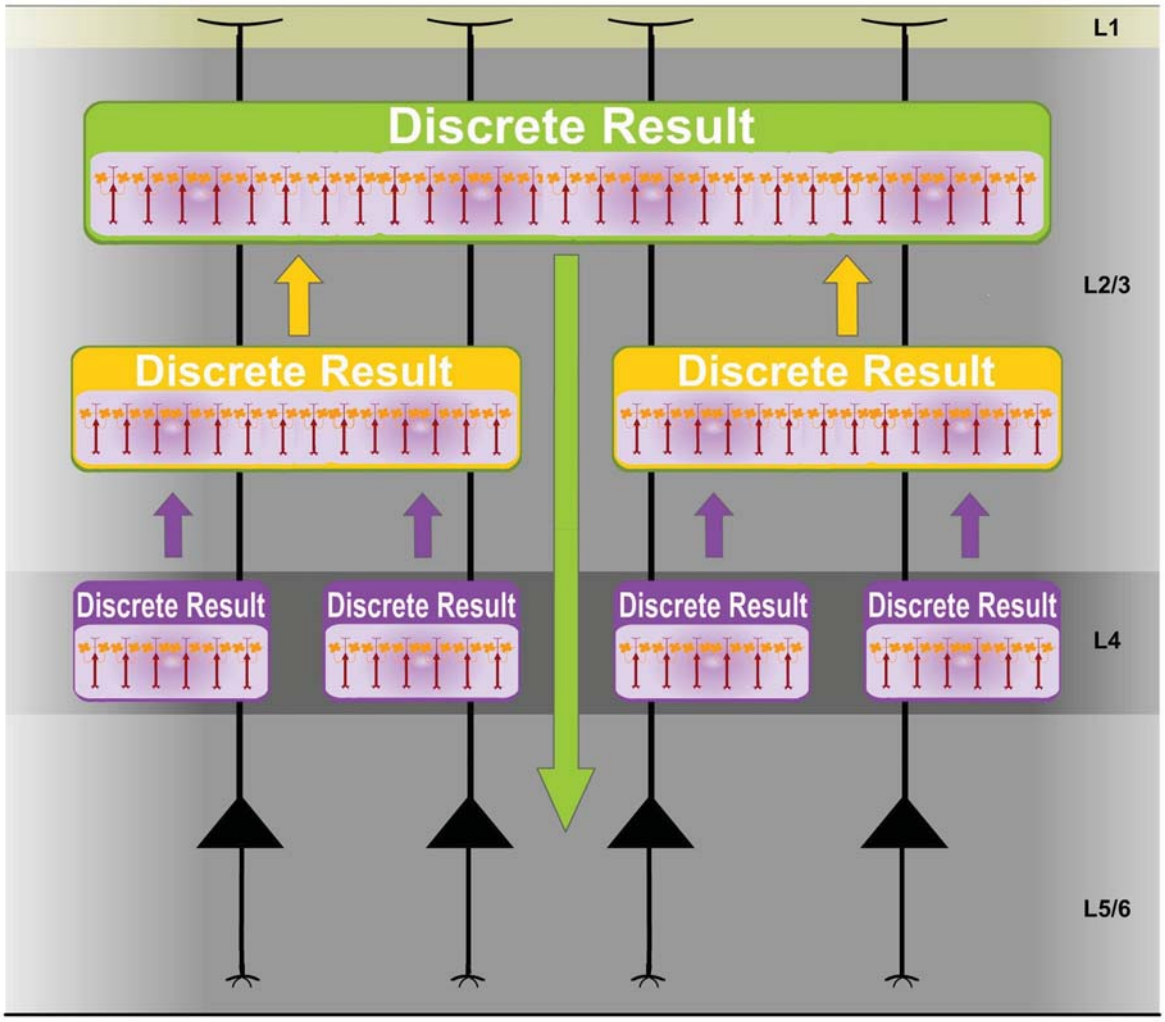


FIGURE 5 | Discrete Results: functional units of neural computational integration. Different sets of possible Ensembles are created by different synchronized networks of FS cells along the cortical processing hierarchy. Experimental studies support this idea. Distinct clusters of FS interneurons have been identified in the cortex. For example, in the rat barrel cortex, one layer 4 FS interneuron type has an axonal domain strictly confined to a barrel (Koelbl et al., 2015). Accordingly, the cortex performs computations using multiple Ensembles in parallel creating a multitude of Discrete Results simultaneously. Discrete Results at higher levels integrate computational results from previous stages. Therefore, each Discrete Result constitutes a functional unit that has the ability to process, integrate and represent specific content (Discrete Results) from previous computations. Consequently, in sensory processing, they functionally contribute to unified stimulus codification. Therefore, the Discrete Result concept could explain the binding of separate features enabling perceptual unity. Experimental data provide support for this proposal. Highly distributed representations of tactile information have been described in the cortex (Nicolelis et al., 1997). Moreover, the auditory cortex is dominated by broad scale dynamics in which a complete representation of sounds emerges only at a global scale (Bathellier et al., 2012).

processing, they functionally contribute to unified stimulus codification.

The Discrete Result concept has the ability to explain how complex neural computations underlying cortical processing could be temporally discrete. Consequently, we propose that sensory information would need to be quantized to be computed by the cerebral cortex. Therefore, processing of sensory information must be temporally discrete and information flow in the cortex must be quantized allowing for the formation of Discrete Results. Therefore, in sensory processing, they can be defined as each neural computational functional unit resulting in quantization of the continuous flow of sensory information (**Figure 6**). Increasing the number of Discrete Results per temporal unit allows resolution enhancement. It could be dynamically adjusted by sensory input or by top-down influence to meet the finest processing resolution depending on perceptual, task or attentional demands.

NEURAL COMPUTATION BY DYNAMIC SEQUENCE OF DISCRETE RESULTS

Multineuronal activity structured in temporal sequences has been suggested since long ago (Lorente de Nó, 1938; Hebb, 1949; Abeles, 1991). Experimental studies have increased our knowledge about how this sequential activity is generated in the brain (Harris et al., 2003). However, untangling its functional computational significance is still a formidable challenge today.

We propose that precise sequences of Discrete Results are the mechanism used by the cortex to perform computations. The computation of the Discrete Results sequence is the mechanism used by the cortex to extract, code, memorize and transmit neural information. This proposal is a neuronal population mechanism to compute and code. Dynamic sequences of Discrete Results generate representations. Different sequences codify different contents.

The rhythmic functioning of the synchronized inhibitory network creates a sequence of Discrete Results (**Figure 7**). Computations between successive Discrete Results in the sequence produce the power of the cortical processing. Experimental data provide support for this hypothesis. Sequential activity of multineuronal spiking has been well described in the cortex (Fujisawa et al., 2008; Crowe et al., 2010; Harvey et al., 2012; Carrillo-Reid et al., 2015) and in the hippocampus (O'Keefe and Burgess, 1996; Fyhn et al., 2004; Foster and Wilson, 2006; Pastalkova et al., 2008; Wikenheiser and Redish, 2015).

Moreover, cortical processing by dynamic sequences of Discrete Results could be the neural source of some rhythmic signals observed at population level. This hypothesis of neural processing could be applied to other structures and nuclei of the brain.

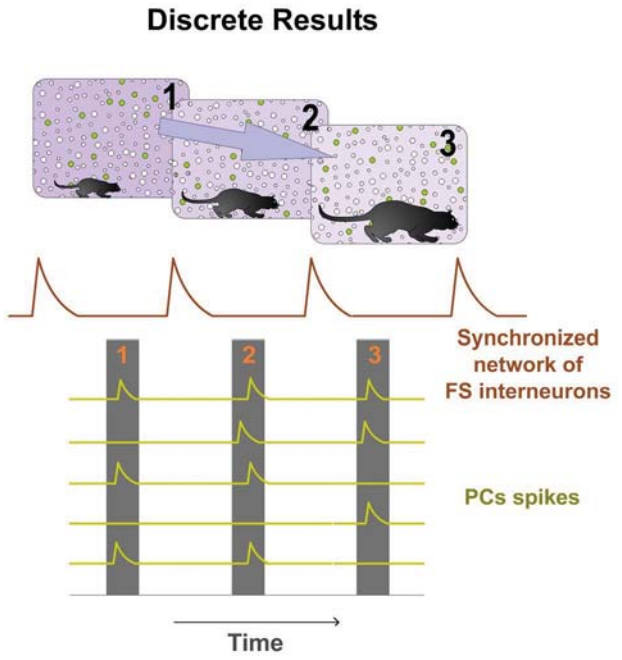


FIGURE 6 | Discrete Results in cortical sensory processing. Hypothetical spatial maps of cortical neurons for each computational event resulting in quantization of the continuous flow of sensory information are shown. Green cells show a representative Ensemble of PCs organized by a specific synchronized network of FS interneurons. Individual spikes of these PCs take functional relevance inserted into a temporal structure forming discrete spatiotemporal functional units (Discrete Results). In this hypothetical example, relevant sensory information (predator movements) can be extracted by computing differences between the Discrete Results. Increasing the number of Discrete Results per temporal unit allows resolution enhancement.

NEURAL UNDERPINNINGS OF DISCRETE RESULTS HYPOTHESIS: SPATIO-TEMPORAL INTEGRATION BY FAST-SPIKING CELLS SYNCHRONIZED NETWORK

Our Discrete Results hypothesis suggests that complex neural computations underlying cortical processing could be temporally discrete. Moreover, we propose that cortical processing is produced by the computation of discrete spatio-temporal functional units. But what could be the neuronal elements underlining this computation? The cerebral cortex is composed of many types of neurons. Although all of them play a key role in cortical processing, our hypothesis suggests that there must be a specific type of inhibitory cell that may be implicated in the creation of a spatio-temporal structure supporting discrete cortical computation. We propose that FS interneuron may be a key element in our hypothesis providing the basis for this computation. These cells forming a synchronized spatially distributed cortical network may impose a temporal spike restriction in PCs creating functionally coupled units of computation. Their rhythmic activity may create a sequence of spatio-temporal functional units (Discrete Results), discretizing the information processing. In sum, we propose that they are able to

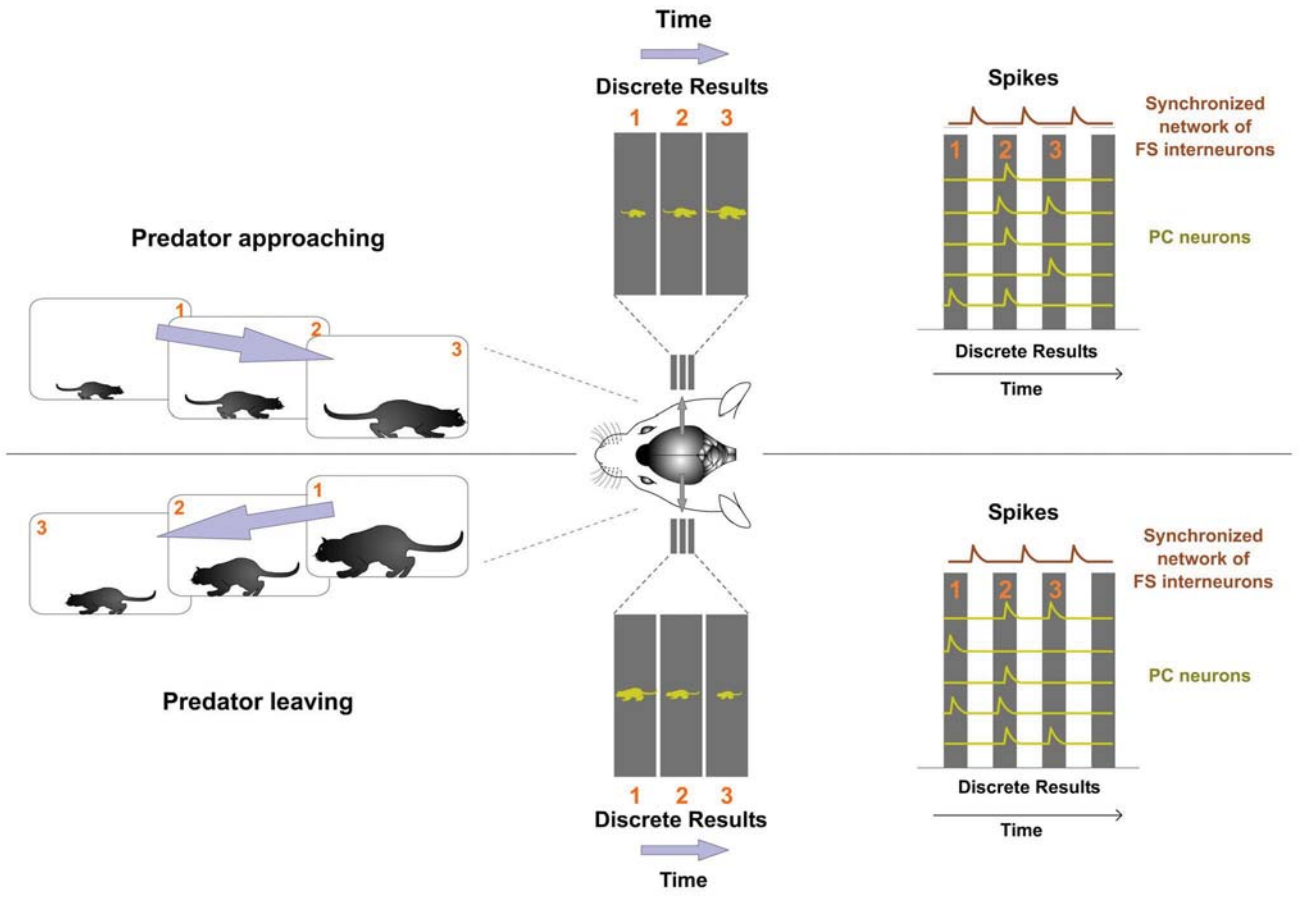


FIGURE 7 | Dynamic sequences of Discrete Results are the mechanism used by the cortex to perform computations. Different sequences of Discrete Results codify different content. In this example, both types of predator trajectories involve different sequentially activated Discrete Results. One sequence codifies the predator approaching and the other leaving. By computing the successive Discrete Results in the sequence the prey's cortex is allowed to extract predator trajectory in order to respond appropriately.

integrate the spatial and temporal dimension of cortical computation.

FS cells (Kawaguchi and Kubota, 1997) are the largest population of interneurons in the neocortex. They play a key role as pacemakers for oscillations (Whittington et al., 1995; Traub et al., 1996) and in shaping multineuronal activity (Cardin et al., 2009). However, it is still unclear how these cells functionally contribute to the operations performed by the cortex.

It is known that they form dense matrices covering PCs (Packer and Yuste, 2011) extending a blanket of inhibition onto them (Karnani et al., 2014). They strategically innervate the axon initial segment (chandelier cells) or soma/proximal (basket cells) dendrites of PCs (Klausberger and Somogyi, 2008). They shape the precise timing and dynamic range of action potentials produced by PCs (Pouille and Scanziani, 2001; Cardin et al., 2009; Sohal et al., 2009; Li et al., 2015). They generate synchronized networks by mutual chemical and electrical connections (Galarreta and Hestrin, 1999; Gibson et al., 1999; Sohal et al., 2009). Accordingly, they fire in high synchrony (Jones et al., 2000) at high-frequency firing pattern without a significant spike adaptation (Kawaguchi and Kubota, 1997). They have narrow spike-waveform, fast kinetics (Atallah et al., 2012) and high synchronous release of GABA (Hefft and Jonas, 2005). Furthermore, they show broader tuning than other neurons (Kerlin et al., 2010; Hofer et al., 2011; Li et al., 2015). Thus, in accord with our proposal, these properties render them well suited for a structural role in cortical processing. Our hypothesis suggests that these cells create a temporal structure or scaffold (Temporal Structures of Spikes) providing alternating windows of no spiking (Silent Gaps) in the emergent Ensemble of PCs. Since different classes of FS cells have distinct properties in their temporal pattern of discharge (Gupta et al., 2000; Dehorter et al., 2015), it is then likely that they create diverse temporal restriction in

PCs firing forming different Temporal Structures of Spikes. Moreover, we propose that different synchronized networks of FS interneurons create different sets of possible Ensembles. Experimental work supports this idea. Distinct clusters of FS interneurons have been identified in the cortex. For example, in the rat barrel cortex, one layer 4 FS interneuron type has an axonal domain strictly confined to a barrel (Koelbl et al., 2015).

Our hypothesis suggests that precise dynamic sequences of Discrete Results is the mechanism used by the cortex to extract, code, memorize and transmit neural information and that FS cells could play a key role in this discrete cortical processing. In agreement with that proposal, these cells are essential for perception, cognition, attention, memory and behavior (Isomura et al., 2009; Letzkus et al., 2011; Yizhar et al., 2011; Courtin et al., 2014; Hu et al., 2014; Kim et al., 2016). They are also implicated in plasticity and learning (Hensch, 2005; Yazaki-Sugiyama et al., 2009; Letzkus et al., 2011; Donato et al., 2013) and have been implicated in psychiatric disorders such as epilepsy and schizophrenia (Powell et al., 2003; Lewis et al., 2012). A prediction of our hypothesis would be that silencing of these neurons will disrupt normal cortical processing. Recently, it has been shown that silencing of these interneurons disrupts attentional processing (Kim et al., 2016). Furthermore, our hypothesis suggests that discrete cortical processing can be dynamically adjusted to meet the finest processing resolution depending on perceptual, task or attentional demands. We propose that increasing the number of Discrete Results per temporal unit allows resolution enhancement. Accordingly, experimental data show that perceptual coding and discrimination are improved by increased spiking of these cells (Lee et al., 2012). Moreover, in agreement with our proposal, increases in task difficulty and attentional requirements are accompanied by an enhancement of FS cells firing (Chen et al., 2008).

CONCLUSION

There is increasing evidence that most neuronal activity in the cortex occurs in the form of coactive groups of cells defining neuronal ensembles. However, it is unclear what exactly an ensemble functionally means. These ensembles of neurons can form emergent functional units. In this Hypothesis and Theory article, we propose a new functional theoretical framework to explain the computational roles of these ensembles in cortical processing. We suggest that complex neural computations underlying cortical processing could be temporally discrete and that sensory information would need to be quantized to be computed by the cerebral cortex. Accordingly, we propose that cortical processing is produced by the computation of discrete spatio-temporal functional units that we have called Discrete Results. For example, perceptual integration, processing resolution and information coding can be now explained by our hypothesis.

The Discrete Result concept explains how complex neural computations underlying cortical processing could be temporally discrete. This novel concept has the ability to integrate the physiological and computational aspects of cortical processing defining the traditional idea of cells ensemble limiting their spatio-temporal dimension and differentiating their membership and relations between the members. Moreover, the Discrete Results hypothesis constitutes a conceptual advance with special relevance for neuroscience and computer sciences.

AUTHOR CONTRIBUTIONS

CC: conceived the hypothesis. CC and AN: conceptually developed and wrote this article.

FUNDING

Work was supported by a grant from Ministerio de Economia y Competitividad (BFU2012-36107).

REFERENCES

Abeles, M. (1991). Corticonics: Neural Circuits of the Cerebral Cortex. Cambridge, UK: Cambridge University Press.

Allport, D. A. (1968). Phenomenal simultaneity and the perceptual moment hypothesis. Br. J. Psychol. 59, 395–406. doi: 10.1111/j.2044-8295.1968.tb01154.x

Atallah, B. V., Bruns, W., Carandini, M., and Scanziani, M. (2012). Parvalbumin-expressing interneurons linearly transform cortical responses to visual stimuli. Neuron 73, 159–170. doi: 10.1016/j.neuron.2011.12.013

Ayzenshtat, I., Meirovithz, E., Edelman, H., Werner-Reiss, U., Bienenstock, E., Abeles, M., et al. (2010). Precise spatiotemporal patterns among visual cortical areand their relation to visual stimulus processing. J. Neurosci. 30, 11232–11245. doi: 10.1523/JNEUROSCI.5177-09.2010

Baldauf, D., and Desimone, R. (2014). Neural mechanisms of object-based attention. Science 334, 424–427. doi: 10.1126/science.1247003

Bartos, M., Vida, I., and Jonas, P. (2007). Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. Nat. Rev. Neurosci. 8, 45–56. doi: 10.1038/nrn2044

Bathellier, B., Ushakova, L., and Rumpel, S. (2012). Discrete neocortical dynamics predict behavioral categorization of sounds. Neuron 76, 435–449. doi: 10.1016/j.neuron.2012.07.008

Baumgarten, T. J., Schnitzler, A., and Lange, J. (2015). Beta oscillations define discrete perceptual cycles in the somatosensory domain. Proc. Natl. Acad. Sci. U S A 112, 12187–12192. doi: 10.1073/pnas.1501438112

Brown, P. (2007). Abnormal oscillatory synchronisation in the motor system leads to impaired movement. Curr. Opin. Neurobiol. 17, 656–664. doi: 10.1016/j.conb.2007.12.001

Busch, N. A., and VanRullen, R. (2010). Spontaneous EEG oscillations reveal periodic sampling of visual attention. Proc. Natl. Acad. Sci. U S A 107, 16048–16053. doi: 10.1073/pnas.1004801107

Buschman, T. J., and Miller, E. K. (2009). Serial, covert shifts of attention during visual search are reflected by the frontal eye fields and correlated with population oscillations. Neuron 63, 386–396. doi: 10.1016/j.neuron.2009.06.020

Buschman, T. J., and Miller, E. K. (2010). Shifting the spotlight of attention: evidence for discrete computations in cognition. Front. Hum. Neurosci. 4:194. doi: 10.3389/fnhum.2010.00194

Butts, D. A., Weng, C., Jin, J. Z., Yeh, C. I., Lesica, N. A., Alonso, J. M., et al. (2007). Temporal precision in the neural code and the timescales of natural vision. Nature 449, 92–95. doi: 10.1038/nature06105

Buzsáki, G. (2005). Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. Hippocampus 15, 827–840. doi: 10.1002/hipo.20113

Buzsáki, G. (2010). Neural syntax: cell assemblies, synapsembles and readers. Neuron 68, 362–385. doi: 10.1016/j.neuron.2010.09.023

Buzsáki, G., and Draguhn, A. (2004). Neuronal oscillations in cortical networks. Science 304, 1926–1929. doi: 10.1126/science.1099745

Caporale, N., and Dan, Y. (2008). Spike timing-dependent plasticity: a Hebbian learning rule. Annu. Rev. Neurosci. 31, 25–46. doi: 10.1146/annurev.neuro.31.060407.125639

Cardin, J. A., Carlén, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., et al. (2009). Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature 459, 663–667. doi: 10.1038/nature08002

Carrillo-Reid, L., Miller, J. K., Hamm, J. P., Jackson, J., and Yuste, R. (2015). Endogenous sequential cortical activity evoked by visual stimuli. J. Neurosci. 35, 8813–8828. doi: 10.1523/JNEUROSCI.5214-14.2015

Chen, Y., Martinez-Conde, S., Macknik, S. L., Bereshpolova, Y., Swadlow, H. A., and Alonso, J. M. (2008). Task difficulty modulates the activity of specific neuronal populations in primary visual cortex. Nat. Neurosci. 11, 974–982. doi: 10.1038/nn.2147

Courtin, J., Chaudun, F., Rozeske, R. R., Karalis, N., Gonzalez-Campo, C., Wurtz, H., et al. (2014). Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression. Nature 505, 92–96. doi: 10.1038/nature12755

Crowe, D. A., Averbeck, B. B., and Chafee, M. V. (2010). Rapid sequences of population activity patterns dynamically encode task-critical spatial information in parietal cortex. J. Neurosci. 30, 11640–11653. doi: 10.1523/JNEUROSCI.0954-10.2010

Deans, M. R., Gibson, J. R., Sellitto, C., Connors, B. W., and Paul, D. L. (2001). Synchronous activity of inhibitory networks in neocortex requires electrical synapses containing connexin36. Neuron 31, 477–485. doi: 10.1016/s0896-6273(01)00373-7

Dehorter, N., Ciceri, G., Bartolini, G., Lim, L., del Pino, I., and Marín, O. (2015). Tuning of fast-spiking interneuron properties by an activity-dependent transcriptional switch. Science 349, 1216–1220. doi: 10.1126/science.aab3415

Donato, F., Rompani, S. B., and Caroni, P. (2013). Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. Nature 504, 272–276. doi: 10.1038/nature12866

Engel, A. K., Fries, P., and Singer, W. (2001). Dynamic predictions: oscillations and synchrony in top-down processing. Nat. Rev. Neurosci. 2, 704–716. doi: 10.1038/35094565

Foster, D. J., and Wilson, M. A. (2006). Reverse replay of behavioural sequences in hippocampal place cells during the awake state. Nature 440, 680–683. doi: 10.1038/nature04587

Fries, P. (2005). A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. Trends Cogn. Sci. 9, 474–480. doi: 10.1016/j.tics.2005.08.011

Fries, P., Reynolds, J. H., Rorie, A. E., and Desimone, R. (2001). Modulation of oscillatory neuronal synchronization by selective visual attention. Science 291, 1560–1563. doi: 10.1126/science.1055465

Fujisawa, S., Amarasingham, A., Harrison, M. T., and Buzsáki, G. (2008). Behavior-dependent short-term assembly dynamics in the medial prefrontal cortex. Nat. Neurosci. 11, 823–833. doi: 10.1038/nn.2134

Fyhn, M., Molden, S., Witter, M. P., Moser, E. I., and Moser, M. B. (2004). Spatial representation in the entorhinal cortex. Science 305, 1258–1264. doi: 10.1126/science.1099901

Galarreta, M., and Hestrin, S. (1999). A network of fast-spiking cells in the neocortex connected by electrical synapses. Nature 402, 72–75. doi: 10.1038/47029

Gibson, J. R., Beierlein, M., and Connors, B. W. (1999). Two networks of electrically coupled inhibitory neurons in neocortex. Nature 402, 75–79. doi: 10.1038/47035

Gray, C. M., Konig, P., Engel, A. K., and Singer, W. (1989). Oscillatory responses in cat visual cortex exhibit inter-columnar synchronization which reflects global stimulus properties. Nature 338, 334–337. doi: 10.1038/338334a0

Gupta, A., Wang, Y., and Markram, H. (2000). Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. Science 287, 273–278. doi: 10.1126/science.287.5451.273

Harris, K. D., Csicsvari, J., Hirase, H., Dragoi, G., and Buzsáki, G. (2003). Organization of cell assemblies in the hippocampus. Nature 424, 552–556. doi: 10.1038/nature01834

Harter, M. R. (1967). Excitability cycles and cortical scanning: a review of two hypotheses of central intermittency in perception. Psychol. Bull. 68, 47–58. doi: 10.1037/h0024725

Harvey, C. D., Coen, P., and Tank, D. W. (2012). Choice-specific sequences in parietal cortex during a virtual-navigation decision task. Nature 484, 62–68. doi: 10.1038/nature10918

Hebb, D. (1949). The Organization of Behavior: A Neuropsychological Theory. New York, NY: Wiley-Interscience.

Hefft, S., and Jonas, P. (2005). Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse. Nat. Neurosci. 8, 1319–1328. doi: 10.1038/nn1542

Hensch, T. K. (2005). Critical period plasticity in local cortical circuits. Nat. Rev. Neurosci. 6, 877–888. doi: 10.1038/nrn1787

Hermes, D., Miller, K. J., Wandell, B. A., and Winawer, J. (2015). Stimulus dependence of gamma oscillations in human visual cortex. Cereb. Cortex 25, 2951–2959. doi: 10.1093/cercor/bhu091

Hofer, S. B., Ko, H., Pichler, B., Vogelstein, J., Ros, H., Zeng, H., et al. (2011). Differential connectivity and response dynamics of excitatory and inhibitory neurons in visual cortex. Nat. Neurosci. 14, 1045–1052. doi: 10.1038/nn.2876

Hommel, B. (2004). Event files feature binding in and across perception and action. Trends Cogn. Sci. 8, 494–500. doi: 10.1016/j.tics.2004.08.007

Hopfield, J. J. (1982). Neural networks and physical systems with emergent collective computational abilities. Proc. Natl. Acad. Sci. U S A 79, 2554–2558. doi: 10.1073/pnas.79.8.2554

Hu, H., Gan, J., and Jonas, P. (2014). Fast-spiking, parvalbumin+ GABAergic interneurons: from cellular design to microcircuit function. Science
345:1255263. doi: 10.1126/science.1255263

Isomura, Y., Harukuni, R., Takekawa, T., Aizawa, H., and Fukai, T. (2009). Microcircuitry coordination of cortical motor information in self-initiation of voluntary movements. Nat. Neurosci. 12, 1586–1593. doi: 10.1038/nn.2431

Jones, M. S., MacDonald, K. D., Choi, B., Dudek, F. E., and Barth, D. S. (2000). Intracellular correlates of fast (>200 Hz) electrical oscillations in rat somatosensory cortex. J. Neurophysiol. 84, 1505–1518.

Karnani, M. M., Agetsuma, M., and Yuste, R. (2014). A blanket of inhibition: functional inferences from dense inhibitory connectivity. Curr. Opin. Neurobiol. 26, 96–102. doi: 10.1016/j.conb.2013.12.015

Kawaguchi, Y., and Kubota, Y. (1997). GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb. Cortex 7, 476–486. doi: 10.1093/cercor/7.6.476

Kerlin, A. M., Andermann, M. L., Berezovskii, V. K., and Reid, R. C. (2010). Broadly tuned response properties of diverse inhibitory neuron subtypes in mouse visual cortex. Neuron 67, 858–871. doi: 10.1016/j.neuron.2010.08.002

Kim, H., Ährlund-Richter, S., Wang, X., Deisseroth, K., and Carlén, M. (2016). Prefrontal parvalbumin neurons in control of attention. Cell 164, 208–218. doi: 10.1016/j.cell.2015.11.038

Klausberger, T., and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science 321, 53–57. doi: 10.1126/science.1149381

Ko, H.-K., Poletti, M., and Rucci, M. (2010). Microsaccades precisely relocate gaze in a high visual acuity task. Nat. Neurosci. 13, 1549–1553. doi: 10.1038/nn.2663

Koelbl, C., Helmstaedter, M., Lübke, J., and Feldmeyer, D. (2015). A barrel-related interneuron in layer 4 of rat somatosensory cortex with a high intrabarrel connectivity. Cereb. Cortex 25, 713–725. doi: 10.1093/cercor/bht263

Lee, S. H., Kwan, A. C., Zhang, S., Phoumthipphavong, V., Flannery, J. G., Masmanidis, S. C., et al. (2012). Activation of specific interneurons improves V1 feature selectivity and visual perception. Nature 488, 379–383. doi: 10.

Letzkus, J. J., Wolff, S. B., Meyer, E. M., Tovote, P., Courtin, J., Herry, C., et al. (2011). A disinhibitory microcircuit for associative fear learning in the auditory cortex. Nature 480, 331–335. doi: 10.1038/nature10674

Lewis, D. A., Curley, A. A., Glausier, J. R., and Volk, D. W. (2012). Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci. 35, 57–67. doi: 10.1016/j.tins.2011.10.004

Li, L., Xiong, X. R., Ibrahim, L. A., Yuan, W., Tao, H. W., and Zhang, L. I. (2015). Differential receptive field properties of parvalbumin and somatostatin inhibitory neurons in mouse auditory cortex. Cereb. Cortex 25, 1782–1791. doi: 10.1093/cercor/bht417

Lorente de Nó, R. (1938). Analysis of the activity of the chains of internuncial neurons. J. Neurophysiol. 1, 207–244.

Lundqvist, M., Rose, J., Herman, P., Brincat, S. L., Buschman, T. J., and Miller, E. K. (2016). Gamma and beta bursts underlie working memory. Neuron 90, 152–164. doi: 10.1016/j.neuron.2016.02.028

Mainen, Z. F., and Sejnowski, T. J. (1995). Reliability of spike timing in neocortical neurons. Science 268, 1503–1506. doi: 10.1126/science.7770778

Markram, H., Lübke, J., Frotscher, M., and Sakmann, B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science 275, 213–215. doi: 10.1126/science.275.5297.213

Miller, J. E., Ayzenshtat, I., Carrillo-Reid, L., and Yuste, R. (2014). Visual stimuli recruit intrinsically generated cortical ensembles. Proc. Natl. Acad. Sci. U S A 111, E4053–E4061. doi: 10.1073/pnas.1406077111

Nicolelis, M. A. L., Ghazanfar, A. A., Faggin, B., Votaw, S., and Oliveira, L. M. O. (1997). Reconstructing the engram: simultaneous, multisite, many single neuron recordings. Neuron 18, 529–537. doi: 10.1016/s0896-6273(00)80295-0

Nuñez, A., Amzica, F., and Steriade, M. (1992). Voltage-dependent fast (20–40 Hz) oscillations in long-axoned neocortical neurons. Neuroscience 51, 7–10. doi: 10.1016/0306-4522(92)90464-d

O'Keefe, J., and Burgess, N. (1996). Geometric determinants of the place fields of hippocampal neurons. Nature 381, 425–428. doi: 10.1038/381425a0

Okun, M., Steinmetz, N. A., Cossell, L., Iacaruso, M. F., Ko, H., Barthó, P., et al. (2015). Diverse coupling of neurons to populations in sensory cortex. Nature 521, 511–515. doi: 10.1038/nature14273

Packer, A. M., and Yuste, R. (2011). Dense, unspecific connectivity of neocortical parvalbumin-positive interneurons: a canonical microcircuit for inhibition? J. Neurosci. 31, 13260–13271. doi: 10.1523/JNEUROSCI.3131-11.2011

Pastalkova, E., Itskov, V., Amarasingham, A., and Buzsáki, G. (2008). Internally generated cell assembly sequences in the rat hippocampus. Science 321, 1322–1327. doi: 10.1126/science.1159775

Pitts, W., and McCulloch, W. S. (1947). How we know universals: the perception of auditory and visual forms. Bull. Math. Biophys. 9, 127–147. doi: 10.1007/bf02478291

Pouget, A., Dayan, P., and Zemel, R. (2000). Information processing with population codes. Nat. Rev. Neurosci. 1, 125–132. doi: 10.1038/35039062

Pouille, F., and Scanziani, M. (2001). Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. Science 293, 1159–1163. doi: 10.1126/science.1060342

Powell, E. M., Campbell, D. B., Stanwood, G. D., Davis, C., Noebels, J. L., and Levitt, P. (2003). Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy and behavioral dysfunction. J. Neurosci. 23, 622–631.

Roelfsema, P. R., Lamme, V. A., and Spekreijse, H. (2004). Synchrony and covariation of firing rates in the primary visual cortex during contour grouping. Nat. Neurosci. 7, 982–991. doi: 10.1038/nn1304

Schroeder, C. E., and Lakatos, P. (2009). Low-frequency neuronal oscillations as instruments of sensory selection. Trends Neurosci. 32, 9–18. doi: 10.1016/j.tins.2008.09.012

Shmiel, T., Drori, R., Shmiel, O., Ben-Shaul, Y., Nadasdy, Z., Shemesh, M., et al. (2005). Neurons of the cerebral cortex exhibit precise interspike timing in correspondence to behavior. Proc. Natl. Acad. Sci. U S A 102, 18655–18657. doi: 10.1073/pnas.0509346102

Siegel, M., Donner, T. H., and Engel, A. K. (2012). Spectral fingerprints of large-scale neuronal interactions. Nat. Rev. Neurosci. 13, 120–134. doi: 10.1038/nrn3137

Singer, W., and Gray, C. M. (1995). Visual feature integration and the temporal correlation hypothesis. Annu. Rev. Neurosci. 18, 555–586. doi: 10.1146/annurev.neuro.18.1.555

Sohal, V. S., Zhang, F., Yizhar, O., and Deisseroth, K. (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 459, 698–702. doi: 10.1038/nature07991

Steriade, M., Nuñez, A., and Azmica, F. (1993). A novel slow (<1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. J. Neurosci. 13, 3252–3265.

Thiele, A., and Stoner, G. (2003). Neuronal synchrony does not correlate with motion coherence in cortical area MT. Nature 421, 366–370. doi: 10.1038/nature01285

Traub, R. D., Whittington, M. A., Stanford, I. M., and Jefferys, J. G. (1996). A mechanism for generation of long-range synchronous fast oscillations in the cortex. Nature 383, 621–624. doi: 10.1038/383621a0

VanRullen, R., and Koch, C. (2003). Is perception discrete or continuous? Trends Cogn. Sci. 7, 207–213. doi: 10.1016/S1364-6613(03)00095-0

VanRullen, R., Reddy, L., and Koch, C. (2005). Attention-driven discrete sampling of motion perception. Proc. Natl. Acad. Sci. U S A 102, 5291–5296. doi: 10.1073/pnas.0409172102

Varela, F. J., Toro, A., John, E. R., and Schwartz, E. L. (1981). Perceptual framing and cortical alpha rhythm. Neuropsychologia 19, 675–686. doi: 10.1016/0028-3932(81)90005-1

Whittington, M. A., Traub, R. D., and Jefferys, J. G. (1995). Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. Nature 373, 612–615. doi: 10.1038/373612a0

Wikenheiser, A. M., and Redish, A. D. (2015). Hippocampal theta sequences reflect current goals. Nat. Neurosci. 18, 289–294. doi: 10.1038/nn.3909

Woodruff, A. R., McGarry, L. M., Vogels, T. P., Inan, M., Anderson, S. A., and Yuste, R. (2011). State-dependent function of neocortical chandelier cells. J. Neurosci. 31, 17872–17886. doi: 10.1523/JNEUROSCI.3894-11.2011

Yazaki-Sugiyama, Y., Kang, S., Câteau, H., Fukai, T., and Hensch, T. K. (2009). Bidirectional plasticity in fast-spiking GABA circuits by visual experience. Nature 462, 218–221. doi: 10.1038/nature08485

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., et al. (2011). Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477, 171–178. doi: 10.1038/nature10360

Yuste, R. (2015). From the neuron doctrine to neural networks. Nat. Rev. Neurosci. 16, 487–497. doi: 10.1038/nrn3962

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Artículo científico nº 3

Higher-order thalamic implication in the codification of bilateral sensory events

Carlos Castejon* and Angel Nuñez

Department of Anatomy, Histology and Neuroscience, Autónoma de Madrid University, Madrid, Spain

*For correspondence:
castejon.neuro@gmail.com

Abstract

In the rodent whisker system, it is well assumed that VPM and POm encode stimulations of the contralateral whisker pad. However, during tactile exploration usually whiskers are stimulated bilaterally. Accordingly, the integration of tactile information from the two sides of the body seems to be fundamental in the codification of these events. Here, to investigate whether POm could be able to codify these bilateral dynamics, whisker-evoked responses in this thalamic nucleus were examined by *in vivo* extracellular recordings in anesthetized rats using contralateral and ipsilateral stimuli. Strikingly, we found that POm is also able to respond to tactile stimulation of ipsilateral whiskers. Our findings reveal the implication of POm in the representation of bilateral tactile events by integrating simultaneous signals arising from both whisker pads and demonstrate the implication of the higher-order sensory thalamus in the codification of bilateral sensory events. This can have important implications in bilateral perceptual function.

Introduction

Rodents have an array of whiskers on each side of the face. In these animals, whisker information is processed by two main parallel ascending pathways towards the cortex (Diamond et al., 1992; Veinante et al., 2000; Wimmer et al. 2010; Casas-Torremocha et al. 2019). The lemniscal pathway includes the ventral posteromedial thalamic nucleus (VPM). The paralemniscal pathway includes the posteromedial thalamic complex (POm). It is well assumed that VPM and POm encode stimulations of the contralateral whisker pad. However, during tactile exploration usually whiskers are stimulated bilaterally. Accordingly, the integration of tactile information from the two sides of the body seems to be fundamental in the codification of these events in bilateral perceptual function. The somatosensory cortical implication in the processing of bilateral stimuli has been much more studied (Armstrong-James and George 1988; Shuler et al. 2001; Debowska et al. 2011). However, the implication of the thalamus in these tactile interactions remains unknown.

Although the function of VPM has been largely studied, the function of POm is still unclear. Here, to investigate whether the POm could be able to codify these bilateral dynamics, whisker-evoked responses in POm were examined by *in vivo* extracellular recordings in anesthetized rats using contralateral and ipsilateral stimuli. We found that POm is also able to respond to tactile stimulation of ipsilateral whiskers. Moreover, our results showed that sensory information from both whisker pads is integrated by the POm. The integration of contra- and ipsilateral whisker inputs has been described previously in the barrel cortex (Shuler et al. 2002). However, the implication of the thalamus in this integration had not been described before. Here, our observations show that POm mediates bilateral sensory processing and demonstrate a thalamic implication in bilateral tactile perception.

Results

1. POm responses to ipsilateral whisker stimulation

First, whisker-evoked responses in POm were examined using contralateral stimuli. In agreement with previous findings (Chiaia et al. 1991; Ahissar et al. 2000), we found robust whisker-evoked responses with very short latencies (mean response onset latency: 11.78 ± 0.71 ms, n = 85; Fig. 1C). These short latency spikes are consistent with direct ascending driving inputs from trigeminal nuclei. POm responses lasted the duration of the stimuli (Castejon et al. 2016; Fig. 1C). Then, ipsilateral stimulation was applied and unexpectedly, we found that POm was also able to respond to tactile stimulation of ipsilateral whiskers. Ipsilateral responses were less strong in magnitude than contralateral responses (-35 %; mean contralateral response magnitude: 7.93 ± 0.53 spikes/stimulus; mean ipsilateral response magnitude: 5.12 ± 0.37 spikes/stimulus; $p<0.001$, Wilcoxon matched-pairs test, n = 85; Fig. 1F) and longer in latency (ipsilateral mean response onset latency: 22.40 ± 1.3 ms; Fig.1C, D). These findings were consistent across all animals (n = 8). Importantly, we found that POm responses also lasted the duration of the ipsilateral stimulus (Fig. 1C, F).

To complement the analyses described above, and as a control, we also characterized VPM thalamic responses delivering the same ipsilateral multiwhisker activation in 6 rats. In contrast to POm, VPM did not respond to ipsilateral stimuli (Fig. 1G). Since the integration of tactile information from the two sides of the body is fundamental in bilateral perception, our results suggest a different implication of these thalamic nuclei in this function.

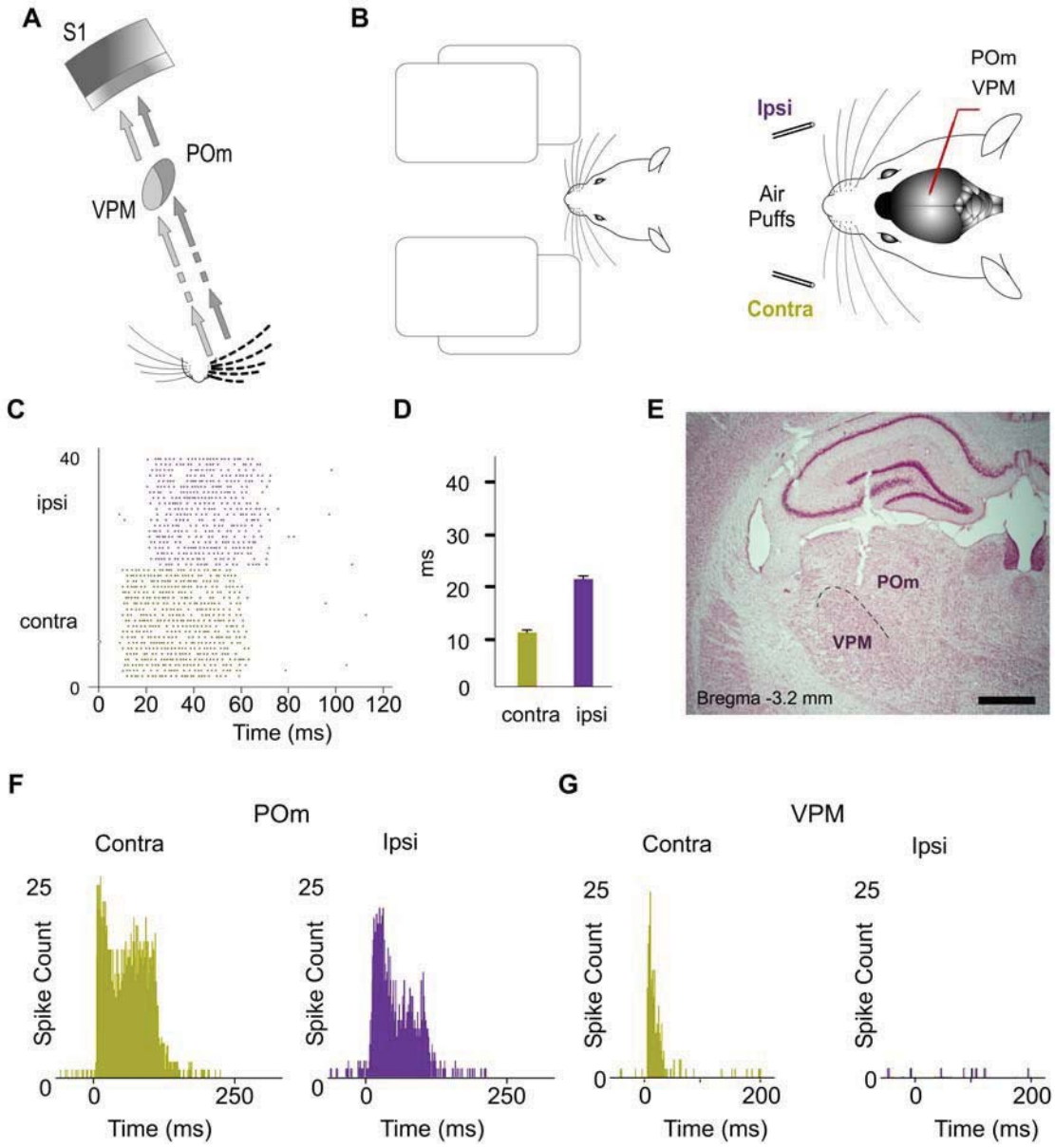


Fig. 1. POm responses to tactile stimulation of ipsilateral whiskers. (A) Schematic illustration of the lemniscal and paralemniscal pathways. (B) Usually tactile sensory events are characterized by bilateral whisker patterns. Schematic drawing displaying the sensory stimulation via patterns of contra- and ipsilateral deflections to investigate whether the thalamus could be implicated in the processing of these bilateral events. Recordings were made in the POm and VPM nuclei. (C) Raster plots showing POm responses evoked by contra- and ipsilateral stimuli (stimulus duration 50 ms; 20 trials shown for each stimulus). Note that response onset latency was longer for ipsilateral than contralateral whisker stimulation. (D) Mean response onset latencies of contra- and ipsilateral responses are shown (ipsilateral, n = 85; contralateral, n = 85). (E) Nissl stained coronal section displaying the location of the recording site in the dorsolateral part of POm and the track left by the electrode. Bregma anteroposterior level is indicated. Scale bar, 1 mm. (F) PSTHs showing POm responses evoked by contra- and ipsilateral stimulation (stimulus duration 100 ms; 30 trials shown for each stimulus). Note that although the ipsilateral response was less strong in magnitude, the response remained robust and also lasted the duration of the stimulus. (G) PSTHs showing VPM responses evoked by contra- and ipsilateral stimulation (30 trials shown for each stimulus). VPM did not respond to ipsilateral stimuli

2. POm integration of bilateral inputs

Finally, since bilateral sensory events usually occur simultaneously producing the overlapping of contra- and ipsilateral inputs, the next question to investigate was whether POm could be able to codify this bilateral interaction. It would require the precise integration of contralateral and ipsilateral sensory inputs. To examine the implication of POm in this computation, we applied bilateral sensory stimulation (Fig. 2). We measured the responses of this nucleus to simultaneous contra- and ipsilateral inputs and found that POm integrates tactile events from both sides increasing its response magnitude. Contralateral POm responses exhibited significant increases in firing rate when contra- and ipsilateral whiskers were activated concurrently (Mean response magnitude variation: 54 %, $p<0.001$, Wilcoxon matched-pairs test, n = 78; Fig. 2). These increments in POm activity seem to represent the bilateral overlapping of simultaneous signals. Similar effects were found stimulating identical (mirror) or different whiskers (nonmirror whiskers) at both sides. These findings were consistently found across animals (n = 8).

In sum, our results show that sensory information from both whisker pads is integrated by the POm (Fig. 2C) and demonstrate that POm is implicated in the codification of bilateral tactile events.

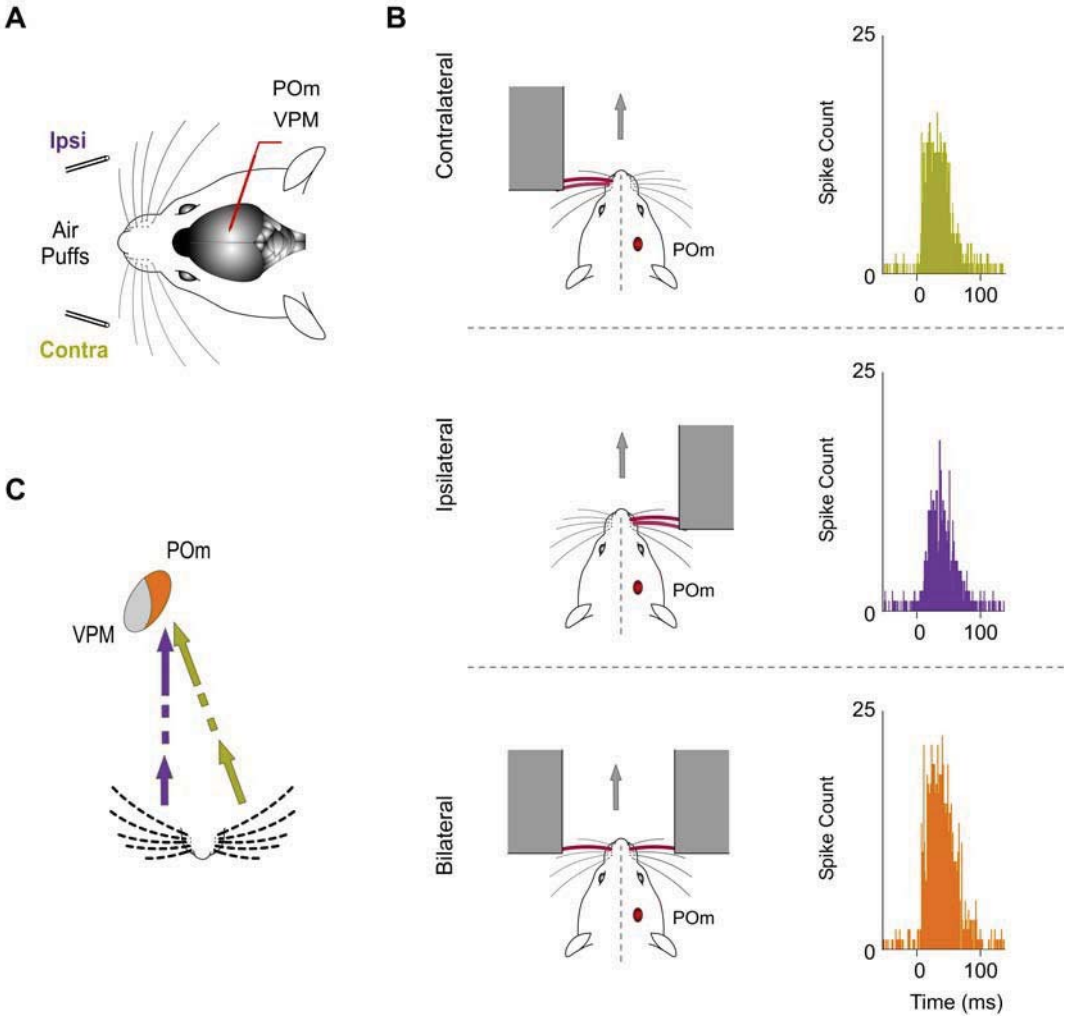


Fig. 2. POm integration of bilateral events. (A) Bilateral tactile events were applied via patterns of contra- and ipsilateral simultaneous deflections to investigate whether POm could be implicated in the integration of these signals. (B) PSTHs of representative POm responses evoked by contra-, ipsi- and bilateral whisker stimulations (stimulus duration 40 ms; 30 trials shown for each stimulus) and their corresponding schematic illustrations of their simulated possible real occurrence during the exploration of different objects and apertures in natural conditions are shown. Note the significant increase in firing rate when contra- and ipsilateral whiskers were activated concurrently. (C) These findings indicate that sensory information from both whisker pads is integrated by the POm.

Discussion

POm mediates bilateral sensory processing

Although it is well described that POm encodes stimulations of the contralateral whisker pad, here we found that POm is also able to respond to tactile stimulation of ipsilateral whiskers. Since the processing and integration of tactile information from the two sides of the body is central in perceptual function (for example, perceptual tasks requiring bilateral integration), our results show that POm has a fundamental role in bilateral tactile perception.

The difference between ipsi- and contralateral response onset latencies (10 ms, Fig. 1C, D) suggests that contra- and ipsilateral sensory activities are mediated by different pathways. How ipsilateral information reaches this nucleus remains a question to investigate.

POm integration of bilateral inputs

Bilateral integration is needed when rodents are exploring in tunnels, discriminating holes and apertures or detecting their width and shape. Our findings revealed the implication of POm in the representation of bilateral tactile patterns by integrating overlapping information arising from both whisker pads.

The implication of the somatosensory cortex in the processing of bilateral stimuli has been much more studied (Armstrong-James and George 1988; Shuler et al. 2001; Debowska et al. 2011). Moreover, the integration of contra- and ipsilateral whisker inputs has been described previously in the barrel cortex (Shuler et al. 2002). However, the implication of the thalamus in this integration had not been described before. Here, our findings demonstrate the functional implication of the higher-order sensory thalamus in the computation of tactile interactions between body sides.

Differences between VPM and POm

We also characterized VPM thalamic responses delivering the same ipsilateral activation in 6 rats. In contrast to POm, VPM did not respond to ipsilateral stimuli (Fig. 1G). Since the integration of tactile information from the two sides of the body is fundamental in bilateral perception, our results suggest a different implication of these thalamic nuclei in this function.

POm responses to ipsilateral stimulation are highly sensitive to anaesthesia

It has been reported that during wakefulness POm is strongly more active than during anesthetized state (Masri et al. 2008; Sobolewski et al. 2015). Moreover, under light sedation, POm activity is significantly higher than during general anesthesia (Zhang and Bruno 2019). This evidence indicates that POm is highly sensitive to anaesthesia.

In our experiments, we found that POm responses to ipsilateral stimulation were highly affected by the level of anaesthesia (data not shown). Increasing this level by supplementary doses of urethane abolished POm responses to ipsilateral whisker stimulation. Therefore, high levels of sedation impair the real dynamics of POm functioning. This can explain why these thalamic responses to ipsilateral stimulation had not been described before.

Materials and Methods

Ethical Approval

All experimental procedures were carried out under protocols approved by the ethics committee of the Autónoma de Madrid University and the competent Spanish Government agency (PROEX175/16), in accordance with the European Community Council Directive 2010/63/UE.

Animal procedures, anesthesia and electrophysiology

Experiments were performed on both sexes (5 males and 9 females) adult Sprague Dawley rats (220-300 g). Animals were anesthetized (urethane, 1.3 – 1.5 g/kg i.p.) and placed in a Kopf stereotaxic frame. Local anaesthetic (Lidocaine 1%) was applied to all skin incisions. The skull was exposed and then openings were made to allow electrode penetrations in the thalamus.

Extracellular recordings were made in the posteromedial thalamic complex (POm; P 2.5-4.5, L 2-2.5, D 5-6.5) and in the ventral posteromedial thalamic nucleus (VPM; P 2.8-4.6, L 2-3.5, D 5.5-7; Paxinos and Watson 2007). Tungsten microelectrodes (2–5 MΩ) were driven using an electronically controlled microdrive system. Importantly, recordings of neuronal activity in these brain structures were performed several hours after the application of urethane (typically 4 - 5 h) to obtain a lower level of sedation but in the absence of whisker movements and pinch withdrawal reflexes. The body temperature was maintained at 37°C with a thermostatically controlled heating pad.

Sensory stimulation

Sensory stimulation was characterized by contra-, ipsi- and bilateral multiwhisker deflections using a pneumatic pressure pump (Picospritzer) that delivers air pulses through polyethylene tubes (1 mm inner diameter; 1-2 kg/cm^2). We applied 20-70 trials per stimulus condition at low frequency (0.5 Hz).

Histology

After the last recording session, animals were deeply anesthetized with sodium-pentobarbital (50 mg/kg i.p.) and then perfused transcardially with saline followed by formaldehyde solution (4%). After perfusion, brains were removed and postfixed. Serial 50 µm-thick coronal sections were cut on a freezing microtome (Leica, Germany). These sections were then prepared for Nissl staining histochemistry for discrimination of thalamic nuclei.

Data acquisition and analysis

Data were recorded extracellularly from VPM and POm. Raw signal of these extracellular *in vivo* recordings was filtered (0.3–5 kHz band pass), amplified via an AC preamplifier (DAM80; World Precision Instruments, Sarasota, USA) and digitized at 10 kHz. From each recording, we extracted the activity of several clusters of multi-units that were collected by amplitude sorting with the aid of commercial software Spike2 (Cambridge Electronic Design, Cambridge, UK).

We defined response magnitude as the total number of spikes per stimulus occurring between response onset and offset from the peristimulus time histogram (PSTH, bin width 1 ms). Response onset was defined as the first of three consecutive bins displaying significant activity (three times higher than the mean spontaneous activity) after stimulus and response offset as the last bin of the last three consecutive bins displaying significant activity. Response duration was defined as the time elapsed from the onset to offset responses. In all figures, raster plots represent each spike as a dot and each line corresponds to one trial. Spikes were aligned on stimulus presentation (Time 0 ms).

Statistical analysis was performed using GraphPad Prism software (California USA). All data are expressed as the mean ± standard error of the mean (SEM). Error bars in the figures correspond to SEM.

Additional information

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Carlos Castejon designed and conducted the experiments, analyzed the results and wrote the paper.
Angel Nuñez designed and conducted the experiments, analyzed the results, reviewed and edited the paper.

Funding

This work was supported by a Grant from Spain's Ministerio de Economia y Competitividad (SAF2016-76462 AEI/FEDER).

Competing interests

The authors declare that no competing interests exist.

References

Ahissar E, Sosnik R, Haidarliu S (2000) Transformation from temporal to rate coding in a somatosensory thalamocortical pathway. Nature 406:302–306.

Armstrong-James M, George MJ (1988) Bilateral receptive fields of cells in rat Sm1 cortex. Exp Brain Res 70:155-165.

Castejon C, Barros-Zulaica N, Nuñez A (2016) Control of somatosensory cortical processing by thalamic posterior medial nucleus: a new role of thalamus in cortical function. PLoS One 11:e0148169.

Chiaia NL, Rhoades RW, Bennett-Clarke CA, Fish SE, Killackey HP. (1991) Thalamic processing of vibrissal information in the rat. I. Afferent input to the medial ventral posterior and posterior nuclei. J Comp Neurol. 314:201–216.

Casas-Torremocha, D. et al. (2019) Posterior thalamic nucleus axon terminals have different structure and functional impact in the motor and somatosensory vibrissal cortices. Brain Struct. Funct. 224,1627-1645.

Debowska, W., Liguz-Lecznar, M. & Kossut, M. (2011) Bilateral plasticity of Vibrissae SII representation induced by classical conditioning in mice. J. Neurosci., 31, 5447–5453.

Diamond ME, Armstrong-James M, Ebner FF (1992) Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus. J Comp Neurol 318:462– 476.

Masri, R., Bezdudnaya, T., Trageser, J.C., and Keller, A. (2008). Encoding of stimulus frequency and sensor motion in the posterior medial thalamic nucleus. J. Neurophysiol. 100, 681–689.

Paxinos G, Watson C. (2007) The rat brain in stereotaxic coordinates. San Diego: Academic Press.

Shuler MG, Krupa DJ, Nicolelis MAL (2001) Bilateral integration of whisker information in the primary somatosensory cortex of rats. J Neurosci 21:5251–5261

Shuler MG, Krupa DJ, Nicolelis MAL (2002) Integration of bilateral whisker stimuli in rats: role of the whisker barrel cortices. Cereb Cortex 12:86–97

Sobolewski A, Kublik E, Swiejkowski DA, Kaminski J, Wrobel A. (2015) Alertness opens the effective flow of sensory information through rat thalamic posterior nucleus. Eur J Neurosci. 41:1321–1331.

Veinante P, Jacquin MF, Deschenes M. (2000) Thalamic projections from the whisker-sensitive regions of the spinal trigeminal complex in the rat. J Comp Neurol. 420: 233–43.

Wimmer VC, Bruno RM, de Kock CP, Kuner T, Sakmann B (2010) Dimensions of a projection column and architecture of VPM and POm axons in rat vibrissal cortex. Cereb Cortex 20:2265–2276.

Zhang, W., and Bruno, R. M. (2019). High-order thalamic inputs to primary somatosensory cortex are stronger and longer lasting than cortical inputs. Elife 8:e44158.

Artículo científico nº 4

Thalamic codification of complex sensory patterns and its possible role in cognition

Carlos Castejon1*, Jesus Martin-Cortecero1,2 and Angel Nuñez1

1 Department of Anatomy, Histology and Neuroscience, Autónoma de Madrid University, Madrid, Spain

2 Institute of Physiology and Pathophysiology, Medical Biophysics, Heidelberg University, 69120 Heidelberg, Germany

*For correspondence:
castejon.neuro@gmail.com

Abstract

The function of the higher-order sensory thalamus remains unresolved. Here, POm nucleus was examined by in vivo extracellular recordings across a range of complex sensory patterns. We found that POm was highly sensitive to multiwhisker stimuli involving complex spatiotemporal interactions. The dynamical spatiotemporal structure of sensory patterns and the different complexity of their parts was accurately reflected in precise POm activity changes. Importantly, POm was also able to respond to ipsilateral stimulation and was implicated in the representation of bilateral tactile events by integrating simultaneous signals arising from both whisker pads. We found that POm nuclei are mutually connected through the cortex forming a functional POm-POm loop. We unravelled the nature and content of the messages travelling through this loop showing that they were 'structured patterns of sustained activity'. These structured messages were transmitted preserving their integrated structure. The implication of different cortical areas was investigated revealing that S1 plays a protagonist role in this functional loop. Our results also demonstrated different laminar implication in the processing of sustained activity in this cortical area and its transmission between hemispheres. We propose a theoretical model in which these 'structured patterns of sustained activity' generated by POm may play important roles in perceptual, motor and cognitive functions. From a functional perspective, this proposal, supported by the results described here, provides a novel theoretical framework to understand the implication of the thalamus in cognition. In addition, a profound difference was found between VPM and POm functioning. The hypothesis of Complementary Components is proposed here to explain it.

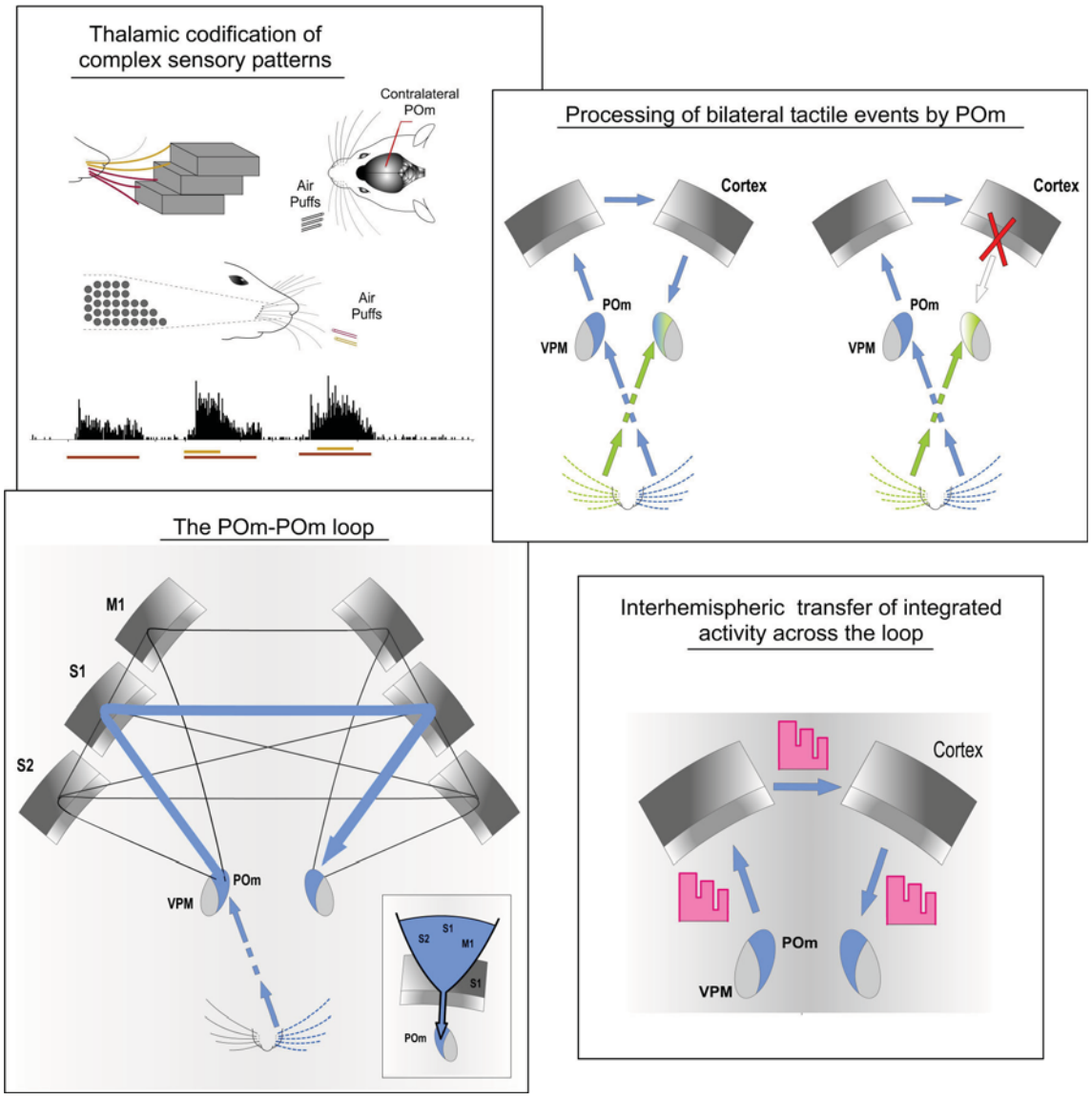
Highlights

POm is implicated in the representation of complex sensory patterns.

POm is implicated in the codification of bilateral tactile events.

POm nuclei are mutually connected through the cortex forming a functional POm-POm loop.

'Structured patterns of sustained activity' travelling through the loop



Introduction

Traditionally, sensory systems have mostly been studied using simple and discrete stimuli. However, in natural conditions, sensory events usually have complex and dynamical spatiotemporal structures and normally multiple sensory signals occur simultaneously with different onsets, offsets and overlappings, challenging the computational capacities of sensory systems. However, it is still unclear how sensory systems generate a representation of these dynamics. Moreover, how sensory systems extract relevant patterns from the raw sensory flow is poorly understood. Here, we propose the hypothesis that higher-order sensory thalamus has a protagonist role in that function.

The rodent whisker system has an extraordinary ability to extract patterns and regularities from the environment and provides a perfect model in which to test our proposal. Rodents have an array of whiskers on each side of the face and during tactile exploration, multiple whiskers are stimulated simultaneously. Accordingly, the activation of individual whiskers strongly overlaps. These multiple contacts with the whiskers generate complex patterns of sensory information. How the somatosensory system transforms these merged raw sensory signals into reliable neural representations and extracts information from that apparent noise is still unclear.

In these animals, tactile information from whiskers is processed by two main parallel ascending pathways towards the cortex (Diamond et al., 1992; Veinante et al., 2000a; Ahissar et al. 2000). The lemniscal pathway includes the principal trigeminal nucleus (Pr5) and the ventral posteromedial thalamic nucleus (VPM). The paralemniscal pathway includes the spinal trigeminal subnucleus interpolaris (Sp5i) and the posteromedial thalamic nucleus (POm). Although the function of VPM has been broadly studied, little is known about the function of POm. POm is powerfully driven by simultaneous activation of multiple whiskers. However, the functional implication of this characteristic remains unknown. Moreover, the content of POm representations and the nature of the messages that POm transfers to and receives from the cortex remain unclear.

In addition, sensory events are usually characterized by complex bilateral sensory patterns. Therefore, the integration of tactile information from the two sides of the body seems to be fundamental in the codification of sensory patterns in bilateral perceptual function. Although, somatosensory cortical implication in the processing of bilateral stimuli has been much more studied, the implication of the thalamus in these tactile interactions remains unknown.

The following experiments were thought to study the implication of POm in the codification of these complex phenomena.

Results

1. Sustained activity in POm

Whisker-evoked responses in POm were examined by in vivo extracellular recordings using stimuli with different durations within the range used by these animals during their natural explorations (Fig. 1 and 2). While studying multi-unit activity (n = 119), we found that this nucleus was able to generate sustained responses even to long-duration stimuli. This capacity was consistent across all animals (n = 12). The duration of the stimulus did not alter the mean onset latency of responses ($p = 0.52$, One-way ANOVA; Fig. 2B).

To analyse how this multi-unit activity replicated the response profile of individual POm neurons, we also characterized this phenomenon studying POm single-units (n = 91). We found that POm neurons responded homogeneously tending to generate sustained responses. This indicated that the population response formed by the combination of spikes from these neurons allowed for the codification of stimuli duration by POm sustained activity (Fig. 2C). Thus, we mainly used POm multi-unit responses for further analyses.

Confirming previous findings (Castejon et al. 2016), these results show that POm has the capacity to sustain its activity to encode and represent tactile input duration with high accuracy. Given that POm can generate sustained responses greatly outlasting the duration of multiple whisk cycles, this capacity can be used to codify

sensory patterns and sequences of stimuli. The following experiments were designed to study the implication of POm in the codification of these phenomena.

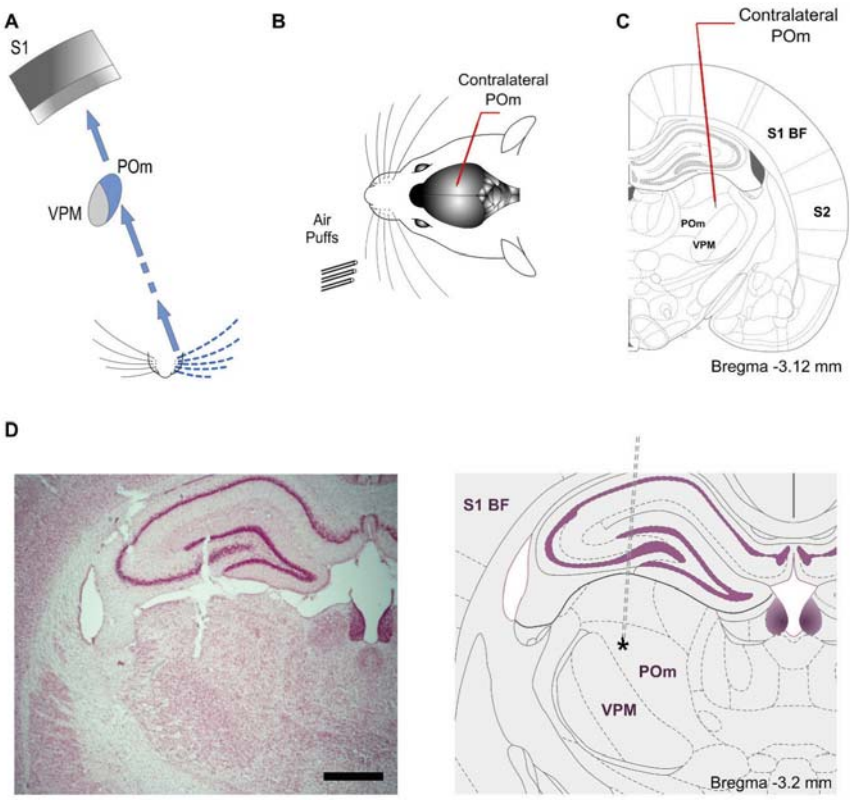


Fig. 1. Experimental paradigm. (A) Illustration of the paralemniscal pathway. (B) Schematic drawing displaying the sensory stimulation via patterns of multiwhisker deflections. Recordings were made in the contralateral POm (C). Coronal section illustrating a recording electrode inserted into POm. Bregma anteroposterior level is indicated. (D) The left panel shows a representative Nissl stained coronal section displaying the location of the recording site in the dorsolateral part of POm and the track left by the electrode. An atlas schematic reconstruction of this recording site within POm is shown in the right panel (Paxinos and Watson 2007). Tip position is indicated by an asterisk. Scale bar, 1 mm. S1 BF, primary somatosensory cortex barrel field. S2, secondary somatosensory cortex.

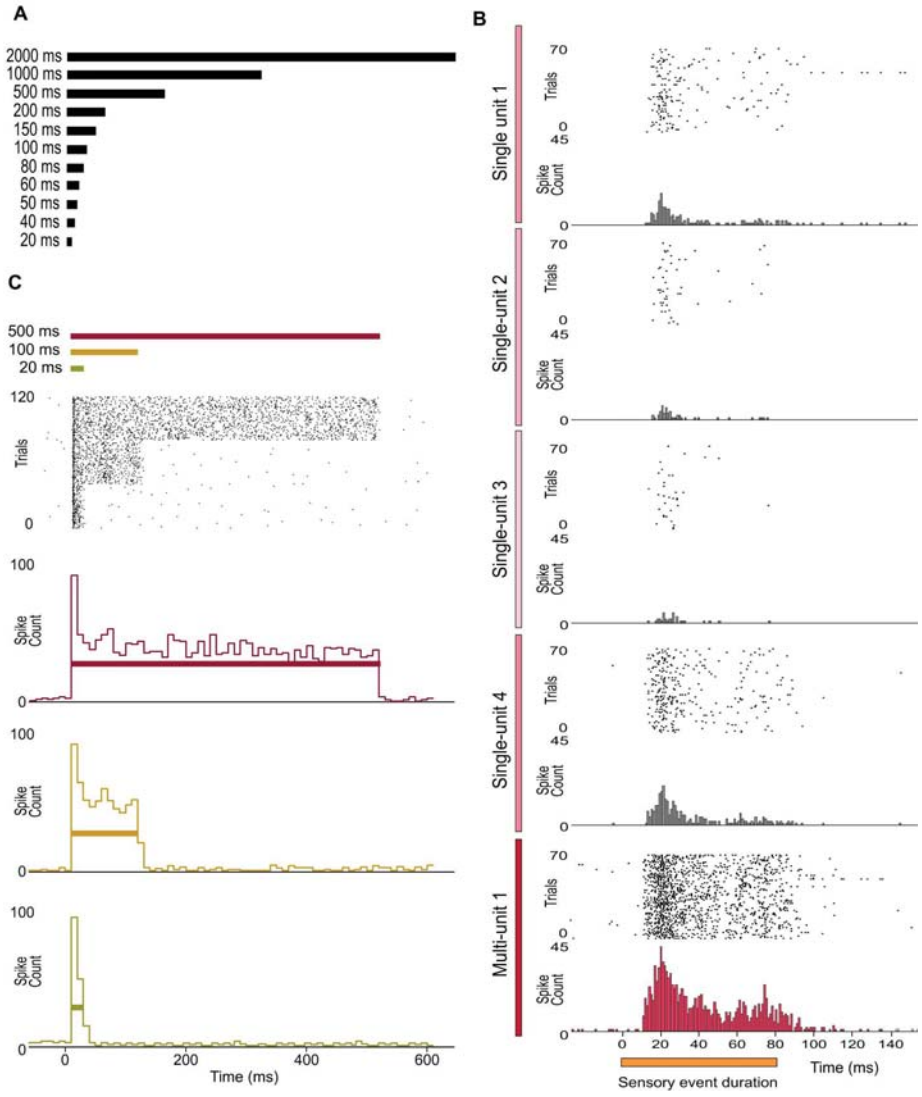


Fig. 2. Sustained activity in POm. (A) Air-puffs used for sensory stimulation varied in duration from 20 ms to 2 seconds. (B) The codification of the duration of sensory events by POm was studied at single-unit and multi-unit level. Raster plots and PSTHs showing one multi-unit and four single-units POm responses extracted from the same recording and evoked by a sensory pattern of 80 ms duration. Note that the robustness of this form of codification by POm sustained activity is obtained from population response formed by the superposition of spikes from individual neurons. The gradual color intensity of vertical lines represents a simulated contribution of each single-unit in this example to the codification of stimulus duration. (C) POm responds throughout the entire duration of the stimuli. Raster plot and peristimulus time histograms (PSTHs; bin width 10 ms) showing sustained multi-unit POm responses evoked by different stimulus duration (40 trials shown for each stimulus). Color lines indicate the duration of the stimulus. Time 0 indicates the onset of the stimulus. Note that the duration of the stimulus did not alter the onset latency of responses.

2. POm codification of spatiotemporal sensory patterns

During whisking rats integrate signals from many whiskers to obtain accurate tactile information from their environment. Our next experiments were designed to map how complex sensory information, produced when multiple whiskers are activated simultaneously during a tactile event, is encoded in the activity of POm.

2.1. POm spatial integration of multiwhisker stimulation

First, we characterized the response properties of POm to simple spatial overlapping stimuli precisely delivering simultaneous air-puffs to different whiskers at different locations across the whisker pad. Consistent with published data (Diamond et al. 1992; Ahissar et al. 2000), we found large multiwhisker receptive fields (mean receptive field size: 10.9 ± 3.1 whiskers; n = 42 units). We observed that POm responses exhibited sustained increases in firing rate when different whiskers were activated simultaneously. To investigate this POm capacity in detail, we studied the integration of signals by the POm across a range of reproducible spatial overlapping combinations (Fig. 3A). Across them, we found that POm multi-unit responses showed sustained increases, as reflected in response magnitude, during overlappings of spatial signals (quantified in Fig. 3C). The spatial integration was also observed between remote whiskers. The mean onset latency and duration of POm responses to different signals were not changed by their overlapping (Fig 3C). We also investigated these effects studying POm single-units (quantified in Fig. 3B). Their responses showed similar dynamics to multi-unit responses. These findings were consistent across all animals (n = 15). Importantly, the robustness of this capacity of codification seems to be obtained from population response (Fig. 3C). Accordingly, we used POm multi-unit responses for further analyses of POm integration.

Together, these results showed that POm was activated more strongly by complex stimuli than by simple ones. This was produced by a facilitative integration of overlapping spatial signals by POm.

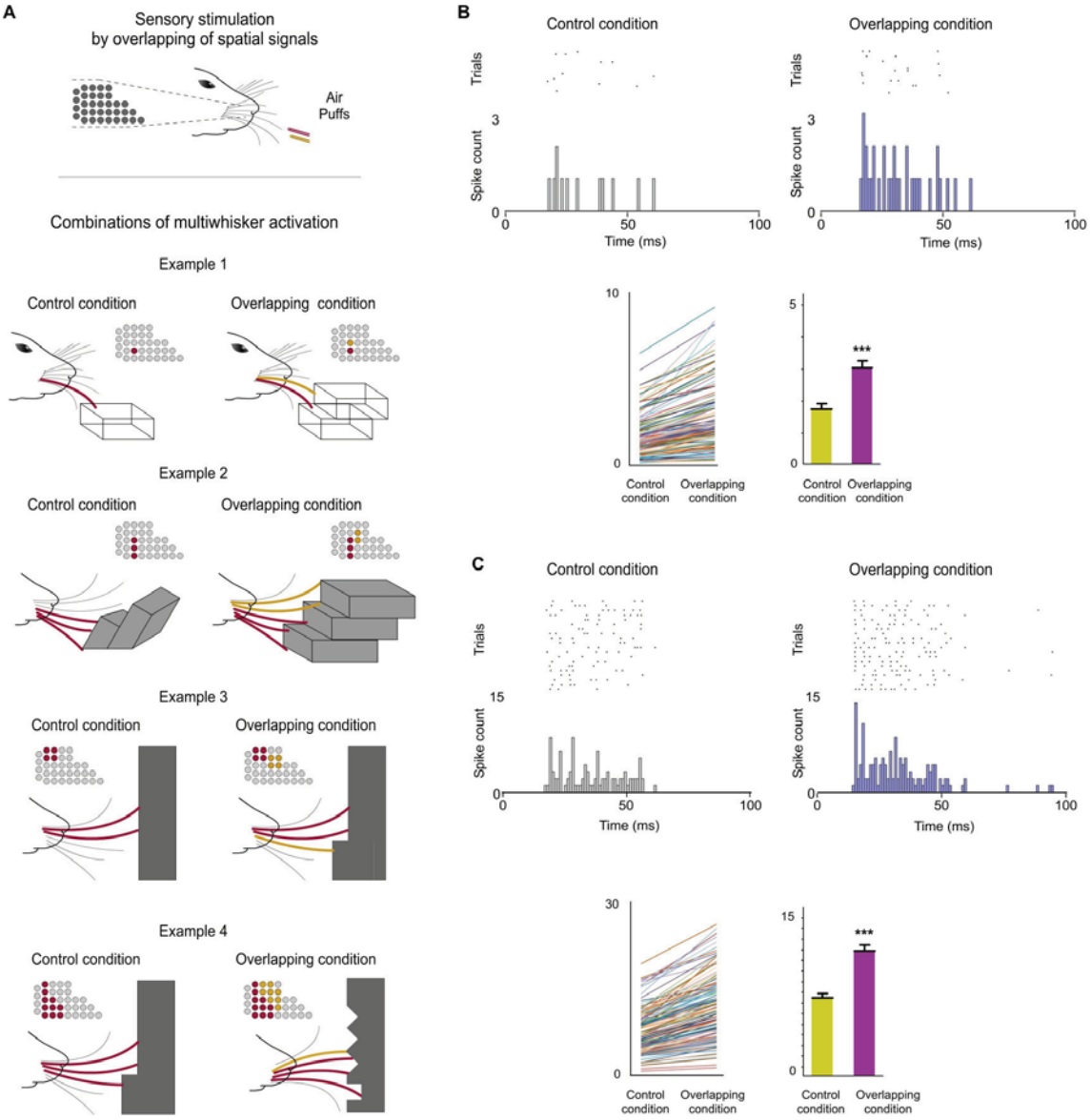


Fig. 3. POm integration of spatial signals. (A) Sensory stimulation was produced by the application of individual (Control Condition) or simulataneous air puffs (Overlapping Condition) activating different whiskers and producing diverse combinations of multiwhisker activation. To simulate natural stimuli, overlappings produced by the activation of whiskers in different directions were also included. The number of whiskers activated by each air puff was varied to generate different combinations. They were repeated multiple times (range 20–70 repeats) and POm responses to them were studied and quantified by comparison between Control and Overlapping conditions. Four examples of these combinations, the whiskers activated (depicted in different colors in the schematic representations of the whisker pads) and their corresponding illustrations of their simulated possible real occurrence during the exploration of different objects, surfaces and textures in natural conditions are shown. (B) POm response magnitude increased when whiskers were activated simultaneously. This facilitative integration during overlappings of spatial signals showed that POm was activated more strongly by complex patterns than by simple ones as can be appreciated in the peri-stimulus time raster plots and histograms of a representative POm single-unit response for 20 trials evoked by the example 2 in A. Note the significant increase in the spike count during Overlapping condition. Data comparing the spike rate in Control and Overlapping conditions of single-units (n = 102, depicted in different colors) across stimulation combinations and the total mean response magnitude in both conditions are also shown. The mean firing rate of all single-units was significantly increased in the Overlapiing condition (72 %; $p < 0.001$; Wilcoxon matched-pairs test). (C) Same as in B, but for multi-unit activity. Raster plots and PSTHs of a demonstrative POm multi-unit response for 40 trials evoked by the example 3 in A. The spike rate in Control and Overlapping conditions of multi-units (n = 136, depicted in different colors) across stimulation combinations and the total mean response magnitude in

both conditions are shown. The mean firing rate of all multi-units was significantly increased in the Overlapiing condition (56 %; $p < 0.001$; Wilcoxon matched-pairs test) Note that response duration was not altered by the overlapping.

2.2. POm integration of spatiotemporal overlapping dynamics

Next, since tactile events typically have spatiotemporal structures that change dynamically in time, we studied how spatial integration (spatial dimension) occurs throughout the duration of the sensory pattern (temporal dimension) in 18 rats (Fig. 4). Interestingly, when overlappings of spatial signals were produced by delivering simultaneous air-puffs with different durations or with the same duration but applied at different times (Fig. 4A middle panel), increases in sustained responses were only observed during the overlapping time between them (data showing the quantification of this effect are described in Fig. 4D). The spatial integration of multiwhisker activation was only produced during the temporal overlapping. Importantly, the increases of POm activity were sustained along the temporal overlappings (Fig.4C). Therefore, the time shared by overlapping signals is also encoded by POm activity.

Then, we studied these effects using sensory patterns formed by diverse temporal overlappings (Fig. 4A bottom panel). We designed these patterns to simulate possible real complex stimuli or sequences of stimuli similar to those occurring in natural circumstances, such as sequential activation of multiple whiskers with partial temporal overlapping between them, simultaneous and delayed activation of different whiskers with different durations producing sequences with diverse spatiotemporal overlappings and precise sequences of long sustained activation of several neighbouring whiskers overlapped with repetitive brief stimulations of remote whiskers. Across patterns, precise changes in POm sustained activity consistent with the spatiotemporal structure of the patterns were observed. Accurate increases in POm sustained activity were produced during the overlapping time between spatial signals across the pattern. These changes in POm activity reflected the spatiotemporal structure of the sensory pattern.

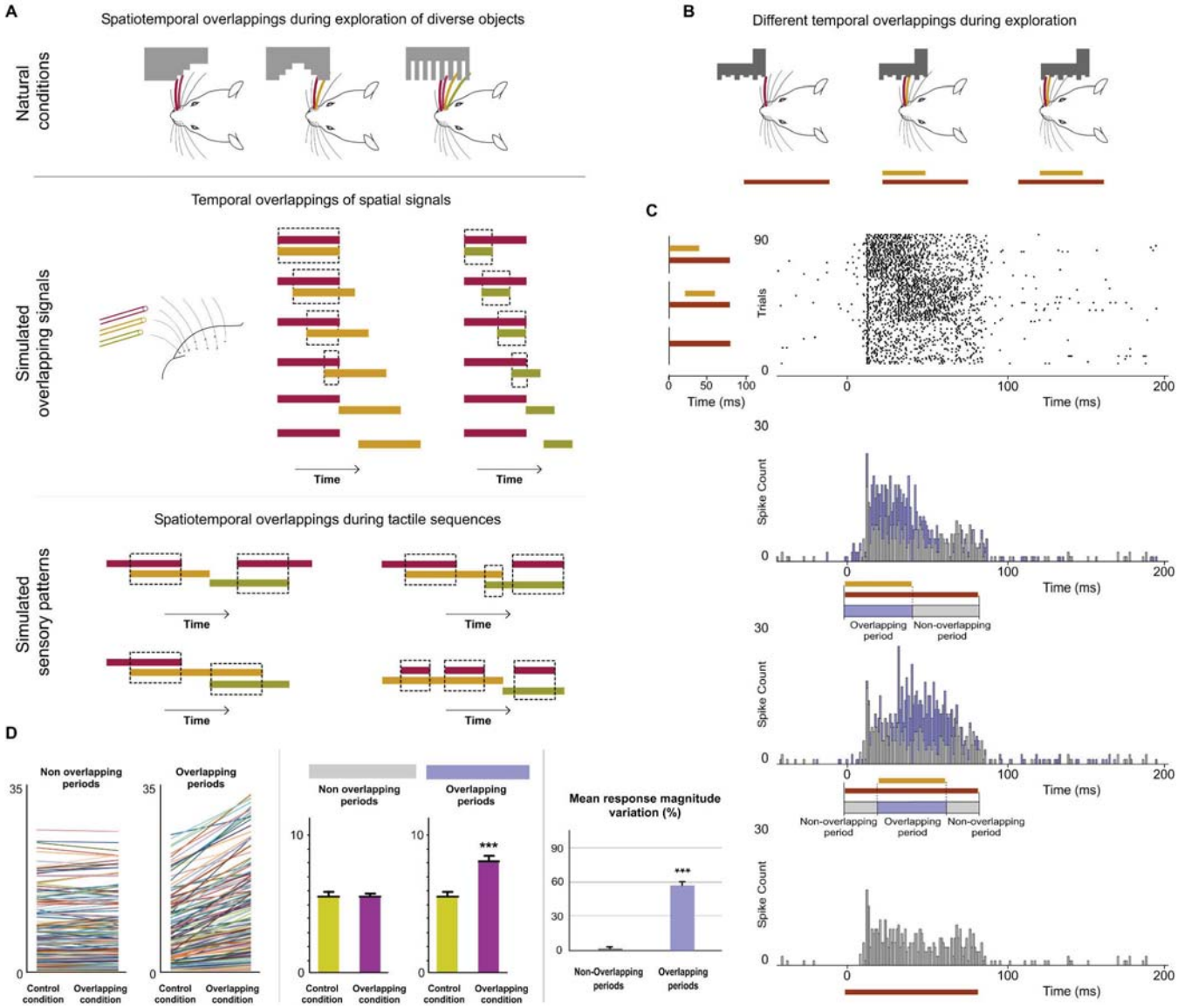


Fig. 4. POm integration of spatiotemporal overlapping dynamics. (A) In natural conditions, diverse shapes and textures generate different sequences of multiwhisker activations and different dynamical overlappings between them during their exploration. Natural sensory patterns can be simulated by simultaneous activation of different whiskers and by varying the number of whiskers activated, their combination, order and duration of air pulses to produce different spatiotemporal overlappings. A variety of spatiotemporal patterns were designed to simulate possible real complex stimuli or sequences of stimuli using controlled multiwhisker deflections performed by overlapping air puffs of different durations applied to different whiskers including neighbouring whiskers or whiskers farther apart across the whisker pad. The duration of air puffs and the temporal overlapping between them were varied to generate different combinations (protocols). The number of whiskers activated by each air puff was varied to generate different sensory patterns using the same protocol. Short duration (20 ms) stimuli and short temporal overlappings between whiskers were also included to study the precision of POm in codifying sensory patterns. Some examples are shown. Air puffs are represented by color lines. Their length reflects the duration of the signal. Temporal overlappings between sensory signals are highlighted. (B) Schematic illustration of a simulated simple tactile sequence during the exploration of an object reflecting the generation of different temporal overlappings. During the sequence, different sensory signals occur simultaneously in diverse moments generating different temporal overlappings between them. (C) Raster plots and PSTHs of representative POm responses evoked by temporal overlappings illustrated in the tactile sequence in B are shown. The appearance of a new signal during the presence of an existing signal was integrated by POm. This was reflected in precise increases in POm activity. These increases in sustained responses were only produced during the overlapping time between them (Overlapping period) but not during the non-overlapping time (Non-overlapping period of response). Color lines indicate the duration of the stimuli. The duration of the Overlapping and Non-overlapping periods is also indicated. Note that the increases of POm activity during overlapping

periods were sustained along the temporal overlapping. (D) Plots comparing the spike rate of all recorded units (n = 155, depicted in different colors) during the Overlapping and Non-overlapping response periods in Control and Overlapping conditions across sensory patterns. The mean firing rate was increased in the Overlapping periods ($p<0.001$; Wilcoxon matched-pairs test) but not in the Non-overlapping periods where the mean magnitude of responses did not change ($p = 0.43$; Wilcoxon matched-pairs test). Response magnitude variation (%) between Control and Overlapping conditions in Non-Overlapping and Overlapping periods ($p < 0.001$, Wilcoxon matched-pairs test) is also shown.

Additionally, since in natural conditions the spatiotemporal structure of sensory patterns changes dynamically across the pattern, their complexity is not homogeneous along their duration. As can be appreciated in Fig. 5A, dissimilar overlappings of spatial signals can produce sensory patterns formed by different parts with diverse complexities. To investigate the capacity of POm to represent the complexity of these different parts and to obtain a better quantification of this form of codification, more complex sensory patterns were generated by overlapping additional spatial signals (Fig. 5B). In these stimulation protocols, we selected the most complex parts ('Core') of overlapping periods and divided the POm response to these parts into three subperiods: Pre, Core and Post. Precise POm activity changes were found during these different times of POm response reflecting the diverse complexity between parts along the overlapping (Fig. 5). Across patterns, POm activity was significantly increased in the Core subperiod compared to Pre and Post subperiods when extra signals were temporally overlapped in the Core subperiod of the patterns (described and quantified in Fig. 5C). This demonstrated that when additional signals were overlapped increasing the complexity of the pattern, POm codified this complexity by increasing its activity during the temporal presence of these signals. We found that POm response magnitude in all overlapping periods gradually increased as more inputs were temporally overlapped in the patterns (Fig. 5D, E). Accordingly, increasing the complexity of the pattern by increasing the number of whiskers temporally overlapped, produced an enhancement of POm response magnitude. Therefore, the complexity of spatiotemporal overlappings, represented by the number of whiskers implicated, was reflected in POm activity changes.

Together, these results showed that the dynamical spatiotemporal structure of sensory patterns and the different complexity of their parts was accurately reflected in precise POm activity fluctuations. This indicates that POm uses these effects to encode sensory patterns. Importantly, we observed that POm generated very similar patterns of integrated activity when different whiskers were activated by the same stimulation protocol. This finding is in agreement with the less accurate somatotopy of the nucleus and suggests that the function of POm integration is not the combined representation of specific whiskers but the encoding and extraction of generic sensory patterns from the entire vibrissal array.

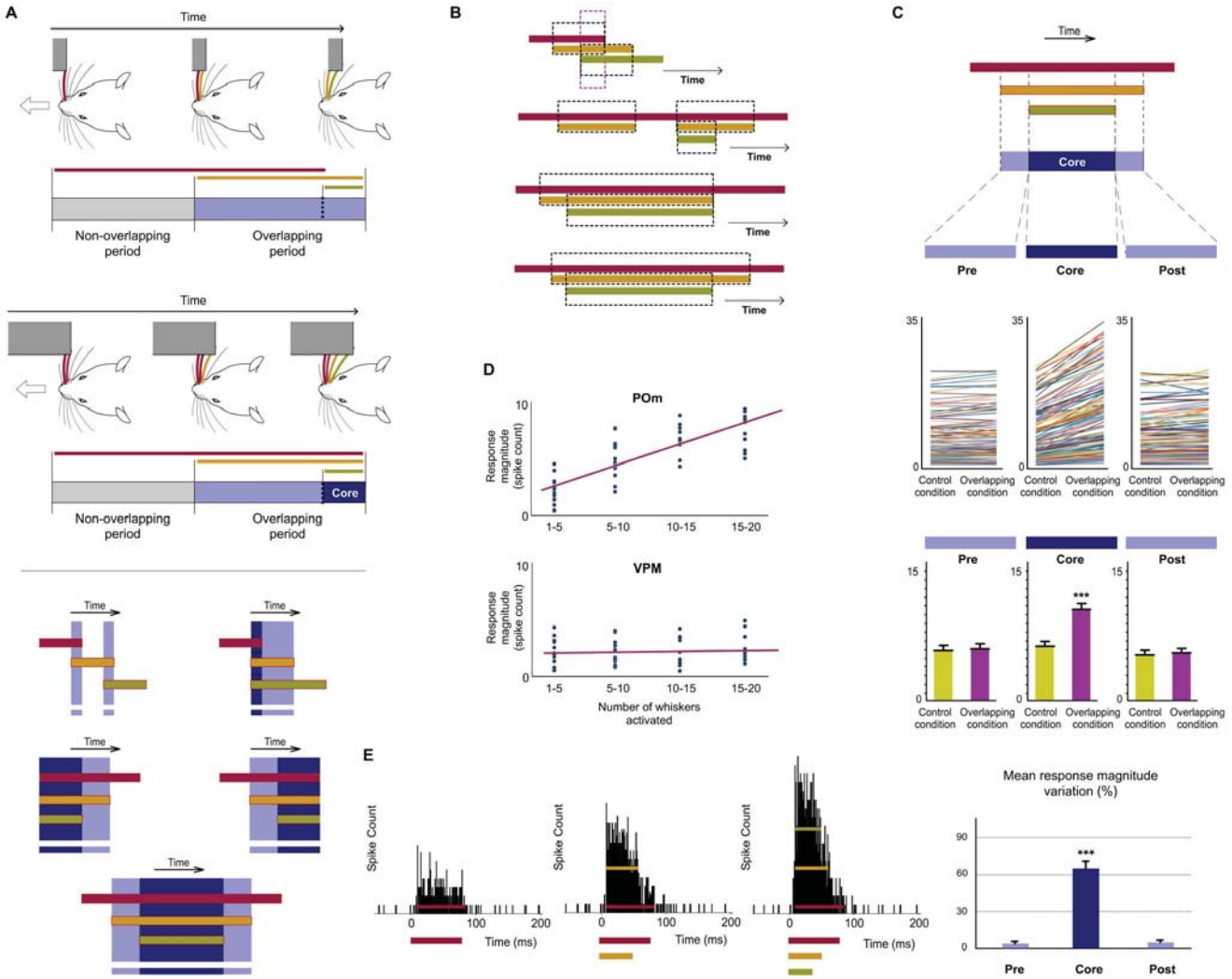


Fig. 5. POm is highly sensitive to the dynamical spatiotemporal structure of sensory patterns and to the different complexity of their parts. (A) Multiple and constantly changing overlappings occur dynamically during natural contact with objects and surfaces during active exploration. This generates sensory patterns formed by overlappings with diverse complexities. The complexity of these overlappings determines the complexity of the patterns. As can be appreciated in these schematic illustrations of two simulated tactile sequences during the exploration of two different objects, the complexity of their corresponding Overlapping periods is different. Simple overlappings (in light blue) and more complex overlappings (in dark blue) were used to understand how POm codifies this complexity (bottom panel). The most complex parts of Overlapping periods were selected and defined as 'Core' parts. (B) Spatiotemporal patterns formed by more complex overlapping periods were produced by the simultaneous application of a third air puff activating additional whiskers. Different complex spatiotemporal overlappings were generated by varying the onset and duration of the third air puff and the number of extra whiskers activated by this air puff. (C) POm responses during Overlapping periods were divided in three subperiods (Pre, Core and Post). They were compared before (Control condition) and after the application of the third air puff (Overlapping condition). Data comparing the spike rate of all recorded units (n = 101, depicted in different colors) in these conditions during Pre, Core and Post subperiods across sensory patterns and the total mean response magnitude in both conditions in these subperiods are described. Mean response magnitude variation (%) in the three subperiods is also shown. POm response magnitude was significantly increased in the Core subperiod compared to Pre and Post subperiods when extra signals were temporally overlapped in the Core subperiod of the patterns ($p < 0.001$, One-way ANOVA). (D) Correlation between POm response magnitude and the number of whiskers simultaneously overlapped. Data from VPM are also shown for comparison. POm: Pearson correlation coefficient, $r = 0.79$, $p < 0.001$, $n = 51$ in 10 rats; VPM: $r = 0.19$, $p < 0.001$, $n = 45$ in 8 rats. Note the profound functional difference between these nuclei. (E) PSTHs for a representative example showing that POm response magnitude in Overlapping periods gradually increased as more whiskers were temporally overlapped. This shows that increasing the complexity of overlappings by increasing the number of whiskers temporally overlapped produced

an enhancement of POm response magnitude. Note that the temporal structure of these different sensory events is reflected in their corresponding POm responses.

2.3. No sustained activity in VPM. Stimuli overlapping did not alter whisker responses in VPM

To complement the analyses described above, whisker-evoked responses in VPM were examined using stimuli with different durations (Fig. 6). Consistent with published data (Diamond et al., 1992; Simons 1995) VPM responses showed high spatial resolution (mean receptive field size: 2.3 ± 0.7 whiskers; n = 44). In contrast to POm responses and in agreement with previous findings (Castejon et al. 2016), VPM responses did not show sustained response patterns. Therefore, response modes differed drastically between these nuclei. POm was persistently activated during whisker stimulation, whereas VPM was only transiently activated at the onset of stimuli (Fig. 7A). Long stimuli usually evoked an onset response at the beginning of the stimulus and an offset response at the end but we did not find sustained responses during stimulus presence in VPM.

Since VPM responses were transient lasting tens of milliseconds, they seem to be excessively short for integrating over multiple whisks or longer sensory events. This suggests that temporal integration in VPM is comparatively weak. To corroborate this, we characterized VPM responses delivering spatiotemporal patterns of multiwhisker activation. Consistently across animals (n = 15), we did not find a significant change of VPM responses by multiwhisker stimuli application (Fig. 7B, see also Fig. 5E).

Together, our results show that VPM responses are different from those of POm neurons and suggest significant functional differences between POm and VPM thalamic nuclei in the processing of complex stimuli.

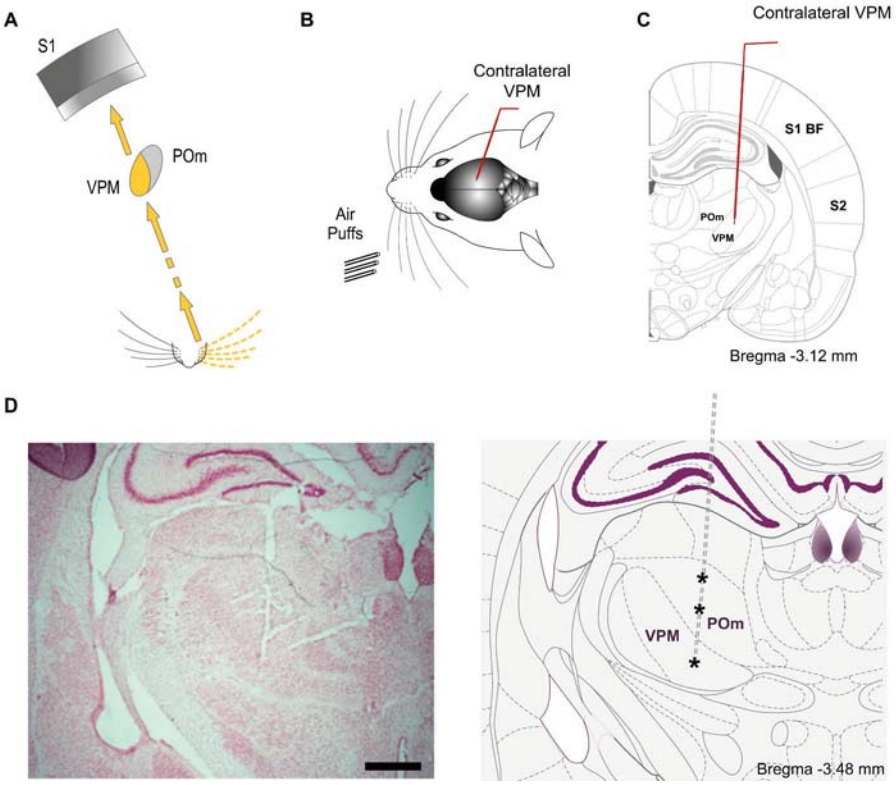


Fig. 6. VPM. (A) Schematic illustration of the lemniscal pathway. (B) Schematic drawing displaying the sensory stimulation via patterns of multiwhisker deflections. Recordings were made in the contralateral thalamic VPM nucleus. (C). Coronal section illustrating a recording electrode inserted into the VPM. (D) The left panel shows a representative Nissl stained coronal section displaying the location of the sequence of recording sites (indicated by asterisks in the other panel) in

POm and VPM and the track left by the electrode. An atlas schematic reconstruction of this coronal section is shown in the right panel (Paxinos and Watson 2007). Scale bar, 1 mm.

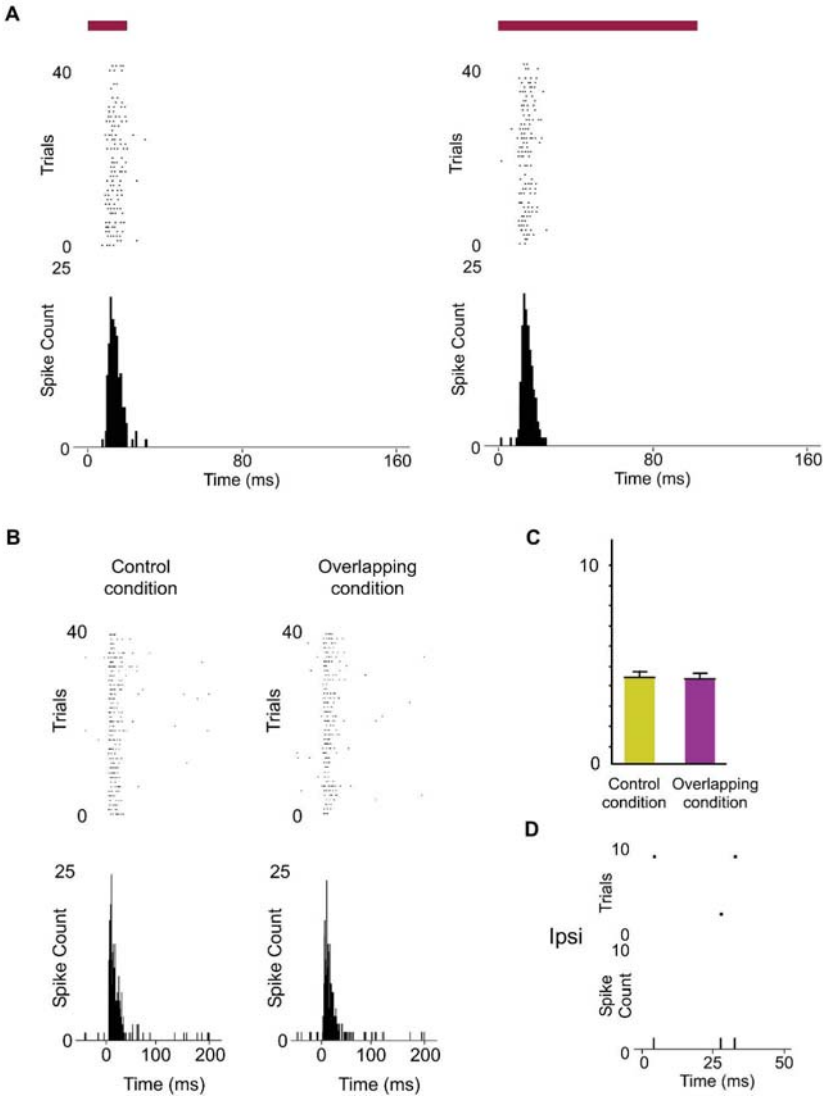


Fig. 7. Response modes differed drastically between VPM and POm. POm sustained versus VPM transient responses. (A) VPM responses were not sustained along stimulus presence. They were transient responses just to the onset of stimuli. Raster plots and PSTHs showing multi-unit VPM transient responses evoked by different stimulus duration (20 ms and 100 ms). Note that VPM responses do not allow the discrimination between different durations of the same stimulus. Red color lines indicate the duration of the stimulus. (B) Raster plots and PSTHs showing that VPM response to simultaneous multiwhisker activation (Overlapping condition) is very similar to individual whisker activation alone (Control condition). (C) The mean response magnitude across units (n = 96) recorded in VPM did not change by the overlapping of spatial signals (-2 %, p = 0.17, Wilcoxon matched-pairs test). (D) In contrast to POm, VPM did not respond to ipsilateral stimuli (described later).

3. POm integration of spatiotemporal overlapping bilateral events

3.1. POm sustained responses to ipsilateral whisker stimulation

Recently, we have described for first time that POm is also able to respond to tactile stimulation of ipsilateral whiskers (Castejon and Nunez 2020). We also showed that this nucleus is implicated in the integration of bilateral signals. Here, to study these phenomena in more detail, we recorded POm responses to ipsilateral and contralateral stimulation and confirmed that POm is able to respond to tactile stimulation of ipsilateral whiskers (Fig. 8). These experiments showed multiwhisker ipsilateral receptive fields (mean receptive field size: 9.3 ± 2.7 whiskers; n = 42) and demonstrated that POm is not only characterized by broad contralateral receptive fields but also by broad ipsilateral ones. Across recorded units (n = 90), the ipsilateral responses were weaker in magnitude than contralateral responses (Fig. 8C, D) and longer in latency (mean response onset latency: 22.31 ± 1.28 ms versus 11.36 ± 0.82 ms). This difference between ipsi- and contralateral response onset latencies (10 ms, Fig. 8F) suggests that ipsilateral sensory information is mediated by different pathways.

Next, using air-puffs that varied in duration, we found that POm has also the capacity to sustain its activity to encode and represent tactile input duration of ipsilateral stimuli. Although the ipsilateral response was weaker in magnitude, the capacity to codify the duration of the stimulus remained robust as reported in the raster plots of a representative response in Fig 8C, E. The duration of the ipsilateral stimuli did not alter the mean onset latency of responses (One-way ANOVA, p = 0.79). These findings were consistent across all animals (n = 10).

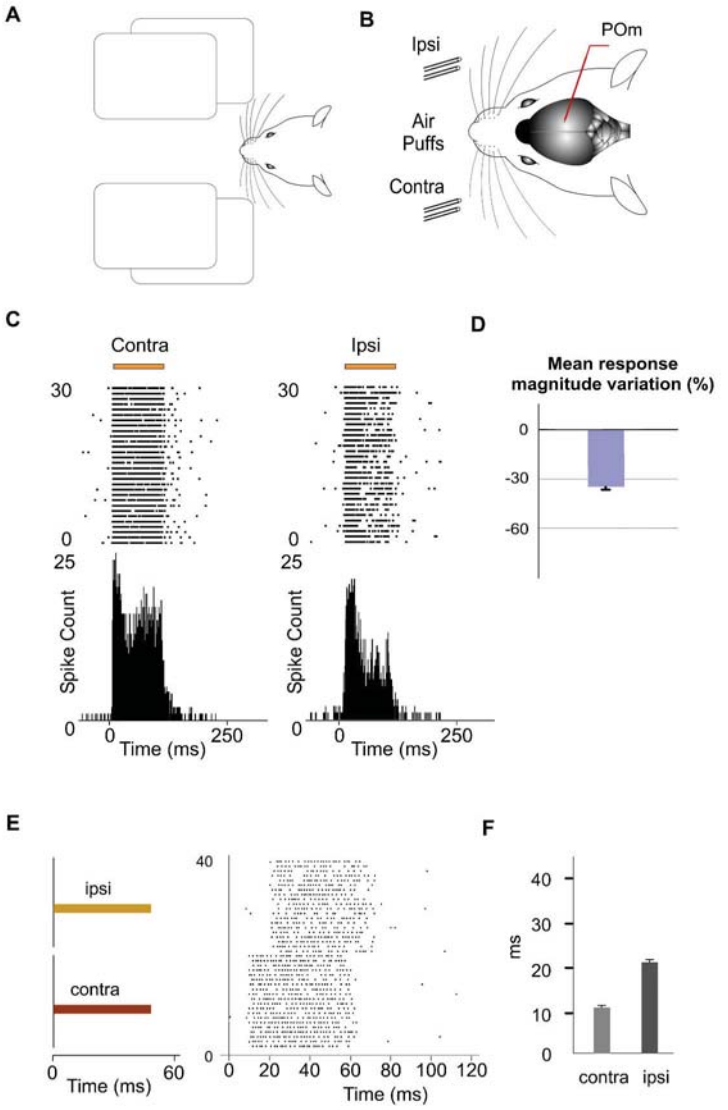


Fig. 8. POm responses to tactile stimulation of ipsilateral whiskers. (A) Tactile information from each side of the face is needed when rodents are exploring tunnels, discerning holes and apertures or detecting their width and shape. (B) Schematic drawing displaying the sensory stimulation via patterns of contra-, ipsi- or bilateral multiwhisker deflections. Recordings were made in the POm nucleus. (C) Raster plots and PSTHs showing sustained POm responses evoked by contra- and ipsilateral multiwhisker stimulation (30 trials shown for each stimulus). Orange lines indicate the duration of the stimulus. Note that although the ipsilateral response was weaker in magnitude, the capacity to codify the duration of the stimulus remained robust. (D) Mean ipsilateral response magnitude was significantly less strong than contralateral one ($p < 0.001$, Wilcoxon matched-pairs test, n = 90 units). (E) Response onset latencies were longer for ipsilateral than contralateral whisker stimulation. Contralaterally evoked responses were 10 ms faster in latency than ipsilaterally evoked ones. Color lines indicate the duration of the stimulus. Note that POm responses also lasted the duration of the ipsilateral stimulus. (F) Mean response onset latencies of contra- and ipsilateral responses are shown (n = 90 units).

3.2. POm integration of spatiotemporal overlapping ipsilateral events

Next, we investigated the implication of POm in the codification of complex ipsilateral stimuli or patterns of stimuli. Accordingly, we studied POm responses to ipsilateral multiwhisker activation protocols producing different sensory patterns of spatiotemporal overlappings. Across these patterns, the firing rate was significantly increased in the Overlapping periods (Fig. 9). These increases were also sustained along the temporal overlappings (Fig. 9D). However, in the Non-overlapping periods, the magnitude of responses did not change.

Again, we found precise changes in POm activity consistent with the spatiotemporal structure of the ipsilateral patterns (Fig. 9 D). These findings were consistent across all animals (n = 11).

We also characterized VPM thalamic responses delivering the same spatiotemporal patterns of ipsilateral multiwhisker activation in 7 rats. In contrast to POm, VPM did not respond to ipsilateral stimuli or to ipsilateral overlapping protocols (Fig. 7D). Since the integration of tactile information from the two sides of the body is fundamental in bilateral perception, our results suggest a different implication of these thalamic nuclei in this function.

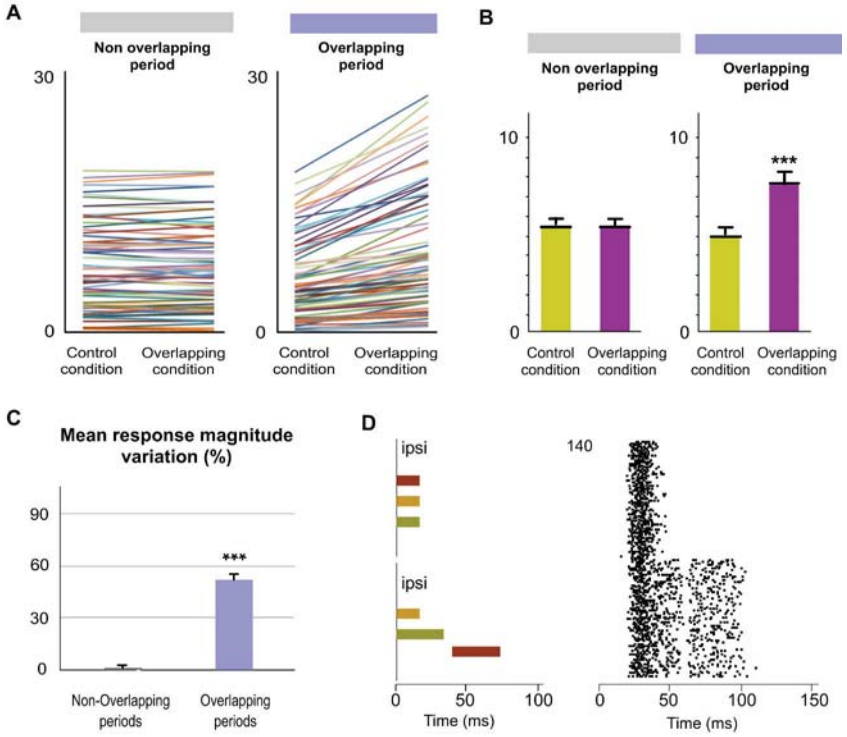


Fig. 9. POm integration of ipsilateral overlapping events. (A) Data showing the quantification of the facilitative integration during overlappings of ipsilaterally evoked signals. Plots comparing the spike rate of all recorded units (n = 80, depicted in different colors) during the Overlapping and Non-overlapping response periods in Control and Overlapping conditions across sensory patterns. (B) The mean firing rate was significantly increased in the Overlapping periods ($p<0.001$; Wilcoxon matched-pairs test) but not in the Non-overlapping periods where the mean magnitude of responses did not change ($p = 0.97$; Wilcoxon matched-pairs test). (C) Mean response magnitude variation (%) between Control and Overlapping conditions in Non-Overlapping and Overlapping periods ($p < 0.001$, Wilcoxon matched-pairs test). (D) Peri-stimulus time raster plots of representative POm responses to two different overlapping protocols of multiwhisker ipsilateral stimulation (70 trials shown for each protocol). An increase in POm response magnitude can be appreciated as more inputs were temporally overlapped in these protocols. Note that the general temporal structure of the sensory pattern, the temporal position of its components (depicted in different colors) and their corresponding overlappings are reflected in the structure of POm response.

3.3. Transmission of integrated sensory activity between both POm nuclei

These findings raise the question of by which route(s) is the ipsilateral information relayed to POm. It is known that POm is subcortically innervated by the Principal (Pr5) and Interpolar nuclei (SpVi) but we did not find evoked responses to contralateral whisker stimulation in these trigeminal nuclei (11 rats), therefore POm responses to ipsilateral whiskers stimulation were not driven by ascending peripheral activity conveyed directly via the trigeminal complex. In agreement with this finding, the difference between latencies in POm to

contralateral and ipsilateral stimuli suggests that ipsilateral information is not received by POm directly from the periphery. The delay (10 ms) that we observed for ipsilateral information suggests the indirect transfer of ipsilateral information between hemispheres from the other POm. To test this possibility, unilateral overlapping sensory patterns were applied while extracellular recordings were performed in both POm nuclei simultaneously in 6 rats (Fig. 10). We found that responses evoked by these unilaterally applied overlapping sensory patterns were similar in both nuclei. In agreement with our data described above, they showed different onset latencies (Fig. 10B) suggesting that evoked activity first arrives at the contralateral POm and is then transferred to the ipsilateral POm in the other hemisphere. This would indicate that both POm nuclei are mutually connected forming a POm–POm loop.

Importantly, precise changes in POm activity caused by the integration of overlapping stimuli reflecting the spatiotemporal structure of the sensory pattern were precisely conserved (Fig. 10B). Therefore, these patterns of integrated information encoded by one POm were transmitted through the loop to the other POm preserving their integrated structure (Fig. 10C).

To confirm that ipsilateral activity reaches one POm from the other POm, we pharmacologically deactivated one of them by muscimol (1 mg/ml) injection (Fig. 10E). We found that evoked responses in the second POm to ipsilateral stimulation were abolished in the majority of cases (4 out of 6 rats; Fig. 10F). However, they were almost abolished but not completely eliminated in 2 cases (-91 %, $p < 0.001$). This residual activity may be attributed to an incomplete deactivation of the opposite POm or a transmission of ipsilateral activity by an alternative pathway (i.e., collicular commissure). However, we observed that the sustained patterns of integrated activity evoked by ipsilateral sensory patterns were abolished in all animals. This indicated that these sustained patterns of integrated information were received from the other POm. Together, these findings demonstrated a transmission of integrated sensory activity between both POm nuclei through a functional POm-POm loop.

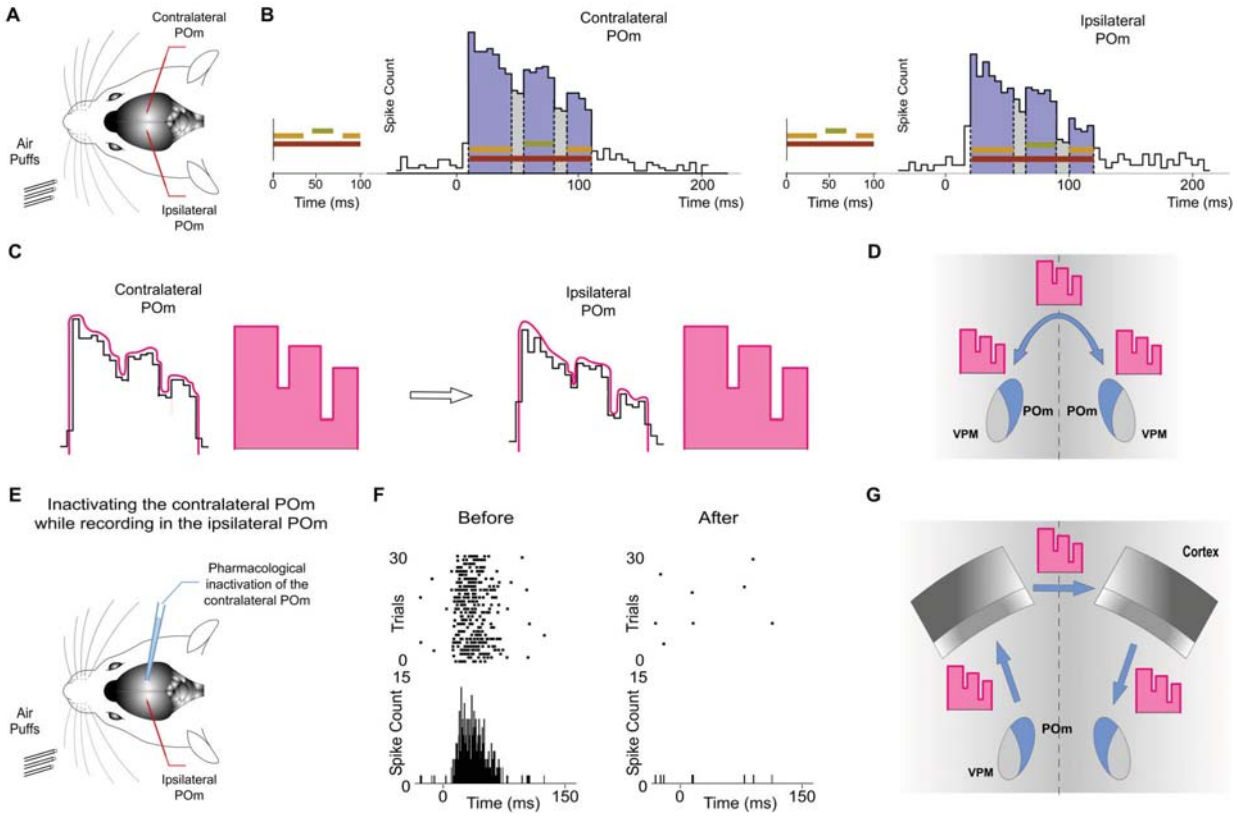


Fig. 10. Ipsilateral sensory information is received by POm interhemispherically from the other POm. This activity was transmitted preserving its integrated structure across the POm-POm loop. (A) Unilateral overlapping sensory patterns were applied while extracellular recordings were performed in both POm nuclei simultaneously. (B) POm responses

evoked by these unilaterally applied patterns showed different onset latencies but similar integrated structure in the contra- and ipsilateral POm nuclei. As can be appreciated in these PSTHs (bin width 5 ms) of POm responses, precise changes in POm activity caused by the integration of overlapping signals were similar in the contra- and ipsilateral POm nuclei. Note that the onset latency was longer in the ipsilateral POm. (C) PSTHs in B show that precise changes (fluctuations represented by lines in pink) in POm sustained activity caused by the integration of overlapping stimuli were precisely conserved. Therefore, these patterns of integrated information encoded by POm were interhemispherically transmitted to the other POm preserving their integrated structure. These fluctuations of POm activity can be considered as 'structured patterns of integrated activity' (represented by schematic patterns in pink). (D) These 'structured patterns' are transmitted across the POm-POm loop. (E) POm responses to ipsilateral stimulation were studied before and after pharmacological deactivation by muscimol (1 mg/ml) injection in the opposite POm. (F) POm responses to ipsilateral stimulation were abolished when the opposite POm was pharmacologically deactivated. (G) The latencies suggest that the POm-POm loop could be formed by a thalamocortical-callosal-corticothalamic route.

3.4. POm integration of spatiotemporal overlapping bilateral events

Finally, since bilateral sensory events occur concurrently producing different overlappings, the next question to investigate was whether POm would be able to codify these complex bilateral dynamics. It would require the precise integration of contralateral sensory inputs from the brainstem and ipsilateral sensory inputs from the other POm. To investigate the implication of POm in these intricate computations, we applied spatiotemporal overlapping patterns of bilateral multiwhisker stimulation simulating possible natural bilateral sensory events (Fig. 11A). We measured the responses of the nucleus to the overlapping patterns and found that POm precisely integrates tactile events from both sides. We found that precise changes in the spatiotemporal structure of bilateral events evoked different patterns of POm integrated activity. Across sensory patterns, the firing rate was increased in the Overlapping periods (quantified in Fig. 11) but not during the non-overlapping time. This indicates that the time shared by overlapping ipsi- and contralateral stimuli is encoded by POm activity. These increases in firing rate were sustained along the Overlapping periods (Fig. 11B). Similar effects were found stimulating identical (mirror) or different whiskers (nonmirror whiskers) on both sides. This is in agreement with the less accurate somatotopy of the nucleus and again indicates that the function of POm integration is not the combined representation of specific whiskers but the generic codification of sensory patterns integrated from both whisker pads. Moreover, during Overlapping periods, facilitation of responses was found as more ipsilateral, contralateral or bilateral temporal overlapping inputs were added showing that the complexity of the bilateral spatiotemporal overlappings is replicated in POm activity variations. These findings were consistently found across animals (n = 17).

Crucially, we found that the temporal interval between bilateral stimuli is critical to bilateral integration of sensory information. The delay (10 ms) that we observed for ipsilateral information determines the interaction between bilateral stimuli (Fig. 11C).

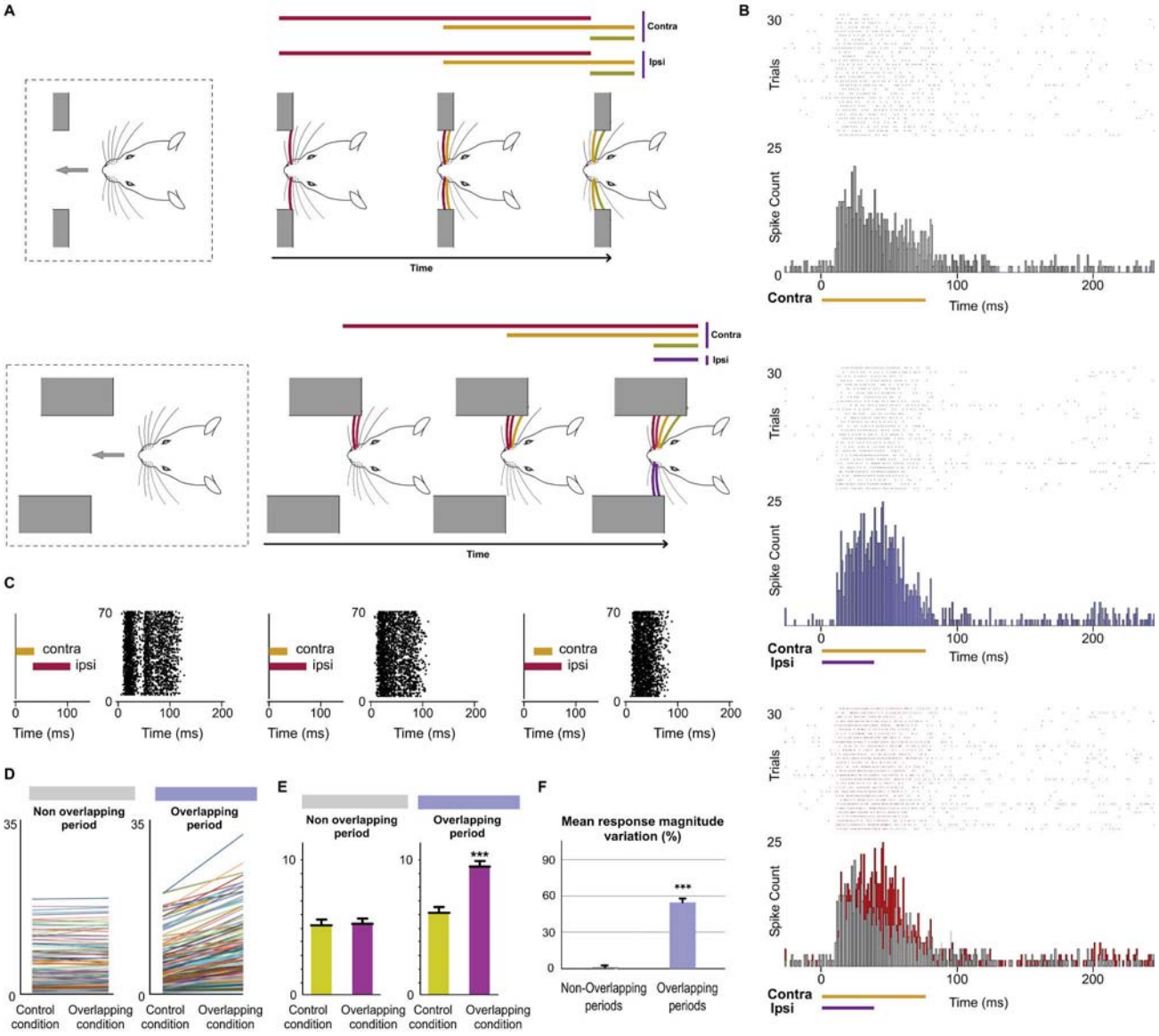


Fig. 11. POm integration of bilateral events. (A) As can be appreciated in these two simulated exploratory tactile sequences, many whiskers on both sides are sequentially deflected simultaneously. In our experiments, bilateral sensory patterns were generated by the application of contralateral and ipsilateral stimuli activating different subsets of whiskers on both sides of the face at mirror (matched whiskers on each side) and nonmirror positions. (B) Raster plots and PSTHs showing demonstrative POm responses evoked by contralaterally (top) and bilaterally (middle) evoked overlapping stimulation using stimuli with different durations (color lines indicate the duration of the stimuli). Note that the time shared by these bilateral overlapping inputs is encoded by a precise increase in POm activity. This increment is depicted in red in the bottom panel. (C) The time interval between contralateral and ipsilateral stimuli was varied to produce different overlappings and to study their integration. Raster plots of representative POm responses to different bilateral overlapping stimulation protocols are shown. Note that since the onset latency of ipsilateral responses is ~10 ms longer than contralateral responses, the temporal interval between contralateral and ipsilateral signals determines their integration and the structure of the response. As can be appreciated in the left panel, no integration was observed when the contra- and ipsilateral stimuli were not temporally overlapped. (D) Data showing the quantification of the facilitative integration during overlappings of contralaterally and ipsilaterally evoked signals. Plots comparing the spike rate of all recorded units (n = 187, depicted in different colors) during the Overlapping and Non-overlapping response periods in Control and Overlapping conditions across bilateral sensory patterns. (E) The mean firing rate was significantly increased in the Overlapping periods ($p<0.001$; Wilcoxon matched-pairs test) but not in the Non-overlapping periods where the mean magnitude of responses did not change ($p = 0.41$; Wilcoxon matched-pairs test). (F) Mean response magnitude variation (%) between control and overlapping conditions in Non-Overlapping and Overlapping periods ($p < 0.001$, Wilcoxon matched-pairs test).

3.5. POm nuclei are mutually connected through the cortex

Our results prompt the question of by which route(s) is sensory information transferred from one POm to the other. Cortical responses to ipsilateral whisker stimulation have been described in the somatosensory cortex (Shuler et al. 2001; Debowska et al., 2011). Therefore, ipsilateral activity seems to arrive at the contralateral POm by crossing the corpus callosum and descending from the cortex. Since POm receives strong innervation from corticofugal projection neurons in S1 (Hoogland et al., 1991; Bourassa et al., 1995; Veinante et al., 2000b), it is then possible that ipsilateral sensory stimulation could produce the activation of these descending corticofugal projections. This could have important implications on the integration of cortical inputs by POm and suggests that ipsilateral stimulation can be used to study the nature and content of the messages travelling through these corticofugal projections. Moreover, it has been proposed that functioning of higher-order nuclei, including POm, are determined by these descending corticofugal projections. To confirm that ipsilateral activity reaches POm via corticothalamic axons and to investigate whether these thalamic capacities are generated or mediated by cortical influence, we studied POm response properties before and after pharmacological deactivation of S1 (of the same hemisphere) by lidocaine (10%) or muscimol (1 mg/ml) application. As a control, we simultaneously recorded neuronal activity in the injected area. The electrodes were placed in the infragranular layer and the inactivation was confirmed by the absence of spontaneous and evoked activity. We found that POm responses to ipsilateral stimulation were almost abolished when S1 was inactivated (-89 %, $p < 0.001$, paired t-test, $n = 8$ rats; Fig. 12B). However, since the attempt to pharmacologically inactivate S1 can produce its partial deactivation and also can affect surrounding cortical areas, we confirmed our result by cortical lesion. The lesion was restricted to S1 and included superficial and deep layers of this area. This approach showed similar results. POm responses to ipsilateral stimulation were almost eliminated (-92. $p < 0.001$, paired t-test, $n = 6$ rats).

POm also receives cortical projections from M1 and S2 (Alloway et al. 2008; Liao et al. 2010). However, we did not find a significant reduction of ipsilateral responses when only M1 and S2 were lesioned (-7 %, $p = 0.32$, paired t-test, $n = 4$ rats). Together, these findings are in agreement with previous studies showing that cortical 'driver' input (Sherman and Guillery 1998) to POm originates almost exclusively from S1 (Veinante et al. 2000b).

When a wider cortical extension was lesioned including S1, M1 and S2, POm responses to ipsilateral stimulation was completely abolished in the majority of cases (4 out of 6 rats). However, we still found very small responses to ipsilateral stimulation in 2 cases (-94 %, $p < 0.001$). Importantly, we found robust contralateral whisker-evoked responses in POm even when the cortex of the same hemisphere was inactivated, which indicates a direct ascending input from the periphery. Cortical inactivation slightly affected the magnitude of POm responses to contralateral whiskers. We only found a minimal reduction of spikes (-8 %; paired t-test, $p = 0.06$, $n = 6$ rats; Fig. 12C). Furthermore, using air-puffs that varied in duration to stimulate contralateral whiskers, we found that cortical inactivation did not affect the capacity of POm to sustain its activity to codify stimuli duration Fig. 12D). We also measured POm responses to contralateral overlapping sensory patterns in this condition and found that cortical inactivation did not affect the capacity of POm to integrate overlapping stimuli (Fig. 12D). On the basis of these results, we conclude that POm does not inherit these capacities from cortical influence.

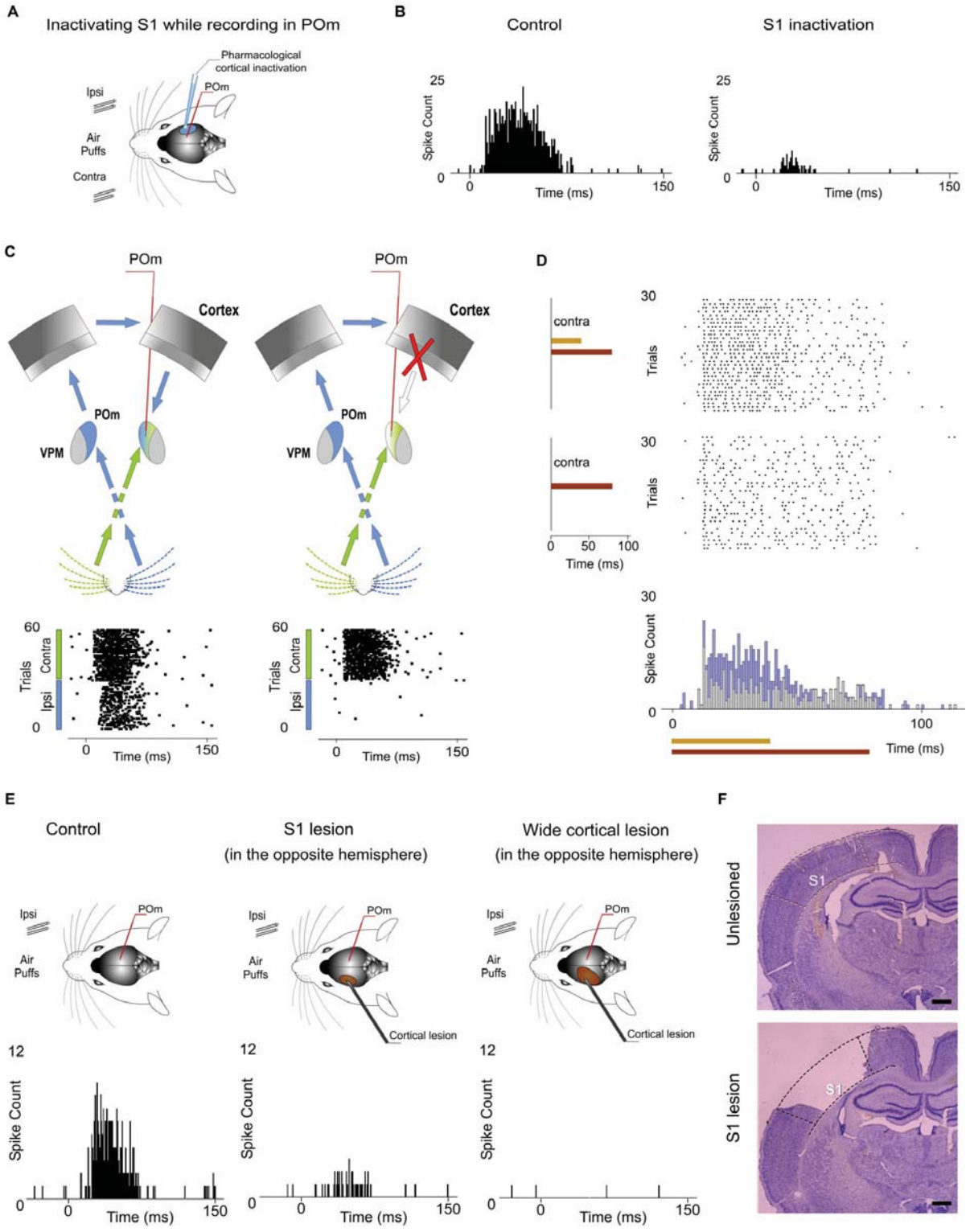


Fig. 12. The POm-POm loop. Ipsilateral and contralateral POm nuclei are mutually connected through the cortex. (A) POm responses were recorded before and after S1 inactivation. (B) POm responses to ipsilateral whisker stimulation were almost abolished when S1 was inactivated or lesioned. PSTHs of representative POm responses before and after S1 inactivation. (C) Schematic illustration representing the transmission of contralateral and ipsilateral information to POm. As can be appreciated in the corresponding raster plots, POm response to ipsilateral whisker stimulation was eliminated when the POm-POm loop was interrupted by cortical removal. Note that POm response to contralateral stimulation was still present in this condition. (D) Cortical removal did not affect the capacity of POm to sustain its activity to codify stimuli duration and

its capacity to integrate overlapping stimuli as can be appreciated in these raster plots and PSTHs of POm responses recorded during this condition. (E) PSTHs of representative POm responses in control, S1 lesion (in the opposite hemisphere) and wide cortical lesion (in the opposite hemisphere) conditions are shown. An almost complete reduction but not a total elimination of ipsilateral responses was produced by the S1 lesion. POm responses to ipsilateral stimulation were completely eliminated only when a wider cortical extension was lesioned. (F) Example histology showing the removal of S1 from a lesioned animal. An unlesioned example is also shown for comparison. Scale bars, 1 mm.

Together, these results demonstrated that ipsilateral and contralateral POm nuclei are mutually connected through the cortex by showing that ipsilateral activity reaches POm via descending parallel corticofugal projections mainly from S1 of the same hemisphere. But, by which route(s) is the ipsilateral information relayed to S1? Since projections from POm to the cortex in the other hemisphere have not been described and since it is known that corticocortical transmission between hemispheres via callosal projections is the main route for ipsilateral sensory inputs (Shuler et al. 2001; Petreanu et al., 2007), it seems that the POm-POm loop could be formed by a thalamocortical-callosal-corticothalamic route. To test this, we studied POm responses to ipsilateral stimulation before and after deactivation of S1 in the other hemisphere by lidocaine (10%) or muscimol (1 mg/ml) application. We found an almost complete reduction but not a total elimination of ipsilateral responses (-86 %, $p < 0.001$, paired t-test, $n = 6$ rats). This was confirmed by S1 lesion ($p < 0.001$, paired t-test, $n = 5$ rats; Fig. 12E). Again, when a wider cortical extension was lesioned including S1, M1 and S2, POm responses to ipsilateral stimulation was completely abolished in the majority of cases (4 out of 5 rats). We still found residual responses to ipsilateral stimulation in one case. This remaining activity could be attributable to other cortical areas projecting to POm or to subcortical interhemispheric pathways such as the collicular commissure.

Finally, to investigate the implication of cortical layers in the processing and transmission of sustained activity between the thalamus and the cortex in the POm-POm loop, evoked responses across recorded multi-units in supra, granular and infragranular layers of S1 were examined ($n = 117$, $n = 88$ and $n = 102$ units respectively) using ipsi- and contralateral stimuli with different durations in 19 rats. Examination of the laminar profile of evoked activity across layers showed profound differences between them. We only found sustained responses lasting the duration of the stimulus in the infragranular layer (Fig. 13). Similar to VPM responses, supra- and granular responses were only transiently activated at the onset of stimuli (Fig. 13D). Long stimuli usually evoked an onset response at the beginning of the stimulus and an offset response at the end but we did not find sustained responses during stimulus presence in these layers. These findings demonstrated different laminar profile of cortical responses. Moreover, using ipsilateral stimuli with different durations to examine the implication of these layers in the interhemispheric transfer of sustained activity, we found that the infragranular layer showed evoked sustained responses to ipsilateral stimulation. These results demonstrated different laminar implication in the processing of sustained activity and its transmission between hemispheres.

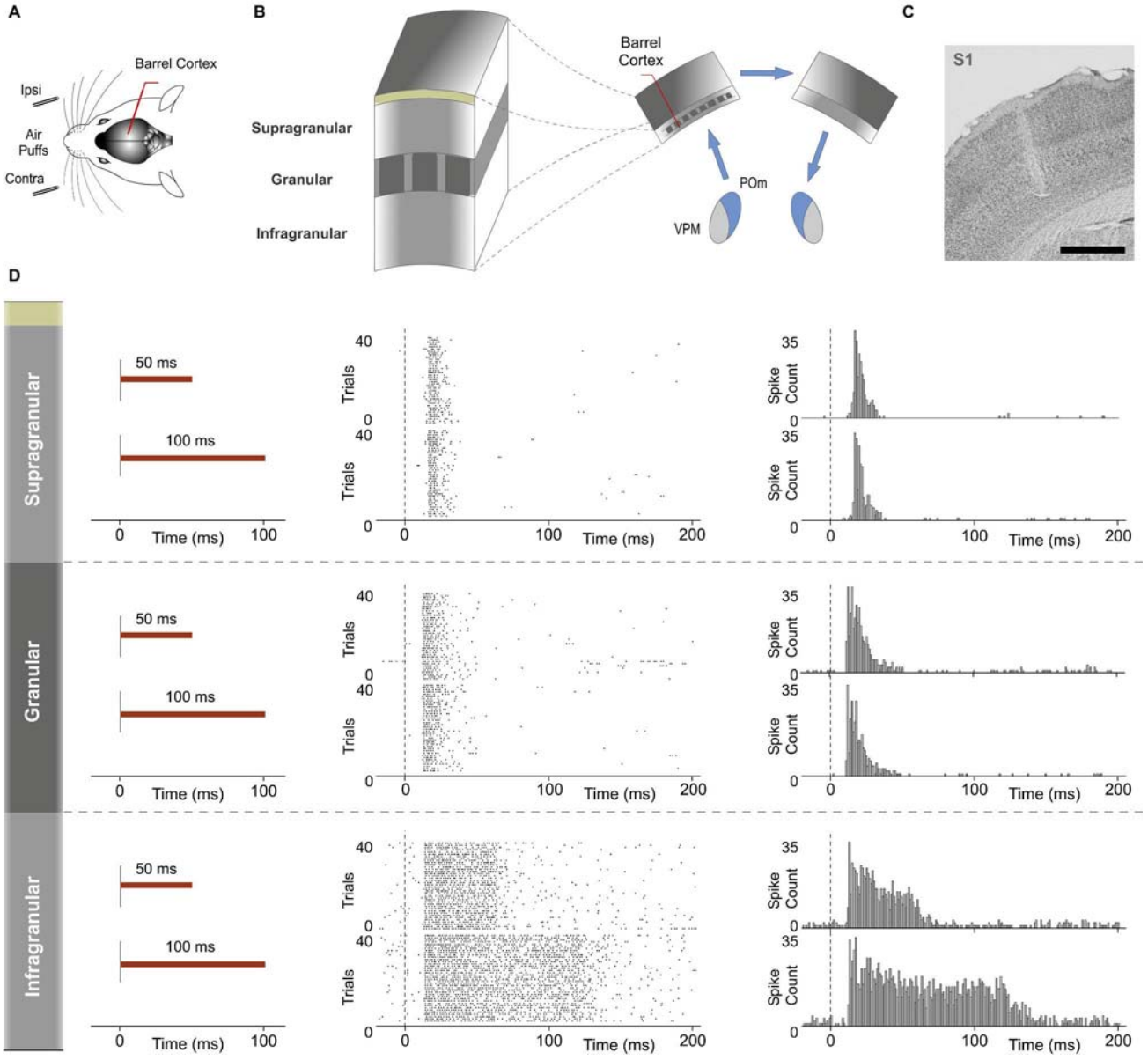


Fig. 13. Response modes differed between cortical layers in S1. Sustained responses along stimulus presence were found in the infragranular layer but not in granular and supragranular layers. (A) Recordings were made in the barrel cortex in S1 using ipsi- and contralateral stimuli with different durations. (B) Evoked responses in supra-, granular and infragranular layers of the barrel cortex were examined to study the laminar implication in the processing of sustained activity and its transmission across the POm-POm loop. (C) Histological section displaying the location of the sequence of recording sites across cortical layers in S1 and the track left by the electrode. Scale bar, 1 mm. (D) Raster plots and PSTHs showing typical responses in supragranular, granular and infragranular layers evoked by contralateral stimuli with different durations (50 ms and 100 ms). Note that evoked responses in granular and supragranular layers were transient just to the onset of stimuli and that they do not allow the discrimination between different durations of the same stimulus. Color lines indicate the duration of the stimulus.

Discussion

1. The role of higher-order sensory thalamus in the codification of complex sensory patterns

The function of the higher-order sensory thalamus remains unclear. Here, we propose the hypothesis that an important function of higher-order thalamic nuclei could be the codification of complex sensory patterns. Their functional implication may allow the processing and extraction of patterns and regularities from the sensory input. They could allow sensory systems to generate a representation of these dynamics. Our findings describing the different implication of POm and VPM in the processing of complex stimuli are in agreement with this proposal. This also needs to be confirmed in other sensory modalities.

2. The capacities of POm to sustain and integrate activity

How does the brain discriminate between different durations of identical stimuli? Here, we confirm previous findings (Castejon et al. 2016) by demonstrating that POm has the capacity to sustain its activity to represent tactile event duration with high accuracy. Extracting this temporal information from the sensory input is essential for the optimal extraction of information. In addition, our results show that POm is functionally implicated in the computation of overlapping spatiotemporal signals. This computation was performed by a precise facilitative integration of these signals.

Our findings show that the capacities of POm to sustain and integrate activity allow the representation of complex tactile events. Moreover, these POm capacities were still present during cortical inactivation. Accordingly, we conclude that they are not inherited from cortical influence. Importantly, these functional POm capacities are obtained from population activity (Fig. 2B). The presence of these functional capacities could be a consistent feature of higher-order thalamic nuclei.

3. Functional significance of POm capacities

POm activity fluctuations to codify patterns

Varying the spatiotemporal structure of sensory patterns, we found that POm is highly sensitive to multiwhisker activation involving complex spatiotemporal interactions. Our results show that the dynamical spatiotemporal structure of sensory patterns and the different complexity of their parts was accurately reflected in precise POm activity changes. Therefore, these precise fluctuations of POm integrated activity generate representations of these dynamics. This finding prompts a fundamental question: What could be the function of these precise thalamic activity fluctuations? Since they are composed by integrated activity generated by POm reflexing the spatiotemporal structure of the sensory input, POm integration may provide a mechanism for detecting spatiotemporal landmarks in the continuous flow of incoming sensory signals. It could be used to precisely decode sequence boundaries allowing for extraction of regularities and patterns from the flow of raw sensory information. Moreover, these fluctuations can also serve as relevant cues in sensorimotor adjustment, pattern recognition, perceptual discrimination and decision-making.

From this functional perspective, active whisking and palpation movements can intentionally optimize the number, frequency and variation of overlappings to maximize the extraction of information (i.e., regularities) from objects, surfaces and textures during their exploration. It is known that rodents use different whisking strategies changing from large-amplitude whisker movements during wide exploration to small-amplitude whisker movements at higher frequencies to increase the resolution in the extraction of detailed information. In agreement with this, we found that POm has the ability to integrate overlapping inputs with high precision even when very short duration stimuli were used (20 ms). This finding is compatible with the range of frequencies described in these animals: between 4-12 Hz (large whisker movements) and 12-25 Hz (small movements; Carvell, and

Simons 1990). Accordingly, adapting the active generation of precise overlappings of specific subsets of whiskers and their frequency would allow these animals to obtain the optimal resolution necessary to solve different perceptual or tasks requirements.

POm is an encoder of patterns

Rodents and other mammals have the ability to detect complex patterns embedded in a continuous stream of sensory activity. Across protocols, we found that varying the spatiotemporal structure of the sensory input produced different patterns of POm integrated activity. We observed that POm generates very similar patterns of activity when different whiskers were activated by the same stimulation protocol. This finding is in agreement with the less accurate somatotopy of this nucleus and suggests that the function of POm integration is not the combined representation of specific whiskers but the codification of generic sensory spatiotemporal patterns from the array of whiskers. Accordingly, our findings suggest that POm is a general encoder of patterns.

4. POm mediates bilateral sensory processing

POm integration of spatiotemporal overlapping bilateral events

Although it is well described that POm encodes stimulations of the contralateral whisker pad, our results show that POm is also able to respond to tactile stimulation of ipsilateral whiskers. This finding challenges the notion that the somatosensory thalamus simply computes contralateral whisker stimuli. In addiction, we found that POm integrates signals from both whisker pads and described how this integration is generated. Importantly, we found that precise changes in the spatiotemporal structure of bilateral events evoked different patterns of POm integrated activity.

These findings can explain why the presence of tactile input from one side affects tactile processing of the other side (for example, in sensory interference paradigms) and demonstrate the implication of the higher-order sensory thalamus in the codification of bilateral events.

The POm-POm loop: POm nuclei are mutually connected through the cortex

Our results show that ipsilateral activity reaches one POm from the other POm (Fig. 10). Moreover, these findings demonstrate a transmission of integrated activity between both nuclei through a functional POm-POm loop formed by thalamocortical, interhemispheric and corticothalamic projections. We confirmed this interhemispheric pathway by inactivating different areas of the cortex in both hemispheres (Fig. 12) and demonstrating that ipsilateral activity mainly reaches POm via S1 but not exclusively. Therefore, POm nuclei are mutually connected forming a complex network of parallel thalamocortical, interhemispheric and corticothalamic projections (Fig. 14A). In agreement with this finding, it is anatomically well described that MI and S2 also receives thalamocortical projections from POm (Ohno et al. 2012), that they are respectively interhemispherically connected (Carvell and Simons, 1987, Kinnischtzke et al. 2014) and that they have corticothalamic projections to POm (Alloway et al. 2008; Liao et al. 2010). Moreover, POm is a strong driver of activity in S2 and M1 (Theyel et al., 2010; Castejon et al. 2016; Casas-Torremocha et al. 2019) and bilateral sensory responses in S2 have also been described (Debowska et al., 2011). However, it is important to note that the remaining ipsilateral activity that we found after S1 deactivation did not allow for the encoding and represention of ipsilateral tactile event duration or its spatiotemporal structure with high accuracy. This indicates that although different cortical areas are parallelly implicated in the POm-POm loop, the robustness of this codification is supported by S1. Furthermore, since S1 receives intra- and interhemispheric projections from these cortical areas (Porter and White 1983; Carvell and Simons, 1987; Kinnischtzke et al. 2014), it is also possible that S1 could collect activity from different cortical regions (mostly from the other S1) and funnel this information via corticothalamic projections to POm of the same hemisphere (Fig. 14A). Consistent with this idea, it is known that motor and S2 cortical regions elicited strong direct input to L6b and L5 in S1 (Mao et al. 2011; Zolnik et al., 2020). Moreover, corticofugal projections

from L6b to POm have also been confirmed (Bourassa et al. 1995; Hoerder-Suabedissen et al. 2018). L6b receives substantial innervation from the contralateral sensorimotor cortical areas producing a contralateral drive to this layer (Zolnik et al. 2020). Here, we found that VPM did not respond to ipsilateral stimuli. Unlike POm, VPM does not receive cortical input from L5 or L6b corticofugal projections in S1 (Hoogland et al. 1991; Hoerder-Suabedissen et al. 2018).

Importantly, our results also demonstrated different laminar implications in the processing of sustained activity and its transmission between hemispheres (Fig. 13). We only found sustained responses lasting the duration of the stimulus in the infragranular layer. Similar to VPM responses, supra- and granular responses were only transiently activated at the onset of stimuli but we did not find sustained responses along stimulus presence. Using ipsilateral stimuli with different durations to examine the implication of these layers in the interhemispheric transfer of sustained activity, we found that only the infragranular layer showed evoked sustained responses to ipsilateral stimulation. These findings demonstrate that the transmission of sustained activity across the POm-POm loop is supported by the infragranular layer (Fig. 14B).

In addition, we found that POm constantly integrates bilateral sensory information and that ipsilateral activity reaches POm via corticofugal projections mostly from S1. This in agreement with previous findings showing the convergence of ascending driver inputs from the periphery and descending driver inputs from L5 of S1 in POm (Groh et al. 2014; Castejon et al. 2016). We found that the temporal interval between contra- and ipsilateral stimuli was critical to bilateral integration of sensory information. The delay (10 ms) that we observed for ipsilateral information determined the interaction between bilateral stimuli (Fig. 11C). Also consistent with our findings is that the activation of corticofugal projections from L5 in S1 by optogenetic stimulation increases ascending sensory responses within a well-defined time window (Groh et al. 2014). In our study, we took advantage of the fact that ipsilateral sensory stimulation produced the activation of these corticothalamic fibers to investigate, in more physiological conditions, the functional interaction of these two streams in the integration of bilateral events and the implication of these corticothalamic projections in POm functioning.

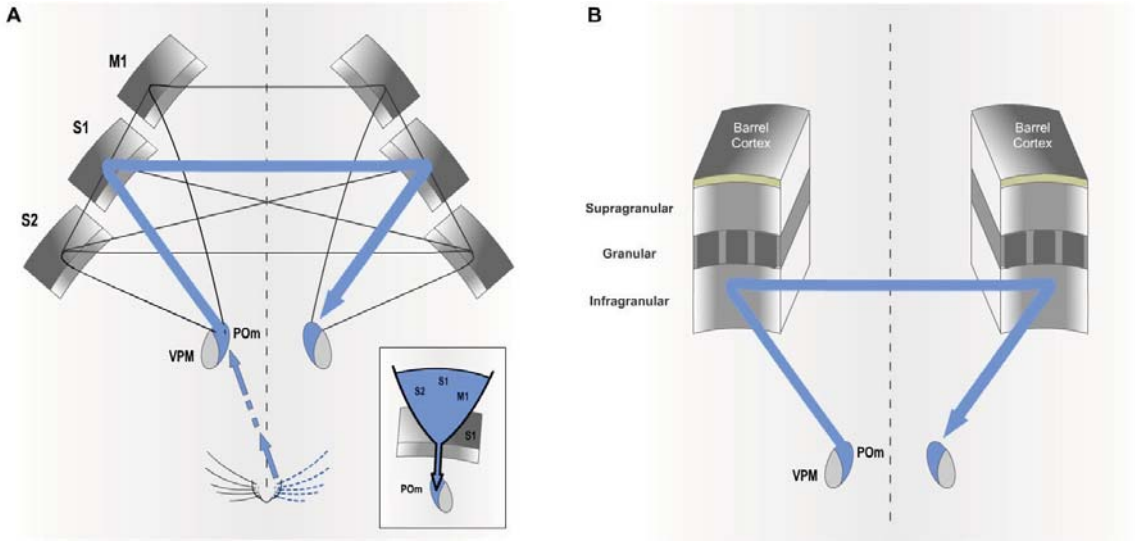


Fig. 14. The POm-POm loop is formed by a functional network of parallel thalamocortical, interhemispheric and corticothalamic projections. (A) Although different cortical areas are implicated, our results revealed that S1 plays a protagonistic role in this functional loop. The inset illustrates the idea that S1 may act as a funnel collecting activity from different areas and sending this information via corticothalamic projections to POm of the same hemisphere. This complex interhemispheric loop allows bilateral integration in the thalamus and is implicated in the bidirectional transmission of sustained activity between the higher-order thalamus and the cortex. This transmission of sustained activity can be functionally implicated in cognitive processes. (B) Our results indicate that the transmission of sustained activity across the POm-POm loop is supported by the infragranular layer. This loop could be also present in other sensory modalities.

5. Structured patterns of integrated activity as 'Templates' for cognitive functions

Our results raise additional questions of interest with regard to the possible functional implications of the sustained thalamic patterns of integrated activity. We found that the sequences of precise fluctuations of POm activity constitute 'structured patterns of integrated activity'. We suggest that the patterns of structured activity generated by POm may be functional building blocks of perception and information extraction. Accordingly, they may serve the function of transforming raw sensory input into useful information by allowing the extraction of relevant patterns from the continuous sensory flow (Fig 15). In agreement with this idea, rodents have the capacity to locate, identify and discriminate shapes, objects and other sensory patterns in their environment with very high precision. But what functional roles could these integrated patterns play apart from perception? We propose that these patterns of structured activity may allow the conversion of sensory information into functional representations relevant to current or future cognitive tasks. Therefore, they could be selected, acquired and used as 'Templates' for cognitive functions. From this perspective, one important function of higher-order sensory thalamic nuclei may be the generation of perceptual units extracted from the sensory flow and converted into functional units which could be used as Templates for current or future cognitive requirements. This functional proposal provides a novel theoretical model to understand the implication of the thalamus in perception and cognition.

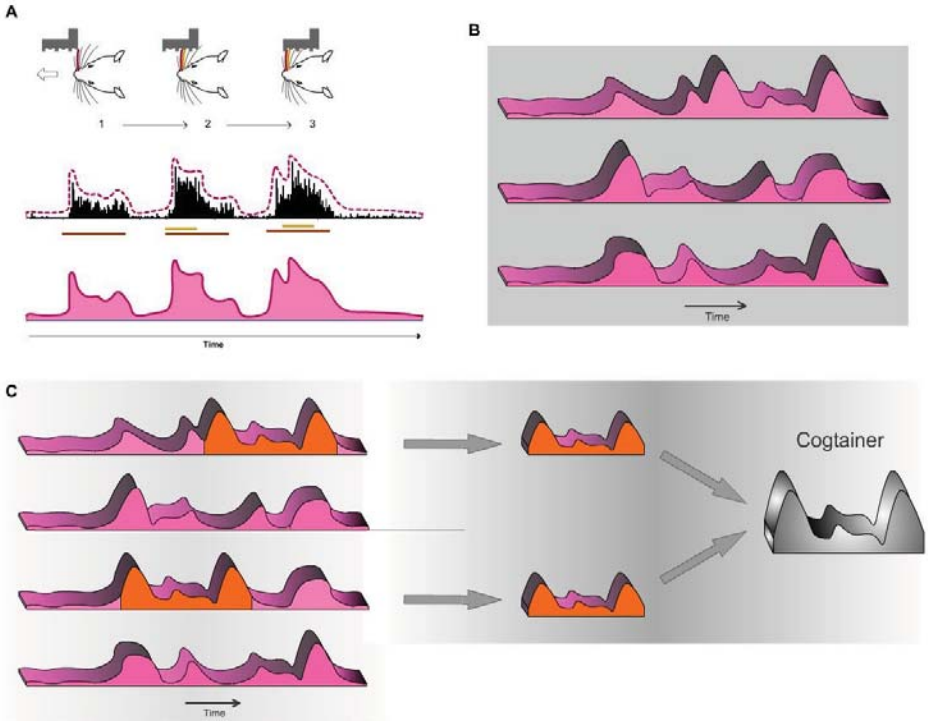


Fig. 15. Thalamic implication in the process of extracting meaningful patterns from raw sensory information and their possible relevance in different brain functions. (A) During the tactile exploration of objects or surfaces different spatiotemporal overlappings are sequentially produced. The codification of these overlappings by POm generates temporal sequences of activity fluctuations (1→2→3 in the example illustrated here). Functionally, the dynamical spatiotemporal structure of different sensory patterns would be accurately represented by different sustained thalamic patterns of integrated activity. Accordingly, different tactile events with diverse spatiotemporal structures would produce different 'structured patterns of integrated activity'. Some simulated examples are shown in (B). (C) This form of codification may provide a mechanism for detecting spatiotemporal cues, landmarks or boundaries allowing for extraction of regularities and functionally relevant patterns from the continuous flow of incoming sensory signals. By this mechanism, raw sensory input derived from the environment may be transformed into meaningful information. From this functional perspective, selected patterns of structured activity (depicted in orange) could be acquired and used as 'Templates' for different brain functions.

Here, we propose that when these functional units are used as Templates for current or future cognitive requirements, they could be called 'Cogtainers'.

6. Transmission of 'structured patterns of integrated activity' and its possible implication in cognition

Transfer of structured patterns of integrated activity across the loop

Sustained activity has been traditionally related to cognitive processes such as working memory (Fuster, 1982; Goldman-Rakic et al 1987). However, how this activity is generated, maintained and transferred is still unknown. Moreover, although interhemispheric communication during perceptual and cognitive processes is well assumed, as far as we know, no studies had been performed to define the nature of sustained activity through the thalamocortical-callosal-corticothalamic loops. These pathways have usually been studied separately. We investigated the nature and content of the activity carried by these projections and found a transmission of structured activity through this thalamocortical-callosal-corticothalamic route. Importantly, precise fluctuations in sustained activity caused by the integration of overlapping stimuli were precisely conserved (Fig. 10). Therefore, these patterns of integrated information encoded by POm were transmitted through the loop preserving their nature and their integrated structure. This finding is in agreement with our functional proposal suggesting that structured thalamic patterns of sustained activity could be transmitted and used as 'Templates' for different brain functions. Therefore, they must be transmitted preserving their integrity to be functionally useful.

Several important comments can be suggested. First, since POm neurons have 'multispecific' thalamocortical axons (Clasca et al. 2016) innervating several cortical areas including S1, S2 and M1 with different laminar profiles, our results suggest that the same 'structured patterns of integrated activity' generated by POm can be sent in parallel to different cortical targets. Therefore, the same message can be used by these areas and layers for different functions (i.e., perceptual, attentional and motor). Second, since POm also projects to different brain structures including the amygdala, basal ganglia, insular or ectorhinal cortex (Ohno et al. 2012), our results suggest that the same 'Templates' generated by POm can be used by these targets for diverse functions such as perceptual discrimination, familiarity, behavioral relevance, motivational meaning or decision-making. Accordingly, we propose that the transmission of these structured patterns of integrated activity (Cogtainers) across circuits (i.e., thalamocortical, cortico-cortical and corticothalamic circuits) supports the representation and maintenance of information during cognitive processes. From a functional perspective, this proposal provides a novel theoretical model to understand the implication of the thalamus (including POm) in cognition.

Since reciprocal interactions between the cortex (i.e., prefrontal cortex) and thalamus play a critical role in cognition, thalamic nuclei with anatomical and functional characteristics similar to those of POm may support cognitive and executive processes by the generation and transmission of Cogtainers. Indeed, thalamic responses sustaining prefrontal representations during different cognitive processes have been described (Bolkan et al., 2017; Guo et al., 2017; Schmitt et al., 2017; Rikhye et al. 2018). We propose that these effects could be the consequence of the transmission of sustained thalamic patterns of integrated activity by thalamic nuclei to their targets. The novel concept of Cogtainers could be used to functionally explain these findings.

POm responses to ipsilateral stimulation, POm sustained activity and its interhemispheric transmission are highly sensitive to anaesthesia

Experimental evidence has shown that during wakefulness or under light sedation, POm activity is significantly higher than during anesthetized state (Masri et al. 2008; Sobolewski et al. 2015; Zhang and Bruno 2019). This suggests that normal POm functioning can be affected in these conditions. Our findings are in agreement with this idea. In our experiments, we did not find POm responses to ipsilateral stimulation during the first hours after the application of urethane (1.3 – 1.5 g/kg i.p.). These responses were only found after sufficient time (typically 4 - 6 h) after the application of anaesthesia. Moreover, supplementary doses of urethane abolished

POm responses to ipsilateral whisker stimulation (data not shown). These observations indicate that the transmission of sensory activity between hemispheres across the POm-POm loop could be highly sensitive to anaesthesia. In agreement with this, it has been previously shown that increasing the level of sedation produces the elimination of evoked responses in S1 to ipsilateral stimulation (Armstrong-James and George, 1988). This fact could explain why POm responses to ipsilateral stimulation had not been reported before. In addiction, we found that POm sustained activity was also highly affected by the level of anaesthesia (data not shown). Increasing this level by supplementary doses of urethane strongly reduced or even abolished sustained activity in POm. These findings suggest that anaesthesia reduces the interhemispheric transmission of sustained activity between cortical areas. This can have important implications in higher cognitive processes such as consciousness. Accordingly, we propose that a possible hypothesis regarding the action of anesthesia could be the suppression of thalamic sustained activity and its transmission between brain structures.

Together, this evidence indicates that high levels of sedation impair the real dynamics of POm functioning.

Different laminar implication in S1 in the processing of sustained activity and its transmission between hemispheres

The laminar analysis revealed that sensory-evoked responses in S1 had different temporal structures across layers (Fig. 13). We only found sustained responses lasting the duration of the stimulus in the infragranular layer. However, not all responses in the infragranular layer were sustained. This in agreement with the complexity of this layer formed by different sublayers. Sustained responses were mostly observed in the superficial part of the infragranular layer corresponding to layer 5. In addition, similar to VPM responses, supra- and granular responses were only transiently activated at the onset of the contralateral stimuli but we did not find sustained responses along stimulus presence. This is in agreement with previous findings that show higher sensory-evoked firing rates in L5 neurons (de Kock et al., 2007; Castejon et al. 2016) and sparse firing to sensory stimulation in supragranular layers of vibrissal cortex in S1 (de Kock et al., 2007; Petersen and Crochet 2013; Clancy et al. 2015; Peron et al. 2015).

In sum, our findings reveal different laminar profile of cortical responses and demonstrate different laminar implication in the processing of sustained activity and its transmission between hemispheres. This could be a common characteristic of the sensory cortex.

7. Differences between VPM and POm

Different but complementary functional roles

Important differences were observed in our study between the response modes of VPM and POm. In agreement with previous findings (Castejon et al. 2016), POm was persistently activated during whisker stimulation, whereas VPM was only transiently activated (Fig. 7A). This indicates that the effect of the stimulus duration on the response was totally different for the two nuclei. Moreover, when delivering the same spatiotemporal patterns of multiwhisker activation, we did not find a significant change of VPM responses by multiwhisker stimuli application (Fig. 7B). This is in agreement with previous findings showing that VPM response to simultaneous multiwhisker activation is very similar to individual whisker activation alone (Aguilar and Castro-Alamancos 2005). However, our results show that POm is activated more strongly by complex stimuli than by simple ones and that POm has a relevant implication in the representation of complex tactile events. Therefore, sufficient complexity is required to capture the functional dynamics of POm. Together, our results show that VPM responses are different from those of POm responses and suggest significant functional differences between POm and VPM thalamic nuclei in the processing of complex stimuli. These findings are in agreement with our hypothesis that an important function of higher-order thalamic nuclei could be the codification of complex patterns.

In addition, our results suggest a functional difference between these thalamic nuclei underlying bilateral sensory processing. In contrast to POm, VPM did not respond to ipsilateral stimuli. Since the integration of tactile information from the two sides of the body is fundamental in bilateral perception, our results suggest a different implication of these thalamic nuclei in this function.

Taken together, these findings must be considered to functionally categorise these thalamic nuclei. The nature (structured versus discrete), type (sustained versus transient) and content (integrated versus segregated) of neural activity processed and transmitted by these nuclei may determine their functional implication and can be used to functionally classify them.

The hypothesis of 'Complementary Components'

As described above, two main parallel ascending pathways convey input from the whiskers to barrel cortex (Diamond et al., 1992; Veinante et al., 2000a). This anatomical segregation suggests a different functional role of these pathways and their corresponding thalamic nuclei in somatosensory processing. Our results show that sensory stimulation protocols with similar spatiotemporal structures produce similar patterns of POm activity fluctuations even when different whiskers were activated by the same protocol. This suggests that accurate somatotopy is not a functional characteristic of this nucleus. Accordingly, we propose that to optimize the extraction of information from the sensory flow and to complement the role of POm as a general encoder of patterns, the paralemniscal system must be complemented with an additional system providing precise somatotopy. This can be the functional role of the lemniscal pathway, phylogenetically more recent and characterized by a precise somatotopy (Diamond 1995; Simons 1995).

Our results, described here, are in agreement with this proposal showing important but functionally complementary differences between POm and VPM. This functional proposal which we have called the hypothesis of 'Complementary Components' can explain why tactile information from whiskers is processed by parallel ascending pathways towards the cortex. This parallel architecture is also present in the majority of sensory systems in the brain and is conserved across animals (Sherman and Guillery 2006). Accordingly, we propose that sensory systems have evolved to optimize the extraction of information from the environment and that the appearance of 'complementary' pathways (as the somatosensory lemniscal pathway) during evolution was essential in that functional optimization.

In addition, our results demonstrate distinct laminar processing of the same stimulus by the cortex. They show that the content, type and nature of the messages that these layers receive, process and transfer is different. Therefore, different 'Components' are also associated with distinct laminar profiles. They may play different but complementary functional roles. This could account for the different profiles of activity in cortical layers.

Materials and Methods

Ethical Approval

All experimental procedures involving animals were carried out under protocols approved by the ethics committee of the Autónoma de Madrid University and the competent Spanish Government agency (PROEX175/16), in accordance with the European Community Council Directive 2010/63/UE.

Animal procedures and electrophysiology

Experiments were performed on adult Sprague Dawley rats (220-300 g) of both sexes (40 males and 56 females). Animals were anesthetized (urethane, 1.3 – 1.5 g/kg i.p.) and placed in a Kopf stereotaxic frame. Local anaesthetic (Lidocaine 1%) was applied to all skin incisions. The skull was exposed and openings were made to allow electrode penetrations to different neuronal stations in the trigeminal complex, thalamus and cortex.

Our recordings were mostly performed several hours after the application of urethane (typically after 5 - 6 h). To assure the absence of whisker movements and pinch withdrawal reflexes, supplementary dosis of urethane were applied if necessary.

Extracellular recordings were made in the Principal (PrV; Posterior from bregma 9–10; Lateral from midline 3–3.5, Depth 8.5–9.5; in mm) and Interpolar trigeminal nuclei (SpVi; P 11.5–14; L 2.5–3.5, D 8.5–9.5) of the trigeminal complex, in the posteromedial thalamic nucleus (POm; P 2.5-4.5, L 2-2.5, D 5-6.5), in the ventral posteromedial thalamic nucleus (VPM; P 2.8-4.6, L 2-3.5, D 5.5-7) and in the vibrissal region of the primary somatosensory cortex (S1; AP 0.5-4, L 5-7). Laminar recordings in supra- (D 150 – 550 μm), granular (D 650 – 850 μm) and infragranular (D > 950 μm) layers of S1 were also performed. Unanalyzed gaps were left between layers to compensate for differences in cortical thickness across this area and as a safeguard against potential errors in laminar localization. Tungsten microelectrodes (2–5 MΩ) were driven using an electronically controlled microdrive system (DavidKopf).

Sensory stimulation and patterns generation

Sensory stimulation was characterized by spatiotemporal patterns of multiwhisker deflections simulating possible real complex stimuli or sequences of stimuli similar to those occurring in natural circumstances. Details of the multiwhisker stimulation patterns are described in Fig 2A, Fig 3A, Fig. 4A and Fig. 5A, B. Anesthetized rats were used to facilitate their application. Using a pneumatic pressure pump (Picospritzer) that delivers air pulses through polyethylene tubes (1 mm inner diameter; 1-2 kg/cm^2), sensory patterns were generated using controlled multiwhisker deflections performed by overlapping air puffs of different durations (20-2000 ms) applied to different whiskers in one or both sides of the face and avoiding skin stimulation. Accordingly, many whiskers were activated simultaneously producing different spatiotemporal overlapping dynamics. The air-puffers were precisely placed and the whiskers were trimmed to a length of 10–30 mm to allow precise overlapping stimulations. Overlappings produced by the activation of whiskers in different directions were also included. A variant order was adopted for delivering the stimulation patterns to avoid possible temporal dependency. We applied 20-70 trials per pattern at low frequency (0.3 - 0.5 Hz). Receptive field sizes were determined by deflecting individual vibrissae with a hand-held probe and monitoring the audio conversion of the amplified activity signal.

Inactivation and lesion of thalamic nuclei and cortical areas

We inactivated the cortex with local infusions of lidocaine (10%) or muscimol (1 mg/ml). To further evaluate the proper level of cortical inactivation, the basal activity and sensory responses to whiskers activation were continuously checked in the inactivated cortical area. Infusions were repeated every 15 min until cortical activity recorded in deep layers was silenced, on average 25 min after the first application. Pharmacological deactivation of POm was performed by injecting 100-200 nL of muscimol (1 mg/ml) in this thalamic nucleus. The drug was slowly delivered through a cannula connected to a Hamilton syringe (1µl) over a one-minute period.

Lesions of different cortical areas were also performed in our experiments. To assure the precision of cortical lesions, the skull was exposed and openings were precisely restricted to the corresponding cortical area according to stereotaxic coordinates (S1, described above; secondary somatosensory cortex (S2), P 0–3.7; L 5.5 – 7.5; primary motor cortex (M1), A 0.5 – 2.5, L 0.2 – 3). Lesions were made by cutting and aspirating the cortical tissue and included superficial and deep layers of these areas.

Histology

After the last recording session, animals were deeply anesthetized with sodium-pentobarbital (50 mg/kg i.p.) and then perfused transcardially with saline followed by formaldehyde solution (4%). After perfusion, brains were removed and postfixed. Serial 50 µm-thick coronal sections were cut on a freezing microtome (Leica, Germany). These sections were then prepared for Nissl staining histochemistry for verification of electrodes tracks, delimitation of cortical lesions and discrimination of thalamic nuclei. Positions of the electrode tips and extensions of cortical lesions were histologically verified by comparing these coronal brain sections with reference planes of the rat brain stereotaxic atlas (Paxinos and Watson 2007).

Data acquisition and analysis

Data were recorded from PrV, SpVi, VPM, POm and S1. The raw signal of the in vivo extracellular recordings was filtered (0.3–5 kHz), amplified (DAM80 preamplifier, WPI) and digitalized (Power 1401 data acquisition unit, CED). Single-unit activity was extracted with the aid of Spike2 software (CED) for spike waveform identification and analysis. Multi-units were collected by amplitude sorting. We defined response magnitude as the total number of spikes per stimulus occurring between response onset and offset from the peristimulus time histogram (PSTH, bin width 1 ms unless noted otherwise). Response onset was defined as the first of three consecutive bins displaying significant activity (three times higher than the mean spontaneous activity) after stimulus and response offset as the last bin of the last three consecutive bins displaying significant activity. Response duration was defined as the time elapsed from the onset to offset responses. In all figures, raster plots represent each spike as a dot and each line corresponds to one trial. Spikes were aligned on stimulus presentation (Time 0 ms). All data are expressed as the mean ± standard error of the mean (SEM). Error bars in the figures correspond to SEM. For normally distributed data (Shapiro-Wilk normality test), statistical analyses were conducted using a Student's t-test. Non-normally distributed data were compared with the Wilcoxon matched-pairs test. Multiple comparisons were evaluated using a One-way ANOVA test. Statistical significance was considered at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Additional information

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

C.C. conceived the theoretical models and hypotheses, designed and conducted the experiments, analyzed the results and wrote the paper.

J.M.C. conducted the experiments, analyzed the results and reviewed the paper.

A.N. designed and conducted the experiments, analyzed the results, reviewed and edited the paper.

Funding

This work was supported by a Grant from Spain's Ministerio de Economia y Competitividad (SAF2016-76462 AEI/FEDER).

Competing interests

The authors declare that no competing interests exist.

Aguilar, J. R., and Castro-Alamancos, M. A. (2005). Spatiotemporal Gating of Sensory Inputs in Thalamus during Quiescent and Activated States. J. Neurosci. 25, 10990–11002.

Ahissar, E., Sosnik, R., and Haidarliu, S. (2000). Transformation from temporal to rate coding in a somatosensory thalamocortical pathway. Nature 406, 302–306.

Alloway, K. D., Olson, M. L., and Smith, J. B. (2008). Contralateral corticothalamic projections from MI whisker cortex: Potential route for modulating hemispheric interactions. Journal of Comparative Neurology 510, 100–116.

Armstrong-James, M., and George, M. J. (1988). Bilateral receptive fields of cells in rat Sm1 cortex. Exp Brain Res 70, 155–165.

Bolkan, S. S., Stujenske, J. M., Parnaudeau, S., Spellman, T. J., Rauffenbart, C., Abbas, A. I., et al. (2017). Thalamic projections sustain prefrontal activity during working memory maintenance. Nature Neuroscience 20, 987–996.

Bourassa, J., Pinault, D., and Deschênes, M. (1995). Corticothalamic Projections from the Cortical Barrel Field to the Somatosensory Thalamus in Rats: A Single-fibre Study Using Biocytin as an Anterograde Tracer. European Journal of Neuroscience 7, 9-30.

Brecht, M., Roth, A., and Sakmann, B. (2003). Dynamic receptive fields of reconstructed pyramidal cells in layers 3 and 2 of rat somatosensory barrel cortex. J Physiol 553, 243–265.

Brecht, M., and Sakmann, B. (2002). Dynamic representation of whisker deflection by synaptic potentials in spiny stellate and pyramidal cells in the barrels and septa of layer 4 rat somatosensory cortex. The Journal of Physiology 543, 49–70.

Carvell, G. E., and Simons, D. J. (1987). Thalamic and corticocortical connections of the second somatic sensory area of the mouse. Journal of Comparative Neurology 265, 409–427.

Carvell, G. E., and Simons, D. J. (1990). Biometric analyses of vibrissal tactile discrimination in the rat. J. Neurosci. 10, 2638–2648.

Casas-Torremocha, D., Porrero, C., Rodriguez-Moreno, J., García-Amado, M., Lübke, J. H. R., Núñez, Á., et al. (2019). Posterior thalamic nucleus axon terminals have different structure and functional impact in the motor and somatosensory vibrissal cortices. Brain Struct Funct 224, 1627–1645.

Castejon, C., Barros-Zulaica, N., and Nuñez, A. (2016). Control of Somatosensory Cortical Processing by Thalamic Posterior Medial Nucleus: A New Role of Thalamus in Cortical Function. PLOS ONE 11, e0148169.

Castejon, C., and Nunez, A. (2020). Higher-order thalamic implication in the codification of bilateral sensory events. bioRxiv https://doi.org/10.1101/2020.05.01.073098.

Clancy, K. B., Schnepel, P., Rao, A. T., and Feldman, D. E. (2015). Structure of a single whisker representation in layer 2 of mouse somatosensory cortex. J. Neurosci. 35, 3946–3958.

Clascá, F., Porrero, C., Galazo, M. J., Rubio-Garrido, P., and Evangelio, M. (2016). Anatomy and development of multi-specific thalamocortical axons: implications for cortical dynamics and evolution. In: Rockland KS (ed) Axons and brain architecture. Elsevier, Amsterdam, pp 69–92.

de Kock, C. P. J., Bruno, R. M., Spors, H., and Sakmann, B. (2007). Layer- and cell-type-specific suprathreshold stimulus representation in rat primary somatosensory cortex. J. Physiol. (Lond.) 581, 139–154.

Debowska, W., Liguz-Lecznar, M., and Kossut, M. (2011). Bilateral Plasticity of Vibrissae SII Representation Induced by Classical Conditioning in Mice. J Neurosci 31, 5447–5453.

Diamond, M. E., Armstrong-James, M., Budway, M. J., and Ebner, F. F. (1992). Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus: dependence on the barrel field cortex. J. Comp. Neurol. 319, 66–84.

Fuster, J. M., Bauer, R. H., and Jervey, J. P. (1982). Cellular discharge in the dorsolateral prefrontal cortex of the monkey in cognitive tasks. Exp. Neurol. 77, 679–694.

Goldman-Rakic, P.S. (1987). Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. In Handbook of physiology, the nervous system, higher functions of the brain (ed. F. Plum), sect. I, vol. V, pp. 373-417. Bethesda, MD: American Physiological Society.

Groh, A., Bokor, H., Mease, R. A., Plattner, V. M., Hangya, B., Stroh, A., et al. (2014). Convergence of cortical and sensory driver inputs on single thalamocortical cells. Cereb. Cortex 24, 3167–3179.

Guo, Z. V., Inagaki, H. K., Daie, K., Druckmann, S., Gerfen, C. R., and Svoboda, K. (2017). Maintenance of persistent activity in a frontal thalamocortical loop. Nature 545, 181–186.

Hoerder-Suabedissen, A., Hayashi, S., Upton, L., Nolan, Z., Casas-Torremocha, D., Grant, E., et al. (2018). Subset of cortical layer 6b neurons selectively innervates higher order thalamic nuclei in mice. Cereb. Cortex 28, 1882–1897.

Kinnischtzke, A. K., Simons, D. J., and Fanselow, E. E. (2014). Motor cortex broadly engages excitatory and inhibitory neurons in somatosensory barrel cortex. Cereb. Cortex 24, 2237–2248.

Liao, C.-C., Chen, R.-F., Lai, W.-S., Lin, R. C. S., and Yen, C.-T. (2010). Distribution of large terminal inputs from the primary and secondary somatosensory cortices to the dorsal thalamus in the rodent. J. Comp. Neurol. 518, 2592–2611.

Mao, T., Kusefoglu, D., Hooks, B. M., Huber, D., Petreanu, L., and Svoboda, K. (2011). Long-range neuronal circuits underlying the interaction between sensory and motor cortex. Neuron 72, 111–123.

Masri, R., Bezdudnaya, T., Trageser, J. C., and Keller, A. (2008). Encoding of stimulus frequency and sensor motion in the posterior medial thalamic nucleus. J. Neurophysiol. 100, 681–689.

Ohno, S., Kuramoto, E., Furuta, T., Hioki, H., Tanaka, Y. R., Fujiyama, F., et al. (2012). A morphological analysis of thalamocortical axon fibers of rat posterior thalamic nuclei: a single neuron tracing study with viral vectors. Cereb. Cortex 22, 2840–2857.

Paxinos, G., and Watson, C. (2007). The rat brain in stereotaxic coordinates. San Diego: Academic Press.

Peron, S. P., Freeman, J., Iyer, V., Guo, C., and Svoboda, K. (2015). A Cellular Resolution Map of Barrel Cortex Activity during Tactile Behavior. Neuron 86, 783–799.

Petersen, C. C. H., and Crochet, S. (2013). Synaptic computation and sensory processing in neocortical layer 2/3. Neuron 78, 28–48.

Petreanu, L., Huber, D., Sobczyk, A., and Svoboda, K. (2007). Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. Nat. Neurosci. 10, 663–668.

Porter, L. L., and White, E. L. (1983). Afferent and efferent pathways of the vibrissal region of primary motor cortex in the mouse. J. Comp. Neurol. 214, 279–289.

Rikhye, R. V., Gilra, A., Halassa, M.M. (2018). Thalamic regulation of switching between cortical representations enables cognitive flexibility. Nature Neuroscience 21, 1753–1763.

Schmitt, L. I., Wimmer, R. D., Nakajima, M., Happ, M., Mofakham, S., and Halassa, M. M. (2017). Thalamic amplification of cortical connectivity sustains attentional control. Nature 545, 219–223.

Sherman, S. M., and Guillery, R. W. (1998). On the actions that one nerve cell can have on another: Distinguishing "drivers" from "modulators." PNAS 95, 7121–7126.

Sherman, S. M., Guillery, R. W., (2006). Exploring the thalamus and its role in cortical function. 2nd ed. Cambridge, Mass: MIT Press.

Shuler, M. G., Krupa, D. J., and Nicolelis, M. A. L. (2001). Bilateral Integration of Whisker Information in the Primary Somatosensory Cortex of Rats. J. Neurosci. 21, 5251–5261.

Sobolewski, A., Kublik, E., Swiejkowski, D. A., Kamiński, J., and Wróbel, A. (2015). Alertness opens the effective flow of sensory information through rat thalamic posterior nucleus. Eur. J. Neurosci. 41, 1321–1331.

Theyel, B. B., Llano, D. A., and Sherman, S. M. (2010). The corticothalamocortical circuit drives higher-order cortex in the mouse. Nat. Neurosci. 13, 84–88.

Veinante, P., Jacquin, M. F., and Deschênes, M. (2000a). Thalamic projections from the whisker-sensitive regions of the spinal trigeminal complex in the rat. J. Comp. Neurol. 420, 233–243.

Veinante, P., Lavallée, P., and Deschênes, M. (2000b). Corticothalamic projections from layer 5 of the vibrissal barrel cortex in the rat. J. Comp. Neurol. 424, 197–204.

Zhang, W., and Bruno, R. M. (2019). High-order thalamic inputs to primary somatosensory cortex are stronger and longer lasting than cortical inputs. eLife 8, e44158.

Zolnik, T. A., Ledderose, J., Toumazou, M., Trimbuch, T., Oram, T., Rosenmund, C., et al. (2020). Layer 6b Is Driven by Intracortical Long-Range Projection Neurons. Cell Reports 30, 3492-3505.e5.