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Psychophysiological reactivity to auditory Binaural Beats stimulation in the alpha and theta EEG brain-wave frequency bands:

A randomized, double–blind and placebo–controlled study in human healthy young adult subjects

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The only way to get out is to get through.

Friedrich Salomon “Fritz“ Perls

* 8th July 1893 (Berlin) † 14th May 1970 (Chicago)

Abstract

Background
Binaural beats are an acoustical illusion of the perception of a “virtual” third tone, fluctuating (i.e. *beating*) in its volume evoked by two carrier–sinusoids of same amplitudes, but slightly different frequencies f_1 and f_2, presented by stereo-headphones. Although this illusion was discovered as early as 1839 by Dove and, after the discovery of the EEG, visual rhythmic stimulation by stroboscopic flicker was known to evoke *photic driving,* until today relatively few works with moreover contradictory results have been published searching for specific Binaural Beat effects on the organism.

Objective
The present investigation aimed, in a highly controlled laboratory study with multimodal measurements, for deciding on the question whether there is or there is not specific psychophysiological reactivity to Binaural Beats auditory stimulation in the EEG alpha and theta frequency bands, striving for the overcoming of unsatisfactory methodology of previous works. If *efficacy* to specifically decrease psychophysiological *arousal* levels would be proven, multiple applications in clinical contexts would be feasible. Moreover, the employed stimuli would be useful for basic research trying to better understand the underlying neurocircuitry of arousal, attention and consciousness regulation.

Methods
After rigorous psychometric screening for *bio–psycho–social* health, N = 12 young university students (5 females) entered in a *within*–subject randomized placebo–controlled design, with 50% of the subjects randomly assigned to the presentation order *Placebo* vs. *Verum,* and the remaining 50% vice versa. *Placebo* and *Verum* sessions took place with more than one week time interval. The 25 min *Verum* stimulus consisted of Binaural Beats carrier frequencies equivalent to a sweep from 10 Hz to 4 Hz embedded into a special dynamic noise mask, while under *Placebo* only this mask was presented without Binaural Beats for 25 min. 63–channel EEG, ECG and respiratory flow by nasal cannulae were continuously recorded at 1024 Hz common sample frequency. Pre– vs. post saliva samples were collected by passive drool technique and immediately *flash frozen* by immersion into liquid nitrogen. Hypnoidal state depth was measured by retrospective psychometry immediately after each experiment using the *Phenomenology of Consciousness Inventory* (PCI) of Pekala (1991).

Results
Logit–transformed EEG relative spectral powers showed only under *Verum* significant *change over time* with distinguishable scalp topographies, a linear increasing trend in slower vs. a linear decreasing trend in faster EEG frequency bands and meaningful correlations with the psychometric effects in hypnoidal state depth. Significant *change over time* in the parasympathetic parameter HRV–HF power derived from ECG was only found under *Verum.* These HRV findings are not confounded by changes in respiratory frequency which showed no significant *change over time*. Pre–vs. post saliva samples revealed significant increases only under Placebo in stress–related biomarkers (Cortisol, α–Amylase and Salivary Secretory Immunoglobulin A, SIgA), but not under *Verum*. Significant increases under *Verum* as compared to *Placebo* were observed in psychometric hypnoidal state depths with a large effect size of r = .513 and difference scores showed a significant and large *Spearman’s* rank order correlation of *rho* = .671 and p_{exact} = .020 with hypnotizability as operationalized as psychometric hypnoidal state depths under *Placebo*. Hypnotizability is thus a predictor of reactivity magnitudes to Binaural Beat auditory stimulation. Presentation order effects could be excluded for all reported effects.

Conclusions
Multimodal evidence was found for the searched specific efficacy of Binaural Beats auditory stimulation in the EEG alpha and theta frequency range. Linear trends in EEG relative spectral powers suggest that only the *Verum* stimulus caused significant decreases in (cortical) arousal. The significant increase of stress–related biomarkers only under *Placebo* could be interpreted as a sign that the *Verum* stimulus inhibited processes which naturally occurred under *Placebo.* Both the *Placebo* and *Verum* of the present investigation should be used as a nonverbal *culture–free* paradigm for neurocientific basic *laboratory studies* on hypnosis and hypnotizability especially, but also on *resting–state networks* and related neurocircuitry generally. The ability of the *Verum* stimulus to decrease arousal levels and induce/boost altered states of consciousness (ASCs) implies applications in several practical–clinical contexts and calls for further *field studies*.

Resumen

Antecedentes

Los pulsos binaurales o *Binaural Beats* son una ilusión acústica que consiste en la percepción de un tercer tono "virtual" que fluctúa (*beating*) en volumen, inducido por dos *ondas portadoras* (f_1 y f_2) de la misma amplitud, pero con frecuencias ligeramente distintas, presentadas en cada uno de los oídos mediante auriculares estéreo. Aunque esta ilusión fue descubierta por Dove en 1838 y después del descubrimiento del EEG se conoce que la estimulación visual por luz estroboscópica provoca *photic driving*, hasta ahora se han publicado pocos trabajos sobre los efectos de los *Binaural Beats* en el organismo, y además con resultados contradictorios.

Objectivos

La investigación en esta tesis tiene como objeto realizar un estudio de laboratorio en condiciones altamente controladas y con registros multimodales para averiguar si hay o no una respuesta psicofisológica a la estimulación auditiva con *Binaural Beats* en las frecuencias *alfa* y *theta* del EEG. La metodología empleada intenta resolver los problemas de investigaciones previas. La demostración de la *eficacia* de los *Binaural Beats* para disminuir específicamente los niveles de *arousal* psicofisiológico daría pie a muchas aplicaciones clínicas. Además, este paradigma de estimulación auditiva puede contribuir a la investigación básica de la neurocircuitería del *arousal*, de la atención y de la regulación de la consciencia.

Métodos

Se reclutaron *N* = 12 jóvenes estudiantes universitarios (5 mujeres) comprobando rigorosamente su salud *bio–psico–social*. El estudio siguió un diseño *intrasujeto*–aleatorizado y controlado por *Placebo*, en que se asignó aleatoriamente a la mitad de los voluntarios un orden de presentación *Placebo* vs. estímulo con *Binaural Beats*, mientras a la otra mitad el orden contrario. Estas dos sesiones experimentales se realizaron al menos con una semana de separación temporal. Los 25 minutos del estímulo con *Binaural Beats* contienen un barrido de 10 Hz a 4 Hz (decreciente) con enmascaramiento auditivo dinámico, mientras que los 25 minutos del *Placebo* contienen solamente la máscara auditiva. Se registró el EEG con 63 canales, el ECG y el flujo respiratorio mediante cánulas nasales a una frecuencia de muestreo común de 1024 Hz. Se tomaron muestras de saliva *pre* vs. *post* mediante la técnica de *passive drool* que fueron inmediatamente ultracongeladas por inmersión en nitrógeno líquido. El nivel del estado hipnótico se midió por psicometría retrospectiva inmediatamente después de cada sesión experimental con el test *Phenomenology of Consciousness Inventory* (PCI) de Pekala (1991).

Resultados

El análisis de la potencia espectral relativa del EEG sometida a la transformación *logit* mostró que existen cambios significativos en su evolución temporal con topografías espaciales distinguibles solamente bajo la estimulación con *Binaural Beats* presentes y no bajo *Placebo.* Además, se observó un incremento de la pendiente de la tendencia lineal en las bandas del EEG más *lentas* y una bajada de la pendiente en frecuencias más *rápidas*. Se encuentraron correlaciones significativas y relevantes con los efectos psicométricos del nivel del estado hipnótico. En el parámetro parasimpático *HRV–HF power* derivado del ECG se encuentron también cambios significativos en su evolución temporal exclusivamente bajo el estímulo con *Binaural Beats*. Estos cambios no se deben a variaciones en la frecuencia respiratoria puesto que en ella no se apreciaron cambios significativos. En las muestras de la saliva *pre* vs. *post* se observaron incrementos significativos en los biomarcadores salivares de estrés (cortisol, α–amylasa e inmunoglobulina A secretora, SIgA) solamente bajo *Placebo.* Se encuentraron incrementos significativos de los niveles del estado hipnótico con un tamaño del efecto r =.513 solamente bajo el estímulo con *Binaural Beats*, pero no bajo *Placebo.* Con un *rho* de *Spearman* de .671 y p_{exact} = .020, las puntuaciones de las diferencias de los niveles del estado hipnótico correlacionan altamente con la *hipnotizabilidad* operacionalizada como nivel del estado hipnótico bajo *Placebo.* Por tanto, el rasgo hipnotizabilidad se identificó como predictor de las magnitudes de las reacciones a la estimulación con *Binaural Beats.* Para todos los efectos mencionados, se pudo descartar la influencia del orden de presentación de los estímulos.

Conclusiones

Esta tesis proporciona evidencia multimodal de la *eficacia* de la estimulación auditiva con *Binaural Beats* en las bandas del EEG *alfa* y *theta.* Las pendientes de las tendencias lineales en las potencias espectrales relativas del EEG sugieren que esta estimulación causa una disminución del *arousal* (cortical). El incremento de los biomarcadores de saliva relacionados con el estrés solo en el caso de *Placebo* sugiere que la estimulación con *Binaural Beats* inhibe procesos que ocurren naturalmente bajo *Placebo.* Tanto el *Placebo* como el estímulo con *Binaural Beats* pueden emplearse como un paradigma no verbal *culture–free* en especial para estudios de neurociencia básica respecto a la hipnosis y la hipnotizabilidad, y, en general respecto a *resting–state networks* y la neurocircuitería relacionada. El hecho de que el estímulo con Binaural Beats puede disminuir el *arousal* e inducir *estados alterados de la consciencia* sugiere aplicaciones en múltiples contextos clínicos y futuros *estudios de campo.*

Contents

1. Introduction

1.1. What is arousal vs. hyperarousal?

Chronic psychophysiological *hyperarousal*, the repeated and prolonged hyperactivation of multiple physiological systems e.g. by stimuli which are perceived as threatening or as exceeding the subject´s available resources (*stress* stimuli), can lead to serious impacts on multiple dimensions of *bio-psycho-social* health outcomes. With regard to impacts on the cardiovascular and immune system (specially infectious diseases and cancer), sufficient evidence has been found that chronic psychophysiological hyperarousal plays an important role as moderator or mediator variable for *morbidity* and even *mortality* (e.g. Broadbent, Petrie, Alley, & Booth, 2003; Dusseldorp, van Elderen, Maes, Meulman, & Kraaij, 1999; Lillberg et al., 2003; Ming et al., 2004; Rosengren et al., 2004 [the *INTERHEART* study]).

Even more alarming is the evidence that exposure to chronic stress can cause damage in specially vulnerable and sensitive brain structures such as the *hippocampus:* Although there is a remarkable body of morphometric MRI observations that e.g. traumatized human war veterans with combat-related *posttraumatic stress disorder* (PTSD) show reduced hippocampi volumes as compared to healthy controls (e.g. evidence for the *parahippocampal gyrus,* Aupperle, Connolly, Stillman, May, & Paulus, 2013)*,* doubts concerning the direction of causality in these correlational observational studies are raised (e.g. Childress et al., 2013): Do these patients just have smaller hippocampi volumes at baselines in the sense of naturally occurring *traits*, i.e. *before* being traumatized, which then makes them more vulnerable to develop PTSD after being exposed to the event, or are the observed hippocampus volume reductions a *consequence* of the chronic stress, produced afterwards by the disease? This question is more likely to be answered with the second option because of evidence from animal studies which also used morphometric MRI: E.g. Lee, Jarome, Li, Kim and Helmstetter (2009) found in a longitudinal within-subjects design significant reductions in rats' hippocampal volumes due to chronic restraint stress which were not observed in control animals. The magnitudes of these reductions were not correlated with baseline hippocampal volumes

while neither other brain regions, specially the forebrain, nor adrenal glands showed any stress related volume changes. These experimental animal data suggest that at least the hippocampus is a brain structure which is differentially vulnerable and sensitive to chronic stress exposure. Interestingly, these hippocampal volume reductions due to chronic stress seem not to be caused by loss of hippocampal neurons itselves, but to dendritic retractions (i.e. reduced dendritic bifurcations and shortening of the total dendritic length) and to loss of synapses, which correlate to magnitudes of chronic stress induced spatial memory deficits in rats (Conrad, 2006; McLaughlin, Gomez, Baran, & Conrad, 2007).

Uchino, Smith, Holt-Lunstad, Campo and Reblin (2007) proposed a path model which explains principle interactions between mediator/moderator processes as reactions to acute and chronic stress stimuli which can trigger a dynamics leading to morbidity and mortality (see figure 1).

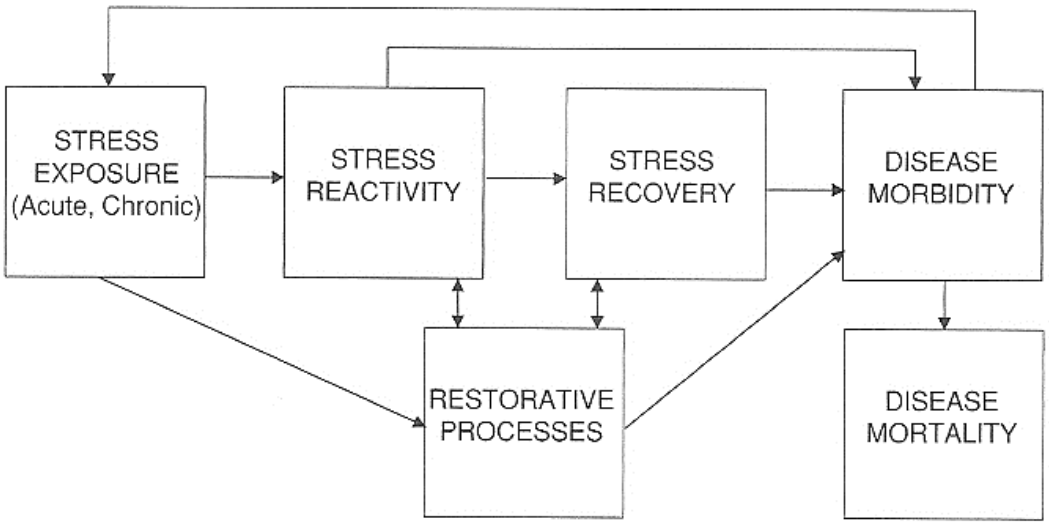


Figure 1. Path model of principle interacting mediators/moderator processes as reactions to acute and chronic stress stimuli which can cause a dynamic leading to morbidity and mortality (Uchino et al., 2007).

Multiple highly interacting physiological systems are involved in these severe consequences of stress. Following Uchino et al. (2007), three of them seem to be the most relevant mediators for pathogenesis in the effector organs: (a) the autonomic nervous system (ANS),

(b) the neuroendocrinological system, especially its hypothalamus–pituitary–adrenal–axis (HPA) and (c) the immune system.

Nevertheless, it is the central nervous system (CNS) which interprets a perceived stimulus as threatening or as exceeding the subjectively available resources of the organism. Although multiple brain subsystems participate in the central coordination of the organism's psychophysiological reactions to stress stimuli, Uchino et al. (2007) report a simplified model of bidirectional interactions, e.g. as efferent and afferent projections or *top-down* vs. *bottom-up* processes, between the most relevant cerebral structures (see figure 2).

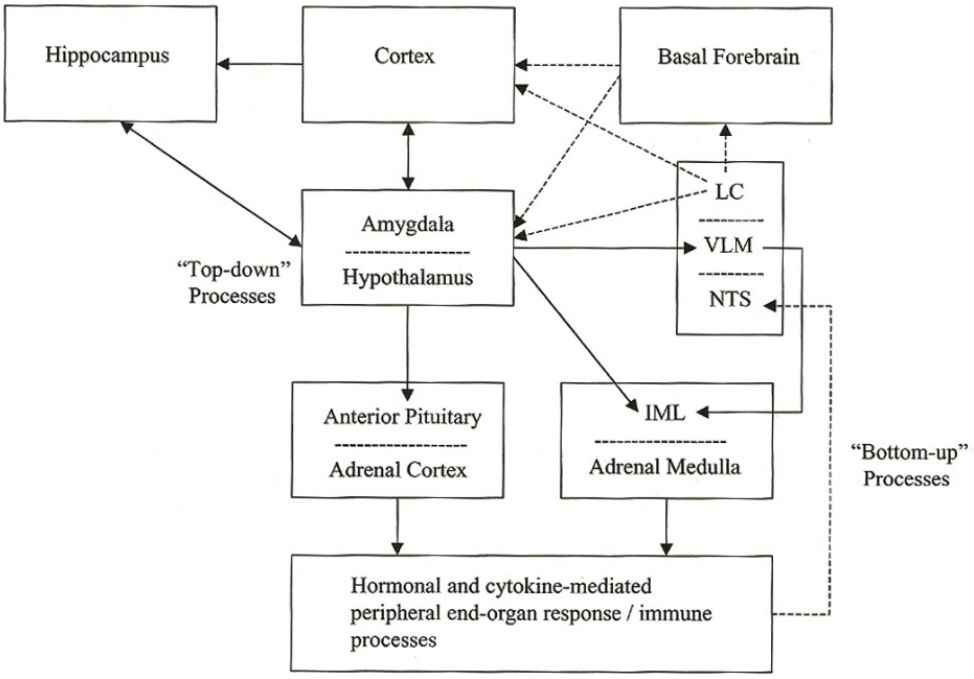


Figure 2. Simplified model of the central coordination of psychophysiological reactions to stress stimuli with bidirectional interactions between brain structures and the organism's peripheral physiologic systems; efferent projections as *solid* lines and afferent as *broken* lines (Uchino et al., 2007). Abbreviations: IML= sympathetic preganglionic neurons of the intermediolateral cell column, LC = locus coeruleus, NTS = nucleus tractus solitarus, and VLM = ventral lateral medulla.

Apart from a wide range of other involved neurotransmitters and hormones, it is the 41-amino-acid-polypeptide *corticotropin-releasing hormone* (CRH) which plays a central role in the organism's response to stress: Experimental central instillation of CRH activates two of the mentioned three most relevant physiological systems responsible for the outcomes of

stress stimuli in the effector organs: (a) the autonomic nervous system (ANS) and (b) the hypothalamus–pituitary–adrenal–axis (HPA) (Irwin, Segal, Hauger, & Smith, 1989).

The principle HPA mechanisms are described in the following: CRHnergic neuronal afferences from the hypothalamus' *nucleus paraventricularis* transduce their electrophysiological input of *action potentials* into a chemical-neuroendocrinological CRH output signal by the process of *neurosecretion* into the system of the *venae portales hypophysiales*, thus reaching directly via the blood stream the target, the CRH1-rezeptor of the basophile cells of the *anterior pituitary* gland in high concentrations without being diluted in the systemic circulation. These cells react with the liberation of the 39-amino-acid polypeptide *adrenocorticotropic hormone* (ACTH) which activates its G protein-coupled receptor (cAMP as a secondary messenger) in the *zona fasciculata* of the adrenal cortex, the *melanocortin receptor 2,* leading to an increase of adrenal steroid hormone secretion, namely the glucocorticoid *cortisol*, by short-term regulations (minutes) and long-term mechanisms. Glucocorticoids inhibit in a negative feedback regulation their own triggers, i.e. the secretion of CRH and ACTH. Cortisol has very broad effects on the organism, which are slower as compared to the *catecholamine* system with its G protein-coupled receptors because the glucocorticoid receptor *NR3C1* is located intracellularly in the cells' cytosol and transmits the cortisol effects mainly by regulation of gene transcription in the cells' nucleus or by inhibiting the expression of proteins into the cytosol. Although the plasma half-life of cortisol is approx. one hour, it has long-term effects mediated by this modulation of gene transcription and its consequences.
In this way, cortisol mainly down regulates immune functions, suppresses inflammatory processes, e.g. by down-regulation of the interleukin-2 receptor (IL-2R) on the helper (CD4+) T-cells which impairs the up-regulation of the humoral Th2 immune response by its ligand interleukin-2 with the consequence of a shift towards cellular Th1 immune dominance with the effect of reduced B-cell antibody production. Furthermore, cortisol increases blood sugar levels by activation of the conversion (*glycogenolysis*) of the polysaccharide energy storage *glycogen*

into glucose (as it also does *adrenaline*) in muscle and liver tissues and by activating the *gluconeogenesis* which synthesizes glucose from non-carbohydrate substrates (e.g. from pyruvate, lactate, glycerol, some amino acids etc.), decreases bone formation etc. As all stress hormones do, cortisol leads to a *catabolic* state of metabolism aiming to release stored energy supporting the organism in a *fight-flight reaction* while at the same time suppressing *anabolic* processes, typically related to the behavioural domain of *rest–digest–reproduction.*

Apart from the described *hypothalamus – pituitary – adrenal* axis (HPA) which uses ACTH as a chemical signal via the systemic blood circulation to modulate the cortisol secretion in the *zona fasciculata* of the adrenal gland within a reaction time of minutes or more*,* the *chromaffin cells* in the *medulla glandulae suprarenalis* can liberate within seconds after a stress stimulus via neurosecretion a mixture of 80% adrenaline and 20% noradrenalin directly into the systemic blood circulation, after receiving direct neuronal input from the autonomic nervous system via innervation by noradrenergic sympathetic fibres. Although adrenaline has with one to three minutes a relatively short plasma half-life, it has very broad effects on multiple physiological systems, all of them are mediated by *adrenorecpetors.* Most important, in the cardiovascular system adrenaline causes a redistribution of the blood volume and rising blood pressure by vasoconstriction of arterioles via α_1-adrenoreceptors namely in the skin and kidneys while it evokes vasodilatation in central and muscle arteries via β_2-adrenoreceptors (*centralization*). The activation of β_1-adrenoreceptors in the heart increases heart rate (*positive chronotropic* effect*),* accelerates neurotransmission in the heart (*positive dromotropic*) while lowering excitation thresholds (*positive bathmotropic*) and it also increases the contractility of the myocardium (*positive inotropic*). Moreover, besides many more effects, adrenaline mainly leads to bronchodilatation, increased liberation and biosynthesis of glucose, increase of lipolysis and inhibits the peristaltic movements of the gastrointestinal system. Noradrenaline, which lacks, as compared to adrenaline, the methyl group at the amino group, causes as a hormone in systemic blood circulation mainly vasoconstriction of arterioles leading to increase of blood pressure.

Interestingly, CRHnergic neurons and CRH receptors are not only found in the *hypothalamus*, but also in the *amygdala* and the *locus coeruleus* (Gray, 1993; Menzaghi, Heinrichs, Pich, Weiss, & Koob, 1993; Valentino, Foote, & Page, 1993), brain structures involved in stress related behaviour such as *freezing* (Swiergiel, Takahashi, & Kalin, 1993), loss of appetite (Krahn, Gosnell, Grace, & Levine, 1986), inhibition of sexual desire and activity (Sirinathsinghji, Rees, Rivier, & Vale, 1983), increase of body care behaviours (Holahan, Kalin, & Kelley, 1997), increase of magnitudes of the *startle response* (Y. Lee & Davis, 1997), etc.

The central role of CRH in the organism's stress responses has been demonstrated in non-human primates by oral administration of the non-peptide CRH-1 receptor blocker *antalarmin* during exposure to stress stimuli, inhibiting the typical stress related behaviours mentioned above (Habib et al., 2000).

One mechanism which can explain the effects of stress stimuli onto the third of the three relevant physiological systems mentioned above, the immune system, is that central *adrenergic* processes increase the secretion of *interleukin IL-6* (also named as *interferon beta-2*) (Soszynski, Kozak, Conn, Rudolph, & Kluger, 1996) which illustrates how stress can not only *suppress* immune functions via e.g. cortisol, but also *increase* inflammatory processes (e.g. allergic reactions).

Readers interested in further details of the endocrinology of the stress response, including effects on thyroid function which were not described here in order to avoid exceeding the scope of the present introduction, are recommended to study the excellent and free available review of Charmandari, Tsigos and Chrousos (2005) and the manifold references quoting it.

Apart from the outlined *top-down* regulations of stress reactions, also many feedback mechanisms from peripheral organs/tissues towards central brain structures (*bottom-up*) have been discovered, mainly mediated by the prosencephalon's/forebrain's cholinergic systems which themselves are bidirectionally connected with the amygdala and by long-distance

connections with the neocortex (Gary G Berntson, Sarter, & Cacioppo, 2003).

Defining *hyperarousal* as the organism's complex and multimodal reaction to stress stimuli, i.e. stimuli subjectively perceived as threatening, which basically is a phylogenetically useful adaptation of the organism to dangerous situations in order to increase the change to survive, there is also *arousal* which refers to activation processes being triggered when the organism is exposed to stimuli subjectively perceived as not yet threatening, but activating/stimulating. The magnitudes of the organism's reactions, the *arousal* evoked by these stimuli still being in the normal range, depend on the one hand of the magnitudes of the sensorial input, on individual levels of psychophysiological reactivity and on diferent individual (cognitive) interpretations, and on the other hand, as moderator processes, on the attentional resources being voluntarily or involuntarily allocated to these stimuli. In contrast to levels of *hyperarousal*, the most relevant effects of different levels of *arousal* onto peripheral effector organs are mediated by the autonomic nervous system's noradrenergic sympathetic and acetylcholinergic parasympathetic innervation (see fig. 3).

At relatively lower levels of arousal, e.g. in a calm resting state, regulation is mainly achieved by withdrawal vs. increase of the so-called *vagal-brake* of the parasympathicus which refers to the perpetual physiologic inhibition of relevant processes, which can be disinhibited for regulation purposes, i.e. the withdrawal of a constant brake. Generally spoken, parasympathetic and sympathetic regulation can interact antagonistically, synergistically and also independently, although predominantly a synergistic mode is preferred (Berntson, Cacioppo, & Quigley, 1993; Berntson, Cacioppo, & Quigley, 1991). Nevertheless, in a calm resting state, normal physiological autonomic regulation is mainly done via parasympathetic modulation, while at higher levels of arousal sympathetic regulation joins more and more importantly.

One of the brain structures most relevant for how much *arousal* is being evoked by how much of an activating, but not threatening sensorial input is the *ascending reticular arousal system* (ARAS), part of the *formatio*

reticularis. While the ARAS is a wide-spread diffuse neuron network reaching from the *medulla oblongata* upto the *diencephalon,* the other parts of the *formatio reticularis* are both the nucl*eus centralis superior* and the nucleus raphe dorsalis, and also the *locus coeruleus* located in the *pons.*

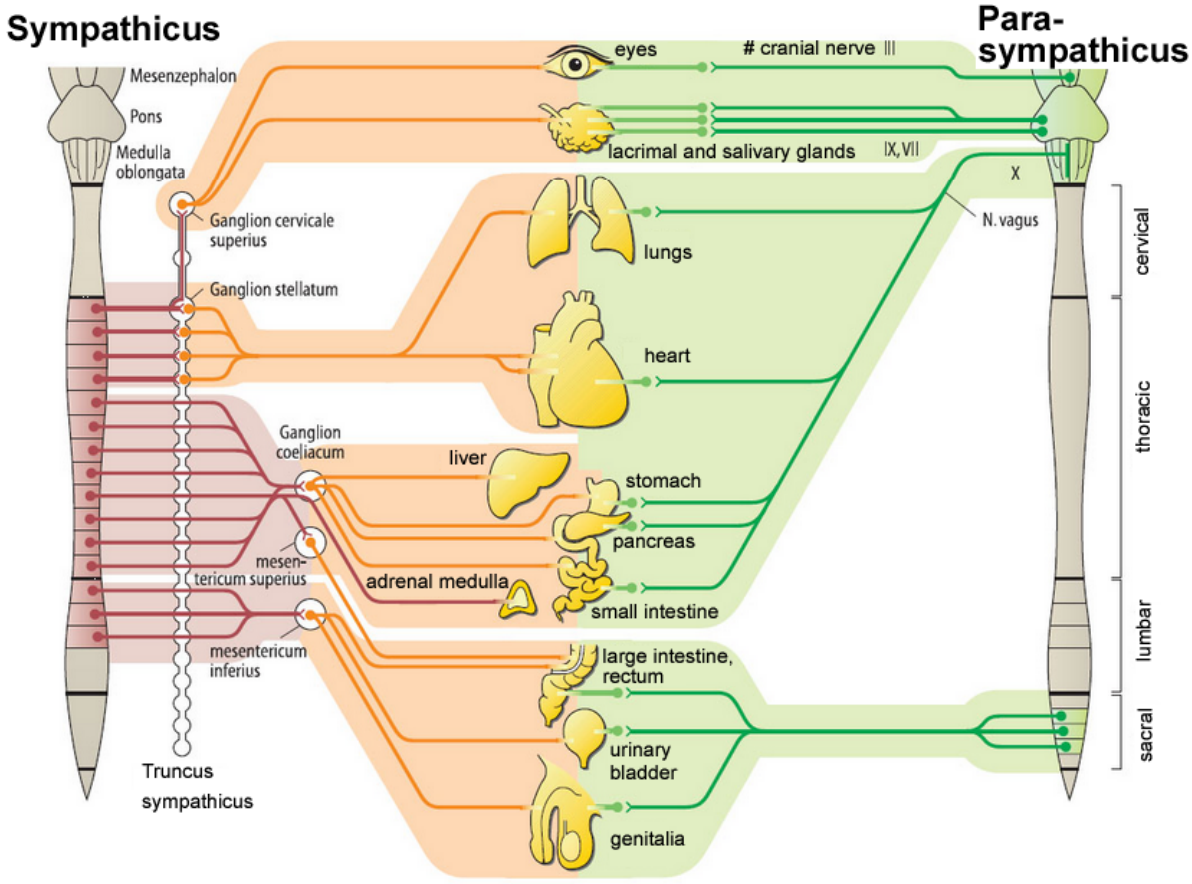


Figure 3. Sympathetic (*fight-or-flight reaction*) vs. parasympathetic (*rest-digest-reproduction*) innervation of the autonomic nerve system's (ANS) effector organs. Red and dark green lines = *pre*ganglionic axons using the neurotransmitter acetylcholine at their synapses both for parasympathicus and sympathicus; orange and light green lines = *post*ganglionic axons using at their synapses acetylcholine for the parasympathicus, while noradrenaline is used for the sympathicus. The following structures are not mentioned in this scheme: Arterial blood vessels are mainly innervated by sympathetic fibres, although some as e.g. the coronary vessels receive also parasympathetic inputs. The eccrine sweat glands being distributed all over the human body use, as the only exception for the sympathicus, acetylcholinergic synapses, whereas the apocrine sweat glands which in humans only exist in the axillae, mamillae and perigenital/perianal regions and which are more important in other mammal species for pheromone signals (being mainly activated by emotions such as excitation, anxiety or anger) use the sympathicus' usual noradrenaline. The musculi arrectores pilorum, responsible for the goose-pimples reaction of the skin, are activated by noradrenergic sympathetic fibres (figure translated into English from Birbaumer & Schmidt, 2006).

The *ARAS* projects e.g. to the *thalamus*, *neocortex* and *basal forebrain* (Edlow et al., 2012) which corresponds to its functions in regulating states of consciousness, e.g. sleep-wake transitions, and regulating levels of attention/activation etc., predominantly corresponding to the magnitudes of sensorial inputs.

Different levels of arousal modulate the entire organism via those described cerebral structures modulated by the ARAS, so not only the activity level of the neocortex is influenced, but also all effector organs of the autonomic nervous system and the neuroendocrinological system, although the further (and more precisely its parasympathetic pathways) plays the most important part in normal resting state conditions.

Generally spoken, changes in arousal levels correspond to changes in the described neuroanatomical structures within the entire body and their complex functioning. To represent the most important domains of physiological systems, changes in arousal level can be observed mainly in changes of *EEG* (higher levels of arousal correspond to predominance of faster brain wave frequencies and phenomena of desynchronization and vice versa for lower levels of arousal), in changes of *heart rate variability* (mainly due to parasympathetic modulation) and decrease of (salivary) cortisol concentrations (Cacioppo, Tassinary, & Berntson, 2007).

As mentioned above, stimuli within the "normal" range of magnitudes can provoke simple psychophysiological activation with increased supply of resources or energies, still away from magnitudes causing an alarm reaction. This range of magnitudes causes *arousal(s)* and is also called *eu*stress, good or healthy stress. When the magnitude of stress stimuli exceeds this range, being evaluated as threatening, now an alarm reaction is triggered which prepares the organism for short-term and stereotypical *fight-or-flight* reactions, but on the long-term leads to decreases of performance with tiredness, then exhaustion and finely fatal collapse of the organism. These levels of activation are called *hyperarousal* or *di*stress, bad, damaging or unhealthy stress. The relationship between levels of the organism's psychophysiological activation and its performance can be

described as an inverse u-shaped function, conceptualized in the famous *law of Yerkes-Dodson,* see figure 4. The optimum point is different for every subject and every situation/paradigm, but some predictors were identified, e.g. personality traits such as extraversion vs. introversion (see e.g. Larsen & Buss, 2008).

In medical practice, in principle, three types of interventions are known in order to decrease states of undesired levels of psychophysiological activation, mostly for treating states of *hyper*arousal/*di*stress with their known serious health and social outcomes when persisting over a prolonged period of time:

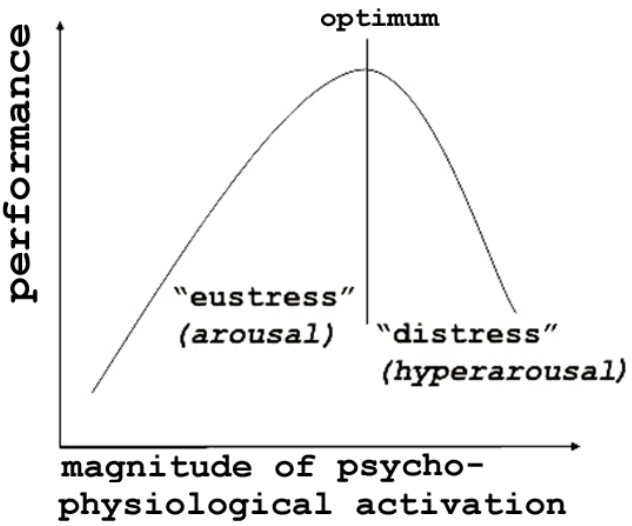


Figure 4. Law of *Yerkes-Dodson* (Yerkes & Dodson, 1908) describing the inverse u-shaped relationship between magnitudes of the organism's psychophysiological activation and its levels of performance. The optimum point is different for every subject and every situation/paradigm, but some predictors were identified, e.g. personality traits such as extraversion vs. introversion (see e.g. Larsen & Buss, 2008).

(i) pharmacological treatments, (ii) rather short-term psychotherapy with cognitive-behavioural background including relaxation or (mindful) meditation techniques and (iii) rather long-term psychotherapy with psychodynamic background aiming to first reactivate/symbolize and then solve/integrate unprocessed or even unconscious intra- and interpersonal

conflicts or situations conceptualized as hidden underlying causes ("*latent*") responsible for the observable symptoms ("*manifest"*), the *hyper*arousal.

Substances for pharmacological treatments of *hyperarousal* are addressed as *sedatives* or *tranquillizers* and basically include barbiturates (obsolete), benzodiazepines, non-benzodiazepines as e.g. buspirone, low potent and low dosed neuroleptics, some antidepressants, first generation H_1-antihistamines such as doxylamine, beta blockers and phytopharmaca as e.g. *valeriana officinalis* or *piper methysticum* ("*Kava Kava"* in polynesian cultures, see e.g. Sarris, Laporte, et al., 2013; Sarris, Stough, et al., 2013; Witte, Loew, & Gaus, 2005). Unfortunately, some of them cause serious addiction and most of them have severe side effects, so the risk-benefit relation is highly problematic, at least for long-term use purposes, i.e. more than e.g. three months. Although useful in some, mainly acute conditions, these pharmacological treatments have the disadvantage that they do not modulate relevant brain structures or peripheral-physiologic subsystems very specifically. Their potential to induce changes in the neuronal circuitry by learning processes is generally low.

On the other hand, all kinds of psychotherapy have the disadvantage that they need highly motivated patients (which in the case of e.g. prison inmates is a complicated issue), relatively high tolerance to ambiguity and a high compliance to stay with the treatment over prolonged periods of time: Psychotherapy stays and falls with the long-term active collaboration of the patient, with his/hers *self-efficacy,* with his/her possibilities to interact within the psychotherapeutic relationship and with his/her possibilities to become conscious/aware about unknown/unconscious processes in his/hers own self and his/her interpersonal relationships.

The development of new therapeutic options for the treatment of hyperarousal to complement the ones described above, aims for methods which (i) require low effort of the patients, (ii) offer acceptable risk-benefit relations and (iii) have the potential to induce learning processes with the possibility to specifically and permanently change problem–relevant neuronal circuitry (neuronal plasticity).

A promising type of interventions offers the hope to fulfill all these three aims, the *neuroregulatory interventions,* which try to help the organism to autoregulate itself towards lower levels of arousal. Using the four main dimensions of consciousness states proposed by Vaitl et al. (2005), the specific aim would be the induction of a state characterized by low psychophysiologicsal activation, a change of attentional focus from the outer surroundings towards to the subject's mental inner world, but generally low consciousness of the own self and a reduced sensorial dynamics.

A classical type of neuroregulatory interventions are *biofeedback* and, more especially, *neurofeedback* techniques (e.g. Gevensleben, Rothenberger, Moll, & Heinrich, 2012; Gruzelier, 2013, 2014a, 2014b; Ruiz, Buyukturkoglu, Rana, Birbaumer, & Sitaram, 2014; Sulzer et al., 2013; Tan, Dao, Farmer, Sutherland, & Gevirtz, 2011). Although these therapeutic strategies have shown a considerable *efficacy* in laboratory studies and also an interesting *effectiveness* in clinical field studies applied to several medical conditions, such as *attention deficit (hyperactivity) disorder* (ADHD/ADD) or substance addiction, these interventions require medical High Tech devices and almost without exception the presence of a medical expert. Another type of neuroregulatory interventions uses Low Tech methods and has been used in healing contexts probably since human kind exists: the introduction of *altered states of consciousness* (ASCs) by rhythmic sensorial stimulation. Almost all indigenous tribes all over the world use auditory (drums), somatosensory (dancing) and visual (flickering fires) stimuli to induce these ASCs. While these rituals often lead to ecstatic *hyperarousal*, for medical contexts the contrary is the aim: the state of consciousness described above within the four main dimensions of Vaitl et al. (2005). Although unfortunately the terminus "altered state" suffers from pseudoscientific or even "esoteric" connotations in uninformed circles, as *hypnosis* and *meditation* often still do, these neurophenomenological domains have been and are today under serious (neuro)cientific research: The most internationally impacting scientific protagonists and scientific journals such as *Nature* work and publish on these topics (e.g. Gruzelier, 2000; Kallio, Hyönä, Revonsuo, Sikka, & Nummenmaa, 2011; Kihlstrom,

2013; Oohashi et al., 2002; Amir Raz & Buhle, 2006; Amir Raz, Shapiro, Fan, & Posner, 2002). Perhaps an analogy from another field of science helps to understand the serious vs. pseudoscientific discourses in one same field of interest: While in astrophysics today one of the most important efforts lays in confirming predictions from theoretical and experimental-laboratory particle physics with data from very large time and space scales (and vice versa), among them the most expensive experiment which human kind ever built, the Large Hadron Collider in Geneva, and the NASA's James Webb Space Telescope for approx. 6.5 billons US-dollars replacing the old Hubble Space Telescope, *astrology* deals with the same domain, but in a pseudoscientific (and superficial) way. Thus, what separates science from pseudoscience (e.g. *astronomy* from *astrology*) is not so much the studied domain of phenomena, but rather the employed methodology and concepts, among them the most important claim of *falsifiability* of the studied hypotheses. The more a phenomenological field under study is contaminated by pseudoscientific discourses, the stronger has to be the applied methodological rigour.

Among the various auditory techniques under question whether to be efficacious to induce ASCs by rhythmical stimulation or not, there is the interesting phenomenon of a special auditory illusion effect, named *Binaural Beats* which will be studied in the present work.

1.2. What are Binaural Beats ?

The most known and ubiquitous auditory stimuli used by humans for modulating mental states, e.g. in terms of *mood management* (see e.g. Hargreaves & North, 1999; Knobloch & Zillmann, 2003; Saarikallio & Erkkilä, 2007), is *music.* Music accompanies all domains of human life, from birth to death, from war to peace, from destruction to healing,

from winning to loosing (think e.g. of sport events), for wakening up vs. promoting sleep, for providing identity in urban or rural subcultures vs. in official state ceremonies, there is underground or revolutionary music vs. there is institutionalized or conservative music. There is contemplative vs. entertaining music (in the sense of distraction), music for our most intimate/private moments of life vs. music for the most public moments, lovers often talk of "their" song, almost no movie in the cinema could work without music, no religion on earth lacks its own characteristic music and songs, every historical century has its own musical style etc. Maybe it is not exaggerated to claim that "*music* makes the world go round!" Perhaps our capacity to produce, to feel and to socially share music is one property which most makes us humans: The production of music seems to be as old as human kind itself, or even maybe older, i.e. instrument using primates such as the *homo erectus* or even the former *homo ergaster*.

Musical stimuli have remarkably broad psychophysiological effects on the organism, Faienza (2005) reviews studies confirming effects on e.g. *heart rate variability* (HRV), *electrodermal activity* (EDA), respiration*,* blood pressure*, electromyography* (EMG), peripheral body temperature, vestibular reactions, blood levels of noradrenaline, endorphin, *LTH, ACTH* and cortisol*,* immunoglobulins and lymphocytes/neutrophils counts etc. It is important to keep in mind that while humans can voluntarily close their eyes, we have no natural possibility to close our ears; hearing is an "unavoidable" human sense, which is phylogenetically useful because auditory stimuli can wake up the organism in situations of danger. Because in the uterus there is no light and somatosensory input is much reduced because of being immersed in the amniotic fluid, *hearing* is one of our first senses in human ontogenesis: Lecanuet, Graniere-Deferre, Jacquet and DeCasper (2000) demonstrated that human fetuses of weeks 36^{th} – 39^{th} can process auditory input, i.e. they are able to differentiate between the low piano tones *C5* and *D4,* 70% of them reacting with expected slowing of their heart rates, as hypothesized by the employed experimental paradigm.

Although there is probably human being (excluding deaf people) which has *never* been exposed to musical stimuli in his/her life, and probably we all

have experienced physiological reactions to musical stimuli, there is a special, less known auditory phenomenon under study whether to be efficacious to induce *altered states of consciousness* (ASCs) by rhythmical stimulation or not, the *Binaural Beats.* They can only be evoked by stereo-headphone stimulations and, to the author's best knowledge, were first discovered by Heinrich Wilhelm Dove (1839), see figure 5.

Figure 5. *Heinrich Wilhelm Dove* (1803 - 1879), German-Prussian physicist and meteorologist, firstly discovered the auditory illusion of Binaural Beats in the year 1839 (picture retrieved from http://upload.wikimedia.org/wikipedia/commons/b/bf/Heinrich_Wilhelm_Dove_1857.jpg)

After studying mathematics, physics, philology and philosophy at the University of Breslau, receiving teaching by the influential philosopher Georg Wilhelm Friedrich Hegel, he became a German-Prussian physicist and one of the founders of modern meteorology, first at University of Königsberg and later at Humboldt University of Berlin. Dove's name is better known for his discovery in 1828 of the opposite rotation spin of tropical cyclones on the northern (counter clockwise) vs. on the southern hemisphere of the earth (clockwise).

Binaural Beats are an auditory illusion, i.e. they are not the representation/perception of a really existing stimulus in the physical outer world, but they are the brain's subjective *illusion* of a "virtual" third tone

which fluctuates (i.e. *beating*) in its volume when two sinusoids of same amplitudes, but slightly different frequencies are dichotically presented by stereo-headphones (Perrott & Nelson, 1969). These two sinusoids are called *carrier frequencies* f_1 and f_2, resulting in a "virtual" illusory interference tone with a perceived principal frequency corresponding to the arithmetic mean of f_1 and f_2 but being *amplitude modulated* by a frequency corresponding to the difference between f_1 and f_2, named Δf. This *envelope* frequency is the perceived *beating,* therefore Δf is the *Binaural Beats frequency.* This superposition effect is a common known interference effect of two waves which slightly differ in their frequencies or wave lengths. What is special in the case of *Binaural Beats* is the fact that the two mechanical oscillations of air molecules of the carrier sounds never interfere mechanically, because the corresponding air volumes are physically separated due to the use of closed headphones. Thus, the superposition of the carrier frequencies happens when the *neuronal correlates* of the carrier sounds interact in the central nervous system, so *Binaural Beats* origin in the neuroanatomical–functional structure of the brain, the brain "makes up" this illusion: Binaural Beats exist "only" in the head. This is why they are, in eyes of the present work's author, so interesting for neuroscience: As other illusions, e.g. the known visual *Müller-Lyer illusion* (Müller-Lyer, 1889), Binaural Beats only exist because of the specific neurocircuitry of the brain. Investigating illusions thus can be an elegant access to better understand its functioning.

A neurophysiological explanation for the genesis of the Binaural Beats illusion refers to neurons which are responsible for *sound location*: It is hypothesized that the firing patterns of those specialized neurons which codify the *phase information* of each ear/cochlea neuroanatomically converge in binaural neurons, located more "above" in the ascending auditory pathway (see figure 6), which analyse *interaural phase differences.*

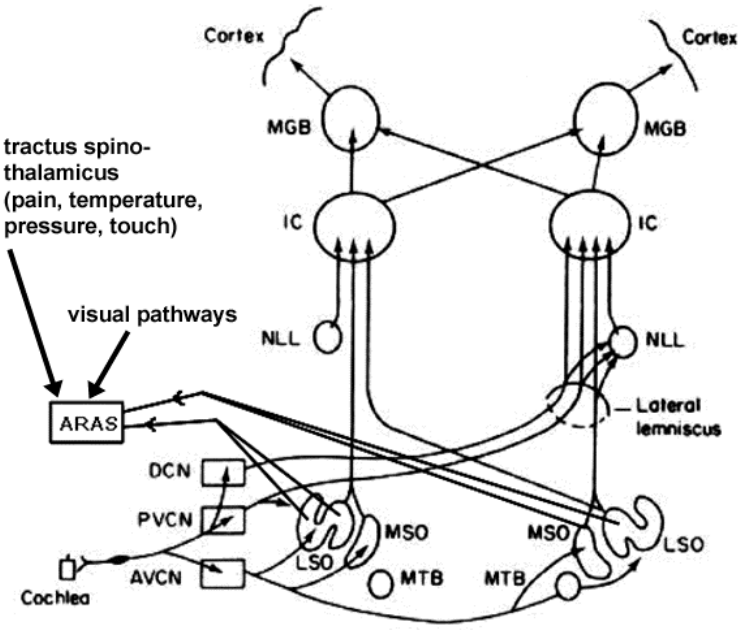


Figure 6. Simplified neurocircuitry of the brain's left and right *auditory pathways* with their afferent projections from the brainstem upto the neocortex (modified following Piccles, 1991). AVCN=anteroventral cochlear nucleus; PVCN=posteroventral nucleus; DCN=dorsal cochlear nucleus; LSO=lateral superior olive; MSO=medial superior olive; MTB=medial nucleus of the trapezoid body; NLL=nucleus of the lateral lemniscus; IC=inferior colliculus; MGB=medial geniculate body; ARAS= ascending reticular arousal system

Indeed, Kuwada, Yin and Wickesberg (1979) recorded cyclic responses in neurons of the cats' inferior colliculus to the periodic, dynamic changes in interaural phase associated with Binaural Beat stimuli.

Psychoacoustic experiments by Feeney (1997) falsified the former theory of Binaural Beats to be due to binaural cross correlation of excitation patterns of f_1 and f_2 occurring within the binaural critical band centred at f_2, i.e. the found data exclude the possibility that the effect could be explained by binaural interaction within a critical band, but support the explication that the Binaural Beats illusion is generated by a *between-channel phase effect*.

Today it is assumed that those mentioned binaural neurons codifying the *phase information* and their following neurocircuitry generate the neurophysiological correlates of Binaural Beats, as early as in the brain stem, demonstrated by unicellular recordings in cats by Wernick and Starr (1968). Scott, Malone and Semple (2009) registered in 283 single neurons

in the auditory cortex of awake macaques discharge rates robustly corresponding to the phase differences of Binaural Beats.

In humans, electrophysiological correlates of the "virtual" third tone in the Binaural Beat paradigm were found on the neocortex as *auditory steady-state response* (ASSR) in MEG recordings by an a extraordinary meticulous study of Karino et al. (2006), but were also found in human EEG recordings (Schwarz & Taylor, 2005), similar to *visually evoked steady state potentials* (SSVEPs) induced by visual flicker stimuli (see e.g. Fernandez-Vargas, Pfaff, Rodriguez, & Varona, 2013). Similar results in the search of the direct neurophysiological correlates of the "virtual" third tone were found in EEG recordings using event-related potentials (ERPs) localized by the *LORETA* technique employing 3 Hz and 6 Hz Binaural Beats with carrier frequencies f_1 of 250 Hz or 1000 Hz mainly over the left lateral and inferior temporal lobe (Pratt et al., 2009).

Crucial for the limits of conscious perception of Binaural Beats are the involved frequencies, while the magnitudes/volumes of the carrier frequencies is less important, because the *beating* of the Binaural Beats is generally detectable at remarkably low magnitudes of the carrier frequencies (Gu, Wright, & Green, 1995): When Δf is too large or too small, then the auditory illusion of Binaural Beats is not evoked. Perrott and Nelson (1969) investigated the maximal detection rate (averaged yes/no ratings) of Binaural Beats of one-second duration and loudness of 12 *sones* at different fixed first carrier frequencies f_1 while manipulating the second carrier frequency f_2 and thus manipulating the Binaural Beats' frequency Δf. They found that the detection rate reaches zero when the first carrier frequency exceeds the upper bound of $f_1 > 1500$ Hz and the maximum was found around $f_1 \approx 500$ Hz. The detection rate's maxima for varying Δf depend on f_1: The lower the first carrier frequency f_1 is, the lower is the Δf of maximal detection rates (see table 1), which implies that for the induction of low frequency Δf Binaural Beats low carrier frequencies should be used.

Table 1. Binaural Beats frequencies *Δf* at constant first carrier frequencies f_1 leading to maximal detection rates (Perrott & Nelson, 1969).

f_1	*Δf* leading to maximal detection rate (yes/no)
250 Hz	5 Hz
500 Hz	10 Hz
1000 Hz	20 Hz
1200 Hz	40 Hz
1500 Hz	80 Hz

These observations lead to the opinion that the auditory system is deaf to interaural time differences at high frequencies. It was an accepted fact that Binaural Beats with relatively high carrier frequencies are generally inaudible, but this opinion was overcome by the discovery of McFadden and Pasanen (1975), published in *Science*, one of the most impacting journals, that when these high carrier frequencies are amplitude modulated by lower frequencies and thus not these frequencies themselves, but their *envelopes* interact, the Binaural Beat illusion can be successfully evoked, although those high frequencies are used.

Apart from research on the neurophysiological correlates of the illusory third *beating* tone (as reported above) in all brain structures of the auditory pathways itself, the fact that the entire *superior olivary complex* (SOC) is the first important structure where auditory information from the left and right ear converge (Oliver, Beckius, & Shneiderman, 1995) and given the neuroanatomical connection between the *lateral superior olive* (LSO) with the *ascending reticular activating system* (ARAS), the hypothesis is justified that Binaural Beats could not only cause a conscious auditory illusion *percept*, but also might rather unconsciously influence the level of (cortical) arousal and thus probably also of peripheral-physiological systems by e.g. rhythmically stimulating the ARAS and/or other mechanisms. That indeed interaural phase differences are extracted by neurons in the SOC was confirmed e.g. in animal experiments with *Mongolian gerbils* (Meriones unguiculatus) by Spitzer and Semple (1995) and with barn owls (Tyto alba) by Carr and Konishi (1990). Because the ARAS modulates/regulates the basic rhythms of the *spontaneous EEG* (see e.g. Empson, 1986) which in turn reflects *cortical* neuronal sum activity without being able to directly

capture activity from subcortical structures, Binaural Beats might be able to modify spontaneous EEG rhythms as a central measure of *arousal* if the above reported hypothesis holds true. While the conscious perception of the third *beating* tone seems to enter into consciousness following the auditory pathway structures, see figure 6, *SOC* → *colliculus inferior* → *corpus geniculatum mediale* (part of the *thalamus*) → *radiatio acustica* → *primary auditory cortex,* as the above reported findings suggest. The hypothesized second effect of *Binaural Beats* on (cortical) arousal levels would act via *SOC* → *ARAS* → diffuse projections on multiple brain structures → entire neocortex. While the first pathway leads to a conscious *percept* as the purpose of the auditory sensorial system, taking into account the knowledge concerning the ARAS, the hypothesized second pathway should not lead to conscious percepts such as the illusion of a tone; this second effect could, at best, only be perceived secondarily by its consequences as e.g. feeling more relaxed etc. While the neurophysiological correlates of the *percept* of the third *beating* Binaural Beats tone are relatively weak, but clearly proven in human subjects by an excellent and meticulous *MEG* study (Karino et al., 2006), the hypothesized effects on (cortical) arousal levels via the mentioned second pathway should be much stronger.

1.3. Current state of research on modulation of arousal levels by Binaural Beats

Searching the database *ISI Web of Knowledge* with "TOPIC: (binaural beat*) Timespan=All years. Search language=English" results in 162 found publications with 2119 *sum of times cited without self-citations*, 15.28 *average citations per item*, the medical data base *PubMed* returns 76 results for the search string "binaural beat*" (both on date 7/02/2014). The published investigations can be structured into *basic* research works searching for neurophysiological correlates of the third illusory/"virtual" tone, as mentioned above, and into *applied* research following the idea of

possible effects on arousal levels, as explained above, mainly by studying EEG recordings.

Unfortunately, only few works were published with regard to this *applied* research, which fulfil an acceptable level of scientific rigour. Most studies lack the standards of *Evidence-based Medicine* for randomized placebo-controlled double-blind trails (see e.g. Sackett, Rosenberg, Gray, Haynes, & Richardson, 1996; Timmermans & Mauck, 2005), did not operationalize and did not test *bio-psycho-social health* of the subjects and most of them neither used sufficient sample sizes nor discussed the problem of statistical test power of the employed research designs at all (Abeln, Kleinert, Strüder, & Schneider, 2013; Carter, 2008; Kasprzak, 2011; Lavallee, Koren, & Persinger, 2011; Le Scouarnec et al., 2001; Wahbeh, Calabrese, Zwickey, & Zajdel, 2007; Waldkoetter & Sanders, 1997). One pilot field study with children diagnosed with *attention deficit hyperactivity disorder* (ADHD), although using a randomized placebo-controlled double-blind design, did not include any physiological measures and could not essentially contribute to neither a strong falsification nor to a verification of possible Binaural Beats effects applied to this disease (Kennel, Taylor, Lyon, & Bourguignon, 2010).

There are some intents to investigate Binaural Beats which lack almost any modern professional-scientific background, i.e. the basics of operationalizing hypotheses, constructing adequate experimental designs, rudimentary statistical knowledge (especially the need of contrasting hypotheses contra an adequate null hypothesis by inferential statistics to minimize spurious random findings), widely accepted standards or rules for causal attribution from experimental data and the neuroanatomical/neurofunctional basics to be able to interpret neurocientific data seem to be absent, leading to "results" which unfortunately do not offer any contribution about the effects of Binaural Beats under question; these publications mainly come from engineering faculties (e.g. Puzi, Jailani, Norhazman, & Zaini, 2013). But there are other works which, although trying to maintain a sufficient scientific rigour, unfortunately fail in the details of experimental design and EEG analyses, as e.g. Crespo, Recuero, Galvez and Begoña (2013) who could not confirm a Binaural Beat effect: The authors use a

between-subjects experimental design comparing three independent groups, two of them receive distinct stimulation with Binaural beats while the third was the Placebo control group, although in many psychophysiological and pharmacological research contexts the use of an *within*-subjects design is traditionally to be preferred, because here each subject serves as his/here own control. *Within*-subjects designs reduce variability caused by third (mainly trait) variables and allow the use of statistical methodology with higher statistical test power. Moreover, the authors compare only four epochs of extracted 30 sec artifact-free epochs at each 5 min of their 20min lasting stimulus, so they only analyze 2.5% of the entire EEG time series under Binaural Beat stimulation. Because it is probable that every subject shows somehow distinct response trajectories over the time course of the Binaural Beat stimulation, 100% of the EEG time series should be included into the input of the analyses, i.e. only the contained artifacts have to be removed.

Some few *applied* works report *confirmation* of Binaural Beat effects (Brady & Stevens, 2000; Ioannou & Bhattacharya, 2012; Lai, Chao, Yang, & Chen, 2010; Lane, Kasian, Owens, & Marsh, 1998; Padmanabhan, Hildreth, & Laws, 2005; Reedijk, Bolders, & Hommel, 2013), while others found *no specific effects* using their specific employed research designs (e.g. Goodin et al., 2012; Stevens et al., 2003; Vernon, Peryer, Louch, & Shaw, 2012; Wahbeh et al., 2007; Weiland et al., 2011).

Apart from the study of possible modulations of (cortical) arousal by Binaural Beats, Kliempt, Ruta, Ogston, Landeck and Martay (1999) investigated in a randomized double-blind trial the modification of unconscious *nociception* during chirurgical intervention under general anaesthesia (*Propofol* i.v. in combination with inhalation of N_2O 66%, 33% O_2 and the halogenated ether *Isoflurane*) by intraoperative Binaural Beats stimulation with $N = 76$ subjects with three conditions: (i) silence, (ii) classical music and (iii) Binaural Beats. The amount of the needed highly potent i.v. opioid analgesic *Fentanyl* to maintain heart rate and arterial blood pressure within ± 20% of preoperative baselines was used as an indirect measure for intraoperative unconscious nociception, caused by the pain-evoking scalpel and other instruments of the surgeon.

Under stimulation with Binaural Beats patients significantly ($p < .001$) needed much less *Fentanyl* as compared to classical music or silence ($M_{Binaural\ Beats}$ = 28 µg with 95% CI 11.1 – 44.9 µg, $M_{classical\ music}$ = 124 µg with 95% CI 96.8 – 151.2 µg and $M_{silence}$ = 126 µg with 95% CI 99.8 – 152.3 µg). Significance was maintained controlling for age and sex. Placebo effects can be excluded because patients were under general anesthesia and because of the double-blind experimental design, so indeed Binaural Beats seem to have strong analgesic effects when applied under general anesthesia. This highly interesting study was confirmed by a replication of Dabu-Bondoc, Vadivelu, Benson, Perret and Kain (2010) and shows that *specific* Binaural Beat effects can be objectively measured even in patients without consciousness. What makes these findings so interesting is the fact that because conscious mentation is absent under general anaesthesia, which is after all the aim of this kind of pharmacological intervention, these reported Binaural Beats effects are to be considered as free from higher cognitive functioning, e.g. free from interindiviudally different *cognitive styles* and free from e.g. distinct cultural semantic systems. Because neocortical activity is strongly inhibited under general anaesthesia, these reported effects should be hypothezised to be mainly due to Binauarl Beats interacting with *subcortical* brain structures, see figure 6.

In summary, although Lane et al. (1998) came to the same conclusion 15 years before, still today we lack more scientific studies confirming the specific efficacy of Binaural Beats with adequate methodology, mainly whether they are indeed able to significantly modulate the *spontaneous EEG* as a measure of cortical arousal. Nevertheless, since this reported statement we now have some more neuroscientific evidences, especially the extraordinary meticulous study of Karino et al. (2006) confirming that Binaural Beats indeed induce a measurable neurophysiologic cortical correlate of the *percept* of the third illusory "beating" tone. But, unfortunately, studies with strong scientific rigour confirming specific Binaural Beats effects on modulation of arousal levels, as measured by the *spontaneous EEG,* are still rare and unfortunately in parts contradictory.

As consequences from the contradictions among the cited previous investigations, where the crux seems to be in several methodological problems, for the present work, methodological decisions have to be in the focus of attention: First of all, a strict selection of subjects by psychometric test batteries operationalizing bio-psycho-social health seem to be most important. But also a stimulation not by only static but by a dynamic *sweep* of Binaural Beat frequencies, multimodal measures of *arousal* levels (not only EEG) and analysis of the *entire* time series by refined EEG techniques have to be employed, e.g. until today there is (to the best knowledge of the present work's author) no study on Binaural Beats which used *spatial filtering* of EEG biosignals in order to reduce topographical blurring due to volume conduction effects.

1.4. Aims and main hypotheses: Is there a significant *psychophysiological reactivity* to an EEG alpha and theta Binaural Beats *sweep* in multimodal outcome measures?

Given the conflicting results of *applied* research concerning Binaural Beats effects published until today, the primary aim of the present work is to confirm or to reject the hypothesis whether audio stimulation with Binaural Beats can specifically influence levels of *arousal,* apart from the available acceptable evidence for direct neurophysiologic correlates of the "beating" third illusory tone coming from neurocientific *basic* research (Karino et al., 2006).

As explained above, *arousal* implies reactions of *multiple* physiological systems of the organism, but no work on Binaural Beats published until today employed multimodal measurements. Hence, in order to cover the most important systems involved in changes of *arousal* and apart from central *cortical* arousal measured by EEG, reactions of the autonomous nervous system (ANS) will be simultaneously measured by *heart rate variability* and reactions of the neuroendocrinologic system by pre-/post intervention tests of analytes in saliva samples.

Almost all published works on Binaural Beats until today used fixed carrier frequencies and fixed Binaural Beats frequencies *Δf,* but we know from their visual analogy triggered by stroboscopic *flicker*, the *steady state visually evoked potentials* (SSVEPs), that SSVEP magnitudes are different for every human subject (see e.g. Fernandez-Vargas et al., 2013; Herrmann, 2001) while showing common preferred frequencies (10, 20, 40 and 80 Hz) and that subjects' traits can interact/modulate these frequencies, especially the *Big Five* personality trait *Openness to experience* (e.g. Stough, Donaldson, Scarlata, & Ciorciari, 2001; Varona, Fernandez-Vargas, Pfaff, & Rodriguez, 2013). From this analogy of the visual system it is concluded that it has to be hypothesized that every subject might also have more or less different frequencies bands in which he/she is most vs. least reactive to Binaural Beats. This is why applying the same fixed carrier and Binaural Beats frequencies to all subjects, as it has been done in almost all published works until today, is likely to miss –at least for some percentage of the sample– subjects' individual "frequency gates" for Binaural Beat reactivity. From these thoughts emerged the idea not to use *fixed* carrier and Binaural Beats frequencies, but to use a continuous *sweep* of frequencies covering the range of all frequencies of interest. In the case of using for all subjects the same frequency *sweep* it is to be expected that every subject will show reactions in a somehow different moment of stimulation, so a general fixed overall reaction pattern is hardly to be expected. Following this idea, the entire time series of biosignals have to be analysed (excluding only artefacts parts) and hypotheses about the development in time have not to be expressed as *directed* hypotheses (increase vs. decrease vs. staying the same), but as *undirected* hypotheses*:* Is there significant *change over the entire stimulation time* in the biosignal to be analysed? In inferential statistical terms, this question would read as "Has the observed variability over time a pattern which is significantly *not any more* compatible with the null hypothesis that all data would come from the same underlying population, so at least one or more measurement time points have to represent *another* population and thus there would be a significant *change over time*?" This question can be contrasted with a special class of statistical test, the *omnibus* or *overall* or *global* tests. They are not able to detect *where* in the time series a significant change occurs, but they decide over

the question if such an effect exists or not over *all* measurement time points. These tests decide over the existence or not existence of *change over time.* Later, in order to express or to compare the magnitude or *effect size* of this *change over time,* i.e. in order to *quantify* it, the most simple measure *total intrasubject variability over all measurement time points* can be used.

This approach can be understood as the operationalization of the primary research question of the present work whether Binaural Beats indeed can modulate *arousal* levels or not as *psychophysiological reactivity,* i.e. whether they are able to induce *change over time* in multiple relevant physiologic biosignals/measures: Primarily, it is not so important to discover certain *details* or *local information* of the possible response patterns to Binaural Beat stimulation, which are moreover hypothesized to be quite different for every subject, so a general overall-pattern is not likely to be found, but to contrast the *global* or *omnibus* hypothesis whether there is or there is not a *psychophysiological reactivity* which is *specific* to Binaural Beat stimulation, i.e. which is *not* to be observed under an unspecific Placebo condition. In other words, the primary question of the present work is "Is there any reaction at all to Binaural Beats?" and not "How exactly, which temporal pattern etc. has this reaction, *when* during the frequency sweep do these reactions occur more pronouncedly?" This special operationalization of the primary research question has the advantage that it is not important *when* in the sweep a subject reacts, but only *if* and *how much* all subjects taken altogether react over the entire time course of the frequency sweep.

Principally, if *specific* psychophysiological reactivity could be confirmed to be significant, there are two directions of possible Binaural Beat effects on *arousal* levels*:* increasing vs. decreasing. With respect of the possible clinical applications of Binaural Beats explained above, *decreasing* arousal levels is much more interesting than *increasing* them. When the aim is the induction of a psychophysiological state characterized as explained above following Vaitl et al. (2005) (i.e. low psychophysiological activation, a change of attentional focus from the outer surroundings towards the subject's mental inner world, but generally low consciousness of the own

self and a reduced sensorial dynamics), then the stimulation with Binaural Beats with *Δf* in the *lower* EEG frequency bands should be used: Because *increase* or predominance of slower EEG brain wave frequencies and at the same time *decrease* of the faster frequencies is a general electrophysiological marker for *lower* (cortical) arousal levels, Binaural Beat stimulation should be done in the slower EEG frequency bands, i.e. in *alpha* (7.5 – 12.5 Hz) and *theta* (3.5 – 7.5 Hz). Hence, following the idea of a frequency *sweep, Δf* should change continuously from *alpha* to *theta* (while also continuously lowering the carrier frequencies themselves) within an application compatible time, i.e. approx. 30 min.

As a conclusion of the described argument chain, we can now define the primary hypotheses of the present work: For the EEG as reflecting the *arousal* level of the central nervous system the primary hypothesis of the present work is: "Is there any significant *change over time* in at least some EEG frequency bands over all subjects and all measurement time points, as operationalized and contrasted by an inferential statistical *omnibus* or *overall* test, which only occurs under Binaural Beat stimulation with the *alpha – theta sweep* and not under a Placebo condition, i.e. which is *specific*?" For *heart rate variability* as a peripheral-physiologic measure of the autonomous nervous system, the primary hypothesis is: "Is there any significant *change over time* in at least some *heart rate variability* parameters over all subjects and all measurement time points, as operationalized and contrasted by an inferential statistical *omnibus* or *overall* test, which only occurs under Binaural Beat stimulation with the *alpha – theta sweep* and not under a Placebo condition, i.e. which is *specific*?" For the concentrations of stress-related biomarkers in the saliva samples, first of all for cortisol as a measure of the neuroendocrinologic system (*hypothalamus pituitary adrenal axis*, HPA) the primary hypothesis is: "Is there any significant *difference* in salivary cortisol concentrations over all subjects *pre* vs. *post*, as operationalized and contrasted by an inferential statistical test comparing the *central tendencies* of the two underlying populations *pre* vs. *post*, which only occurs under *Placebo* or *Verum* condition, but not under both or none of them, i.e. which is *specific* for only *Placebo* or only *Verum*?"

Apart from these physiological measures, subjective psychometric questionnaires should measure how much subjects report signs of the desired state of consciousness, as explained above following Vaitl et al. (2005), comparing *Verum* vs. *Placebo.* Thus, the primary psychometric hypothesis of the present work is a *directed* hypothesis: "The *central tendencies* of the underlying populations are significantly different with regard to psychometric measures of the desired state of consciousness, comparing *Verum* vs. *Placebo,* being larger under *Verum."*

After testing these main hypotheses, some intent should be made to extract some more information as only the significance of the global *change over time* for EEG and heart rate variability. For the reasons explained above, determining the exact local information e.g. by *post-hoc tests* is problematic, but at least the *linear trend* over all time points should be determined in order to complete the information of the global hypotheses, i.e. is there an *increase* vs. *decrease* vs. *near-constant* linear development over time? Moreover, in the sense of an integrated *psychophysiological* study, results in the physiological domain and those in psychometric dimensions should be investigated for possible associations, e.g. by *correlation* measures.

One important advantage of operationalizing the research hypotheses as *changes over time* and *linear trends* is that a problematic correction for baseline states is not necessary because not absolute values are contrasted, but only their intraindividual *change* or *variability* which in itself implies leaving out the information of baseline levels.

2. Methods

2.1. Subjects

Posters advertising the study of the present work as "reactions of the human body on auditory/musical stimulation" were distributed in all faculties of *Universidad Autónoma de Madrid* which invited volunteers to enter an *online questionnaire* for recruitment. Before responding the psychometric instruments, at the first page, subjects were briefly informed about the study's nature and risks, that it had been approved by the university's ethical review board, that the participation would be completely voluntarily, not associated with any academic activity of their careers, that subjects could leave the experiments at any time without further explanation and that for their own security answering as honestly as possible would be necessary. Accepting the statements at the first page and going on with the questionnaire corresponds to a *written informed consent.* After successfully passing the first test battery of approx. 45 min, volunteers were invited via email to a second online questionnaire of approx. 45 min and if passed, they were invited to participate. This two-step recruitment process was chosen as a filter, so only highly interested and motivated persons would take part and others would give up before being physically present in the experiments. The test batteries tried to operationalize the construct *bio-psycho-social health.*

The first test battery operationalized as *inclusion criteria* age between 19 and 30 years, academic level university degree or university student and as *exclusion criteria* the following*:* any medication or drug (ab)use including alcohol, any neurological (especially photosensitive epilepsy), any psychiatric diagnosis or any major physical disease, any intents of suicide, past or present major life events, left-handedness as measured by the *Edinburgh Handedness Inventory* (Oldfield, 1971) and recent or present pregnancy. Moreover, deviations more than ± two standard deviations from Spanish normal samples in the following psychometric instruments in their Spanish adaptation leaded to exclusion: *Dickman Impulsivity Inventory*

(DII) (Chico, Tous, Lorenzo-Seva, & Vigil-Colet, 2003), subscales exploratory excitability, novelty seeking and self-directedness from *Cloningers Temperament and Character Inventory (TCI-R-67)* (Pedrero-Pérez, 2009), *Composite Scale of Morningness (CSM)* (Adan, Caci, & Prat, 2005), Behavioral Inhibition System (*BIS*) and Behavioral Activation System (*BAS*) following Gray's biopsychological theory of personality (Barranco-Jiménez, Rodarte-Acosta, & Medina-Cuevas, 2009), *Schizotypal Personality Questionnaire* (Mata, Mataix-Cols, & Peralta, 2005), *Dissociative Experiences Scale-II (DES-II)* (Icarán, Colom, & Orengo-García, 1996), *Epworth Sleepiness Scale (ESS)* (Izquierdo-Vicario, Ramos-Platón, Conesa-Peraleja, Lozano-Parra, & Espinar-Sierra, 1997) and *Experiences in Close Relationships-Revised (ECR-R)* following Bowlbys' and Ainsworth's theory of human attachment (Alonso-Arbiol, Balluerka, Shaver, & Gillath, 2008).

The second test battery contained the following psychometric instruments, as for the first test battery, deviations more than ± two standard deviations from Spanish normal samples lead to exclusion of the subject: *Beck's Depression Inventory (BDI-II)* (Sanz, Perdigón, & Vázquez, 2003), *Toronto Alexithymia Scale (TAS-20)* (Páez et al., 1999), *Rosenberg Self-Esteem Scale (RSES)* (Martín-Albo, Núñez, Navarro, & Grijalvo, 2007), *Symptom Checklist-90-Revised (SCL-90-R)* (De las Cuevas et al., 1991), *Parental Bonding Instrument (PBI)* (Gómez-Beneyto, Pedrós, Tomás, Aguilar, & Leal, 1993), *Multidimensional Scale of Perceived Social Support (MSPSS)* (Edwards, 2004) and the *NEO Five-Factor Inventory (NEO-FFI)* (Manga, Ramos, & Morán, 2004).

The first questionnaire was answered by 122 subjects correctly, but only 52 subjects passed the *inclusion/exclusion criteria* and were invited to the second questionnaire which answered all of them correctly. Sixteen subjects had to be excluded in this second step, so at the end 36 suitable subjects remained. From this population seven males and five females were randomly selected and this final sample of $N = 12$ was invited into the laboratory.

Considering the *Big Five* personality dimensions as measured by the *NEO-FFI*, the sample showed for *neuroticism* $M = 17.17$ and $SD = 4.914$, for *extraversion* $M = 29.33$ and $SD = 7.981$, for *openness to experience* $M = 35.08$ and $SD = 5.869$, for *conscientiousness* $M = 31.17$ and $SD = 5.828$ and for *agreeableness* $M = 29.92$ and $SD = 5.178$. Comparing these values with the published normal values for Spanish university population in the test's manual, significant differences were found using the *Z*-test as *one-sample location test*, i.e. comparing the mean of a sample to a given/known population constant with known population variance: The employed sample of $N = 12$ shows significant lower *neuroticism* with $z = -3.852$ and $p = .00012$ and significant higher *openness to experience* with $z = 3.271$ and $p = .0011$ as normal Spanish university population. These findings in *openness* can be explained with self-recruitment processes, i.e. higher *openness* predestines subjects to be interested in taking part in scientific experiments as volunteers. The findings in *neuroticism* can be explained by the rigorous selection process with the aim to only invite very healthy subjects. The results in the *Big Five* dimensions imply that the employed $N = 12$ sample seems to be a *subpopulation* of Spanish university population and that the recruitment process successfully selected persons with high levels of health.

Subjects in this final $N = 12$ sample had age $M = 23.92$ years and $SD = 3.965$, body size of $M = 176.25$ cm and $SD = 5.207$, body weight of $M = 75.50$ kg and $SD = 10.388$ and resulting *body-mass indices* (BMI) of $M = 24.27$ and $SD = 2.809$ which is to be classified as at the upper bound of the range for normal/healthy weight following the definitions of the *World Health Organization* (WHO) of normal *BMI* range = 18.5 – 25 (WHO, 2006). All subjects had *BMIs* ≤ 25, with exception of three persons which were in the range of *pre-obese* with *BMI* ≥ 25, but ≤ 30.

2.2. Experimental design

Because the primary aim of the present work is to confirm or to reject the hypothesis whether audio stimulation with Binaural Beats can specifically influence levels of *arousal,* which is a causal hypothesis of the *efficacy* of Binaural Beat stimulation, i.e. how well the treatment works in laboratory studies (as compared to *effectiveness* with the question how well a treatment works in the practical clinical field), highly selected subjects under highly controlled conditions have to be investigated. As mentioned above, most works on Binaural Beats published until today unfortunately do not follow the standards of *Evidence-based Medicine* (e.g. Sackett et al., 1996; Timmermans & Mauck, 2005). These standards try to oblige investigators to use research designs which maximize the possibilities of valid *causal* attributions of the found effects to the employed treatment conditions. Following these standards, the research design to be employed needs to be an *explanatory crossover-randomized placebo-controlled double-blind trial:* Explanatory trials investigate efficacy as opposed to pragmatic trials which investigate effectiveness; randomization minimizes subject allocation biases, crossover means a *within*- or *intra*subject design, i.e. that every participant receives both *Verum* and *Placebo* while the presentation order is randomized over the subjects; placebo-control makes the investigation of treatment specificity contra *Placebo* possible, i.e. comparison of effect sizes of unspecific (e.g. setting etc.) vs. specific treatment effects; double-blinding minimizes Placebo-, Nocebo-, expectation-, habituation-, *Rosenthal*- (Rosenthal & Jacobson, 1968) and self-report biases.

The consequences from this necessary experimental design are that subjects had to come for two experimental sessions to the laboratory: Randomly selected 50% of them were exposed in the first session to the *Verum* condition and then in the second session to *Placebo,* while the remaining 50% were assigned to the vice versa presentation order, i.e. first *Placebo,* second *Verum.* The temporal distance between these two sessions was kept more than one week to reduce habituation and learning effects.

2.3. Justification of sample size *N* = 12

Before performing any experiment *N* times, the *statistical design* has to be checked with regard to *statistical test power,* i.e. estimating the optimal sample size *N* for a given statistical test with presumed effect size so that desired α *and β error levels* are reached*.* Experiments can only decide adequately about the maintenance of *null hypotheses,* if statistical test power *1–β* is sufficient, i.e. if they allow to conclude in the case of non-significant results that the searched effect indeed probably does not exist in the underlying population and that this failure is primary not to be attributed to insufficient statistical experimental design, but can be interpreted as a valid *null finding*. Apart from the widely accepted standard α*–error level* of α = .05, Cohen (1988) proposed for the *β–error level* in the context of behaviour studies a value of four times the standard α*–error level*, i.e. *β* = .20 which means a statistical test power of *1–β* = .80. In the same publication he moreover defined general effect sizes with *Cohen's d* between .2 to .3 as to be considered as *small effects,* around .5 as *medium effects* and above .8 up to positive infinity as *large effects.* These values are to be understood as only orientative, because what is *small* and what is *large* always is a relative issue, so effect sizes should be presumed from already known data in the specific research field or sometimes can also be determined by pilot studies. With fixed α– and *β–error levels* within the same statistical test, it is the presumed effect size which determines the optimal *N,* i.e. the smaller the effect the more observations are needed to maintain the desired fixed α– and *β– error levels* for the inferential statistical decision about the *null hypothesis.*

Primary, mainly two nonparametric statistical tests will be used in the present work, the *Wilcoxon signed-rank test* for comparing inferential statistically the *central tendencies* of *k* = 2 distributions and the *Quade test* as *overall* or *omnibus* test for comparing the *central tendencies* of *k* > 2 distributions. Further details of both tests are described in section *Statistical Methods,* see below.

Statistical test power analyses are problematic for non-parametric tests, because test power depends on the shape of population distribution (which is not always known), but this is just the advantage of these *distribution-free* tests, they do not need Gaussian normal distribution. Assuming an example of a simple non-normal distribution, the *Laplace* distribution (also named *double-exponential distribution*), the *Wilcoxon signed-rank test* has a sufficient test power for *large* effect sizes: *N* = 12 is fully sufficient to adequately decide over the effects when they are *medium–large* (*Cohen's d* = .7), as calculated by the program *G*power 3.1.7* (Faul, Erdfelder, Ang, & Buchner, 2007), see figure 7.

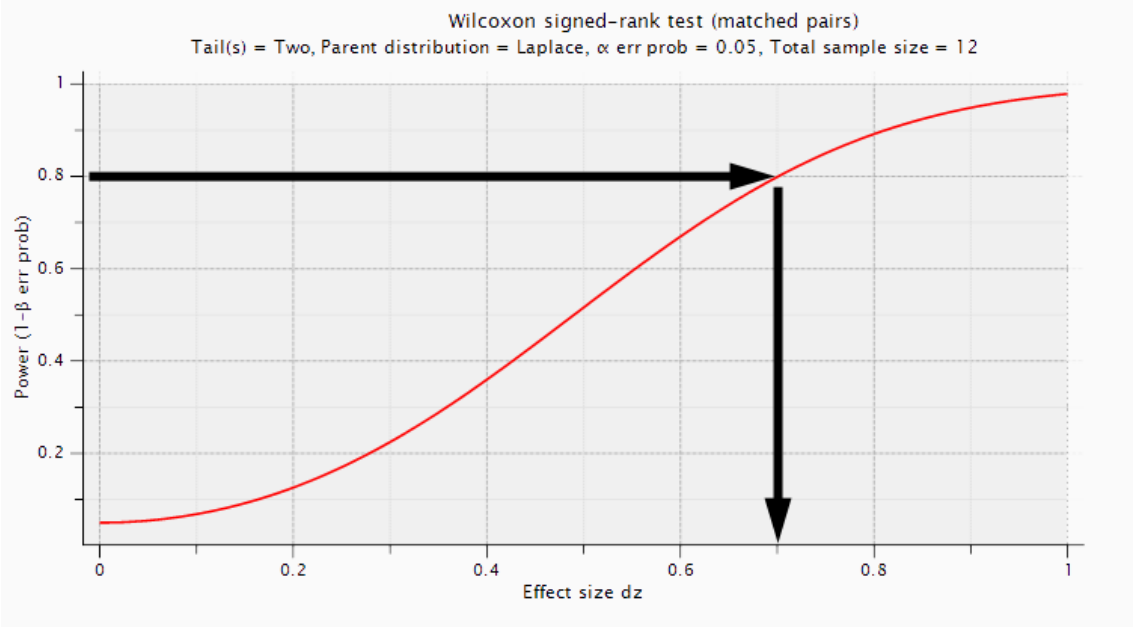


Figure 7. The Wilcoxon signed-rank test has sufficient test power for *large* effect sizes, assuming an example of a simple non–normal distribution, the *Laplace* or *double expotential* distribution: *N* = 12 is sufficient to adequately decide over the effects when they are *large* (Cohen's d = .7), as calculated by the program *G*power 3.1.7* (Faul et al., 2007).

To the best knowledge of the present work's author, until today there is no approach available to calculate test powers for the nonparametric *Quade* tests for different shapes of population dirstribution (other than simulation studies), so the parametric alternative, *repeated measures ANOVA* is used for statistical test planning which assumes normality of the data. As to be seen in the graph of figure 8, detecting changes within five repeated

measures at the desired standard α and β levels is possible down to a *medium* effect size of $f = .25$ with a sample size of $N = 12$.

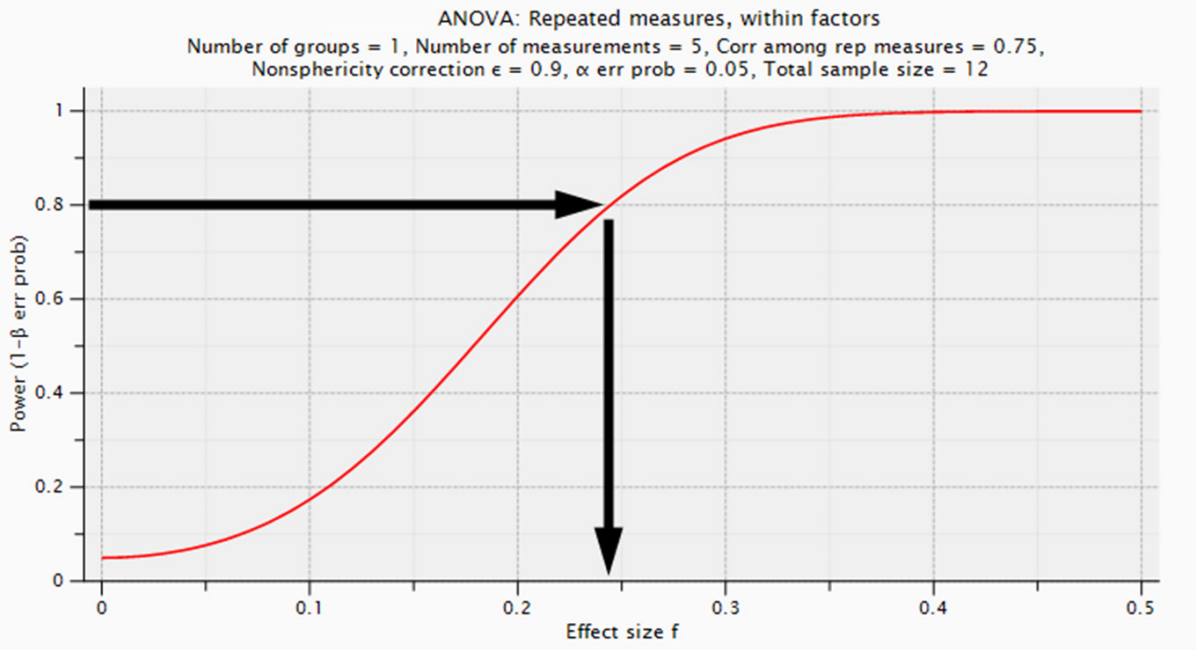


Figure 8. Because there is no approach available to calculate test powers for the nonparametric *Quade* test, the parametric alternative is used to estimate statistical test powers, *repeated measures ANOVA*. Detecting changes within five repeated measures at standard levels for α and β is possible down to a *medium* effect size of $f \approx 0.25$ with a sample size of $N = 12$, calculated by the program *G*power 3.1.7* (Faul et al., 2007).

Taken altogether, although a sample size of $N = 12$ might perhaps seem too small and inadequate to some readers, statistical testpower analyses demonstrate that inferential decisions on the null hypotheses can be made at desired standard α and β error levels. Due to the multimodal approach of the present work, a tradeoff between statistical test power vs. budget (especially for expensive biochemical saliva analyses) but also available time had to be found (EEG manual–visual artifact correction is very time consuming; only one single experimental session could be realized in one day and all experimental work was only done by the author of the present work without help from assistants, etc.). This found tradeoff with sample size $N = 12$ offers in combination with the employed *within*-subjects design fully adequate statistical test powers.

2.4. Auditory stimuli

For the decisions how to design the Binaural Beat stimulus to be employed in the present work, previous studies were revised. Brady and Stevens (2000) and Stevens et al. (2003) used in both studies the same complex Binaural Beat stimulus with *Δf* in the theta EEG frequency band generated by multiple carrier frequencies arranged in the form of musical chords: 0-3 minutes *C-minor*^{7} (292 Hz, 330 Hz, 392 Hz, 466 Hz); 3-6 min *C-major* (292 Hz, 330 Hz, 392 Hz, 523 Hz); 6-10 min *G-major* (196 Hz, 247 Hz, 294 Hz, 392 Hz); 10-15 min *D-minor* (294 Hz, 349 Hz, 440 Hz); 15-20 min *C-major* (292 Hz, 330 Hz, 392 Hz, 523 Hz). *Δf* was designed in a dynamic way such that it swings during 4 seconds from 5.5 Hz to 8.5 Hz and back to 5.5 Hz. The authors used these complex stimuli with oscillating *Δf* because of their opinion that this could facilitate allocation of attentional resources to the Binaural Beats and reduce habituation effects. These Binaural Beats were masked by *pink noise* for the *Verum* stimulus and for *Placebo* only the *pink noise* without embedded Binaural Beats was used. Unfortunately, both studies came to contradictory results. This is one reason why in the present work a *distinct* stimulus should be used. First of all, using multiple carrier frequencies could cause too complex interactions, so Binaural Beats will be generated by only two carrier frequencies. Second, from the findings of Perrott and Nelson (1969) concerning the maxima of detection rates in function of f_1 and *Δf* (see table 1) it is to be concluded that for a *Δf* in the alpha and theta EEG frequency range carrier frequencies < 250 Hz are most appropriate. Moreover, the concept of a rapidly and periodically changing *Δf* will not be employed, because it is likely that the central nervous system needs time to react and this could be *impaired* by constructing *Δf* as the above mentioned authors did which could be a reason for the contradicting results of their studies. Hence, all changes of carriers and also *Δf* should be *continuous* and rather *slow,* but never periodic.

With these considerations in mind, the following sweep of decreasing carrier frequencies and decreasing *Δf* was invented for the total stimulus length of 25 min, starting in the first 10 min with *Δf* in the alpha EEG frequency band, continuing the decrease covering the theta EEG frequency band in the following last 15 min (see table 2, figure 9 and figure 10).

Table 2. Design of the Binaural Beats frequency sweep by the author of the present work, to be studied in the present dissertation.

time [min]	carrier f_1[Hz] *left ear*	carrier f_2[Hz] *right ear*	Binaural Beat frequency Δf [Hz]
0 (start)	**160**	**150**	**10.000**
1	156.8	147	9.800
2	153.6	144	9.600
3	150.4	141	9.400
4	147.2	138	9.200
5	144	135	9.000
6	140.8	132	8.800
7	137.6	129	8.600
8	134.4	126	8.400
9	131.2	123	8.200
10	**128**	**120.0**	**8.000**
11	124.8	117.1	7.734
12	121.6	114.1	7.467
13	118.4	111.2	7.200
14	115.2	108.3	6.934
15	112	105.3	6.667
16	108.8	102.4	6.400
17	105.6	99.5	6.134
18	102.4	96.5	5.867
19	99.2	93.6	5.600
20	96	90.7	5.334
21	92.8	87.7	5.067
22	89.6	84.8	4.800
23	86.4	81.9	4.534
24	83.2	78.9	4.267
25 (end)	**80**	**76.0**	**4.000**

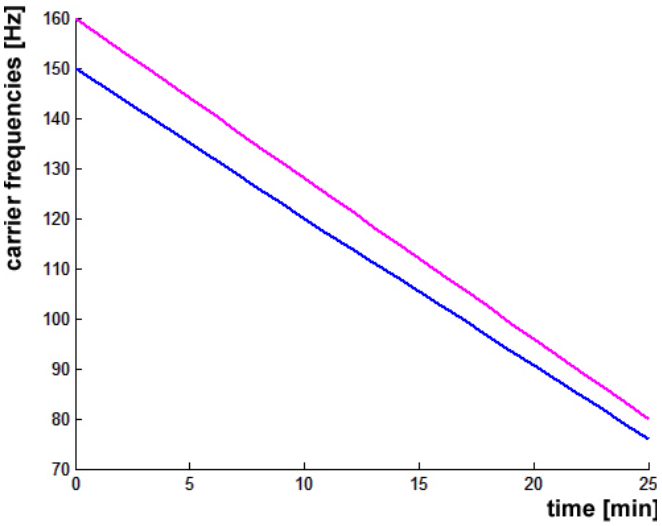


Figure 9. Sweep of the *carrier frequencies* with continuous linear decrease for the Binaural Beat stimulus studied in the present work. Magneta = left ear, blue = right ear.

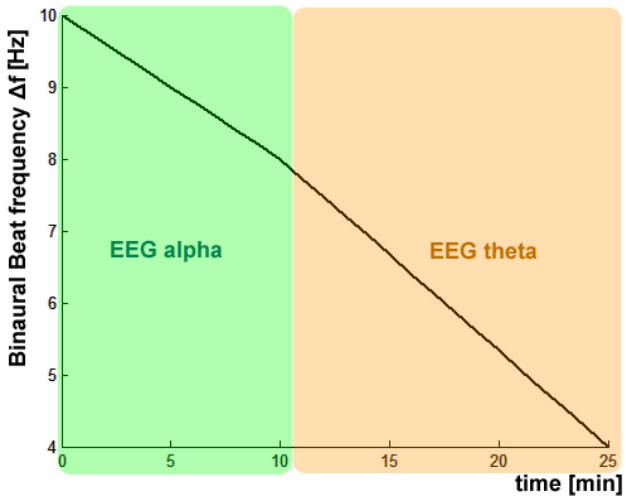


Figure 10. Sweep of *Δf* with continuous decrease for the Binaural Beat stimulus studied in the present work with *Δf* covering EEG alpha and theta frequency bands.

For the *left* ear, carrier frequency f_1 decreases within the 25 min of stimulus length linearly from 160 Hz to 80 Hz, i.e. a slow linear decrease of 3.2 Hz per minute. For the *right* ear, carrier frequency f_2 decreases linearly from 150 Hz to 120 Hz within the first 10 minutes, i.e. a slow decrease of 3.0 Hz per minute. Then the decrease of f_2 continues to be linear, but with a different slope, so at 10 min there is a bend: At 10 min f_2 decreases linearly from 120 Hz down to 76 Hz at the end of the stimulus, i.e. a slow decrease of 2.93 Hz per minute. This design means for the Binaural Beat frequency Δf a linear decrease in the first 10 min from 10 Hz to 8 Hz which corresponds to a sweep from the middle alpha EEG frequency band down to the beginning of theta. Decrease of Δf happens with a velocity of .2 Hz per minute. After 10 min, Δf decreases from 8 down to 4 Hz corresponding to the entire theta EEG frequency band, i.e. with a velocity of .266 Hz per minute.

Because the Binaural Beat illusion would be *aversive* when presented over longer periods of time, it has to be covered by an auditory *mask.* Although other studies used *white* or also *pink* (i.e. *1/f* noise) stationary noise, for the present work a special dynamical auditory mask is designed. All audio mastering was done with the digital multitrack professional sound studio program *Adobe Audition* ("Adobe Audition 3.0," 2007) which is a world-wide used standard tool for professional music and radio journalism production. The design of this special dynamical auditory mask aims first to homogenize *cortical auditory maps* and second to introduce some slow continuous changes thought to avoid habituation effects. First of all, a *red* noise (i.e. $1/f^2$ noise) was digitally generated. Many naturally occurring acoustical noises in nature follow this spectral $1/f^2$ distribution, such as the noise caused by the wind, but also many non-acoustical phenomena show this kind of spectral distribution, e.g. the *Brownian motion* which can be described as a *Wiener process* with its interesting property of *self-similarity* over varying time-scales.

In order to change this artificially generated *red* noise from being stationary to dynamical, two digital audio effects were applied, one after the other. The first one is the *Graphic Phase Shifter* which processes both stereo audio

tracks in such a way that (using headphones) the illusion is created that the noise would be rolling in space around the head; rolling-velocity was set to one minute for an entire period of 360°. This applied first digital effect leads to a continuous change of the *phase space* of the stereo signal, i.e. *interaural phase differences* change slowly and continuously and thus stimulate those neurons which analyse phase differences, but without a specifically structured stimulation pattern in order to cause homogenization in the sense of a unstructured, uniform stimulation field (*Ganzfeld effect*, see Metzger, 1930; Wackermann, Pütz, & Allefeld, 2008). Moreover, this illusion of a noise rolling around the head is meant to direct attentional resources to the noise and especially to its spatial illusion and thus for *phase differences* between the two ears. Because Binaural beats are theorized to work by phase differences, this continuous, but *non-specific* stimulation of brain subsystems analysing sound localizations by this kind of special auditory mask is meant to make the *specific* stimulation by Binaural beats more *salient.* This idea was inspired by the phenomenon of *stochastic resonance* (see e.g. Moss, Ward, & Sannita, 2004). Another explication is that while the noise implies continuous and fast random changes in phases and frequencies, the very slowly changing Binaural Beats become more *salient* in it, as known from the visual domain as the so-called *figure-ground principle* (see e.g. Rubin, 2001).

After applying this digital *Graphic Phase Shifter* sound effect to the artificially generated *red* noise, as a second treatment, the *Stereo Sweeping Phase Shifting Effect* was used: Simplifying, a copy of the original signal is delayed and then remixed with the original, which leads to phase differences of 180° for special frequencies, so *extinction* of waves is the consequence. The resulting filter effect can also be described as a *multiple notch filter* or *comb filter.* When the delay of the described filter is continuously changed, then every time *other* frequencies are affected by extinction which means that these *multiple notches* move in a sweep over the auditory spectrum. The *Graphic Phase Shifter* effect is frequently used in pop and techno music and creates the subjective impression that the sound becomes more "muffled" and then "sharper" again. This effect was adjusted for periodic cycles from "muffled" and then "sharper" of 20 seconds and synchronized to the first applied digital effect, such that one

cycle is finished when the noise seems to have moved for 90° around the head. While the first digital effect achieves a periodic continuous sweep in the *phase domain* of the stereo signal, the second *Graphic Phase Shifter* produces a cyclic continuous sweep in its *frequency domain.* The described digital sound processing makes the auditory mask *dynamical* as intended for the above explained reasons.

After creating the Binaural Beats with the described frequency sweep at 45 dB(a) RMS sound level, multitrack environment of *Adobe Audition 3.0* was used to mix them with the auditory mask to create the *Verum* stimulus. The mixture was adjusted for a sound level of 65 dB(a) RMS. The *Placebo* stimulus was the bare auditory mask without embedded Binaural Beats at also 65 dB(a) RMS. The *signal-to-noise distance* of approx. 10 dB between carrier frequencies and noise was chosen to be quite low, so presence or absence of Binaural Beats was not consciously detected by any person with the only exception of a professional musicologist working as a professional audio producer who moreover had the rare gift of *absolute pitch*. Relatively low *signal-to-noise distance* was chosen (see figure 11) because following Gu, Wright and Green (1995) magnitudes of carrier frequencies can be surprisingly low and still trigger Binaural Beats. This does not surprise when we take into account that very small phase differences at very low magnitudes are sufficient for the brain to detect the localization of sound sources. It has to be assumed that the ability of sound source localization was so much important to survival in the evolution that the brain developed this amazing sensitivity to reliably localize in space even very quiet sound sources in very noisy and complex auditory surroundings.

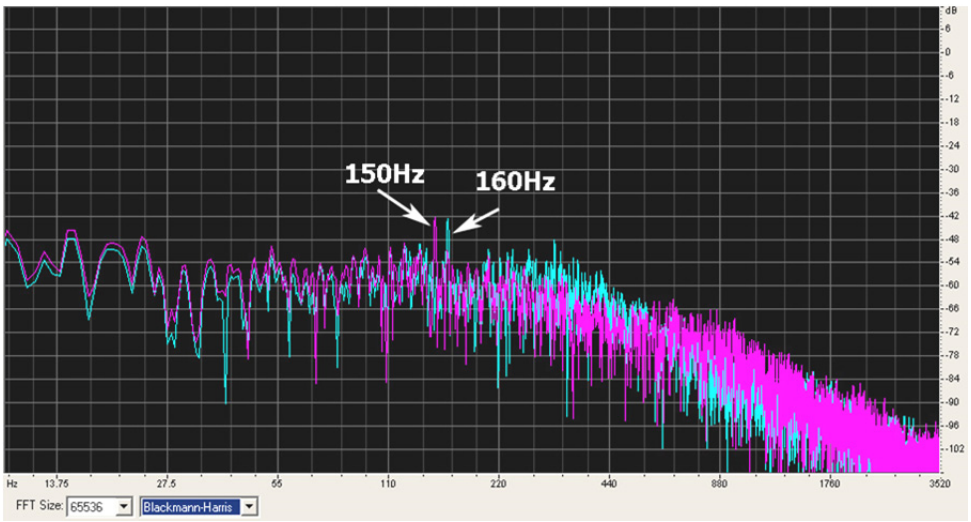


Figure 11. Power Density Spectrum at time point 0 of the *Verum* stimulus, *x*-axis in Hz and *y*-axis in dB. The two carrier frequencies (left ear magenta, right ear cyan) are embedded into a special auditory noise mask. *Signal-to-noise distance* is approx. 10 dB within the frequency band 55 – 220 Hz.

The property of the *Verum* vs. *Placebo* stimulus that they cannot be distinguished by the subjective impression of the participants has the important advantage that all cognitive processes concerning the nature of the stimuli cannot reflect the reality of presence vs. absence of embedded Binaural Beats: Because the stimulus under question is quasi inaudible, possible differences in the reactions to *Verum* vs. *Placebo* cannot be attributed to conscious perceived differences in the auditory stimulation.

Audio stimuli and all instructions were digitally stored on a SD card in **.wav* audio format at standard CD quality 44.1kHz sample frequency and 16 bit resolution without any compression. Sound was played back by the battery-driven professional digital audio processor *Zoom H2* (see figure 12; ZOOM Cooperation, 4-4-3 Kanda-surugadai, Chiyoda-ku, Tokyo 101-0062, Japan) using special closed HiFi headphones *Ultrasone HFI 680* used in professional audio production (Ultrasone AG, Gut Raucherberg 3, 82407 Wielenbach, Germany).

Figure 12. Battery-driven professional digital audio processor *Zoom H2* with 2.0 GB SD card for audio **.wav* file storage used to play–back of all instructions and stimuli in the present work via magnetically shielded headphones *Ultrasone HFI 680* or loudspeakers. A battery-driven sound source offers the advantage that ground-loops are avoided and electromagnetic interferences (EMI) are minimized.

Apart from the very high sound quality, these headphones are magnetically shielded by *mu-metal* fulfilling the *Ultra-Low Emission* standard: Because dynamic headphones and loudspeaker electrically are a coil, fed with *AC*

voltage, they produce an electromagnetic alternating field which in the case of headphones is located very close to the EEG electrodes; thus, without very effective electromagnetic shielding, direct electromagnetic interference could disturb the EEG signal. As some previous experiments confirmed, no interferences in the EEG could be induced by the *Ultrasone HFI 680* headphones, but not-shielded headphones as e. g. the *Sennheiser HD 429* do produce small interference. EEG research involving auditory stimulation not done by special shielded headphones or special air conduction headphones should be considered with reservations. Sound level output by the *Zoom H2 – Ultrasone HFI 680* system was calibrated using a sound level meter (*Voltcraft SL-200,* Conrad Electronic SE, Klaus-Conrad-Str. 1, 92240 Hirschau, Germany) positioned exactly one centimeter where in its normal use the human auditory canal would be situated using multiple frequency and noise types, so for both *Verum* and *Placebo* a presentation loudness of 65 dB(a) RMS was calibrated.

2.5. Measures

2.5.1. Psychometric instruments: domain of subjective introspective experience

Thoroughly developed psychometric instruments measuring *altered states of consciousness* (ASCs) are seldom, so expert advice was asked for from collaborators of Dr. Ulrich Ott, Bender Institute of Neuroimaging (Justus-Liebig-Universität Gießen, Otto-Behaghel-Str. 10H, 35394 Gießen, Germany), head of the institute's working group on ASCs and author in the milestone publication of Vaitl et al. (2005). They recommend to capture psychometric *state* changes concerning ASCs by the use of the Phenomenology *of Consciousness Inventory (PCI)* of Pekala and Levine (1982) and Pekala (1991). The inventory aims to retrospectively assess consciousness states in reference to a particular immediately preceding stimulus condition. The PCI has been empirically verified to be able to psychometrically reliably and psychometrically validly map phenomenological experiences to stimuli conditions such as eyes open and closed sitting quietly, hypnotism, progressive relaxation, breathing

techniques, drumming and trance postures, and even fire-walking (Forbes & Pekala, 1993, 1996; Maurer, Kumar, Woodside, & Pekala, 1997; Pekala & Ersek, 1992; Pekala & Levine, 1981, 1982; Pekala, Steinberg, & Kumar, 1986; Pekala, 1991, 1995a, 1995b; Pekala & Wenger, 1983; Woodside, Kumar, & Pekala, 1997). These findings were confirmed and expanded also by other researchers for other stimuli conditions such as meditation (Venkatesh, Raju, Shivani, Tompkins, & Meti, 1997), ego boundaries (Rock, Wilson, Johnston, & Levesque, 2008), religious narratives (Wildman & McNamara, 2010), virtual-reality environment (Huang, Himle, & Alessi, 2000), partial epileptic seizures (Johanson, Valli, Revonsuo, Chaplin, & Wedlund, 2008), etc.

These decades of developing the PCI were inspired by the aim of *quantifying consciousness* which the author stated in his dissertation as follows (Pekala, 1980):

> "[…]. Just as behaviorism replaced introspective psychology, and cognitive psychology is now replacing behaviorism, it may be that the scientific study of consciousness and its various dimensions will be the new frontier to which many psychologists will turn when the approach of cognitive psychologists has been found wanting.
>
> An empirical methodology which brings together phenomenological observation with psychological research and theorizing must form the basis for such an endeavor, since consciousness, as subjectively experienced, can be best known, not through neurophysiology, but through introspection or phenomenological observation that is coupled with and related to a psychoneurophysiological data base.
>
> Although electrophysiological, neurochemical, and neuroanatomical data can help, a science of consciousness must be grounded in an empirical psychophenomenology of consciousness by which the phenomenological attributes of subjective, conscious experience are empirically observed, assessed, and evaluated in conjunction with biological, psychological, and physiological variables. Such a psychophenomenology, if found reliable and valid, would investigate consciousness through empirical, phenomenological methodologies and relate such observation to more traditional psychological, physiological, and biochemical approaches. […] "

The PCI consists of 53 bipolar–opposing items with 7-point answer scale in the sense of a *semantic differential* (Osgood, Suci, & Tannenbaum, 1957), i.e. the extreme poles of the scale (0 vs. 6) correspond to the opposing statements to rate. The PCI contains 12 psychometric main scales with 14 subscales and a special reliability index of 6 items which leads to exclusion of the subject due to unreliable answer pattern when this index exceeds

≥ 2.3 (Pekala, 2009). Subjects answer the PCI immediately after stimulus presentation, i.e. *retrospective phenomenological observation*. Several analyses of psychometric properties, repeated over years, over different populations and over distinct stimuli conditions by Pekala (1991, 2009) and co-workers showed sufficient *reliability* of the scales as measured by *internal consistency* and *test–retest* correlations and excellent evidence for *validity* with regard to *factorial structure,* convergence and discrimination behaviour with other psychometric measures and stimulus specificity. The 12 main scales with its most important subscales in parenthesis are: *Positive Affect, Negative Affect, Altered Experience (Altered Body Image, Altered Time Sense, Altered Perception, Altered Meaning), Imagery, Attention, Self-Awareness, Altered State of Awareness, Internal Dialogue, Rationality, Volitional Control, Memory* and *Arousal*. Scale raw scores are computed by dividing scale sums by each total item number of the scale.

Moreover, the PCI offers the *Hypnoidal State Score (HSS)* which was developed by co-administration of the *Harvard Group Scale of Hypnotic Susceptibility, Form A (HGSHS:A)* (Shor & Orne, 1962) and the use of *multiple linear regression* modelling, with the aim to predict *HGSHS:A* scores from the PCI scales. These empirical investigations lead to the following regression equation *HSS* = .35**Altered experience* –.07**Body Image* +*.13***Time Sense* +.19**Absorption* –.27**Self-awareness* + .31**Altered State* –.11**Internal Dialogue* +.23**Rationality* –.28**Volitional Control* –.14**Memory* + *4.51* (Pekala, 2009). The authors showed that with this *HSS* the depth of a hypnoidal state can be measured reliably and validly. *HSS* in the range 1.00 – 3.00 is considered as *non-hypnoidal state,* 3.01 – 5.00 as *mild hypnoidal state,* 5.01 – 7.00 as *moderate hypnoidal state* and 7.01 – ≥ 9.00 as *high hypnoidal state.* The authors report *HSS* baseline values for healthy normal population determined by simple sitting with eyes closed of around 3.4.

A Spanish version of the PCI, translated by a Spanish–English psychophysiologist was obtained by personal communication with the author of the original version. Generally, every psychometric instrument has to be re-investigated after translation into other languages or for the use in

different cultural settings (e.g. Spain vs. Latin America). Although the Spanish PCI has been used in unpublished works, no publication concerning its psychometric properties is available until today. Given that the re-investigation of the *German* version resulted in no mayor differences in reliability and validity (Rux, 2002), that the measured domain concerns the field of *general* psychology which refers to relatively *cultural-free* phenomena and that a complete Spanish re-investigation would be an entire large research project itself, the Spanish version of the PCI has to be used without re-investigation of its psychometric properties. Nevertheless, in the framework of multimodal measures in the present work, its validity can be concluded indirectly interpreting the PCI results in the context of the possible findings in the other measures. The PCI's author does not permit full reprint of its instrument to maintain control over its responsible use, so only the most important main scale for the *HSS* regression equation can be reported here for illustration purposes, *Altered Experience* (see figure 13).

4. He tenido una experiencia que denominaría religiosa, espiritual o trascendental.	0 1 2 3 4 5 6	No he tenido una experiencia que denominaría religiosa, espiritual o trascendental.
11. Mi cuerpo terminaba en el límite entre mi piel y el mundo.	0 1 2 3 4 5 6	He sentido que mi cuerpo se expandía en gran medida más allá de los límites de mi piel.
15. Ha cambiado drásticamente mi percepción del flujo del tiempo.	0 1 2 3 4 5 6	No he percibido cambio alguno en mi percepción del flujo del tiempo.
17. Ha cambiado drásticamente mi percepción del mundo.	0 1 2 3 4 5 6	No he notado cambios en mi percepción del mundo.
23. He sentido sobrecogimiento y reverencia hacia el mundo.	0 1 2 3 4 5 6	No he tenido la sensación de sobrecogimiento y reverencia hacia el mundo.
26. Mis sensaciones corporales parecían expandirse hacia el mundo que me rodeaba.	0 1 2 3 4 5 6	Mis sensaciones corporales estaban confinadas a la zona del interior de la piel.
29. El mundo externo se ha vuelto muy diferente en cuanto a formas o colores.	0 1 2 3 4 5 6	No he notado cambios en el color o la forma del mundo externo.
30. El tiempo parecía acelerarse o ralentizarse mucho.	0 1 2 3 4 5 6	He experimentado el tiempo sin cambios en su ritmo.
32. No he tenido una comprensión profunda de las cosas diferente de mi entendimiento habitual.	0 1 2 3 4 5 6	He tenido una comprensión muy profunda y esclarecedora de determinadas ideas o cuestiones.
39. No he notado cambios en el tamaño, la forma o la perspectiva de los objetos del mundo externo.	0 1 2 3 4 5 6	Los objetos del mundo externo han cambiado de tamaño, de forma o de perspectiva.
43. No he tenido sensación de eternidad; el tiempo fluía tal como suelo experimentarlo.	0 1 2 3 4 5 6	El tiempo se ha detenido; el tiempo no se movía en absoluto.
47. No he tenido la sensación de que la existencia fuera sagrada o que tuviera un significado profundo más allá de lo	0 1 2 3 4 5 6	La existencia se ha convertido en algo muy sagrado o con un significado profundo.
51. He mantenido constantemente una sensación intensa de separación entre mí mismo y el entorno.	0 1 2 3 4 5 6	He sentido una unidad intensa con el mundo; se han disuelto los límites entre mí mismo y el entorno.

Figure 13. 13-item psychometric main scale *Altered Experience* of the *Phenomenology of Consciousness Inventory* (PCI) in its Spanish version by Pekala (1991). Reprint of the entire instrument is not permitted by its author in order to maintain control over its responsible use.

After explaining the PCI, now the psychometric main hypothesis of the present work can be specified: "If the *Verum* stimulus would indeed have relevant and specific efficacy, the *central tendencies* of the distributions of *Hypnoidal State Scores (HSS)* under *Verum* vs. *Placebo* must show significant differences as contrasted by the inferential statistical non-parametric test for related samples *Wilcoxon signed-rank test* and moreover, the central tendency under *Verum* must be *higher* as under *Placebo,* i.e. comparing their *medians."*

2.5.2. Physiological measures

Apart from the domain of subjective introspective experience measured by psychometry, physiological biosignals and its parameters are measured continuously in the experiments by an in-house made data acquisition setup: *Electroencephalogram* (EEG), *Electrocardiogram* (ECG) and *Respiratory Nasal Flow* (RNF). Subjects sit in a special lounger with 45 ° inclined back rest (*IKEA Systems B.V.*), allowing maximal relaxation with subject's feet put up, but still staying in an upright position. A large grounded shield hand-made from steel wire mesh in the shape of a rectangular cuboid covers the lounger with the subject and the data acquisition devices in order to protect against *electromagnetic interference* (EMI), see figure 14. The electrical mains for driving the *PC* computer (Intel i7-950 quad core, 4*3.06GHz) and the monitor are galvanically separated from earth by a special medical *isolation transformer* (*IMEDe 2000*, Noratel Germany AG, Elsenthal 53, 94481 Grafenau, Germany), thus offering maximal electrical security: Even if the output voltage of 230 V_{RMS} would reach accidentally the subjects' body, no current would result, thus protecting the lives of the subjects. Although there are multiple galvanic separations between the computer and the subject's body, the use of this special *isolation transformer* avoids even theoretical risks, making the setup very secure.

All biosignals are sampled at 1024 Hz, visualized in real time on a flat screen for control purposes outside the shield and stored as one entire data matrix in form of an *ASCII*-file on a *Linux* (*Ubuntu)* system by in-house

code written in *C.* ECG and RNF are analogically amplified and filtered by battery driven preamplifiers situated in the grounded shield, personally designed and constructed by the author of the present work, as described below. Still in the shield, their output voltages in the typical range of ± 5 V are connected via coaxial cables to the input rack of a professional isolated DAQ data acquisition board (*NI-PCI-6251*, National Instruments, Building B, 11500 N Mopac Expy, Austin, TX 78759, USA), see figure 15,

Figure 14. Self-made shield with 1.5m height/width and 2.15m length consisting of a wooden frame offering mechanic stability for the attached 0.25mm diameter steel wire mesh with 1.5mm mesh opening. The steel mesh is connected to the isolated common ground of the isolation transformer for potential equalization. The lounger for the subject and a table for battery driven preamplifiers and DAQ data acquisition rack are located inside the shield, while the recording computer with flat screen and the chair for the experimenter are placed outside.

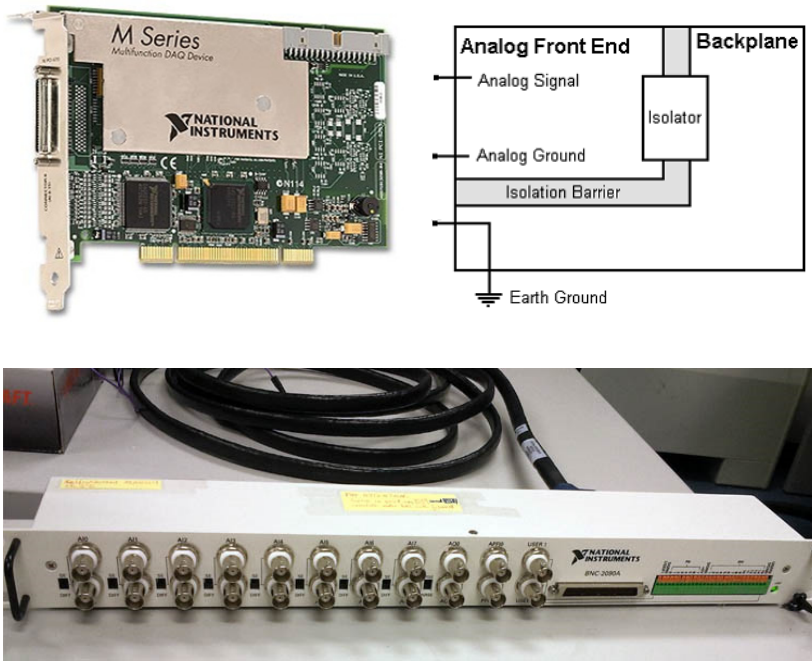


Figure 15. Data acquisition board *PCI-6251* (16 channel, 16-bit) of the manufacturer *National Instruments* used for analog-to-digital conversion of all biosignals (except EEG) at 1024 Hz sample frequency. It provides an internal isolation barrier between its analogue and digital circuitry based on an inductive coupling technology (*iCoupler*, Analog Devices) to insure electrical safety. The connection to the analogue preamplifiers is realized by a *BNC* socket rack, which is placed onto the table inside the steel mesh shield and connected to the outside located PC card by means of a shielded cable (black thick wire).

whose shielded connection cable leaves the described shield towards the computer located outside reaching the plugged-in PCI card (*Peripheral Component Interconnect*). The DAQ board is controlled under *Linux* by the open source driver library *COMEDI* (linux control and measurement device interface, see http://www.comedi.com). EEG is amplified and digitalized by the isolated 64-channel commercial system *Brainbox® EEG-1166* (Braintronics B.V., Gildemark 130, 1351 HL Almere, Netherlands) driven by in-house written software at 1024 Hz sample frequency. Both data streams coming from the DAQ board and the EEG devices are synchronously written into the same data matrix of the *ASCII* output file.

2.5.2.1. Electroencephalogram EEG and electrooculogram EOG – hardware

A sampling frequency of 1024 Hz allows to analyze frequencies up to 512 Hz due to the *Nyquist-Shannon sampling theorem* (Shannon, 1949). Given that the scalp EEG signal contains frequencies maximally up to 130 Hz, this is more than sufficient. But the EEG is not only sampled in *time domain*, but also in *spatial domain* using a multichannel sintered Ag/AgCl EEG cap *Aegis array* (Sands Research, 955 N Resler Drive Suite 104 #113 El Paso, TX 79912, USA) using international standard electrode positions following the 10-20 system (Jasper, 1958). Not commonly known, the *aliasing* problem occurs also in the *spatial* domain of the EEG when it is not measured with a sufficient spatial resolution, so based on simulation studies Nunez and Srinivasan (2005) recommend at least approx. 64 electrodes to avoid spatial aliasing. In the employed setup, 63 electrodes with nose tip as reference are used as depicted in figure 16, leaving the 64^{th} as an analogue event synchronization channel. After placement of the EEG cap, skin abrasion by smooth twisting movements of the blunt steel needle, and injection of conductive gel in every electrode, impedances are measured and contacts checked to keep them ≤ 10 kΩ. EEG is amplified and digitalized by the isolated 64-channel commercial system *Brainbox® EEG-1166* (Braintronics B.V., Gildemark 130, 1351 HL Almere, Netherlands) driven by in-house written software at 1024 Hz sample frequency and connected to the computer via galvanically isolated *Ethernet.*

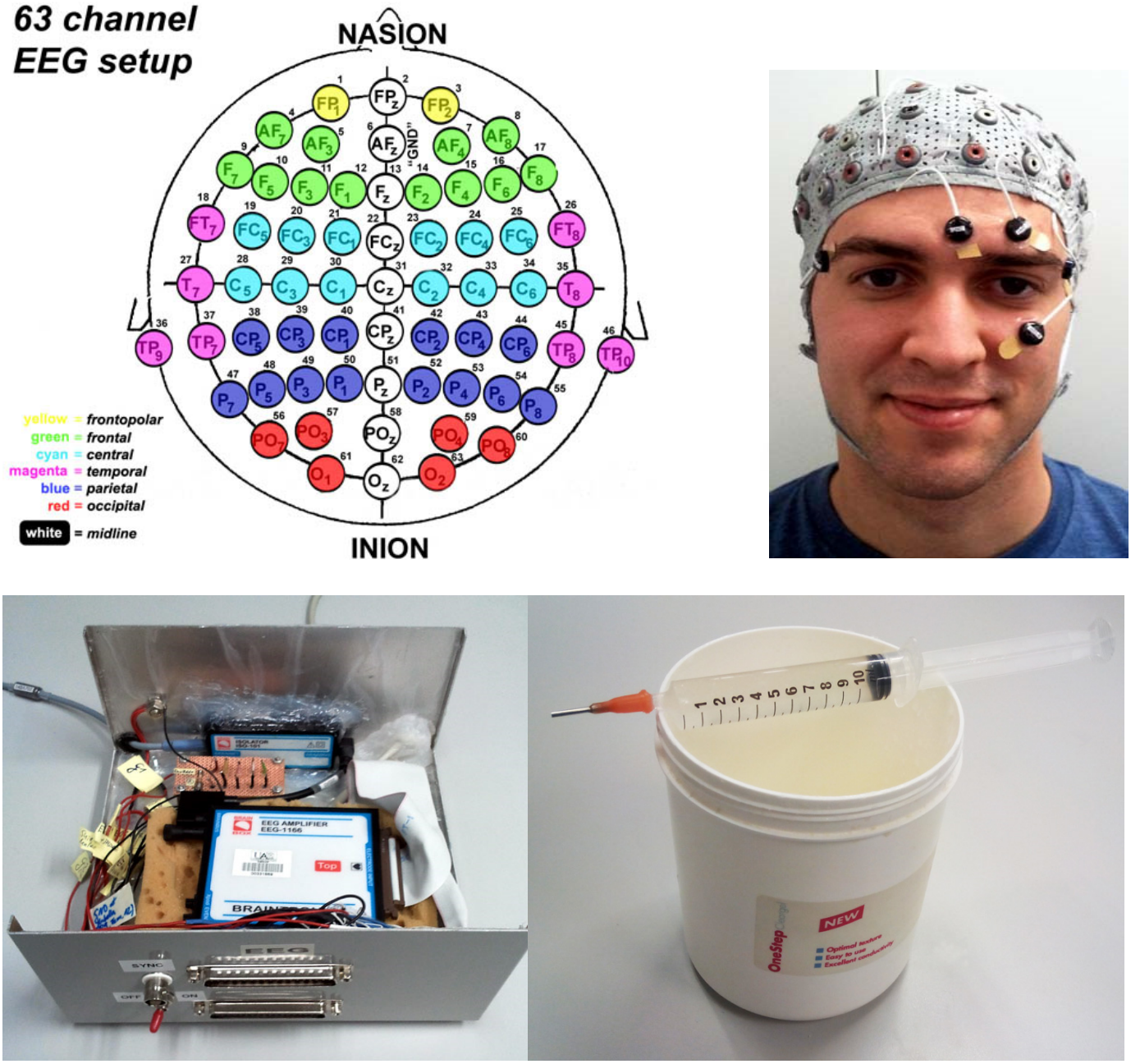


Figure 16. 63 channel EEG setup following the international 10-20 standard electrode locations (Jasper, 1958), leaving the 64th channel as an analogue event synchronization channel. Sintered Ag/AgCl EEG cap *Aegis array* (Sands Research, 955 N Resler Drive Suite 104 #113 El Paso, TX 79912, USA) is used with medium size (56 – 59 cm head circumference). Sintered Ag/AgCl electrodes with double-sided adhesive rings are placed above/below the left eye and right/left *cantus* while ground is placed at *glabella* for measurement of electro-oculogram (EOG). Reference electrode is placed to *nose tip* (not shown) and each channel is amplified and digitalized as *unipolar montage* by the commercial isolated 64-channel *Brainbox® EEG-1166* amplifier (Braintronics B.V., Gildemark 130, 1351 HL Almere, Netherlands) connected to the computer via isolated *Ethernet*.

OneStep electrolytic gel (MedCaT B.V., Fürstenriederstr. 279a, 81377 München, Germany) is injected into the electrodes by means of standard disposable 10ml syringes with blunt steel tips 15G 0.5'' Luer-Lock and twisted to abrade skin in order to keep electrode impedances < 10kΩ.

After each use, the EEG cap is thoroughly cleaned by a soft jet of water, then disinfected for 30 min in 10% *GigaseptFF neu* (Schülke & Mayr GmbH, Robert-Koch Str. 2, 22851 Norderstedt, Germany), rinsed again with sterile distilled water and stored for drying. *GigaseptFF neu* is a high-performance but anti-corrosive disinfectant used in hospitals for chirurgical and endoscope instruments.[1]

[1] *GigaseptFF neu* was approved by the *Robert Koch Institute* (Nordufer 20, D-13353 Berlin, Germany) which is part of the German Federal Ministry of Health, but also part of the European Centre for Disease Prevention and Control (ECDC), the EU Directorate General Health and Consumers (DG SANCO) and the World Health Organization (WHO). International laws claim *approved* disinfection methods of EEG electrodes after each use, contravention is legally defined as *attempted murder* and judges usually apply long imprisonment without probation in cases of dysfunctional disinfection. This very strict practice is mainly meant to avoid the highly infectious and persistent *Hepatitis B virus,* causing a disease with still relatively high mortality. The simple buy of EEG hardware implies the complete knowledge and acceptance of *medical device legislation*, so common arguments such as "others do not disinfect either" or "we are not in a hospital, so medical legislation does not apply" is legally irrelevant and can even cause *increase* of penalties because of proof for *intent*. Murder and its attempt do never become statute-barred, in opposite to *manslaughter*.

The EEG is unavoidably interfered by electro-oculogram (EOG) signals, they have to be measured in an isolated manner in order to later use special EOG artifact correction algorithms on the EEG signals. EOG is captured with Ag/AgCl electrodes fixed by adhesive rings placed above/below the left eye and right/left lateral *canthi* while ground is placed at *glabella,* after cleaning the skin with 70% isopropanol and cotton wool, abrasion with a commercial standard sponge (*Scotch-Brite*, 3M) and repeated cleaning with 70% isopropanol and cotton wool. Vertical and horizontal EOG signals (vEOG / hEOG) are amplified and filtered by a battery-driven preamplifier personally constructed by the author of the present work following circuitry developed by Usakli and Gurkan (2010), see figure 17 and figure 18. Both filtered and amplified vEOG and hEOG output signals in the range of ±5 V are connected by coaxial cable to the data acquisition rack, just as the other analogue preamplifiers, digitalized at 1024 Hz and written into the final *ASCII* data-matrix.

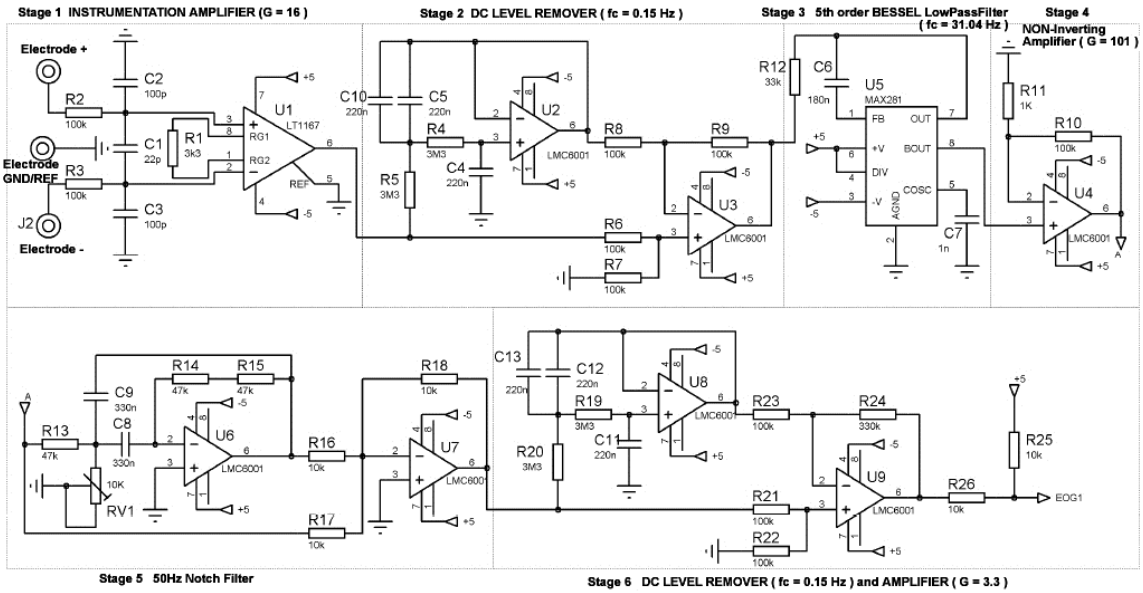


Figure 17. The EOG amplifier circuit kindly supplied by Usakli and Gurkan (2010) after personal communication. Only one channel signal-chain is shown, because the circuits for horizontal and vertical EOG are identical copies.

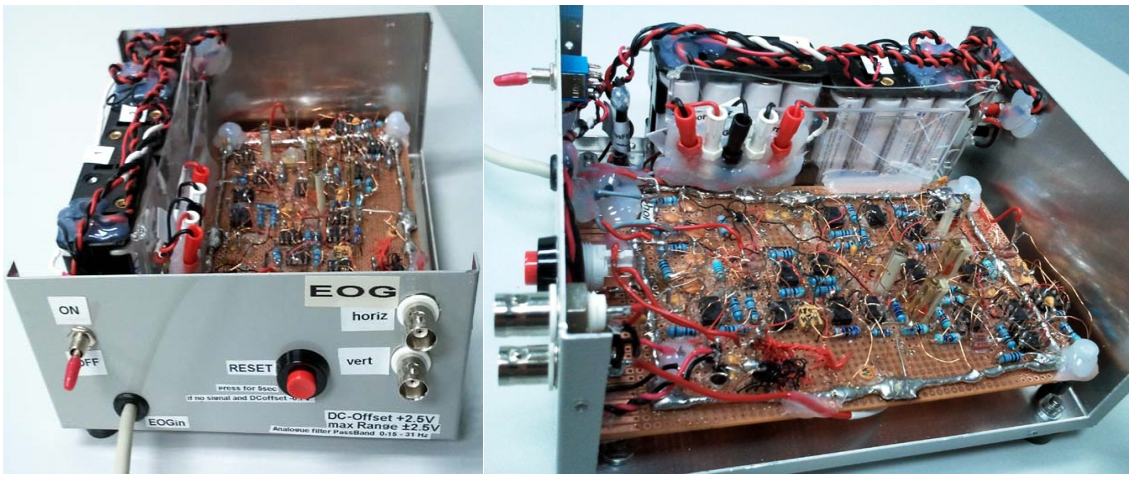


Figure 18. The battery-driven EOG amplifier personally constructed by the author of the present work by air wiring technique usually used in electronic prototyping with the two *BNC* output connectors. Time for total handiwork needed approx. 150 hours.

In the **first stage** the EOG signal passes a passive RC low-pass filter (R2, C2 and R3, C3) with a f_{Cutoff} = 15.9 kHz in order to cut off radiofrequency *EMI* and other high frequency interferences as e.g. *PC* monitors or fluorescent lamps. In the integrated circuit *instrumentation amplifier* LT1167 manufactured by *Linear Technologies* the two glabella referenced voltages are subtracted from each other and 16 times amplified, so only the voltages common to the two entrances, i.e. 50 Hz interferences etc. which affect the two entrances almost in the same proportion, are highly suppressed: The LT1167 has an extraordinary *common-mode rejection ratio* (CMRR) of > 120dB.

In the **second stage**, the DC offset of the EOG signal is removed by adding the .015 Hz low-pass filtered output of the instrumentation amplifier to the original output signal, because they are in opposite phases, thus they are differentiated, the constant, the DC offset, is removed.

In the **third stage**, the signal passes a 5^{th} order *Bessel* low-pass filter in form of the integrated circuit MAX281. At this stage, the EOG signal is reduced to the bandwidth at interest .15 – 31 Hz, so e.g. remaining 50 Hz interferences are filtered out.

The **fourth stage** contributes the main part of the entire amplification process. Here, the author of the present work modified the design of

Usakli and Gurkan (2010) by replacing R10 by a 100kΩ trimmer, so the gain can be easily modified in e.g. a calibration process.

The **fifth stage** subtracts from the output of stage four its 50 Hz notch-filtered (10 Hz bandwidth) derivate, so remaining 50 Hz interferences are highly suppressed another time.

The **sixth and last stage** is a copy of stage two and removes any DC offset which may be introduced to the signal as artifacts from the amplification stages. Horizontal and vertical EOG signals are amplified by two identical amplifiers, but in this sixth stage the last amplification step is set to gain G = 3.3 for the vertical and G = 4.7 for the horizontal EOG, due to its slightly different signal amplitudes. At the end, if R10 is set at 100 kΩ, the EOG signal was amplified with a total gain of $G = 16 \cdot 101 \cdot 3.3 = 5332.8$ times for the vertical or $G = 16 \cdot 101 \cdot 4.7 = 7595.2$ times for the horizontal EOG.

Examples of EOG signals measured by the described circuitry are shown in figure 19.

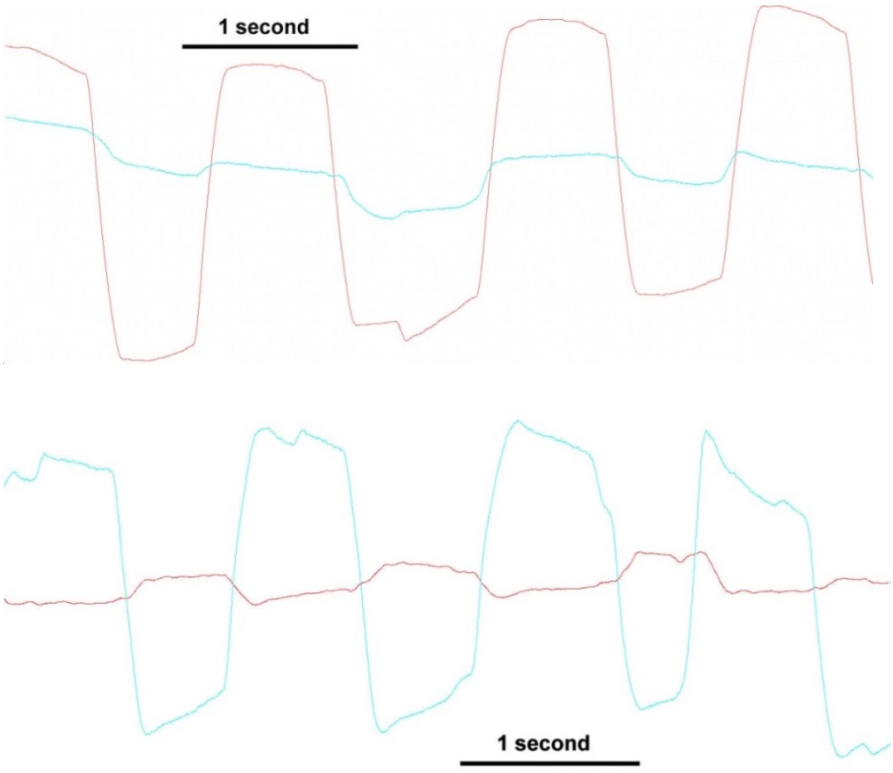


Figure 19. Horizontal (above) and vertical (below) *eye movements* represented in the vertical (blue) and horizontal (red) electro-oculogram (EOG) signals, digitally filtered by a digital 5th order *Butterworth* low-pass filter with f_{cutoff} = 30 Hz to reject remaining 50 Hz interference without changing the original signals. Because this example is an uncalibrated test recording, the y-axis is left without scale.

2.5.2.2. Electrocardiogram ECG – hardware

The electrocardiogram (ECG) are voltage changes of the cyclically repeating electrical excitations of the heart muscle primarily produced by the *sinoatrial node*, which form an oscillating electromagnetic dipole. Because voltage is measured as the potential difference between two points, this dipole is observed as the spatial projection of its vector to the axis which is formed by these two electrodes. An illustrative example is a figure placed on a spinning plate on which light is projected falling on a screen placed behind, thus forming a cyclic changing shadow: The angle from where the light is projected and where the screen is placed determine the shadow figures. Hence, the form of the measured ECG signal depends on the location of the projection axis with regard to the heart's electromagnetic dipole formed by the two active measuring electrodes, which are both referenced to a common *ground* electrode. Hence, not only one unique ECG electrode location is possible, but their number is theoretically infinite.

There are some distinct systems where to place ECG electrodes on the body, depending on the purpose or emphasis of the researcher or physician, generally named by its inventors' names. *Psychophysiologists* generally do not want to diagnose pathological cardiological conditions, but they are interested in the healthy ECG as a measure of the modulation of the heart beat due to psychologically induced changes in the outflow of the autonomic nervous system (ANS). Although also other elements of the electrical heart cycle can be modulated by the ANS, the most reactive measure is the temporal distance between two heart beats, more precise, just the moment between the beginnings of two following contractions of the ventricles, the so-called *interbeat interval* (IBI). The beginning of the ventricular contraction is electrically reflected in the ECG by the so called *R peak* (see figure 20), hence psychophysiologists generally chose ECG positions which maximize the amplitude of the R peak to facilitate its automatic recognition for IBI calculation. The ECG electrode configuration normally used in psychophysiology and also used in the present work is the so-called *Nehb lead* (see figure 21) with R peaks typically in the range of ≈1 mV. Economical cheap standard disposable adhesive Ag/AgCl ECG electrodes are used after degreasing the skin with 70% isopropanol.

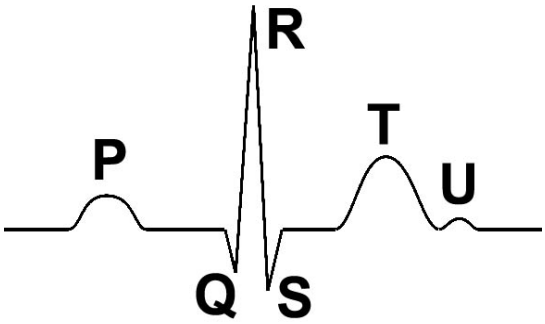


Figure 20. Nomenclature of ECG peaks in a standard idealized *Einthoven II lead* (right arm, left leg) which is electrically identical as compared to the *Nehb lead*. The electrical correlate to the beginning of the ventricular contraction is the *R* peak which has the largest amplitude of the ECG biosignal, typically ≈ 1 mV.

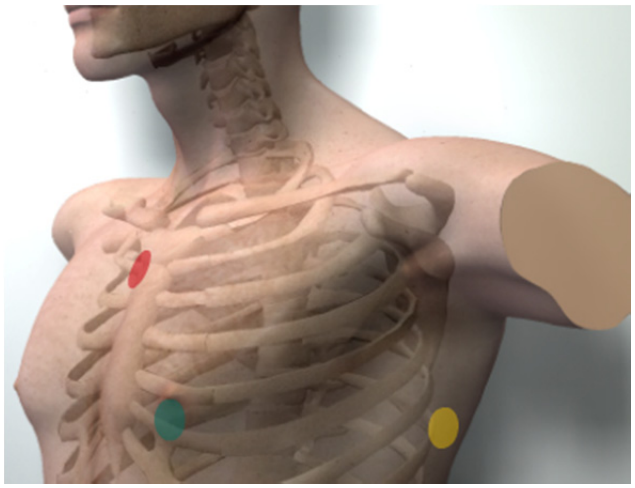


Figure 21. ECG electrode positions of *Nehb lead*, typically used in psychophysiology to maximize *R* peaks. Red and green are the active electrode positions for the difference amplifier, yellow the reference (adapted from http://flexikon.doccheck.com/Ableitung_nach_Nehb).

The circuit of the battery driven ECG preamplifier is the same as for the EOG (see figure 17), but with modifications designed by the author of the present work: Firstly, in the G = 101 amplification stage, R10 is replaced by a 50kΩ trimmer because the ECG has larger amplitudes than the EOG, so not as high gains as for the EOG are needed. Moreover, the two DC remover stages are modified in order to reliably reject any baseline drifts, a common analogue problem in ECG recordings: C10, C5, C4 and C13, C12, C11 are replaced by 47 nF condensers, increasing f_{Cutoff} to .24 Hz.

Because the circuit proved excellent functioning in the prototyping construction for the EOG setup, for the ECG a *printed circuit board* construction was preferred. Using the common *EAGLE PCB Design Software* 5.0 (CadSoft Computer, 19620 Pines Blvd., Pembroke Pines, FL 33029, USA), the author of the present work first copied the modified circuit scheme, then manually chose the positions of the components on the board and as the last step used the *autorouter* algorithm to design the wiring. Some manual changes were needed and in the end four wires had to be left for manual wiring. The layout was etched by phototransfer technique and holes were drilled manually. The board was then assembled by manual soldering by the author of the present work in approx. 100 hours (see figure 22).

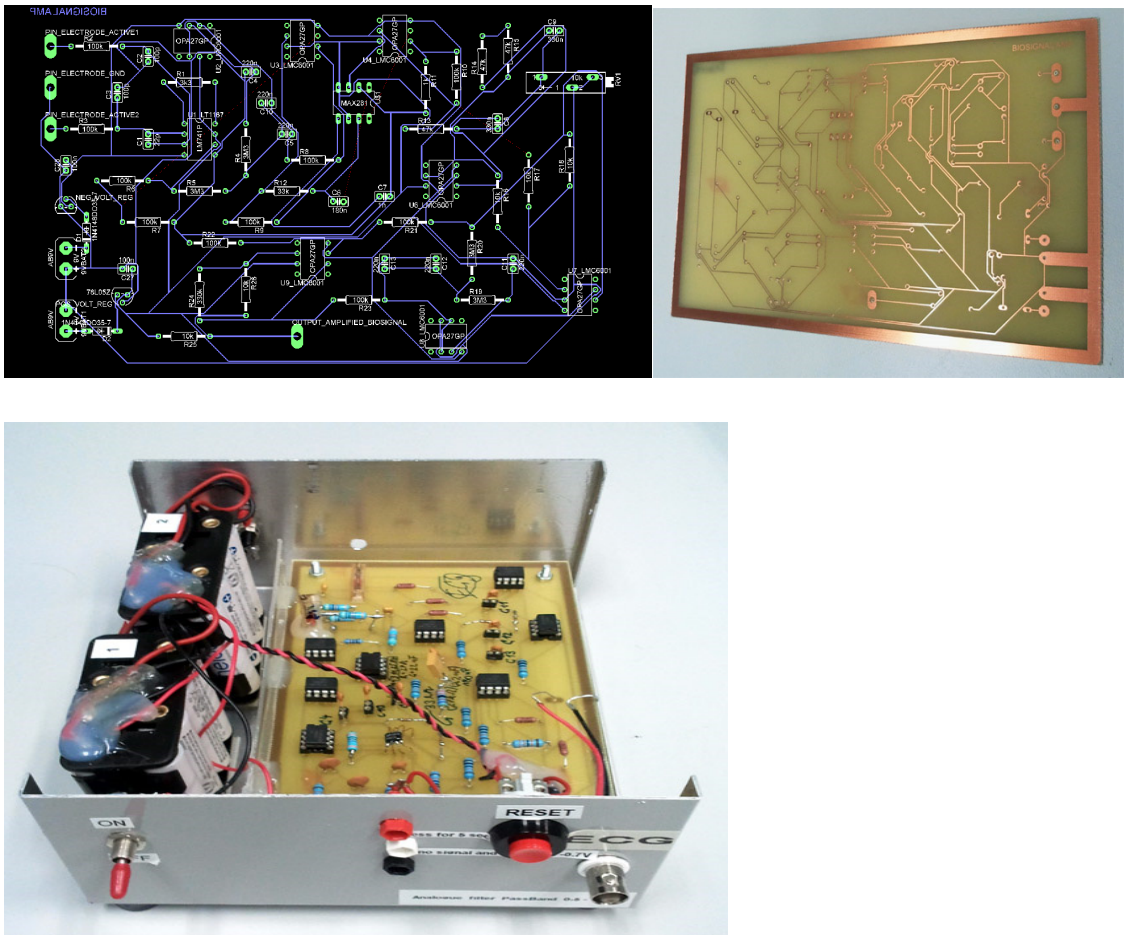


Figure 22. Development and assembly of the battery-driven ECG preamplifier. All design work and handicraft was done personally by the author of the present work in approx. 100 hours.

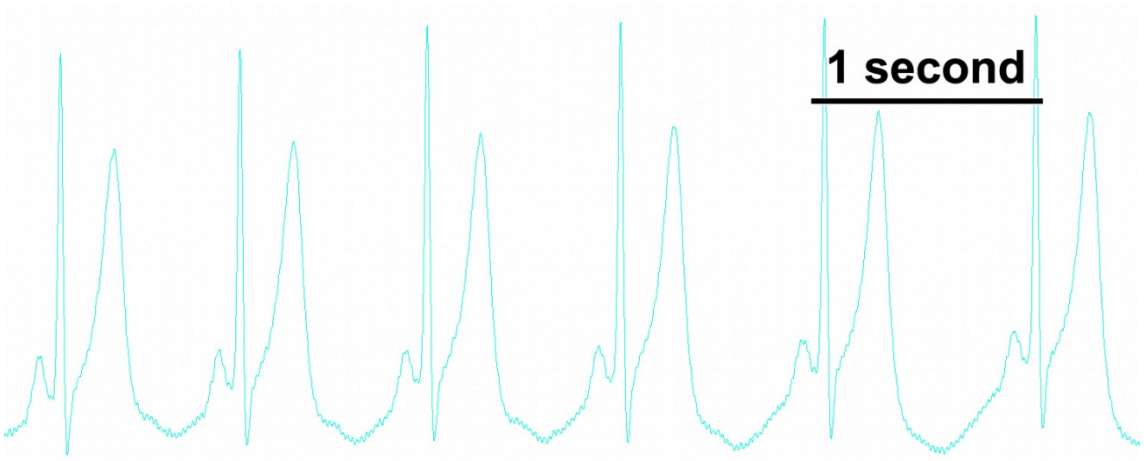


Figure 23. Typical real-world *Nehb lead* – ECG digitally filtered by a digital 5th order *Butterworth* low-pass filter with f_{Cutoff} = 30 Hz to reject the few remaining 50 Hz interference without changing the original signal. Because this example is an uncalibrated test recording, the y-axis is left without scale.

A typical *Nehb lead*-ECG measured with the described battery-driven ECG preamplifier is shown in figure 23. The output signal in the range of ± 5V is connected to the rack of the data acquisition board using coaxial cable.

2.5.2.3. Respiratory Rate V_f – hardware

The respiratory system is a complex system with many distinct parameters which are constantly regulated by the organism. Most of them can only be observed with quite invasive methodology, as e.g. fixing a *spirometry mask* on the face of the subject, placing the person into a spirometry *whole body plethysmograph* which is as big as a public telephone call box and moreover is very expensive, or extracting capillary blood from the ear lobes to measure the gas saturations in the blood. In the psychophysiology of subjects in a resting state without higher metabolic demands, only two respiratory measures are from general interest, the respiratory rate V_f and the so-called *tidal volume* V_T which is the amount of air being moved from normal inspiration to normal expiration. Unfortunately, precise measurements of V_T need the described more invasive methods of spirometry, but V_f can be measured almost non-invasively by *nasal cannulae* fixed into subjects' nostrils connected to a *pressure-to-voltage* transducer (see figure 24), the commercial highly sensitive differential pressure transducer *HCLA02x5EB* for the range of 0 … ±2.5mbar

(First Sensor AG / SensorTechnics, Niederlassung München, Boschstrasse 10, 82178 Puchheim, Germany). Following the physical principle of a *pitot-tube* (Pitot, 1732), velocity of air flow in the nostrils is proportional to the dynamic pressure induced in the *nasal cannulae* system. The same principle is used to measure velocity of air crafts. Because air flow velocity changes cyclically in the inspiration and expiration process, respiratory rate V_f can be precisely and quite easily determined from this continuous dynamic pressure signal by *offline* spectral analyses. A typical respiratory signal measured with the described setup is shown in figure 25.

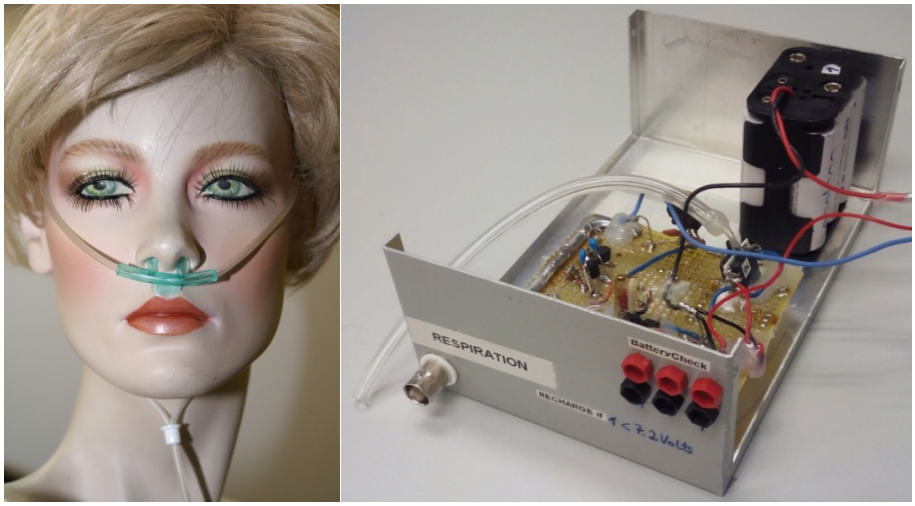


Figure 24. Nasal cannulae used to measure *respiratory rate* V_f (picture taken from http://hempel-sauerstoff.de). The cannulae's open end is plugged into a battery-driven *pressure-to-voltage* transducer based upon the commercial highly sensitive differential pressure transducer *HCLA02x5EB* for the range of 0 … ±2.5mbar (First Sensor AG / SensorTechnics, Niederlassung München, Boschstrasse 10, 82178 Puchheim, Germany). The output signal of 2.25 ± 2V is connected to the data acquisition rack by coaxial cable.

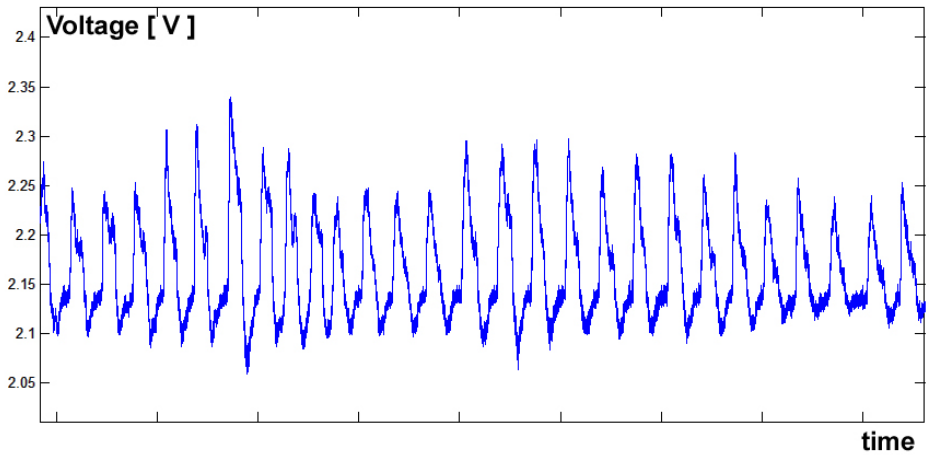


Figure 25. Typical respiratory signal as measured by the described setup with peaks corresponding to *ex*pirations and valleys to *in*spirations.

2.5.2.4. Saliva sampling and analyses

In order to measure changes in concentrations or activities of substances of the neuroendocrinological system, psychophysiologists usually prefer to use *saliva samples,* because they do not require painful blood taking by injection needles. Just before (*pre*) and just after (*post*) each experiment, saliva samples are obtained by *passive drool technique* collecting unstimulated saliva into special *Salivette*® test tubes (SARSTEDT AG & Co., Sarstedtstraße, Postfach 1220, 51582 Nümbrecht, Germany): Participants incline their heads and spit out repeatedly the accumulating saliva via small plastic tubes into the *Salivette*®, until sufficient saliva is collected (> 2ml) while the exact time needed is measured by a chronometer and protocolled. Saliva flow rates in ml / min are later calculated by measuring each total saliva volume and dividing it by the protocoled times in minutes. Salivia flow rates were later used to normalize *Salivary Secretory Immunoglobulin A* (SIgA) and α–Amylase concentrations in order to be expressed as secretory velocities, while for cortisol non-normalized concentrations are used. Immediately after recollection, samples are *flash frozen* by immersion into *liquid nitrogen* at -196°C and then immediately stored into a freezer at -20°C. After the end of all experiments, saliva samples were translated in a polystyrene foam box cooled by CO_2 *dry ice* to the unit of clinical analyses of University Hospital *La Paz* (Paseo de la Castellana, 261, 28046 Madrid) associated with the university of the author of the present work, *Universidad Autónoma de Madrid.* Samples were thawed at room temperature and centrifuged for 15 min at 1500 x g in order to separate mucins and other particulate matter which otherwise might interfere with the assays. Commercial *ELISA* microtiter plate assay kits, especially developed and validated for saliva specimen, were used to measure *Cortisol* and SIgA: Item No. 1-3002 and Item No. 1-1602 of *Salimetrics Europe Ltd.* (Unit 7 Acorn Business Centre, Oaks Drive, Newmarket, Suffolk, CB8 7SY, United Kingdom). Developing the kits was done strictly following the manufacturer's instructions.[1] After developing was finished, absorbances/optical densities of the microtiter

[1] downloadable at http://www.salimetrics.com/assets/documents/1-3002.pdf and http://www.salimetrics.com/assets/documents/1-1602.pdf

plates were measured by the commercial automated photometric mircotiter plate reader system *Minilyser* with its photometric unit *Sunrise* (Tecan Group Ltd., Seestrasse 103, CH-8708 Männedorf, Switzerland) by an optical filter of 450 nm both for cortisol and SIgA kits. Absorbances of the standards with known concentrations, measured in doublets, were modelled by four parameter logistic curve fitting (4PL)[2] and with this model the unknown concentrations were calculated from absorbances. Because the amount of free wells to measure unknown concentrations on the 96-well microtiter plate was not sufficient to measure all saliva samples in doublets and purchasing a second microtiter plate would have been far too expensive, five randomly selected subjects were measures only in singlets. Intra-Assay Coefficients of Variability (Intra-Assay CV), as measures of goodness of practically carrying out the manufacturer's kit instructions, were calculated over the remaining doublet measurements by averaging over all results from individual doublets each time calculated as *SD*/*M* * 100. Overall Intra-Assay CV was for *cortisol* 7.65 % and for *SIgA* 5.32% which fulfils the quality requirements that Intra-Assay CV should be < 10%.

α–Amylase enzyme kinetics was measured by first diluting saliva 1:5 with standard 0.9% NaCl solution. This dilution then was measured at 37°C temperature by the hospital's own very well calibrated system for measuring serum α–Amylase activity, e.g. for diagnostics of acute pancreatitis. Because decisions concerning life or death of patients depend of these measurements and because of frequent *round robin tests* as obliged by law, accuracy was trusted and measurement in doublets was not considered to be necessary. The measurement principle for α–Amylase is a chromogenic substrate, 2-chlor-4-nitrophenol, which is bound to maltotriose. α–Amylase hydrolyses this substance, which liberates time-, temperature- and concentration-dependently 2-chlor-4-nitrophenol which is measured at 405nm by spectrum–photometry. The quantity of generated 2-chlor-4-nitrophenol at 37 °C is a direct measure of the α–Amylase activity in the saliva sample under physiological temperature conditions.

[2] using a free MATLAB-script downloadable at http://www.mathworks.com/matlabcentral/fileexchange/38122-four-parameters-logistic-regression-there-and-back-again

α–Amylase activities were finally multiplied by five (to take into account the 1:5 dilution) and then normalized by multiplication with saliva flow rates.

That cortisol is valid a marker of the activity of the *hypothalamus – pituitary – adrenal cortex* stress axis (HPA), was outlined in the introduction. Cortisol concentrations show a satisfactory concordance comparing serum vs. salivary specimen with $r = .600$ and $p < .001$, although saliva sampling has been proven to be even superior to serum samples for measuring HPA, because the influence of the third variable *cortisol-binding globulin* (CBG) is eliminated (Gozansky, Lynn, Laudenslager, & Kohrt, 2005). Thus, saliva sampling is not only fully feasible for cortisol, but strongly to be preferred as compared to traditional blood samples. α-Amylase and SIgA have been discovered as new salivary stress biomarkers reflecting sympathetic neuromodulation of saliva glands: Many studies showed significant and pronounced *increases* of α-Amylase under standardized psychosocial stress paradigms (e.g. Nater & Rohleder, 2009; Nater et al., 2005; Noto, Sato, Kudo, Kurata, & Hirota, 2005), while for SIgA acute stress was shown to trigger *increases* (e.g. Takatsuji et al., 2008), whereas chronic stress caused *decreases* (e.g. Ng et al., 2003).

2.5.3. Digital offline biosignal-processing

2.5.3.1. EEG and EOG

All digital offline biosignal-processing was done in *MATLAB 7.11.0.584 64bit* (The MathWorks, Inc., 3 Apple Hill Drive Natick, MA 01760-2098, USA) under *Windows 7* on a Intel *i7-950 quad core* 4*3.06GHz *PC* with 16GB *RAM*. In a first step, all recorded data are imported into MATLAB and all relevant epochs are extracted following the event markers in the data matrices generated by the experimenter's manual keystrokes during the recordings while he hears the same audio channels as played to the subject's headphones. All relevant epochs are then merged and the following prefiltering treatments are applied for each of the 63-channels raw EEGs and for vEOG/hEOG biosignals sampled at 1024 Hz, see figure 26.

The *MATLAB* script which automates the preprocessing (described in figure 26) puts out a final plot with *x*-axis representing all 63 EEG channels and two curves which represent *linear Pearson correlations* of entire vEOG vs. hEOG biosognals with each entire EEG channel. Reductions of correlations both for vEOG and hEOG indicate the suspicion of *bad channels,* i.e. channels in which electrical contact between electrode and scalp worsened e.g. due to cap shift due to relevant head movements of the subject and thus contain too much electronic noise or interferences: EEG biosignals contain a mixture of both EEG and EOG, thus all EEG electrodes will show some correlation with pure EOG signals recorded separately. If this correlation is strongly reduced for both EOG channels, contamination with electronic noise and interferences is probable. These probably *bad channels* identified as described above are noted down to help later final visual decision whether a channel is considered to be *bad* or not.

This visual decision is taken after visualizing the 63–channel EEG data by *EEGLAB 12.0.2.3b,* a MATLAB toolbox distributed under the free GNU GPL license (Delorme & Makeig, 2004). Channels identified to be *bad* are excluded and data matrix is exported without them, but still containing *vEOG* and *hEOG* channels.

Figure 26. Digital offline pre-processing of raw EEG and EOG biosignals under *MATLAB 7.11.0.584 64bit,* raw sample frequency of 1024 Hz is finally reduced to 256 Hz by downsampling.

Now, *EOG* artifacts have to be removed/reduced because they have magnitudes up to approx. 10 times larger than EEG. Here the *REG–ICA* method is used (Klados, Papadelis, Braun, & Bamidis, 2011): First *blind source separation* (BSS) is computed by *independent component analysis* (ICA), then found EEG components which contain EOG artifacts are adaptively filtered by *H-infinity optimal filtering* using the information of vEOG and hEOG channels. Which ICA component contains EOG artifacts is determined by comparing the *fractal dimensions* of all ICA components with

those of vEOG/hEOG biosignals. After adaptive filtering, all ICA components are mixed again with the result that EEG signals are almost not affected, but that EOG artifacts are eliminated or remarkably reduced. Unfortunately, *REG-ICA* is computationally very expensive, processing a typical recording of the experiments of the present work took 1.5 hours on the high performance *64bit* machine Intel *i7-950 quad core* 4*3.06GHz *PC* with 16GB *RAM*. Without the previous step of downsampling from 1024 Hz raw sample frequency to 256 Hz, computing *REG-ICA* on the described system is impossible leading without any exception to excess of the available computational resources with the consequence to produce failures of the entire computer operating system ("computer crash"). The MATLAB script for *REG–ICA* (adapted and developed by the author of the present work) automatically outputs a plot of *linear Pearson correlations* for *vEOG* vs. *hEOG* with each treated vs. non–treated EEG channel and thus offers a visual control of *REG–ICA's* efficacy which can be quite different among distinct data sets.

Now, the formerly excluded *bad channels* have to be reconstructed to complete the *REG–ICA* treated EEG matrix to the final 63 channels. This is done by interpolation of the missing *bad channel* from the information of its nearby-located channels by *spherical spline interpolation* (Perrin, Pernier, Bertrand, & Echallier, 1989, 1990), as implemented in *EEGLAB 12.0.2.3b.*

This preprocessed 63–channel EEG matrix together with vEOG/hEOG signals is separated into single epochs, *Verum* and *Placebo* stimuli epochs are cut into 5x5min epochs. Epochs are then visualized in *EEGLAB 12.0.2.3b* and manually–visually all remaining EOG, EMG or movement artifacts are cut out, i.e. are *rejected.* This manual process is very time consuming and

requires advanced experience and concentration, for one experiment approx. four hours are needed. After this manual artifact rejection process, all relevant artifacts are completely excluded.

These pre-processed and artifact–cleaned epochs of EEG data are now merged again and a *Laplacian spatial filter* is applied using the MATLAB toolbox *Current Source Density (CSD) 1.1* (Kayser & Tenke, 2006; Kayser, 2009). Expressing scalp EEG signals from original *Volt*–changes in time now as Amperes/m^2– changes in time offers reduction of "spatial smear" due to *volume conduction* and thus remarkably improves resolution of scalp topographies (Kayser & Tenke, 2006). This technique aims to approximate the *local current density* flowing into the scalp perpendicularly to the skull. The physics behind is that *scalp current density* (SCD) with units Amperes/square meter can be calculated by assuming scalp conductivity (units are Siemens/meter) and calculating surface *Laplacian–transform* of scalp potentials (resulting units of *Laplace*–transform are Volts/square meter) using the following equation: SCD = -1 * scalp conductivity * Laplacian of scalp potential (Perrin, Bertrand, & Pernier, 1987).[3]

The resulting time domain EEG signals (now expressed in Amperes/m^2– changes in time) are now converted into *frequency domain* by *sliding-window FFT transform* with window size of 1024 sample points which corresponds to 4 sec at 256 Hz sample frequency and a sliding shift of 8 sample points which corresponds to 32 points per sec in the output signals. Before calculating each *FFT,* first a linear detrending and second a *Hann–window function* is applied each time to each set of 1024 data points in each analysis window. FFT coefficients are normalized to their number, i.e. divided by 512, squared to obtain the *power spectrum* and then summed up in each EEG frequency band of interest, see table 3.

[3] A short and comprehensible explanation of the physics of *scalp current density* (CSD) was published online by Dr. Darren L. Weber at http://psdlw.users.sourceforge.net/career/dweber_docs/eeg_scd.html

Table 3. EEG frequency bands of interested for which relative *spectral power* will be calculated by sliding–window FFT transform. Definition of frequency bands follows the EEG standard text book of Zschocke and Hansen (2012).

name	EEG frequency band [Hz]
delta	0.5 – 3.5
deltaRECHkales	0.5 – 2.0
theta	3.5 – 7.5
thetaLowNarrow	4.5 – 5.5
thetaLow	3.5 – 6.6
thetaHigh	6.5 – 7.5
theta1	3.5 – 4.5
theta2	4.5 – 5.5
theta3	5.5 – 6.5
theta4	6.5 – 7.5
alpha	7.5 – 12.5
alphaLow	7.5 – 9.0
alphaHigh	9.0 – 12.5
alpha1	7.5 – 8.5
alpha2	8.5 – 9.5
alpha3	9.5 – 10.5
alpha4	10.5 – 11.5
alpha5	11.5 – 12.5
sleepSpindleRangeALL	11.0 – 16.0
sleepSpindleRange12Hz	11.5 – 12.5
sleepSpindleRange14Hz	13.5 – 14.5
betalow	12.5 – 18.0
betamid	18.0 – 24.0
betahigh	24.0 – 30.0
gamma1	30.0 – 40.0
gamma2	40.0 – 46.0
gamma3	54.0 – 120.0
gammaNarrow	38.5 – 41.5
totalSpectrumWide	0.5 – 120.0
totalSpectrumNarrow	0.5 – 70.0

Frequency bands are defined as recommended in the EEG standard text book of Zschocke and Hansen (2012) and because *alpha* and *theta* bands are of special interest in the present work, they are also subdivided into 1 Hz bands.

In order to express the *frequency domain* transformed signals as *relative spectral powers*, every resulting data point (32 points per sec) of every specific EEG frequency band is divided by its corresponding data point in *totalSpectrumNarrow* (0.5 – 70 Hz). Thus, spectral powers are expressed as *proportions* of total power, i.e. as amount of contribution to total power in

every moment of the 32 points per sec. Because *relative spectral powers* usually show a *skewed* statistical distribution, in the advanced EEG literature a *logit–transformation* is recommended to achieve approx. Gaussian normal distributions (see Fernández et al., 1995; *most relevant:* Gasser, Bächer, & Möcks, 1982; John et al., 1980; Pollock, Schneider, & Lyness, 1991):

$$logit = ln\left(\frac{p}{1-p}\right)$$

$$= 2 * artanh\,(2 * p - 1)$$

The entire *sliding–window FFT transform* MATLAB script (entirely developed and programmed by the author of the present work) needs for a typical experiment (*Verum* or *Placebo*) approx. four hours computation time under *Windows 7 64bit* on the Intel *i7-950 quad core* 4*3.06GHz *PC* with 16GB *RAM,* thus for all *N* = 12 subjects approx. 96 hours. Taking into account that for every electrode for every subject's *Verum* or *Placebo* condition approx. 60 000 *FFT* calculations are performed for each EEG channel and the final MATLAB workspace for the output of results for all subjects contains a data volume of approx. 30 GBytes (yet compressed into a binary **.mat* – file), so these remarkable computation times are understandable.

Now, for each subject, for *Verum* vs. *Placebo,* for each EEG electrode, each epoch and each EEG frequency band *arithmetic means* of the *logit*-transformed EEG power time series are computed and used as input for further statistical analyses, i.e. EEG biosignals under *Verum* and *Placebo* stimuli conditions are represented each time as five *arithmetic means* representing 5·5 min.

2.5.3.2. ECG

The raw *ECG* signal sampled at 1024 Hz is digitally detrended in MATLAB and then imported into the MATLAB-based, but stand-alone program *ARTiiFACT 2.4. 64bit - A tool for heart rate artifact processing and heart rate variability analysis* (Kaufmann, Sütterlin, Schulz, & Vögele, 2011). Under ARTiiFACT, first of all, ECG signal is 4 Hz highpass-filtered, using the built-in filter function of the program, then a threshold is defined following visual inspection in order to detect *R* – peaks of the ECG and in order to obtain the *interbeat–interval* (IBI) time series, see figure 27.

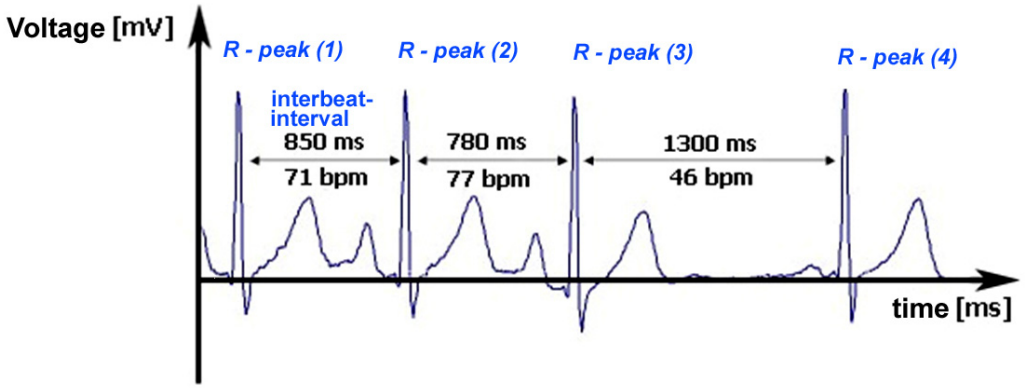
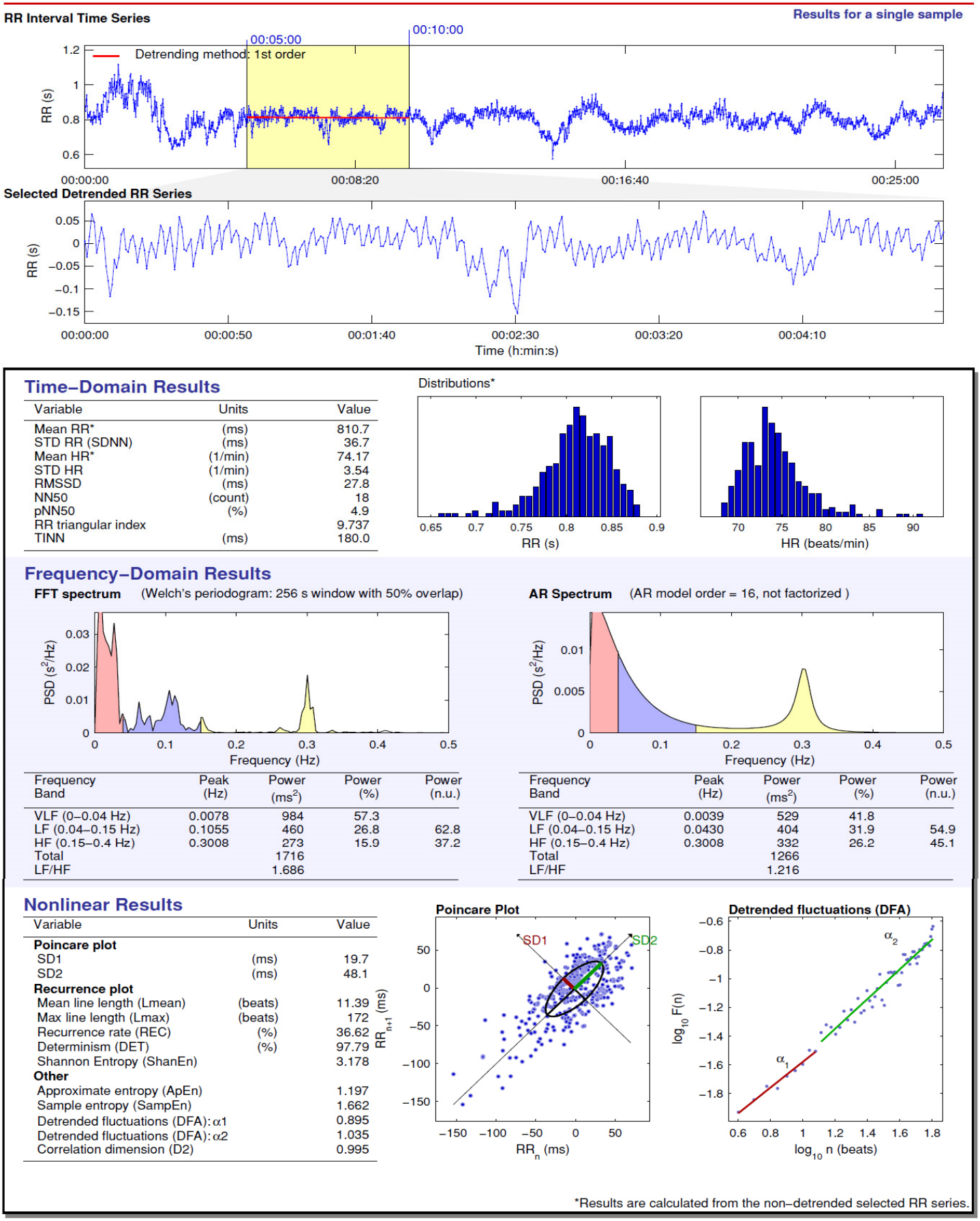


Figure 27. Conversion of ECG signal into raw *interbeat–interval* (IBI) time series by automated detection of *R* –peaks realized with the program *ARTiiFACT 2.4. 64bit - A tool for heart rate artifact processing and heart rate variability analysis* (Kaufmann et al., 2011).

Raw IBI time series are then processed by the program's module *detect and process artifacts in IBI data* which offers automatic artifact detection with the possibility of manual modifications. This step is very important because only a few artifacts in IBI time series can distort the later results remarkably. For finally deriving *heart rate variability* (HRV) parameters, these processed IBI time series are exported in *ASCII*–text files and imported to another MATLAB–based, but stand–alone program *Kubios HRV 2.1* (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2009), a typical analysis output is shown in figure 28.

HRV Analysis Results



Kubios HRV, version 2.1
Department of Applied Physics
University of Eastern Finland, Kuopio, Finland

Figure 28. Typical analysis output of the *heart rate variability* (HRV) program *Kubios HRV 2.1* (Tarvainen et al., 2009) parameterizing *IBI* time series in *time, frequency* and *nonlinear* domain for operationalization of *parasympathetic outflow to the heart* and other phenomena.

Kubios 2.1 is used with parameters set to default values (no artifact correction, frequency definitions *VLF* = 0 – 0.04 Hz, *LF* = 0.04 – 0.15 Hz*, HF* = 0.15 – 0.4 Hz*,* interpolation rate 4 Hz, FFT spectrum window width = 256, window overlap = 50%, AR model order = 16, no factorization) with the exception of *remove trend components* set to *first order.* All epochs, in the case of *Verum* and *Placebo* 5·5 min epochs, are analysed by *Kubios 2.1* with the mentioned parameters and exported as MATLAB binary data files **.mat* for final statistical analyses. Kubios 2.1 outputs parameters describing *heart rate variability* (HRV) in *time, frequency* and *nonlinear* domain, a short explication of each parameter is reported in the *Kubios HRV 2.1* manual[4].

For the present work, only a subset of the most important HRV parameters is used for further analysis (see table 4), the most relevant is *HFpower* normalized to total power being expressed in percentage: Because the acetylcholinergic synapses of the parasympathetic nerves modulating *heart rate* work remarkably faster than the noradrenergic synapses of the sympathicus, and also faster than the relatively slow effect of *adrenaline* on heart rate via β_1–adrenoreceptors liberated due to activation of the *hypothalamic–pituitary–adrenal axis* in the *medulla glandulae suprarenalis* and its transportation to the heart by systemic blood circulation, modulations of HRV above 0.15 Hz can only be caused by parasympathetic modulations. Spectral power of HRV in the range of 0.15-0.4 Hz is defined as *high frequency power* (HF) and is a classical pure parasympathetic parameter, confirmed by many pharmacological and chirurgical experiments: When changes in *HRV–HF power* are observed while respiratory rate V_f and the so-called *tidal volume* V_T do not show relevant changes (see Ritz & Dahme, 2006), then changes in HRV–HF powers validly reflect changes of parasympathetic outflow to the heart. Now, the general hypothesis concerning parasympathetic changes can be further specified:

"If the Binaural Beat stimulus employed in the present work indeed might lead to changes in levels of *arousal* during its duration, then the classical parasympathetic *HRV*–parameter *normalized HF power expressed in percentage* should show significant *change over time.*"

[4] http://kubios.uef.fi/media/Kubios_HRV_2.1_Users_Guide.pdf

Table 4. Subset of the *heart rate variability* (HRV) parameters used in the present work for further statistical analyses. A short explanation of each parameter is given in the manual of the program *Kubios HRV* (Tarvainen et al., 2009), downloadable at http://kubios.uef.fi/media/Kubios_HRV_2.1_Users_Guide.pdf

***HRV*–parameter**	**unit**
MeanRR	*ms*
SDNN	*ms*
RMSSD	*ms*
pNN50	*percentage*
HRVtriangularIndex	*dimensionless*
AR_PeakHF	*Hz*
FFT_PeakHF	*Hz*
FFT_HFpower	ms^2
FFT_HFpower	*percentage*
FFT_LFpower	ms^2
FFT_LFpower	*percentage*
AR_HFpower	ms^2
AR_HFpower	*percentage*
AR_LFpower	*percentage*
AR_LFpower	ms^2

Because percentage is just another form to express *ratios,* the same argumentation given for *relative EEG spectral powers* applies to *HRV-HF powers:* Data transformation by *logit–transform* is recommended to transform skewed statistical distributions into Gaussian normal distributions (see above, most relevant Gasser et al., 1982). *Log–* and *logit–* transformations of *HRV parameters* are frequently used and recommended in cardiology literature. Although maybe this transformation might be perceived as *quite arbitrary,* an excellent justification for these data transformations is given by Kerkhoff and Enquist (2009) published in the *Journal of Theoretical Biology:*

> "[…] The default status of the additive error model is perhaps on the deeper assumption that arithmetic scales are somehow truer or more intuitive, and that log-transformation thus represents a distortion of the 'real' data. However, recent research strongly challenges the assumption that arithmetic scales are more natural. […] Log-transformation is not simply a statistical convenience. It is indeed a non-linear transformation, but it places numbers into a geometric domain in which proportional deviations are represented consistently, independent of the scale and units of measurement. But more importantly, it is often appropriate in biology because many biological phenomena (e.g. growth, reproduction, metabolism, sensation) are fundamentally multiplicative, and likely conform more closely to a geometric error model. […] However, we should not try to force a geometric biological world into an arithmetic box simply because we learn to count on our fingers. Log-transformation is entirely appropriate, indeed necessary, for […] many other problems in biology."

2.5.3.3. Respiratory Rate V_f

Because HRV–*HF power* only purely reflects parasympathetic outflow to the heart under the condition that respiratory rate V_f and the so-called *tidal volume* V_T do not show significant changes, respiration has to be measured and analysed for proper HRV interpretation (see Ritz & Dahme, 2006). While *tidal volume* V_T can only be measured by more invasive spirometry, V_f can be derived quite easily from the nasal cannulae pressure signal by spectral analysis using the *SPTool* from the *MATLAB's* signal processing toolbox using *Welch* as method with *Nfft* = 60*5*1024, *Nwindows* = 30*1024, window type = *hanning* and overlap = 0. Mean respiratory rate V_f can be easily determined by manually measuring the frequency of the corresponding characteristic peak, see figure 29. Mean V_f of every epoch is manually measured and stored in a MATLAB binary **.m* file for final statistical analyses.

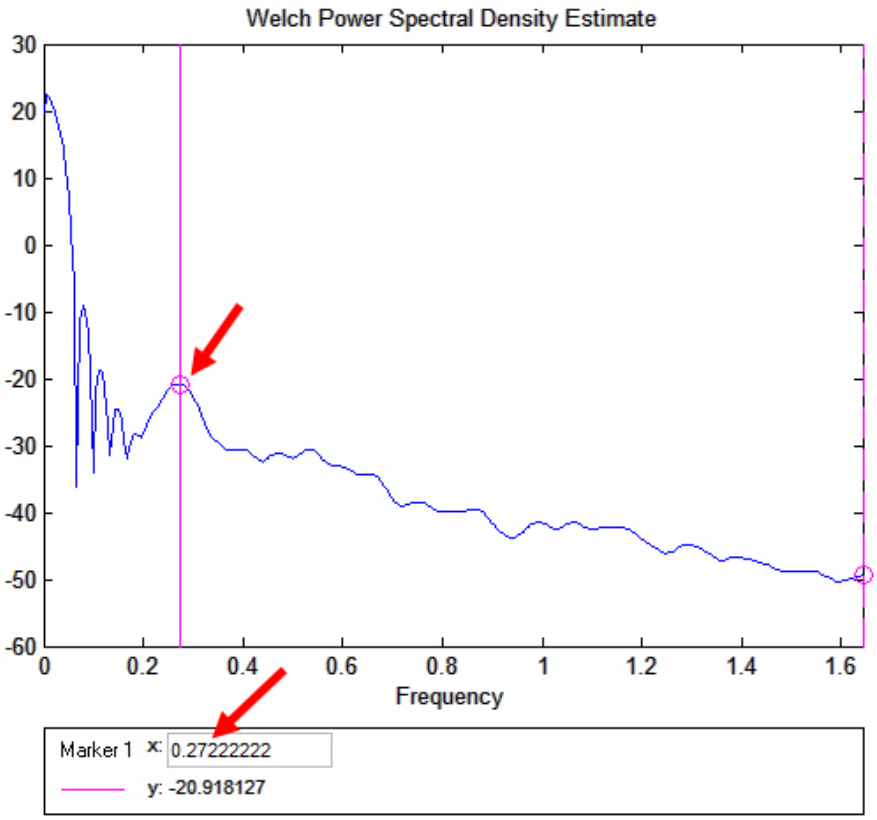


Figure 29. Mean respiratory rate V_f can be easily determined directly in Hz with *spectral analyses* by manually measuring the frequency of the corresponding characteristic peak using *SPTool* from MATLAB's *signal processing toolbox*.

2.6. Experimental procedures

After subjects were invited to the first experimental session, presentation order was determined by a third person, who was not involved in the experiments, a colleague of the experimenter flipping a coin. This third person renamed the prepared audio tracks for *Verum* or *Placebo* to neutral descriptors *subjectIdentifier_A.wav* and *subjectIdentifier_B.wav,* where *A* indicates the first experimental session, and *B* the second. These audio tracks were copied to the *SD card* of the professional audio player. Thus, neither the experimenter nor the subject knew which session contained the *Verum* and which the *Placebo* stimulus (double–blind). After the end of each second session, the experimenter opened a prepared envelope which informed about the presentation order. Presentation order was organized by this third person in such a way that 50% of the subjects received in the first session *Verum* and in the second *Placebo* and the remaining rest of 50% vice versa. Subjects were invited to come at 10:00 o'clock in the morning to the laboratory in order to do the experiments in moments of circadian patterns where falling asleep is minimized and because cortisol and other hormones show a pronounced circadian pattern with peaks in the morning (for a review about circadian pattern of hormone liberation see: Tsang, Barclay, & Oster, 2014). Although done at the same hours of the day, subject's c*hronotypes* (Adan et al., 2005) of *morningness* or *eveningness* could lead to chronobiological biases and thus were excluded in the recruitment screening, so that only the *indifferent* chronotypes remained. Before the first session, subjects were informed roughly about the purpose of the experiments (without explicitly communicating the hypotheses), that differences between *Verum* vs. *Placebo* stimuli cannot be heard consciously and that presentation order will remain double–blinded until the end of the second session.

In order to try to control *unspecific factors* which might influence the arousal level of subjects, in the interpersonal interaction they were treated in a highly empathetic, but in a rather neutral–distant way aiming to establish the feeling of trust in order to reduce possible fears or uncertainties and to maintain attention and interest.

Subjects were treated in a way that they felt in control over the situation all the time. The room with the experimental setup was kept at 22 ° C by air condition.

After connecting all sensors/electrodes, verifying EEG impedances < 10 kΩ and checking quality of biosignals, subjects sat down on the lounger (see figure 14). First, subjects were instructed by a 10 min recording played back via loudspeakers to move their eyes in an exactly defined manner using a fixation point and numbers attached to the walls, as to be seen in figure 30. This first experimental phase aimed to generate standardized EOG artifacts to later entrain the *REG-ICA* offline–algorithm used to correct EEG signals for interfering EOG artifacts by merging this approx. 10 min recording with the experimental phases of interest.

Figure 30. View from the subjects' perspective sitting down on the lounger (see figure 14) in the grounded steel mesh shield protecting for electromagnetic interferences. A fixation point and numbers attached to the door/wall in combination with a 10 min recording played back via loudspeakers indicated how subjects had to move their eyes in order to generate standardized EOG artifacts used in order to later entrain the offline–algorithm used to correct EEG signals for EOG artifacts.

Now, after a short rest of 5 min, headphones were put on subjects' heads and three baseline conditions were recorded by instructions played back via headphones: (i) 3 min *eyes open* fixing the point attached to the wall,

(ii) 3 min *eyes closed* and (iii) 3 min *mindwandering with eyes closed* in which subjects are instructed to vividly imagine/remember their daily way to university or to their work. Without any pause and still with eyes closed, the recorded voice then explained the *pre* saliva sampling in which subjects had to incline their heads and fill a prepared test tube with saliva using a small plastic tube while the experimenter measured the time needed by a chronometer. *Pre s*aliva samples were immediately flash frozen by immersion in liquid nitrogen at −196 °C, then the experimenter left the room for some minutes in order to store the sample into a freezer at −20 °C while subjects waited on the lounger. An overview of the experiments' timeline is given in table 5.

Table 5. Overview of the experiments' timeline for one single session. *Verum* or *Placebo* sessions are identical (with the exception of the employed stimuli), took place with > one week temporal distance, presentation order was randomly assigned (50% of the subjects *Verum* – *Placebo* vs. remaining 50% *Placebo* – *Verum*) and was unknown for subject and experimenter (double – blind).

hour	**duration**	**Event**
10:00	45:00	subject enters the laboratory, in the first session the theoretical background is roughly explained to the subjects without mentioning the exact hypotheses, that *Verum* and *Placebo* cannot be consciously distinguished and that neither experimenter nor subject know the randomly assigned presentation order (randomized double – blind)
10:45	1:00:00	subject and experimenter enter the recording room kept at 22° C by air condition, **connecting and checking all sensors/electrodes**, verifying EEG impedances < 10 kΩ
11:45	10:00	instructions via loudspeakers for standardized **EOG artifact generation phase**
11:55	5:00	short rest
12:00	15:00	initial instructions via headphones, **standardized baselines phases:** **3 min eyes open → 3 min eyes open → 3 min mindwandering with eyes closed**, instructions for saliva sampling
12:15	10:00	**saliva sampling PRE**
12:25	1:00:00	headphones with eyes closed, **experimental/treatment phase (*Verum* or *Placebo*) consisting of:** **30min relaxation exercise, 25min stimulus *Verum* or *Placebo***
13:25	10:00	**saliva sampling POST**
13:35	3:00	headphones with eyes closed, **control condition with 3 min exciting music**
13:38	10:00	**retrospective psychometry** by paper – pencil version of the *Phenomenology of Consciousness Inventory* (PCI) (Pekala, 1991, 2009) in its Spanish translation
13:48	10:00	**non-structured interview** for subjective phenomenology
≈ *14:00*		subject leaves, hair washing in the bathroom because of electrode gel used for EEG

The exact wording of the played back *baseline recording instructions* in Spanish language was as follows, for non–Spanish speaking readers a translation into English is provided in the appendix of the present work:

> " Después de haberte colocado y probado todos los sensores, vamos a empezar ya con el experimento. A partir de ahora, todo lo importante lo vas a escuchar por los auriculares. Los sensores son bastante sensibles, por eso tienes que ayudarnos evitar que haya interferencias..
>
> Por favor, durante el experimento, **no hables nada**, solamente concéntrate en mi voz. – Cierra ahora tus ojos; **tus ojos deben estar cerrados** durante casi todo el experimento, es muy importante: mantén tus ojos cerrados. Aunque estén cerrados, intenta no moverlos mucho. Los movimientos oculares pueden causar interferencias en los sensores. En cualquier caso, no tienes que concentrarte en esto especialmente, solamente **deja tus ojos *cerrados* y *relajados***.
>
> Vamos a hacer una prueba ahora, que *luego* **NO** debes hacer: Mueve tus ojos cerrados AHORA tan rápido como puedas, de derecha a izquierda, de derecha a izquierda, ida y vuelta, sigue…sigue…BIEN, ya está.
> Ahora hacemos lo mismo, pero de arriba a abajo, mueve AHORA tus ojos tan rápido que puedas de arriba a abajo…sigue….sigue…MUY BIEN. Ya sabes, lo que son los movimientos oculares. A partir de ahora, **deja tus ojos cerrados y quietos**; tranquiiiilos; relajados.
>
> Otra cosa que tienes que intentar evitar es…*apretar los dientes*. Vamos a probarlo, aunque *luego* **NO** lo hagas tú: Aprieta ahora tus dientes primero ligeramente, y ya está, ¡suelta! … [3seg pausa] Repítelo, aprieta ahora tus dientes ligeramente, y ya está, ¡suelta! … [3seg pausa] Y por última vez, aprieta ahora tus dientes fueeertemente, y ya está, ¡suelta! … En el experimento, intenta **NO** apretar tus dientes, mover mucho la mandíbula, la lengua o tragar mucho…todo esto puede interferir con los sensores. Si tuvieras que hacerlo, hazlo rápido, pero suavemente. Tampoco te concentres demasiado en esto, solamente deja tu mandíbula quieeeta y relejaaada.
>
> Finalmente, lo más importante es que no muevas tu cabeza, tu cuerpo, tus brazos o tus piernas; intenta quedarte *quieto*, búscate ahora una posición cómoda e intenta **NO** moverte. Si no lo puedes evitar, hazlo rápido, pero suavemente. Si por ejemplo te pica algo, es peor que luches contra el picor que rascarte rápidamente, pero suavemente. A partir de ahora, intenta quedarte quieto.
>
> Desde ahora el experimento durará unos 30 minutos y va a consistir en tres fases. Por favor, no hables nada hasta que te diga que el experimento ha finalizado.
>
> Ya, empezamos con la fase ***uno***: Por última vez en el experimento, abre tus ojos AHORA. Vamos a grabar tu estado habitual, lo que se llama en ciencia "*línea de base".* Mira, hay un punto blanco colgado en la puerta, ¿lo ves? Vale, vamos a hacer una cosa: fija tus ojos en este punto e intenta no mover tus ojos… intenta no parpadear mucho, solamente si te molesta y hazlo rápidamente, pero suavemente. Es un minuto y medio, empezamos **YA**: FIJATE EN EL PUNTO…sigue…30 segundos, muy bien… un minuto, muy bien… **ya está**. Relájate y cierra ahora tus ojos, ciérralos, no los muevas mucho, intenta quedarte quieto.
> [3min pausa]
>
> Muy bien, AHORA, quiero que te imagines una cosa tan real como puedas, cuando diga "YA", pero solamente durante un minuto y medio…Imagínate ahora tu camino diario al *trabajo*, o a la *universidad* o simplemente un camino que conoces muy bien. Imagínate ahora, con todo detalle, los colores, los sonidos, los olores, como dejas tu casa y como empiezas ese camino. Empezamos…**YA**…[3min pausa] Muy bien, ya hemos grabado tu estado habitual con todos los sensores, la *línea de base,* con ojos

cerrados… Mantén tus ojos cerrados y quédate quieto…Necesitamos también analizar los cambios en tus hormonas, la línea de base de tus hormonas. Usamos una tecnología moderna, funciona con una simple muestra de *saliva.* Es bastante simple, solamente necesitamos un mililitro de saliva. Ahora, te explico como lo hacemos: luego, cuando te diga "YA", vas a abrir tus ojos y te vas a inclinar con todo tu cuerpo en la silla hacia adelante, inclinando también tu cabeza un poco hacia abajo. Los científicos te darán una pajita de plástico en una mano y un tubito especial de ensayo en la otra. Cuando el científico te diga "YA", tragarás una vez toda la saliva que estaba en tu boca, para eliminarla, pon rápidamente la pajita en tu boca y el otro extremo en el tubito de ensayo. Mientras el científico toma el tiempo con el reloj, tu tarea es… continuamente recolectar un poco de saliva en tu boca, sin hacer nada más que esperar un rato, echarlo a través de la pajita en el tubo de ensayo, recolectar otro poco y echarlo, hasta que te digan "YA ESTÁ". Así pues, vamos a tomar un mililitro de saliva: el tiempo que necesitas para recolectarlo no será más de 3 minutos. Después de que tendremos este mililitro, vas a dar al científico la pajita y el tubo de ensayo, y tú simplemente te pondrás otra vez cómodo en la silla, buscando una posición cómoda. Vas a cerrar otra vez tus ojos y vas a quedarte quieto como antes. El científico saldrá de la habitación durante unos minutos con tu prueba de saliva, para dejarla en el frigorífico. Mientras estas solo, te imaginaras mentalmente otra vez el camino de tu casa hacia el trabajo, la universidad o lo que te has imaginado antes. Vas a escuchar como el científico regresa a la habitación y después de un rato vas a escuchar otra vez mi voz a través de los auriculares y el experimento seguirá.

Ahora, cuando te diga "YA", vas a abrir tus ojos y te vas a inclinar con todo tu cuerpo en la silla hacia adelante, inclinando también tu cabeza un poco hacia abajo y el científico te dará la pajita y el tubo de ensayo...Prepárate, empezamos…"¡YA!". [El científico marca este momento en el registro como cambio de fase experimental, y marca cuando empieza otra vez la voz] "

After the experimenter returned to the room, now the real experimental/treatment part *Verum* vs. *Placebo* started, instructions and stimuli were again played back via headphones with eyes closed during this entire part. In order to start the stimulation with subjects having reached a maximal relaxed state, a 30 min relaxation exercise was played back which intersected at its end directly with the following 25 min *Verum* vs. *Placebo* stimulus. After it, subjects were again instructed for the *post* saliva sampling. The exact wording of the previous relaxation exercise and *post* saliva sampling in Spanish language was as follows, translation into English is provided in the appendix of the present work:

" Aquí me escuchas otra vez. Simplemente concéntrate a mi voz. Seguimos con el experimento. Ponte cómodo, deja los ojos cerrados, intenta no moverlos mucho y quédate quieto, ya lo conoces. La fase ***uno*** del experimento ya ha terminado y ahora, empezamos la fase ***dos*** del experimento:
Ahora tu tarea es que te relajes tan profundamente como puedas. Disfruta de la tranquilidad aquí, siente el sillón, siente que cómodo es estar en él. Intenta no pensar en nada particular, simplemente déjate llevar un rato con la música, no pienses en nada en especial, intenta dejar tu mente en blanco. Déjate llevar un rato por la música…

Simplemente escucha la música y mi voz. La música cambiará luego a un sonido especial. Vamos, a trabajar con tu fantasía: te voy a pedir que te imagines algunas cosas mentalmente; tu tarea será imaginártelas lo más real que puedas, como si fuera

una película y tú estuvieses dentro de ella. – Puede que mi voz, y lo que vayas a imaginarte, te resulte quizás un poco extraño al principio – solamente espera un poco:
Te vas a acostumbrar poco a poco. Lo importante es que intentes imaginártelo todo tan real como puedas.
No tienes que hacer nada, simplemente estás en el sillón. Ahora, lo importe es mi voz. No tienes que hacer nada, solamente escuchar: Disfruta de la tranquilidad y relájate.

Tu conciencia deja ahora poco a poco la realidad del exterior – tu atención se concentra más y más en tu mundo interior… tu conciencia se sumerge más y más en tu interior.

Todo lo que viene de fuera, ya no tiene ninguna importancia: Los sonidos de fuera, las luces de fuera, - todo lo de fuera te da igual ahora, ya no tiene ninguna importancia: Simplemente estás aquí, tranquiiiiiilo, quieeeeeeto y agradablemente relajaaaaaado. - Déjate llevar – más y más sumérgete en tu interior, más y más profundamente. Déjate llevar.
El tiempo pasa ahora más y más leeento, más y más lento; no tiene ninguna importancia.

Tu cuerpo está relajado y va a dormirse ahora, pero tu mente se queda despierta – en un estado tranquiiiiiilo, quieeeeeeto y agradablemente relajaaaaaado.

Voy a contar ahora hacia atrás – del 6 al 1, cada vez estarás más relajado:

Seis – La frente, los músculos y nervios de la frente se relajan, se dejan llevar.

Cinco – Las mejillas, los músculos y nervios de las mejillas se relajan.

Cuatro – El cuero cabelludo, tus músculos y nervios se relajan. – La cabeza está quieta, ligera y relajada.

Tres – Ahora relaja el cuello, la nuca, los hombros, los brazos y las manos – todos los músculos y nervios se relajan.

Dos – la relajación se extiende desde el cuello, baja por el pecho, el vientre, por las piernas hasta los pies – todos los músculos y nervios se relajan.

Uno – la relajación sube por la espalda, la espalda se relaja, la columna vertebral se relaja – todos los músculos y nervios se relajan.

Todo el cuerpo está relajado, se relaja totalmente. Pero tu mente está despierta – en un estado tranquiiiiiilo, quieeeeeeto y agradablemente relajaaaaaado.

Ahora, deja todos tus pensamientos y sentimientos de lado – mientras estés aquí en el sillón. Los pensamientos y sentimientos pasan como nubes, vienen y se van – vieeeenen...y se van – no tienen ninguna importancia ahora. Si una imagen o un pensamiento se queda, di mentalmente: “Dejo eso ahora, me dejo llevar”. Los pensamientos y sentimientos no tienen ninguna importancia ahora, son nubes, que vienen y se van, déjate llevar. – Ahora el tiempo pasa cada vez más y más lentamente, el tiempo no tiene ninguna importancia. Los pensamientos y sentimientos pasan como nubes, vieeenen y se vaaan, no tienen ninguna importancia, déjate llevar.

Ahora, imagínate vivamente que estás en un prado, - tu estás en un prado – echa un vistazo: es un prado de hierba suave, con flores de todos los colores, - escucha como revolotean las mariposas entre las flores, - es un sonido muy suave, escucha como revolotean y buscan néctar, mira cuan suave son las mariposas, suaves y sensibles – huele como las flores emanan sus aromas: Solo hay paz y tranquilidad… Hace una tarde maravillosa de primavera. El sol brilla, brilla suavemente y hace una temperatura muy agradable para ti.

Ahora estás andando por el prado, lentamente. El prado termina en un lago grande de agua pura y cristalina que reluce azul. Mira qué bello es el lago. A los lejos ves una montaña grande, que se eleva hacia el cielo. El prado, el lago y la montaña son muy antiguos...
Sobre esta montaña ves ahora, bastante lejos, una pantalla enorme. Aunque está lejos, puedes verla bien. - Todas las imágenes, pensamientos y recuerdos, que te llegan, aparecen en esa pantalla sobre la montaña – lejos de ti. Al igual que pasa en la pantalla, en el prado delante del lago estás absolutamente seguro. Es como estar en el cine: Por muchas cosas que pasen en la pantalla, tu estás seguro en tu sillón de cine – el prado es tu sillón de cine. En el prado estás completamente libre. El prado es tu interior, aquí estás a salvo, aquí hay paz.

Siempre hay algo que ver en la pantalla, allí hay una secuencia de imágenes. No son reales, son imágenes, construcciones que el cerebro produce permanentemente. A veces somos conscientes de ello, a veces no – pero la secuencia de imágenes nunca se corta, se produce automáticamente. Las imágenes no son reales, sólo son proyecciones a la pantalla – lejos de ti en las montañas. Esa cascada de imágenes es como la sangre que fluye permanentemente por tu cuerpo.
Aunque siempre que hay algo que ver en la pantalla tú tienes el poder de tomar la decisión de verlo o no. Siempre eres tú el que tiene el control y en el prado delante del lago estás seguro y a salvo. Si algo aparece en la pantalla que no te gusta – no tienes por qué verla, sólo espera a que la imagen se haya ido o cambie.

Deja todos tus pensamientos y sentimientos de lado – durante el tiempo que estés aquí en el sillón. Los pensamientos y sentimientos pasan como nubes, vieeennen y se van, vieeenen y se van. Si una imagen, si un pensamiento se queda, di mentalmente: “Dejo eso, me dejo llevar”. El tiempo pasa más y más lentamente, no tiene ninguna importancia. Los pensamientos y sentimientos pasan como nubes, déjate llevar. Nada importa ahora, déjate llevar.

Ahora la música va a cambiar por un sonido, déjate llevar hasta que mi voz te despierte. Ahora la música va a cambiar por un sonido, que se mueve lentamente alrededor de tu cabeza … déjate llevar por ese sonido. No voy a hablar mientras escuchas. Si una imagen, si un pensamiento se queda, di mentalmente: “Dejo eso, me dejo llevar”. Los pensamientos y sentimientos pasan como nubes, vienen y se van, ninguno tiene importancia ahora. ¡Déjate llevar! ¡Suéltate! ¡Déjate llevar! ¡Suéltate! ¡Dejate llevar por el sonido!

[25 min estimulo *Verum* vs. *Placebo* Binaural Beats]

[con la voz baja] Ya estoy contigo, de nuevo...Ojos cerrados, quédate quieto. [ya con la voz normal] Ya empieza la fase tres del experimento. Ojos cerrados, quédate quieto.

Ahora, vamos a seguir con el experimento. Necesitamos tomar otra muestra de saliva; es exactamente lo mismo que hemos hecho antes. Cuando te digo “YA”, te inclinaras y te daremos la pajita y otro tubo de ensayo…Empezamos…”¡YA!” “

After the experimenter entered the room having *flash frozen* and stored the *post* saliva sample into the freezer, subjects closed again their eyes and the final *control condition* with 3 min *exciting music* was played back. The exact wording of the *control condition* in Spanish language was as follows, translation into English is provided in the appendix of the present work:

" Me escuchas otra vez. Simplemente concéntrate en mi voz. Seguimos con el experimento. [con la voz baja] Ojos cerrados, quédate quieto. [ya con la voz normal] Ya empieza la ***fase cuatro*** del experimento. Ojos cerrados, quédate quieto.

[Música estimulante, 3 minutos: *Junior Senior - Move Your Feet* mesclado con música tecno, mezclada cíclicamente cambiando *stereo –panning* para evocar estrés]
El experimento ya ha finalizado, pero quédate en el sillón, luego te vamos a quitar los sensores.
Abre tus ojos YA, inspira fueeerte y expulsa el aire fuertemente…otra vez… inspira fuerte, expira fuerte y por última vez, inspira fuerte, expira fuerte… **Estás completamente de vuelta en la realidad**, estás en un sillón en un experimento de la Universidad. Gracias por participar, quédate en el sillón. ¡Estás completamente de vuelta en la realidad! El experimento ya ha finalizado. "

Immediately after the end of the played back audio track, headphones were taken off subject's heads for improving comfort and the paper–pencil version of the psychometric instrument *Phenomenology of Consciousness Inventory* (PCI) (Pekala, 1991) was given to the subjects. After they had completed the questionnaire, subjects were interviewed shortly in a *non-structured interview* for subjective phenomenology, with a focus on experience of changes in *time sense, body scheme, mental imagery, convergent vs. divergent thought style etc.* Special interest was paid on signs of *regression to primary object relations,* changes of *ego boundaries* and material related to *primary processes.* Due to international accepted strict rules for safety of psychoanalytic processes, especially for situations with possible *regression to primary object relations,* collaborators were strictly instructed to never enter the experimental room during the experiments, indicated by a sign attached outside the door. Following the psychoanalytic *rule of abstinence,* personal contact with the subjects was strictly avoided after the experiments, except responding eMails asking for the results. Interviews with the subjects were protocolled by paper–pencil for documentation.

After taking off all sensors/electrodes, the experimenter reassured following his academic training that subjects did not show possible signs of *hypnotism* any more*,* that defense mechanisms were again normal and that subjects were completely oriented in reality; then they were said good-bye and left the building.

2.7. Statistical methods

Biomedical data are often non-normally distributed and frequently contain outliers, so the use of parametric inferential statistical tests (e.g. t-test, ANOVA) is limited. The alternative of non-parametric methodology is often regarded as *problematic* due to alleged less statistical power. This common belief is often inadequate as will be explained in the following.

2.7.1. Wilcoxon signed–rank test

The *Wilcoxon signed–rank test* was proposed by the chemist and statistician Frank Wilcoxon at the end of the Second World War to investigate if two dependent samples differ in their *central tendencies* as a non–parametric alternative to the *Student's dependent t-test for paired samples* (Wilcoxon, 1945). The *Wilcoxon signed–rank test* contrasts the null hypothesis H_0 that the median difference between the pairs is zero, while its alternative hypothesis H_1 is that median difference is not zero. Blair and Higgins (1985) researched the statistical test power of the *Wilcoxon signed–rank test* comparing it to the *Student's dependent t-test for paired samples* in multiple *Monte Carlo simulations* under different population shapes assuming a relative small sample size of $N = 10$: Normal, uniform, double, exponential, truncated normal, log-normal, chi-square or Cauchy were tested. Only under normal Gaussian distribution the *Student's t-test for paired samples* had a small, but practically negligible advantage in power, but for all the other shapes, the *Wilcoxon signed–rank test* showed better powers. The authors concluded that when the shape of population distribution is unknown or uncertain, the use of the *Wilcoxon signed–rank test* clearly has more advantages and even if Gaussian normality is given, no relevant disadvantages are accepted. This study of Blair and Higgins (1985) suggests that the *Wilcoxon signed–rank test* should be the first choice to investigate differences in two related samples with regard to their *central tendencies.* In the case of the *Wilcoxon signed–rank test*, for small

sample sizes with $N < 15$, it is important *not* to use the common *asymptotic* estimation of *p*–values as offered by most statistical software packages, but the *exact* procedure has to be used. For this purpose, the *Exact Tests*™ module in *SPSS Statistics 17.0* is used (IBM Corporation, 1 New Orchard Road, Armonk, New York 10504-1722, USA). That the wrong use of *asymptotic* estimation for small sample sizes is not just only a theoretical–mathematical moaning, but unfortunately lead to wrong conclusions in decades of work in entire research fields, was revealed in an important paper of Mundry and Fischer (1998) at Free University of Berlin, Germany.

Although the *Wilcoxon signed–rank test* does not assume Gaussian normality, it still needs some assumptions: First, the dependent variable has to be measured at least at *ordinal* (or *interval* or *ratio*) level. Second, the data have to be *dependent* or *paired*, which is always the case for *repeated measures.* Third, all pairs have to be sampled from this population without statistical dependencies *between* them. Fourth, the differences have to show a distribution which is *symmetric* around its median.

The specific calculation procedure of the *Wilcoxon signed–rank test* is described in the following: First, differences for each pair are calculated, their sign is ignored (absolute values) and finally they are ranked from smallest to largest while average ranks are assigned to ties. If differences are zero, this pair is excluded and *N* respectively reduced. Now, the obtained ranks for *negative* differences are summed, and the same is done for all *positive* differences. The test statistic *W* is the *smaller* of both rank sums and follows for $N > 20$ approximately a Gaussian normal distribution. *Exact* procedures to determine *p*–values for $N < 15$ are reported in Sprent (1993).

2.7.2. Quade–test for *k* > 2 repeated measurements (*omnibus* or *overall* test)

The *Quade test* was proposed by the biostatistician *Dana Quade* (Quade, 1979) as an alternative or improvement of the *Friedman's test* (Friedman, 1937). The *Quade*-test is an extension of the *Wilcoxon signed-rank test* over *u* subjects ("blocks") for *k* > 2 *related* or *paired* samples, i.e. *k* different treatments or, in our case here, *k* different repeated measurement time points, while the Friedman test is an extension of the *sign test* for *k* > 2. The *Quade*-test is more powerful than the Friedman's test in designs with $k \leq 5$ (see Conover, 1999; Iman, Hora, & Conover, 1984; Tardif, 1987), here we use $k = 5$. In contrast to the Friedman's test, *Quade* gives more weight to subjects (or "blocks") whose sample range (maximum – minimum value) is largest. Although less known, the Quade-test was used before in other research analyzing *change over time* of frequency domain representations of EEG signals (Tallon-Baudry, Bertrand, & Fischer, 2001). Both *Friedman's test* and *Quade test* are so–called *omnibus* or *overall* inferential statistical tests, i.e. they contrast the null hypothesis H_0 that there is no difference between all underlying populations for each related sample with regard to their *central tendencies* which is the same as that all samples come from the same population. In the case of *repeated measures* H_0 consists of the assumption that there is no significant *change over all* k *repeated measurement time points.* The alternative hypothesis is that there are at least two (or more, or all) underlying populations which show significant differences with regard to their *central tendencies.* Hence, these tests are called *omnibus* or *overall* tests, because in the case of a statistically significant result they do not reveal *which* sets of measurments are responsible for the found *overall*–difference with regard to *central tendencies,* they only extract the *global information* that at least *one* of them is different. As explained in the introduction of the present work, the main hypotheses primarily require checking *if* there is such significant *change over time* or not; the exact configuration or shape of this *change over time* is not so important for the aims of the present work. This investigation of the exact configuration, *i.e.* at which time points exactly are responsible for the *overall change over time* requires so-called *post–hoc tests,* which usually are applied as *pairwise*

comparisons. Due to necessary corrections of α*–error accumulation* these pairwise comparisons need much larger sample sizes to reach acceptable statistical test power levels. Such large samples could not be measured in the framework of a doctoral dissertation, because experimentation would need several years.

As compared to the Friedman's test, the *Quade* test uses an additional *weighting factor:* The basic idea behind this weighting factor is that measurement time points with larger variability are more likely to reflect the true underlying order, i.e. they are considered to contain more information and thus should receive greater weight in the analysis (Quade, 1979, pp. 680-681).

The assumptions of the *Quade*–Test are that the dependent variable is to be measured in *interval* or *ratio* level as opposed to the Friedman's test which only needs *ordinal* level. This is due to the *range* information within the repeated measurement points which is additionally used by the *Quade* test: A *range* can only be appropriately determined if information of the distance *between* the scale points is available. This also means that *monotonic,* but non–linear data transformations which affect the *range* (such as *log* or *logit* transformations etc.) will influence the *Quade* test, while the Friedman's test would remain unaffected. Further assumptions of the *Quade* test are that the values in the dependent variable are independent over the subjects and *within* time points, i.e. that data from one subject are statistically independent from any other subject, but that they are paired *between* the measurement points which is the case for repeated measurements. Self-evident, being a non-parametric test, the *Quade* test does *not* assume or need Gaussian normality of the underlying populations.

The details of the *Quade* test's seven mathematical steps to derive its test statistic *W* are explained in the following:

Data are organized as described in table 6: There are in total *u* subjects (indexed with *i*) measured in total *k* times (indexed with *j*) at which the only dependent variable *x* is observed:

Table 6. Input data organization for the *Quade* test (Quade, 1979) for all *u* subjects ("blocks") and all *k* related samples ("treatments"), here repeated measurement time points.

subjects *i*	repeated measurement time points *j*			
	1	**2**	**…**	***k***
1	x_{11}	x_{12}	…	x_{1k}
2	x_{21}	x_{22}	…	x_{2k}
3	x_{31}	x_{32}	…	x_{3k}
…	…	…	…	…
u	x_{u1}	x_{u2}	…	x_{uk}

Note: In the following, in the case of *ties,* average ranks are used.

1) For each subject *i*, calculate the *range* of its intraindividual distribution in *x* over all *k* measurement time points:

$$Range_i = Max(x_{ij}) - Min(x_{ij}) \quad for\ j = 1, 2, \dots k$$

2) Rank these *u* ranges and store them into a *weighting*-vector Q_i, the smallest $Range_i$ gets a rank of 1 and the largest a rank of *u*:

$$Q_i = R(Range_i)$$

3) Rank all measurements x_{ij} within each subject *i* over all *k* measurement time points:

$$R(x_{ij})$$

4) Q_i is then multiplied by the difference between each rank and the average rank which gives S_{ij} . Conover (1999, p. 373) explains S_{ij} as "the relative size of each observation within the block adjusted to reflect the relative significance of the block in which it appears", the term *block* is here equivalent to subjects:

$$S_{ij} = Q_i \left\{ R(x_{ij}) - \frac{k+1}{2} \right\}$$

5) Calculate the following based on the weighted ranks for each time point *j* over all *u* subjects:

$S_j = \sum_{i=1}^{u} S(x_{ij})$ for *j* =1, 2,… *k*.

6) Calculate within-subjects variance A_2 and between-time-points variance *B*:

$$A_2 = \sum_{i=1}^{u} \sum_{j=1}^{k} S_{ij}^2 \qquad B = \frac{1}{u} \sum_{j=1}^{k} S_j^2$$

7) The Quade test statistic *W* is now defined as:

$$W = \frac{(u-1)B}{A_2 - B}$$

This test statistics *W* follows the *F* – distribution where $F_{1-\alpha}(k1, k2)$ is the critical *F* quantile with degrees of freedom $k1=k-1$ and $k2=(k-1)\cdot(u-1)$.

The **Quade test's *null* hypothesis** is:

H_0: The ranking of repeated measurement time points effects are equally likely, i.e. all medians *of the underlying populations over the measurement points are inferential–statistically identical.*

The **Quade test's *alternative* hypothesis** is:

H_1: At least at one of the repeated measurement time points the observed values are larger than at least at one other repeated measurement point, i.e. at least one median *of the underlying populations over the measurement points is inferential–statistically different from at least one other.*

In the present work, all *Quade*–tests were calculated using the implementation of Cardillo (2009) under *MATLAB 7.11.0.584 64bit* (The MathWorks, Inc., 3 Apple Hill Drive Natick, MA 01760-2098, USA).

2.7.3. Adjusted rank transform test (ART) as alternative to classical *ANOVA* for the investigation of possible presentation order effects

The concept of statistical *interaction* is generally based on comparing *magnitudes* of effects in a dependent variable in function of different levels of an independent factor. Because most non-parametric methods use *rank transformation* of the raw data, i.e. they work with the only information that something is higher or lower while rejecting the information of *magnitudes* of these differences, investigating interaction effects with non-parametric methodology is problematic. On the other hand, parametric statistics rely on assumptions such as *(multi)normal distribution, homoscedasticity, sphericity* (e.g. in the case of repeated measures with k >2) etc. which are frequently violated by real-world data. With respect to the investigation of interaction effects in the *ANOVA* context, a trade-off between non-parametric vs. parametric methodology, a "middle ground" or "hybrid technology", was proposed and investigated in Monte Carlo simulations by Leys and Schumann (2010): the *adjusted rank transform test* (ART). As the authors state "[…] using the ART is advisable if the sample size is under 30 per experimental condition and the requirement of a normal distribution is not fulfilled, or when heteroscedasticity occurs along with a non–normal distribution".

The basic idea of the ART for studying *interaction* effects is isolating them by removing the main effects via subtracting the sum of the marginal mean of the line and the column from each relevant observation, then aligning all observations in an increasing order, assigning increasing ranks from one to *N* and adapting *ties* by assigning the same averaged rank to each one. As the last step, a usual *ANOVA* is calculated using the data transformed in the described manner and self-evidently, only *interaction* effects are interpreted.

The same procedure is performed to study *main* effects, but now removing interaction effects and thus isolating main effects by subtracting the mean of the two diagonal group means from each observation.

3. Results

3.1. Psychometric instruments: domain of subjective introspective experience

Comparing the PCI main scales, two scales show significant differences between *Verum* vs. *Placebo*: *Altered Experience* shows highly significant differences in the central tendencies of underlying populations as measured by the inferential statistical non-parametric test for related samples *Wilcoxon signed-rank test* with Z = -2.682 and p_{exact} = .006. With *Mdn Verum* = 2.039, *SD* = .927 vs. *Mdn Placebo* = 1.539, *SD* = .848 *Verum* shows higher scores in *Altered Experience* as compared to *Placebo;* the items of this *Altered Experience* main scale can be found in figure 13*.*

Rationality scores are with *Mdn Verum* = 3.667, *SD* = 1.313 vs. *Mdn Placebo* = 2.833, *SD* = 1.267 and *Wilcoxon signed-rank test* with Z = -1.994 and p_{exact} = .047 significantly larger under *Verum* as compared to *Placebo.* The three items of this short main scale *Rationality* can to be seen in figure 31. To correctly interpret this finding, it important to understand that item 2 and item 24 are expressed *inversely,* so the direction of this scale is that *larger* scores indicate *more Rationality,* thus, surprisingly, under *Verum* subjects report *more Rationality* as compared to *Placebo,* while at the same time they report signs of *altered states of consciousness*, as shown above in the main scale *Altered Experience.*

2.	Mis pensamientos eran claros y comprensibles.	0 1 2 3 4 5 6	Mis pensamientos no eran claros ni fáciles de entender.
24.	Desde el punto de vista conceptual, mi pensamiento era claro y definido.	0 1 2 3 4 5 6	Desde el punto de vista conceptual, mi pensamiento era confuso.
36.	Mis procesos mentales no eran racionales y resultaban difíciles de comprender.	0 1 2 3 4 5 6	Mis procesos mentales eran racionales y muy fáciles de comprender.

Figure 31. The three items of the Spanish version of the short main scale *Rationality* of the *Phenomenology of Consciousness Inventory* (PCI) (Pekala, 1991). Item 2 and 24 are inverse.

More relevant are the findings in the *Hypnoidal State Score* (HSS) which was described above in the chapter *methods.* They have to be considered as the psychometric main findings of the present work:

HSS are with *Mdn Verum* = 5.775, *SD* = 1.483 vs. *Mdn Placebo* = 5.120, *SD* = 1.066 and *Wilcoxon signed-rank test* with *Z* = -2.511 and p_{exact} = .009 highly significantly larger under *Verum* as compared to *Placebo.* The *Placebo* induced by its *unspecific* stimuli already a hypnoidal state which is, following the authors, to be classified as a *moderate hypnoidal state* (Pekala, 2009), with a typical baseline HSS in healthy normal population found to be around 3.45 (Rux, 2002). The *Verum* with its additional *specific* Binaural Beat stimulus caused a highly significant *increase* in the same subjects, who did not know as neither the experimenter which session contained which kind of stimulus (double–blind). Thus, the difference in HSS magnitudes between *Placebo* and *Verum* reflects the specific effect of the employed Binaural Beats contributing to the introduction of a *hypnoidal state.* Quantifying the magnitude of this specific psychometric effect of Binaural Beats on hypnoidal state depth is easily done by the *effect size* which for the *Wilcoxon signed-rank test* is calculated as follows:

$$r = \frac{Z}{\sqrt{N}}$$

with *N* = number of total observations, in the case of the present work 24 observations in total. This gives *r* = .513 which is, following the classification of effect size magnitudes proposed by Cohen (1988), to be considered as a *large* effect. Interestingly, predicting difference scores between *Verum* and *Placebo* (which reflect the *specific* psychometric Binaural Beat effects) by *Placebo* scores which reflect the *unspecific* stimuli effects, only for HSS a significant non–parametric *Spearman* correlation of *rho* = .671 and p_{exact} = .020 is found, see figure 32, while doing the same for *Altered Experience* gives *rho* = -.105 with p_{exact} = .743 and for *Rationality rho* = -.443 with p_{exact} = .149.

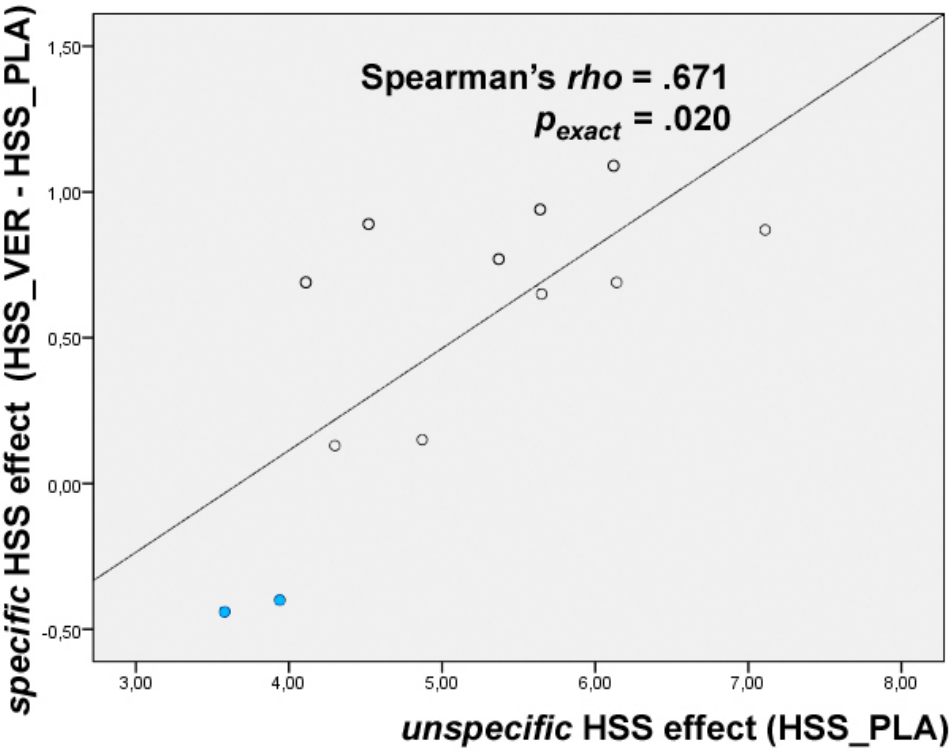


Figure 32. Magnitudes of the *specific* effect of Binaural Beats on psychometric *hypnoidal state scores* (HSS) (Pekala, 2009), as defined as the difference HSS_VER – HSS_PLA, can be predicted by magnitudes of *unspecific* stimuli as defined as HSS_PLA with significant non–parametric *Spearman* correlation of *rho* = .671 and p_{exact} = .020. Doing the same for *Altered Experience* gives *rho* = -.105 and p_{exact} = .743 and for *Rationality rho* = -.443 and p_{exact} = .149. Interestingly, the two *non–responder* subjects (data points with negative difference, plotted in blue) which showed *higher* HSS under *Placebo* as compared to *Verum* are the ones with lowest initial *Placebo* HSS values among all other subjects in the sample.
When HSS_Placebo is understood as a measure of trait hypnotizability, these findings can be interpreted as evidence that the magnitudes of the Binaural Beats' psychometric outcomes can be successfully predicted by trait hypnotizability: The higher a subject's trait hypnotizability is, the stronger will he/she experience subjective introspective Binaural Beat effects, i.e. hypnotizability seems to predict levels of susceptibility to the employed Binaural Beats stimulus.

The HSS reactivity of a subject on *Placebo* stimulation predicts significantly how much will be the increase of *hypnoidal state depth* caused by *Verum* Binaural Beat stimulation: The larger subjects' HSS response is yet under *Placebo,* the more HSS increases will be observed as compared to *Verum,* the magnitudes of the psychometric effects of the present work depend on subject-specific initial values of HSS under *Placebo.* This holds also true for predicting the two *non–responders* which showed higher HSS under *Placebo* as compared to *Verum,* because they are the ones with lowest initial *Placebo* HSS values among all other subjects in the sample. HSS under unspecific *Placebo* seem to reflect levels of *susceptibility* to specific Binaural Beat stimulation.

3.2. Illustrative example of subjective experience in the retrospective unstructured interviews

In order to illustrate participants' subjective experience, to give the reader of the present work a subjective impression, the retrospective unstructured interview after the *Verum* stimulus of the subject who showed the largest psychometric *hypnoidal state score* (HSS) effect is reported in the following in English translation:

> *I remember that I felt very relaxed, there was no moment of being nervous or anxious at all. On the contrary, it was a very pleasant feeling, similar to listening music being in bed before falling asleep, those moments when thoughts begin to flow by themselves or also similar to the experience when you are listening to music walking around and suddenly you notice that you reached your destination without being conscious on the way to it.*
>
> *I felt that my consciousness was somehow broadened, perhaps comparable to when you are looking at clouds in the sky in a somehow meditative way; but it was clearly the opposite of how you feel when calculating mathematics.*
>
> *I remember that I was quite conscious, but not conscious to what happened in the outer world, but conscious to what the voice in the headphones said. I completely forgot that I was in a lounger in an experiment at university, I was completely immersed into my inner world. Before that special noise came, I remember very clearly the field with green grass which the voice told me to imagine, the huge mountain. But it is strange, somehow I lost the sense of distance, I cannot say that it was near or far away? Quite strange, but there was this big, huge mountain… on its top, as told by the voice, I remember vividly the overwhelming big television screen, where I saw everything which happened. It is difficult to describe this state of mind, somehow it is like to be in a dream, but with the difference to be conscious all the time. Somehow I lost almost any sense of time, I mean I was so surprised when the experimenter told me after the experiment what time it was. My time sense was that it has been much less time as compared to how much time passed objectively, like in a good movie when you go out of the cinema and you are surprised how much time has gone. Moreover, I lost the consciousness of my body somehow, mostly of my arms. In the experiment I did not notice what happened with my body, but now remembering, it was like my arms did not exist at all, at least I was not conscious about them at all, I cannot remember them.*
>
> *For the time when this special noise came, I do not remember any details, but I was very, very relaxed and my mind was somehow very far away, but I did not sleep. This was a feeling which I never felt before, it is difficult to describe this in words. Perhaps we can say that my consciousness was far away.*
>
> *All in all, I remember shining bright colors, comparable to trick–film drawings of spring, I mean the green was very green, the blue was very blue, but I do not remember any smells. The green field which I was told to imagine was in silence, totally in silence, but somehow it was audible this silence, this is also very difficult to put in words. Emotionally the experiment was very intense. It was somehow strange to actually see my own thoughts as a movie on the television screen. My thoughts were a bit complex and not so strongly connected, somehow like floating? The strange thing is that I cannot remember details of the time when the special noise came, as I told you it was just like my mind was in a state which I never experienced before, but I felt very, very relaxed, cozy and in peace.*

3.3. Physiological measurements

3.3.1. Domain of parasympathetic modulation of Heart Rate Variability (HRV)

Parasympathetic modulation of *heart rate variability* (HRV) was hypothesized to show significant *change over time* under *Verum,* but not under *Placebo* and the most relevant HRV–parameter to test this hypothesis was considered to be HRV *high frequency spectral power* (HF–power) from 0.15–0.4 Hz, normalized to HRV *total power* by expressing it in percentage. As explained above, due to the different velocities of synaptic neurotransmission in the autonomic nervous system, HRV *HF–power* is exclusively caused by *parasympathetic* modulation of the heart's own pace makers and thus an indirect measure of neural *parasympathetic outflow* to the heart. The 25 min stimuli *Verum* vs. *Placebo* are represented by five epochs of five min length, for each of them the entire subset of the most relevant HRV parameters (see table 4) was determined, ratio HRV parameters were *logit* and other HRV parameters were *log* transformed, and *change over time* over all five epochs in each parameter was tested using *Quade tests*, both for *Verum* vs. *Placebo,* for results see table 7.

Table 7. Subset of the *heart rate variability* (HRV) parameters tested in the present work for significant *change over time* by *Quade* tests over the 25 min *Verum* vs. *Placebo* (five • five min epochs). Because HRV is influenced by *respiratory rate* via *respiratory sinus arrhythmia* (RSA) (Grossman & Taylor, 2007; Ritz & Dahme, 2006) as a *third variable,* it has to be also checked for significant *change over time.* A short explication of each HRV parameter is given in the manual of the program *Kubios HRV* (Tarvainen et al., 2009), downloadable at http://kubios.uef.fi/media/Kubios_HRV_2.1_Users_Guide.pdf. Sign. results highlighted in bold.

***HRV*–parameter**	**Unit**	**data transform**	***p* – value Quade test *Placebo***	***p* – value Quade test *Verum***
MeanRR	*ms*	Log	.791	.375
SDNN	*ms*	Log	.553	.343
RMSSD	*ms*	Log	.206	.971
pNN50	*percentage*	Logit	.210	.476
HRVtriangularIndex	*dimensionless*	Log	.670	.804
AR_PeakHF	*Hz*	Log	.915	.476
FFT_PeakHF	*Hz*	Log	.271	.887
FFT_HFpower	ms^2	Log	.963	.763
FFT_HFpower	***percentage***	**Logit**	**.317**	**.003**
FFT_LFpower	ms^2	Log	.549	.119
FFT_LFpower	*percentage*	Logit	.775	.749
AR_HFpower	ms^2	Log	.484	.647
AR_HFpower	***percentage***	**Logit**	**.994**	**.015**
AR_LFpower	*percentage*	Logit	.746	.164
AR_LFpower	ms^2	Log	.921	.989
Respiratory Rate	*Hz*	Log	.131	.358

As hypothesized, only normalized HRV *HF–power* (percentage) shows highly significant *change over time exclusively under* Verum*,* but not under *Placebo,* both for determining this parameter by classical FFT–*Welch* vs. more advanced *AR* power estimation methodology. Interpreting *HF–power* (percentage) in terms of parasympathetic outflow to the heart requires control of at least the third variable *respiratory rate* or better of also *tidal volume* (Grossman & Taylor, 2007; Ritz & Dahme, 2006): No significant *change over time* was observed for *respiratory rate* and only the *Verum* stimulus lead to significant *change over time* for *HF–power* (percentage)*,* thus it has to be concluded that Binaural Beats specifically caused time–dependent fluctuations of neural *parasympathetic outflow* to the heart over the time course of 25 min length of the *Verum* stimulus. Although no correction for α*–error accumulation* due to multiple testing was employed, with α–level set to standard .05 leading to 1.6 false–positive detections to be expected, it has to be considered to be more than pure random that just the two operationalizations of normalized HRV *HF–power* and moreover both exclusively under *Verum* show significant *change over time.*

Summing up, the hypothesis that the employed Binaural Beat stimulus specifically, i.e. contra null findings under *Placebo* and contra null findings in the third variable *respiratory rate,* induces significant fluctuations or *change over time* in the neural *parasympathetic outflow* to the heart is verified by the observed data.

As explained above, the correct inferential statistical investigation of the exact shape of this *change over time* within the five by five min epochs would require many more observations which was not feasible in the context of a doctoral dissertation. With regard to the interpretation of change of (cortical) *arousal* levels, EEG data offer much more sensitive and informative measures (see below) and results in HRV should better only be interpreted on the level of *global* information as operationalized by the concept *change over time: That* the Binaural Beat stimulus modulates neural *parasympathetic outflow* to the heart has been verified by the present work, but its exact shape of temporal development has to be investigated in further studies with much larger samples.

3.3.2. Domain of cortical arousal as measured by EEG *logit*–transformed relative spectral powers

All 63–channel EEG biosignals of all *N* = 12 subjects during *Verum* and *Placebo* stimulation 2▪12▪25min = 600min, *baselines* 12▪3▪3min = 108min and *EOG artifact generation* 12▪10min = 120min had to be visually checked and corrected for remaing artifacts by manual rejection, after applying automatic REG–ICA methodology (Klados et al., 2011), as described above. At the same time, the author of the present work watched out for possible *sleep signs* in the EEG biosignals following the international standard definitions of the classical Rechtschaffen & Kales manual and its actualization, the AASM manual (Berry et al., 2012; Rechtschaffen & Kales, 1968) based on his practical academic training of polysomnographies at Unidad de sueño, Hospital Universitario de La Princesa, Madrid. Apart from subject's confirmations that they did not felt having fallen asleep during the experiments, no EEG signs of any sleep stage could be observed. Thus, the findings in EEG biosignals presented in the following, cannot be attributed to sleep.

Whether there is significant *change over time* in the different EEG frequency bands over the 5 time epochs represented as 5▪5min means of *logit*-transformed EEG relative spectral FFT powers (after *Laplace* spatial filtering in time domain), comparing each time *Verum* vs. *Placebo* by multiple *Quade* tests, gives the results represented in table 8. α-error accumulation at α-level = .05 due to multiple *Quade* hypothesis testing among 63 tested electrodes leads to expected 3.15 false significant results in each EEG frequency band, so approx. three results have to be attributed to this false discovery rate due to randomness because of no correction for multiple hypothesis testing; findings below this threshold of three are thus represented in parenthesis. Over all 20 analyzed EEG frequency bands, under *Placebo*, 46 electrodes show sign. *Quade* tests, while under *Verum* 328. Given the false discovery rate over all EEG frequency bands $\sum_{total}$ with 20▪3 = 60, for *Placebo* all 46 sign. findings taken together are clearly not more than random discoveries, while the 328 sign. findings under *Verum* exceed ≈5.5 times the false discovery rate.

Hence, overwhelming evidence for *change over time* over the 5·5min epochs in relative spectral powers of multiple EEG frequency bands was found under *Verum* vs. no evidence at all was observed under *Placebo.*

Table 8. Significant *change over time* over the different EEG freq. bands in the 5 time epochs represented as 5·5min means of *logit*-transformed EEG relative spectral FFT powers (after *Laplace* spatial filtering in time domain). *Verum* specific sign. *change over time* is most pronouncedly observed in Delta, ThetaLow, Alpha4, BetaMid and Gamma1 EEG frequency bands, details are plotted in figures 33, 37, 41, 45 and 49.

EEG freq. band	**number of electrodes showing sign. *change over time* (Quade test) at α–level = .05**		**averaged effect size over all sign. electrodes (averg. total intrasub. variance)**
	Verum	***Placebo***	***Verum***
DELTAtotal [0.5-3.5 Hz]	15	0	0.6947
THETAtotal [3.5-7.5 Hz]	28	(2)	0.6137
THETAlow [3.5-6.5 Hz]	30	6	0.7071
THETAhigh [6.5-7.5 Hz]]	14	5	0.2944
THETA1 [3.5-4.5 Hz]	31	5	0.7313
THETA2 [4.5-5.5 Hz]	23	(1)	0.6806
THETA3 [5.5-6.5 Hz]	25	0	0.5513
THETA4 [6.5-7.5 Hz]	14	5	0.2944
ALPHAtotal [7.5-12.5 Hz]	17	(3)	1.0305
ALPHAlow [7.5-9 Hz]	9	(2)	0.3344
ALPHAhigh [9-12.5 Hz]	18	(1)	1.0693
ALPHA1 [7.5-8.5 Hz]	11	(3)	0.2294
ALPHA2 [8.5-9.5 Hz]	7	5	0.4900
ALPHA3 [9.5-10.5 Hz]	10	5	1.0738
ALPHA4 [10.5-11.5 Hz]	25	(1)	0.8681
ALPHA5 [11.5-12.5 Hz]	19	0	0.3186
BETAlow [12.5-18 Hz]	0	0	Ø
BETAmid [18-24 Hz]	19	(2)	0.5843
BETAhigh [24-30 Hz]	5	0	Ø
GAMMA1 [30-40 Hz]	8	0	0.7410
	$\sum_{total}$ = 328	$\sum_{total}$ = 46	

Note. α-error accumulation at α-level = .05 due to multiple *Quade* hypothesis testing leads among 63 tested electrodes to expected 3.15 false sign. results for each EEG frequency band, so approx. three results have to be attributed to this false discovery rate due to random because of no correction for multiple hypothesis testing; findings below this threshold of three are thus represented in parenthesis.
Over all 20 analyzed EEG frequency bands, under *Placebo,* 46 electrodes show sign. *Quade* tests, while under *Verum* 328. Given the false discovery rate over all EEG frequency bands $\sum_{total}$ with 20·3 = 60, for *Placebo* all 46 sign. findings taken together are clearly not more than random discoveries, while the 328 sign. findings under *Verum* exceed ≈5.5 times the false discovery rate.

Given that the same subjects represent *Verum* vs. *Placebo* experimental groups and given that the presentation order was double–blinded and randomized, these findings are to be interpreted as overwhelming evidence for the *specific* (i.e. contra Placebo) efficacy of the employed Binaural Beat stimulus expressed as *change over time* of relative spectral EEG powers over the 5▪5min epochs.

Verum specific significant *change over time* (as investigated by multiple *Quade* tests over the 5 ▪ 5 min epochs) was most pronouncedly observed in Delta, ThetaLow, Alpha4, BetaMid and Gamma1 EEG frequency bands. These bands are now investigated in detail; most important details are plotted in figures 33, 37, 41, 45 and 49. With regard to these figures, note that here the *x*-axes represent the EEG electrodes following the scheme reported in figure 16, while the *y*-axes refer to the *Quade*–tests' *p*–values (i.e. remaining error–probability of null hypotheses), as explained in section 2.7.2.

To facilitate comparisons between these results in different EEG frequency bands, the structure of the presented analyses and figures is each time the same. Note that the found topographic patterns do not represent a single subject, but represent the overall-response of a *group* of subjects as response pattern to the employed Binaural Beat stimulus.

3.3.2.1. Results in delta (0.5 – 3.5 Hz) *EEG relative spectral powers*

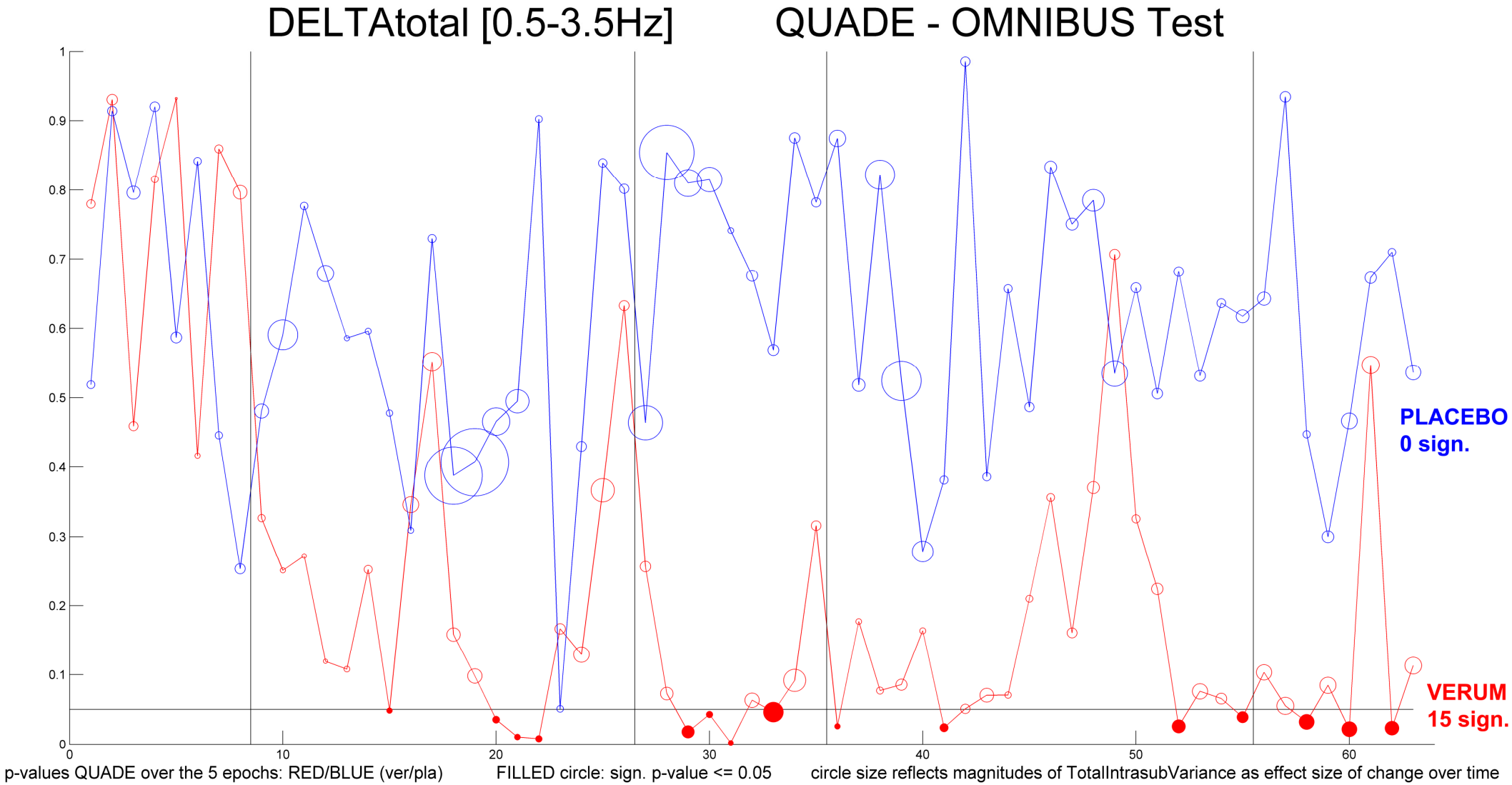


Figure 33. Comparing *Verum* (red) vs. *Placebo* (blue) with regard to significance of *change over time* for each 5 • 5min means of relative spectral EEG powers in delta (0.5 – 3.5 Hz) by multiple *Quade* tests for each of the 63 electrodes (x – axis), *p* – values on y – axis. Due to false discovery rate, *three* false – positive findings are expected under *Verum* and under *Placebo.* Vertical black lines subdivide scalp regions to facilitate the understanding of electrodes' spatial organisation (frontopolar/anterior–frontal, frontal, central, parietal, occipital). Mind the symbology explained under the plot.

Change over time of **delta powers (0.5 – 3.5 Hz)** is statistically significant at the standard α-level = .05 under *Verum* in the *Quade* test at 15 electrodes vs. at none electrode under *Placebo* (see figure 33). As to be seen, there is a large distance of the *Quade* test *p*-values of delta powers between *Verum* and *Placebo.* Moreover, some adjacent electrodes to those which show significant *change over time* at the standard α-level = .05 fail significance only narrowly, but seem to capture the same effects. For further analysis of the *Verum* topographic pattern of effect sizes of *change over time* as expressed as total intrasubject variance at each electrode, in this special case, α-level is thus reasonably raised to = .010. This topographic pattern is to be seen in figure 34. A lateralization to the *right* hemisphere is observed, most pronounced at *central* transversal line, which is, interestingly, just the opposite pattern as compared to the *left* hemisphere lateralization which has been observed in alpha4 powers (10.5 – 11.5 Hz), see below. The effect of *change over time* in delta powers shows relatively smallest magnitudes around *Cz*. Seen altogether, the important part (in terms of effect sizes) of *Verum* specific *change over time* in delta powers seems to have happened at right-central and right-posterior electrodes.

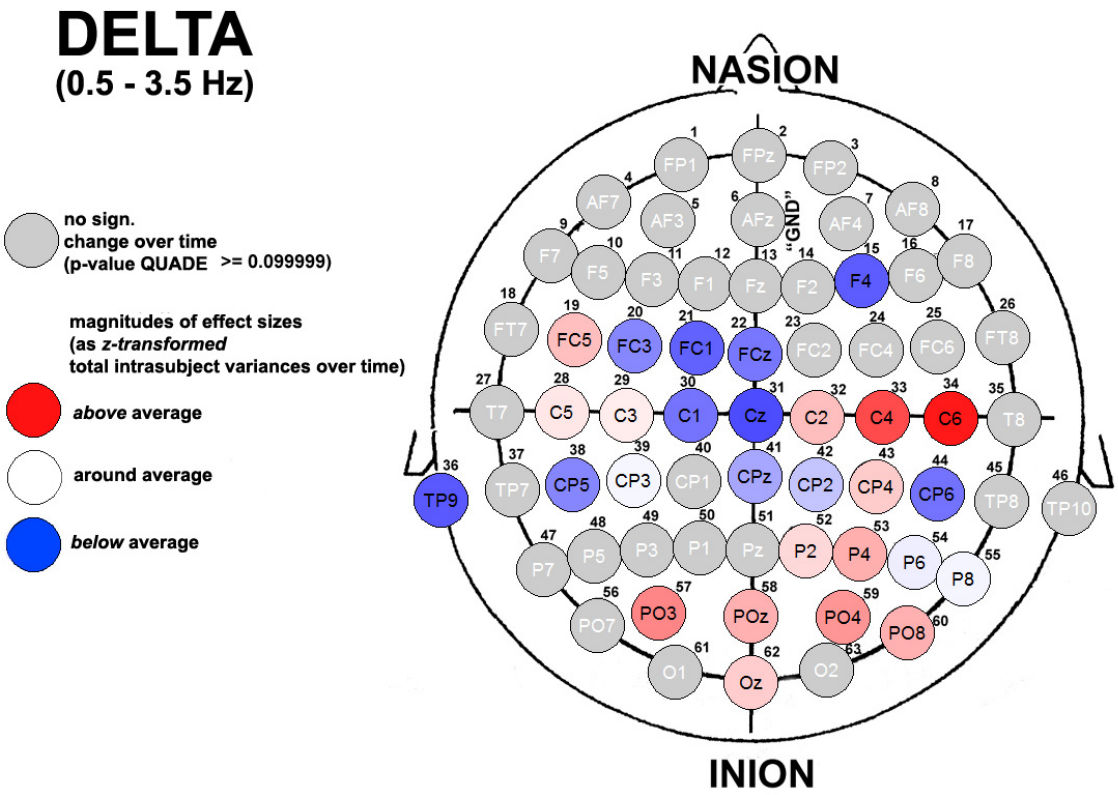
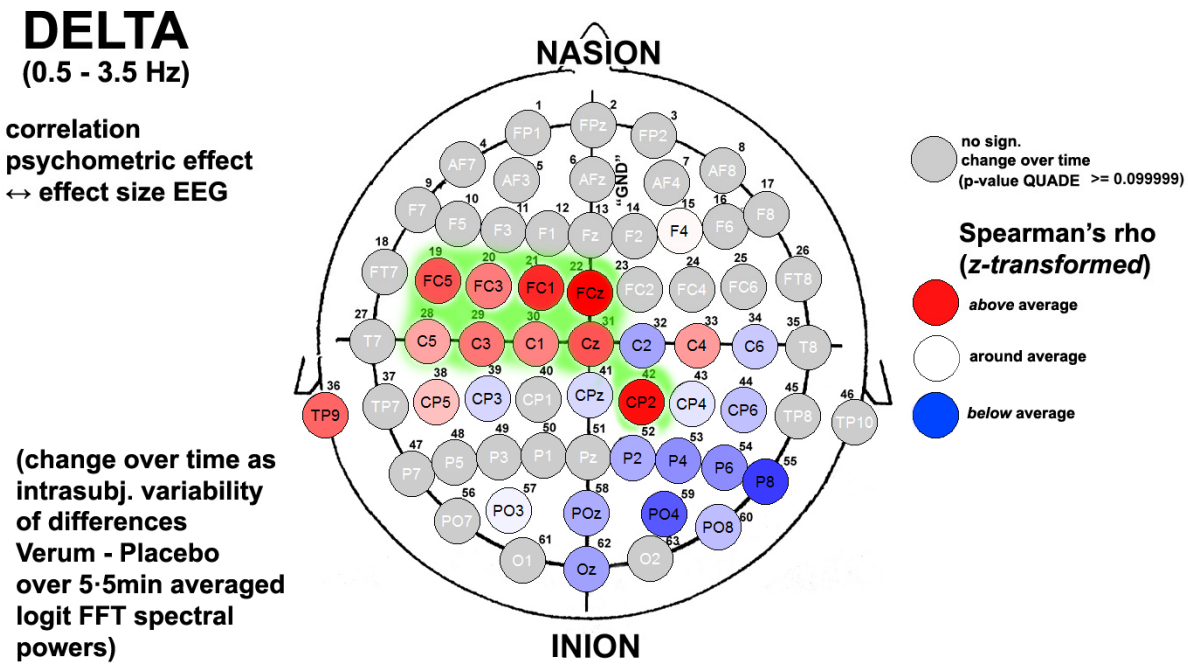


Figure 34. *Verum* topographic pattern of effect sizes of *change over time* as expressed as *total intrasubject variance* at each electrode for delta relative spectral powers (0.5 – 3.5 Hz). Values are *z* – transformed to facilitate visual inspection of the topographic pattern. A *right*–lateralized pattern is observed with maxima at *C6* and *C4.*

Now, possible associations between *psychometric* effect magnitudes (hynoidal state score *Verum* minus hynoidal state score *Placebo*) and magnitudes of effects of *change over time* (total intrasubject variance Verum minus Placebo) of EEG delta relative spectral powers are investigated using *Spearman's* non–parametric correlation, see figure 35.

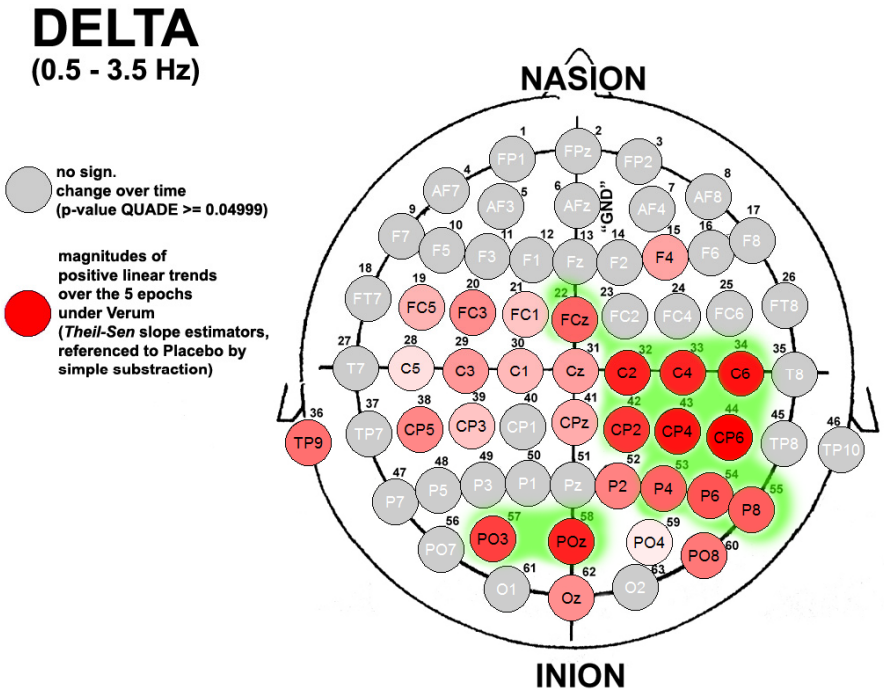


# electrode	Spearman's *rho*	p_{exact}	# electrode	Spearman's *rho*	p_{exact}
15	.405	.216	**42**	**.724**	**.012**
19	**.629**	**.038**	43	.342	.304
20	**.574**	**.065**	44	.269	.424
21	**.688**	**.019**	52	.228	.501
22	**.743**	**.009**	53	.169	.620
28	**.519**	**.102**	54	.164	.630
29	**.583**	**.060**	55	.000	1.000
30	**.565**	**.070**	57	.374	.258
31	**.629**	**.038**	58	.232	.492
32	.214	.527	59	.059	.863
33	.533	.091	60	.264	.432
34	.287	.392	62	.205	.545
36	.606	.048			
38	.483	.132			
39	.314	.346			
41	.319	.339			

Figure 35. Topography of associations between magnitudes of *psychometric* effects (hynoidal state score *Verum* minus hynoidal state score *Placebo*) and magnitudes of effects of *change over time* (total intrasubject variance) of EEG delta relative spectral powers investigated using *Spearman's* non–parametric correlation. A clear *left*–lateralized pattern is observed. Relevant electrodes are shadowed in green and highlighted in bold.

The topographic distribution of delta powers' (0.5 –3.5 Hz) *change over time* as measured as total intrasubject variances under *Verum* (figure 34) compared to the topographic pattern of *relationships* to the *psychometric effect* expressed as Spearman's *rho* (figure 35) reveals roughly spatially–antiproportional patterns: Looking at the most predictive, i.e. meaningful, electrodes in figure 35 a surprisingly clearly *left*-lateralized pattern is found for *frontocentral* and *central* electrodes while the topographic pattern of the scalp distribution of simple *magnitudes* of *change over time* (figure 34) roughly shows a *right*-lateralized and rather posterior pattern. While for alpha4 (10.5 – 11.5 Hz), see below, roughly comparable topographic patterns of distributions of magnitudes of *change over time* vs. those of correlational relationships to the subjectively experienced psychometric effects were found, for delta (0.5 – 3.5 Hz) these patterns seem to relate to each other in a spatially–*antiproportional* fashion. With regard to figure 35, 6 among 28 *Quade* test significant electrodes show significant *Spearman* correlations with the psychometric effect within a *false discovery rate* of 1.4 electrodes, due to α-error accumulation because of multiple hypothesis testing.

Determining the *details* of the time course over all 5 epochs in EEG powers, the exact shape, is problematic as mentioned before. Extracting only the information of the *linear trend* over the 5 epochs, excluding all non-linear information, using the non-parametric *Theil-Sen slope estimator* (Wilcox, 1998) and referencing it to those obtained under *Placebo,* all *Quade* test significant electrodes (at the modified α-level = .010) show without exception only *positive* slopes (see figure 36), in contrast to alpha4 (10.5 – 11.5 Hz) and betaMid (18 – 24 Hz) which showed exclusively *negative* slopes, see below. This means that under *Verum* the faster brainwave frequency bands alpha4 and betamid show a *decrease* in their linear trends over the 5 epochs, while the slowest EEG frequency band delta shows an *increase.*



# electrode	difference *Theil-Sen* slopes Verum – Placebo	# electrode	difference *Theil-Sen* slopes Verum – Placebo
15	.0469	39	.0301
19	.0382	41	.0423
20	.0584	**42**	**.1038**
21	.0294	**43**	**.1213**
22	**.0791**	**44**	**.1304**
28	.0159	52	.0638
29	.0527	**53**	**.0791**
30	.0358	**54**	**.0874**
31	.0491	**55**	**.0828**
32	**.1147**	**57**	**.0982**
33	**.1128**	**58**	**.1132**
34	**.1217**	59	.0098
36	.0734	60	.0688
38	.0615	62	.0575

Figure 36. Extracting only the information of the linear trend over the five epochs, excluding all non-linear information, using the non-parametric *Theil-Sen slope estimator* (Wilcox, 1998) and referencing it to those obtained under *Placebo*, all *Quade* test significant electrodes (at the modified α-level = .010) show without exception only positive slopes, in contrast to alpha4 (10.5 – 11.5 Hz) and betaMid (18 – 24 Hz) which showed exclusively negative slopes, see below. Most relevant electrodes are shadowed in green and highlighted in bold.

3.3.2.2. Results in thetaLow (3.5 – 6.5 Hz) *EEG relative spectral powers*

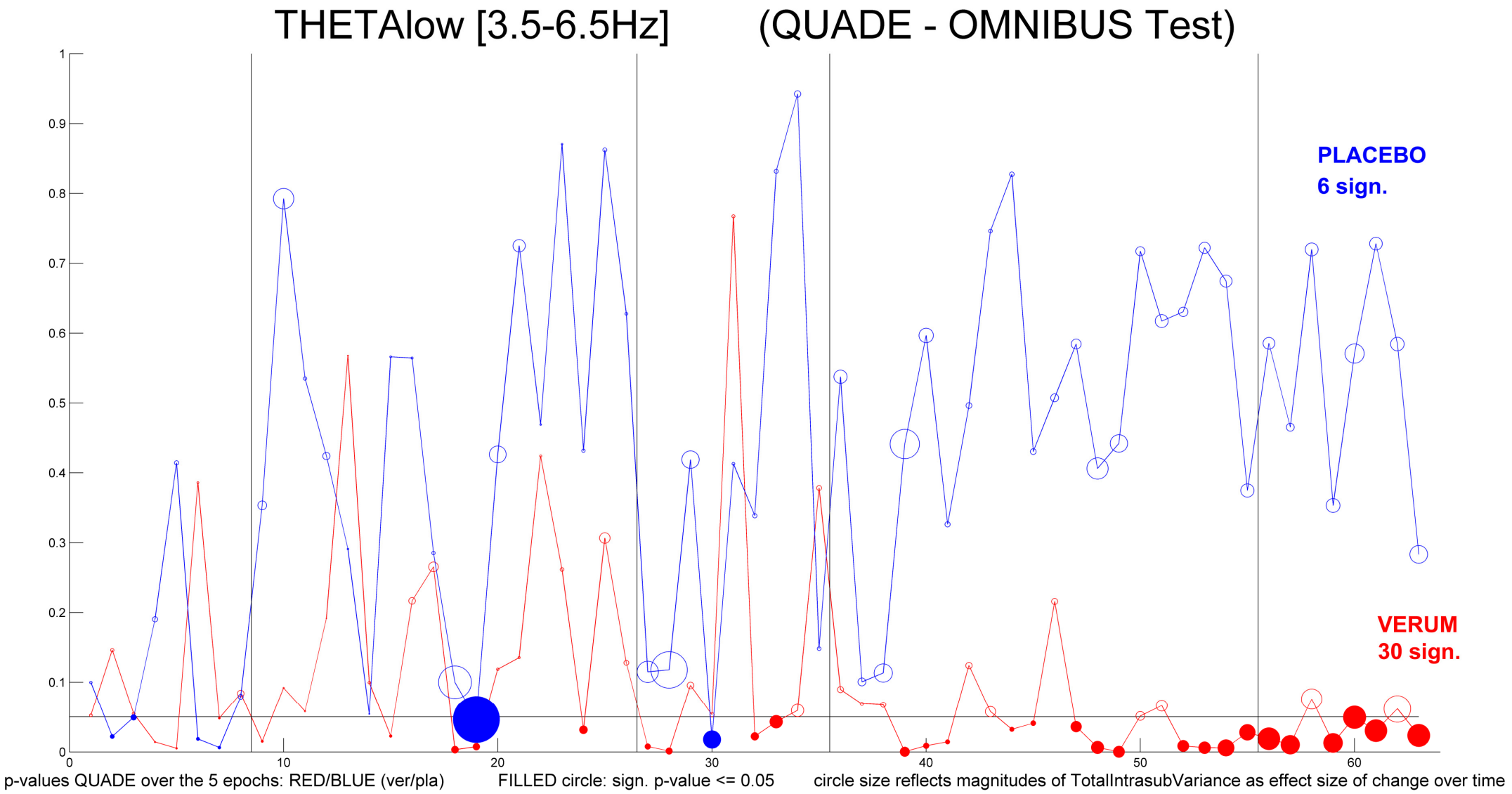


Figure 37. Comparing *Verum* (red) vs. *Placebo* (blue) with regard to significance of *change over time* for each 5 • 5min means of relative spectral EEG powers in thetaLow (3.5 – 6.5 Hz) by multiple *Quade* tests for each of the 63 electrodes (x – axis), *p* – values on y – axis. Due to false discovery rate, *three* false – positive findings are expected under *Verum* and under *Placebo.* Vertical black lines subdivide scalp regions to facilitate the understanding of electrodes' spatial organisation (frontopolar/anterior–frontal, frontal, central, parietal, occipital). Mind the symbology explained under the plot.

The topographic distribution of *change over time* of **thetaLow** relative spectral powers **(3.5 – 6.5 Hz),** see figure 37, is statistically significant under *Verum* in the *Quade* test at 30 electrodes under *Verum* vs. at 6 electrodes under *Placebo* from which three of them are probably due to α – error accumulation because of multiple hypothesis testing and thus are statistical artifacts without any relevant meaning. These findings show that only under *Verum* at least one of the five epochs shows thetaLow powers which come from an underlying population which is significantly different from those corresponding to at least one or more of the other remaining four time points and that this happens at ≈ 50% of all 63 electrodes.

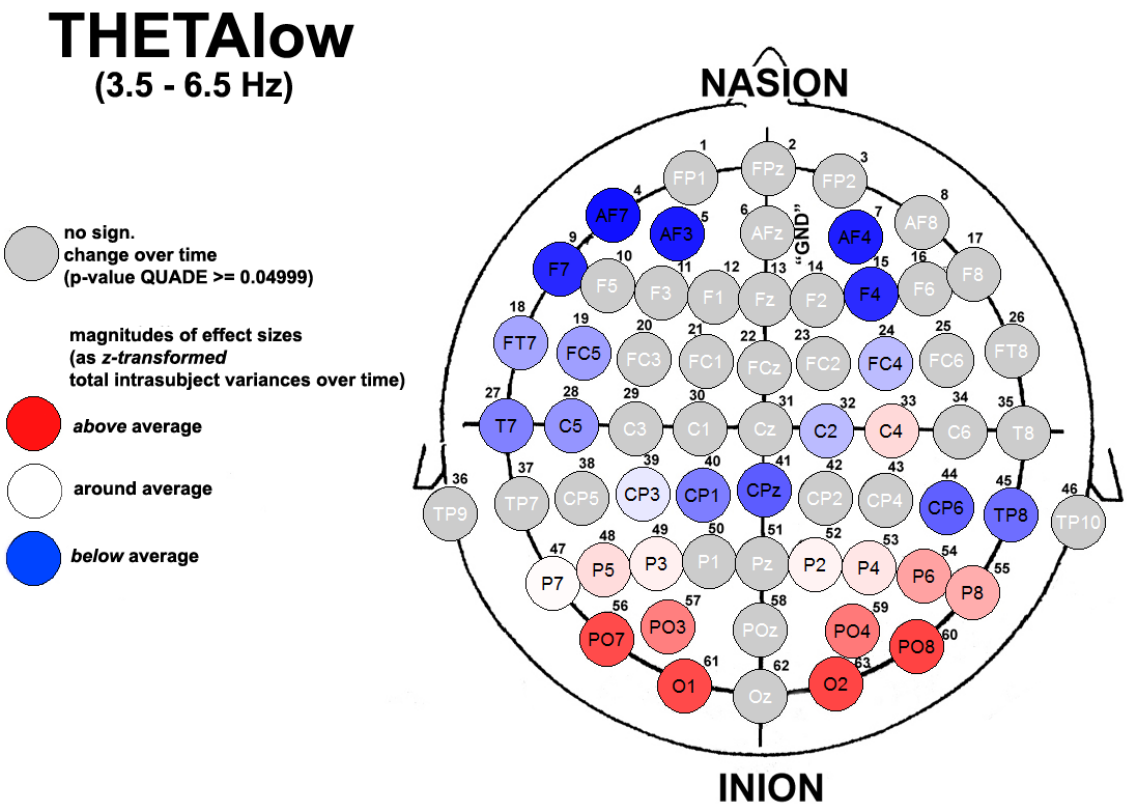
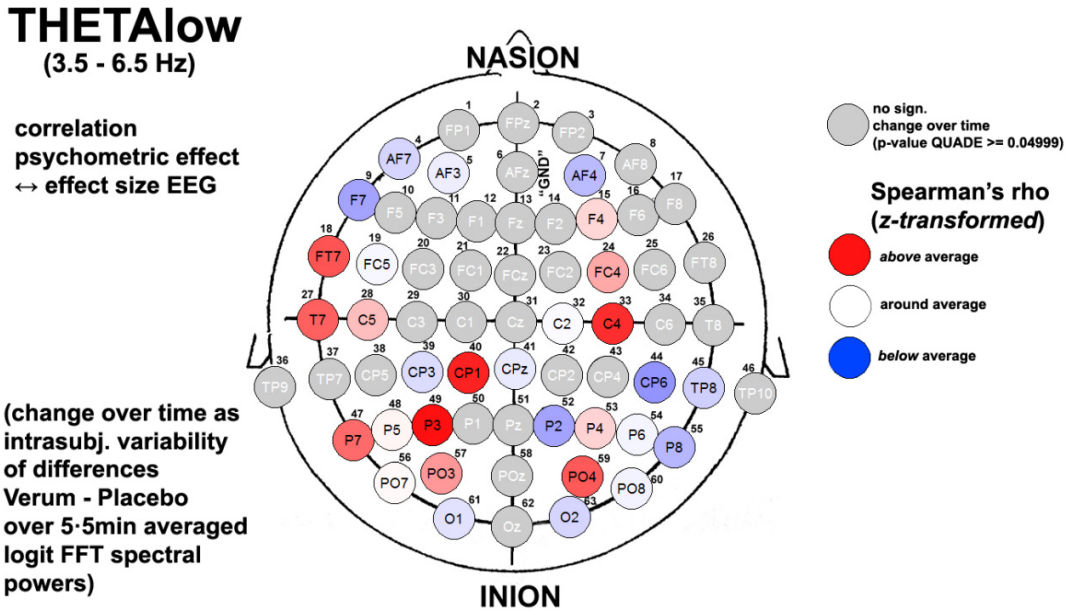


Figure 38. *Verum* topographic pattern of effect sizes of *change over time* as expressed as total intrasubject variance at each electrode for thetaLow relative spectral powers (3.5 – 6.5 Hz). Values are *z* – transformed to facilitate visual inspection of the topographic pattern. Effects are most pronounced at posterior electrodes.

Analyzing the topography of effect sizes of *change over time* (figure 38), as measured as total intrasubject variances (intrasubject variance over all 5 epochs summed up over all subjects), shows that the most pronounced effects of *change over time* are clearly observed at posterior, i.e. parietal, parieto-occipital and occipital electrodes, while central and frontal electrodes show minor *change over time* in thetaLow powers. No signs of hemispheric asymmetry of these effects in thetaLow are observed.

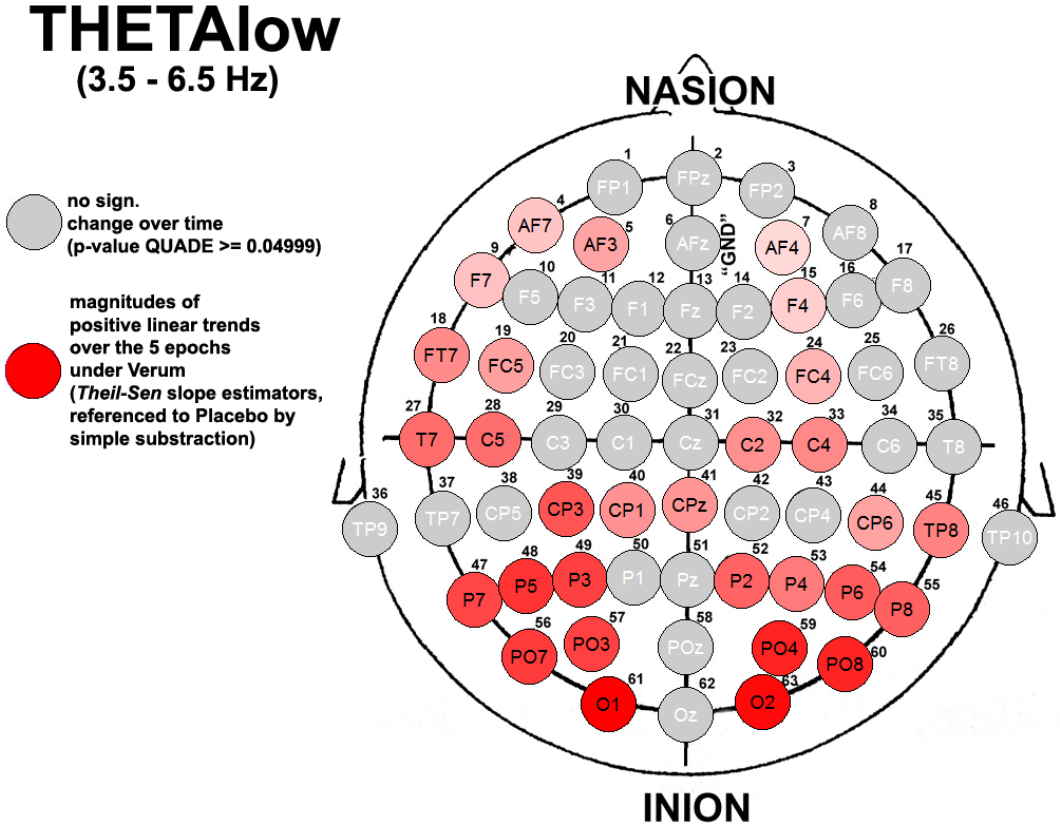


# electrode	Spearman's *rho*	p_{exact}	# electrode	Spearman's *rho*	p_{exact}
4	.292	.384	45	.278	.408
5	.355	.284	47	.560	.073
7	.223	.509	48	.410	.210
9	.164	.630	**49**	**.656**	**.028**
15	.442	.174	48	.410	.210
18	.583	.060	52	.164	.630
19	.378	.252	53	.446	.169
24	.492	.124	54	.374	.258
27	.569	.067	55	.214	.527
28	.469	.145	56	.405	.216
32	.387	.239	57	.510	.109
33	**.624**	**.040**	59	.579	.062
39	.310	.354	60	.369	.264
40	**.638**	**.035**	61	.323	.332
41	.346	.297	63	.292	.384
44	.123	.719			

Figure 39. Topography of associations between magnitudes of *psychometric* effects (hynoidal state score *Verum* minus hynoidal state score *Placebo*) and magnitudes of effects of *change over time* (total intrasubject variance) of EEG thetaLow relative spectral powers investigated using *Spearman's* non–parametric correlation. Relevant electrodes are highlighted in bold. No clear topographic pattern is observable.

The topographic distribution of the effect sizes of thetalow powers' (3.5 – 6.5 Hz) *change over time* as measured as total intrasubject variances under *Verum* (figure 38) compared to the topographic pattern of *relationships* to the *psychometric effect* expressed as Spearman's *rho* (figure 39) shows no consistent correspondence between both patterns. Correlations are found to be most prominent at *P3* and *CP1* and also *C4;* using *Spearman's* correlation at α-level = .05 among the 31 *Quade* test significant electrodes implies a *false detection rate* of 1.55 electrodes, so at least one of the three found significant correlations is *not* due to α-error accumulation because of multiple hypothesis testing.

Extracting only the information of the *linear trend* over the five epochs by the non-parametric *Theil-Sen regression slope estimator* (Wilcox, 1998) excluding all non-linear information, and referencing these linear trends to those of *Placebo* (figure 40) reveals that all *Quade*-test significant electrodes show a *positive* linear trend of the time course of thetaLow with a clear pattern of increase of magnitudes towards posterior sites.



# electrode	difference *Theil-Sen* slopes Verum – Placebo	# electrode	difference *Theil-Sen* slopes Verum – Placebo
4	.0311	44	.0496
5	.0484	45	.0662
7	.0205	47	.0992
9	.0330	**48**	**.1089**
15	.0256	**49**	**.1015**
18	.0618	52	.0830
19	.0509	53	.0708
24	.0395	54	.0864
27	.0785	55	.0850
28	.0771	**56**	**.1047**
32	.0591	**57**	**.1011**
33	.0636	**59**	**.1177**
39	.0908	**60**	**.1168**
40	.0583	**61**	**.1359**
41	.0558	**63**	**.1306**

Figure 40. Extracting only the information of the linear trend over the five epochs, excluding all non-linear information, using the non-parametric *Theil-Sen slope estimator* (Wilcox, 1998) and referencing it to those obtained under *Placebo*, all *Quade* test significant electrodes show without exception only positive slopes for thetaLow (3.5 – 6.5 Hz) as also observed for delta (0.5 – 3.5 Hz), in contrast to alpha4 (10.5 – 11.5 Hz) and betaMid (18 – 24 Hz) which showed exclusively negative slopes, see below. ThetaLow shows a clear pattern of increase of slope magnitudes towards posterior sites.

3.3.2.3. Results in alpha4 (10.5 – 11.5 Hz) *EEG relative spectral powers*

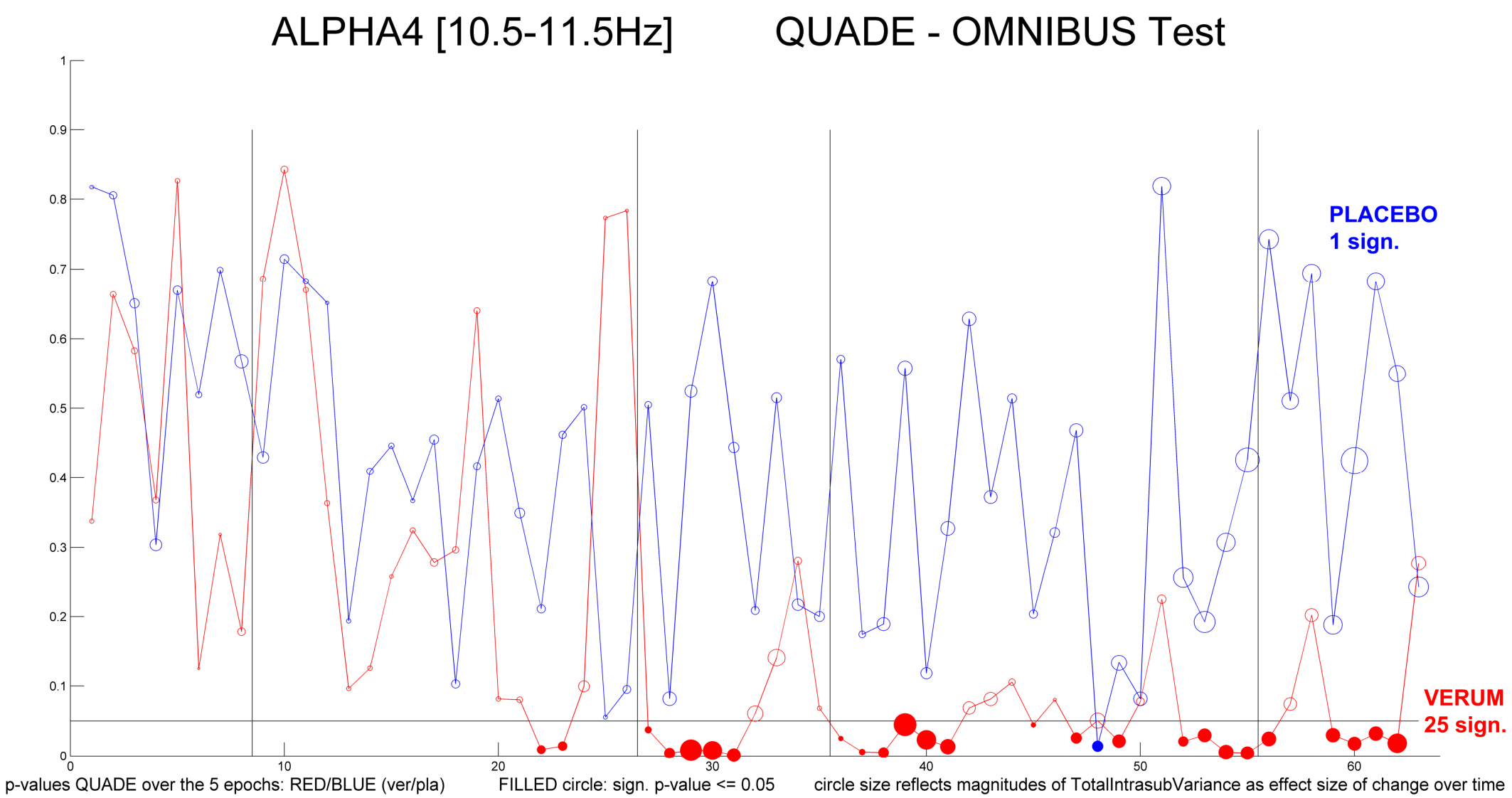


Figure 41. Comparing *Verum* (red) vs. *Placebo* (blue) with regard to significance of *change over time* for each 5 • 5min means of relative spectral EEG powers in alpha4 (10.5 – 11.5 Hz) by multiple *Quade* tests for each of the 63 electrodes (x – axis), *p* – values on y – axis. Due to false discovery rate, *three* false – positive findings are expected under *Verum* and under *Placebo.* Vertical black lines subdivide scalp regions to facilitate the understanding of electrodes' spatial organisation (frontopolar/anterior–frontal, frontal, central, parietal, occipital). Mind the symbology explained under the plot.

The topographic distribution of *change over time* of **alpha4** relative spectral powers **(10.5 –11.5 Hz)** is statistically significant under *Verum* in the *Quade* test at 25 electrodes vs. only at one electrode under *Placebo* (see figure 41) which is still in the range of the false detection rate due to α – error accumulation because of multiple hypothesis testing and thus has no meaning. These findings show that only under *Verum* at least one of the five epochs shows alpha4 powers which come from an underlying population which is significantly different in terms of central tendencies from those corresponding to at least one or more other remaining four time points and that this happens at ≈ 40% of all 63 electrodes, which almost all of them are *not* frontal electrodes.

Analyzing the topography of effect sizes of *change over time* (see figure 42), as measured as total intrasubject variances (intrasubject variance over all 5 epochs summed up over all subjects), shows that there is a clear topographic lateralization of the magnitude of these *changes over time* in alpha4 towards the *left* hemisphere. This pattern in alpha4 is roughly spatially–antiproportional as compared to delta's lateralization to the *right* hemisphere (see above, figure 34).

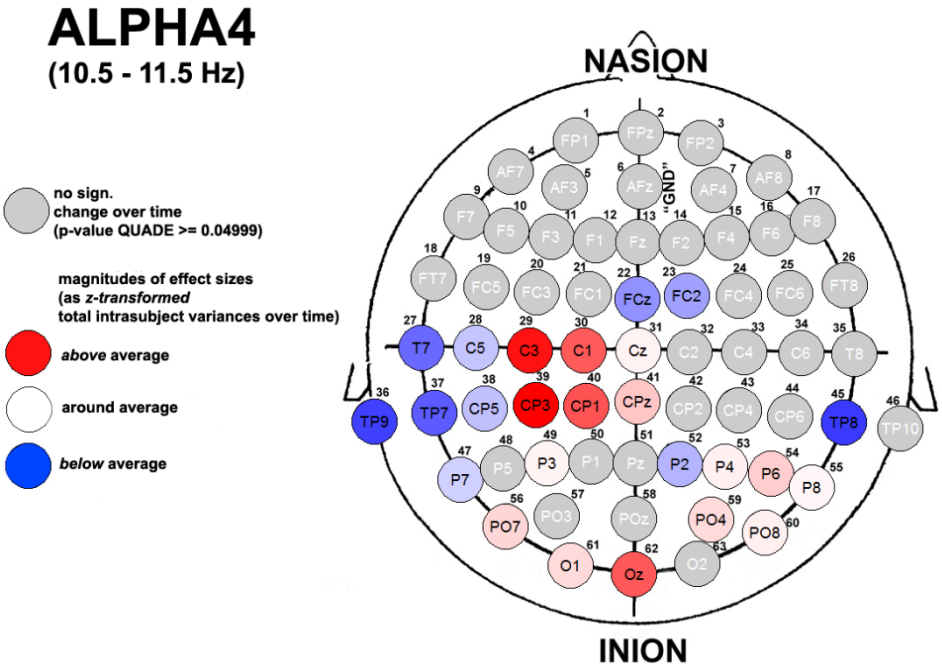
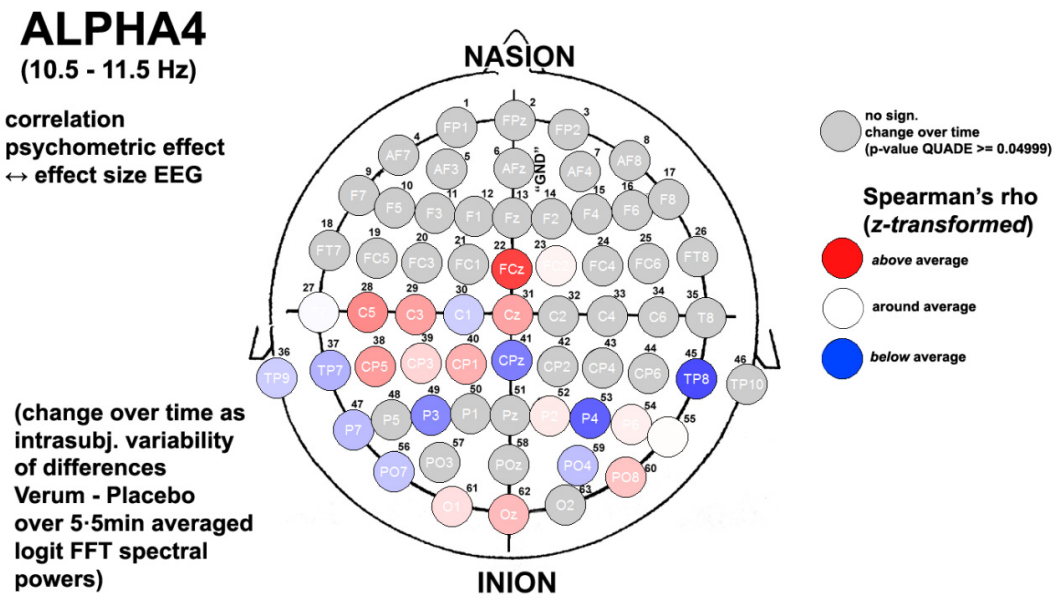


Figure 42. *Verum* topographic pattern of effect sizes of *change over time* as expressed as total intrasubject variance at each electrode for alpha4 relative spectral powers (10.5 – 11.5 Hz). Values are *z* – transformed to facilitate visual inspection of the topographic pattern. A clearly *left*–lateralized pattern is observed.



# electrode	Spearman's *rho*	p_{exact}
22	**.592**	**.055**
23	.282	.400
27	.255	.449
28	**.469**	**.145**
29	**.428**	**.189**
30	.182	.592
31	**.424**	**.194**
36	.178	.601
37	.132	.699
38	**.437**	**.179**
39	**.337**	**.311**
40	**.401**	**.222**
41	.046	.894
45	-.041	.905
47	.150	.659
49	.064	.852
52	.301	.369
53	-.005	.989
54	.292	.384
55	.269	.424
56	.164	.630
59	.155	.649
60	.364	.270
61	.319	.339
62	.383	.245

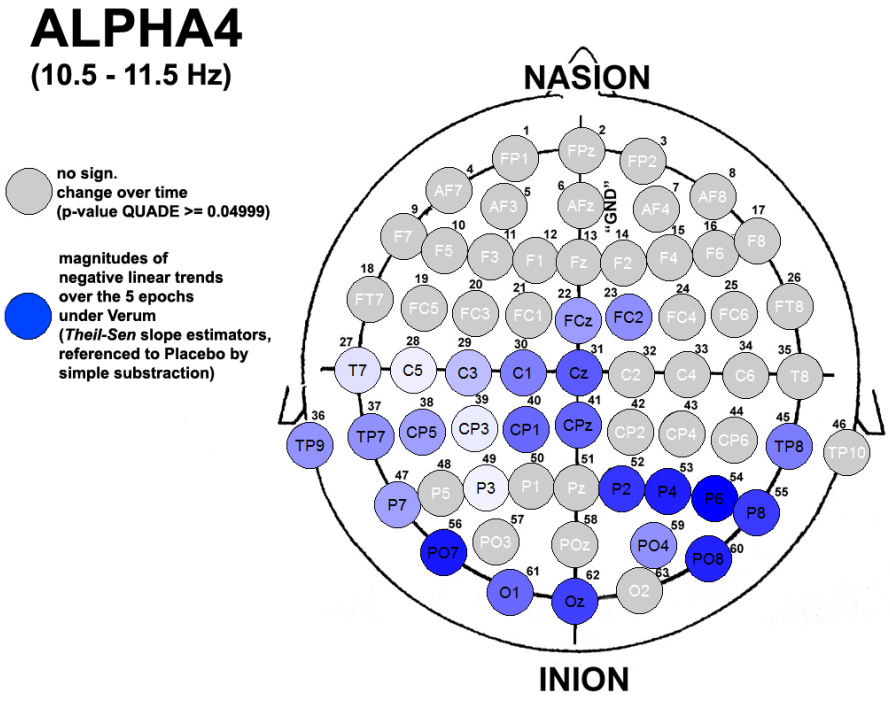
Figure 43. Topography of associations between magnitudes of *psychometric* effects (hynoidal state score *Verum* minus hynoidal state score *Placebo*) and magnitudes of effects of *change over time* (total intrasubject variance) of EEG alpha4 (10.5 – 11.5 Hz) relative spectral powers investigated using *Spearman's* non–parametric correlation. Relevant electrodes are highlighted in bold.

The topographic distribution of alpha4 powers' (10.5 – 11.5 Hz) *change over time* as measured as total intrasubject variances under *Verum* (figure 42) compared to the topographic pattern of *relationships* to the *psychometric effect* expressed as Spearman's *rho* (figure 43) reveals comparable patterns, although *p*–values do not fully reach the standard significance level α = .05, but this seems to be due to relatively small sample size: None among 25 *Quade* test significant electrodes show significant *Spearman* correlations with the psychometric effect within a *false discovery rate* of 1.25 electrodes, due to α-error accumulation because of multiple hypothesis testing.

Taken altogether, not only *Verum* specific *change over time* is observed in alpha4 powers at many electrodes, but this change also shows meaningful relationships with the magnitudes of psychometric effects sharing a similar topographic pattern. Thus, the *Verum* specific *change over time* observed in alpha4 powers can be considered as one electrophysiological correlate of the subjectively experienced psychometric effect.

Determining or analyzing the *details* of the time course (or *trajectories*) over all 5 epochs in EEG powers is problematic, as mentioned above, because, in contrast to *heart rate variability,* it would have to be done not only over the time dimension, but also at the same time over the dimension of space: All time points over all *Quade* test significant electrodes would have to be considered which would imply a quite high-dimensional space of possibilities; but the sample size of *N* = 12 is far away from providing the high amount of observation needed for adequately modelling such a problem. The real nature, i.e. the typical over-all-subject-trajectory and moreover the distribution of possible subtypes of such trajectories over subgroups of subjects, will remain hidden under statistic random "noise".

In order to at least gain some rudimentary access to the nature of the time course, as done above, dimensionality can be decreased by data reduction of simple linear projection, i.e. extracting only the information of the *linear trend* over the 5 epochs, excluding all non-linear information. Although this approach is somehow problematic because of the small sample size of



# electrode	difference *Theil-Sen* slopes Verum – Placebo
22	-.0500
23	-.0575
27	-.0150
28	-.0086
29	-.0335
30	-.0653
31	-.0840
36	-.0549
37	-.0556
38	-.0486
39	-.0103
40	-.0763
41	-.0792
45	-.0666
47	-.0478
49	-.0064
52	**-.1026**
53	**-.1137**
54	**-.1297**
55	**-.0997**
56	**-.1178**
59	-.0561
60	**-.1126**
61	-.0763
62	**-.0974**

Figure 44. Extracting only the information of the linear trend over the five epochs, excluding all non-linear information, using the non-parametric *Theil-Sen slope estimator* (Wilcox, 1998) and referencing it to those obtained under *Placebo*, all *Quade* test significant electrodes show without exception only negative slopes for alpha4 (10.5 – 11.5 Hz) as does also betaMid (18 – 24 Hz), see below, and in contrast to delta (0.5 – 3.5 Hz) and thetaLow (3.5 – 6.5 Hz) which show positive slopes, see above. As to be seen in this figure, alpha4 shows a pattern of increase of slope magnitudes towards posterior sites. Most relevant electrodes are highlighted in bold.

N = 12, results can be improved by robust statistics estimating the simple linear regression slope, e.g. by the non-parametric *Theil-Sen regression slope estimator* (see Dytham, 2011; Wilcox, 1998) which offers the important advantage of robustness to outliers and superior precision in the presence of skewed and heteroskedastic data as compared to standard least squares slope estimators. The five time point linear trajectories under Verum, referenced to Placebo by simple subtraction, gives the topographic pattern as to be seen in figure 44 with alpha4 showing a pattern of increase of slope magnitudes towards posterior sites. All *Quade*-test significant electrodes show a *negative* linear trend of the time course of alpha4 relative spectral powers, but among them, just in the electrodes where stronger *change over time* was observed (expressed as total intrasubject variance) and stronger relationships with psychometric effect magnitudes were found, the linear trend is smallest.

3.3.2.4. Results in betaMid (18 – 24 Hz) *EEG relative spectral powers*

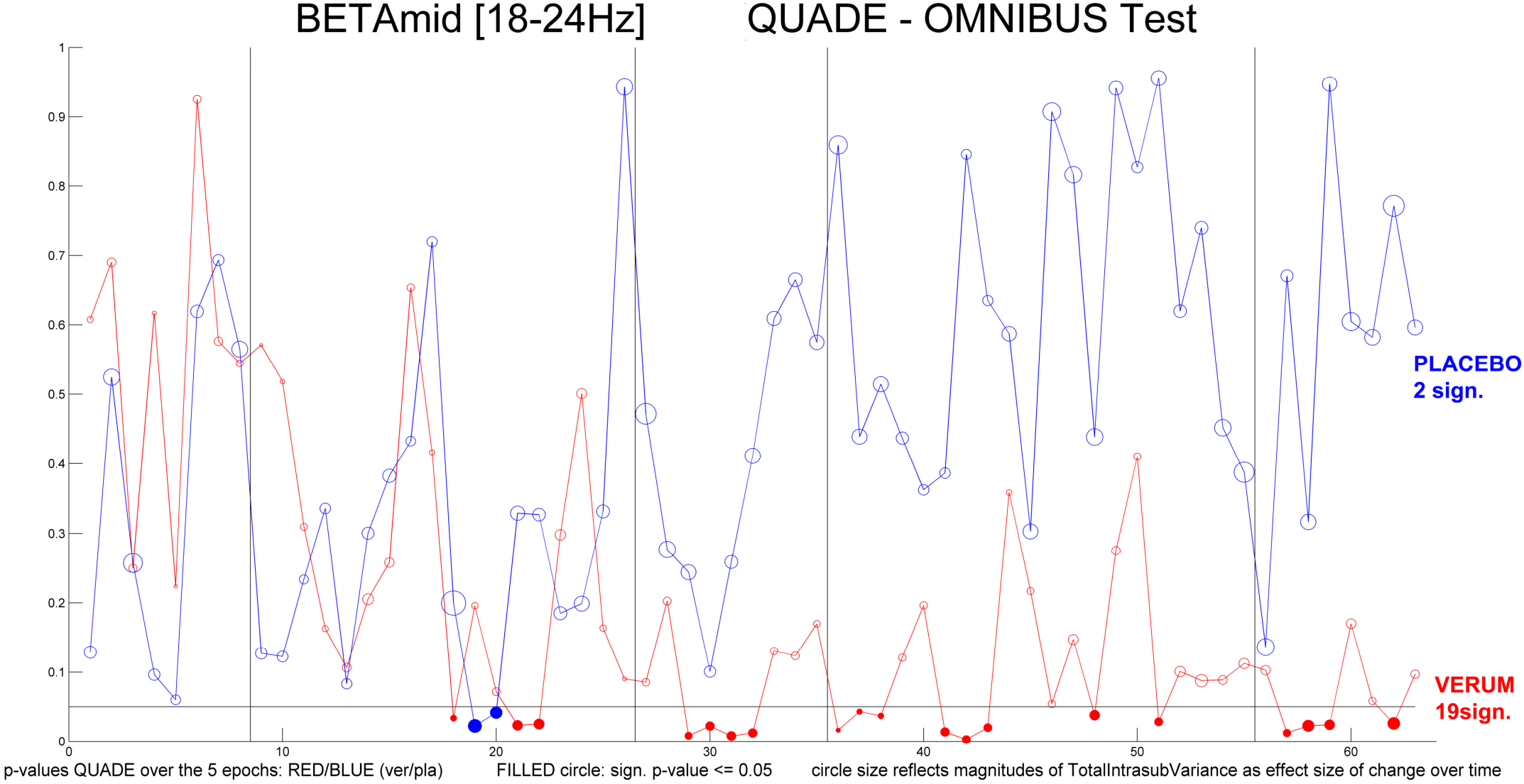


Figure 45. Comparing *Verum* (red) vs. *Placebo* (blue) with regard to significance of *change over time* for each 5 • 5min means of relative spectral EEG powers in betaMid (18 – 24 Hz) by multiple *Quade* tests for each of the 63 electrodes (x – axis), *p* – values on y – axis. Due to false discovery rate, *three* false – positive findings are expected under *Verum* and under *Placebo.*Vertical black lines subdivide scalp regions to facilitate the understanding of electrodes' spatial organisation (frontopolar/anterior–frontal, frontal, central, parietal, occipital). Mind the symbology explained under the plot.

The topographic distribution of *change over time* of **betaMid** relative spectral powers **(18 –24 Hz),** see figure 45, shows statistically significant *Quade* tests under *Verum* at 19 electrodes vs. only at two electrodes under *Placebo* which is still in the range of the false detection rate due to α – error accumulation because of multiple hypothesis testing and thus has no meaning. These findings show that only under *Verum* at least one of the five epochs shows betaMid powers which come from an underlying population which is significantly different from those corresponding to at least one or more other remaining four time points and that this happens at ≈ 30% of all 63 electrodes, all of them are *not* frontal electrodes.

Analyzing the topography of effect sizes of *change over time* (figure 46), as measured as total intrasubject variances (intrasubject variance over all 5 epochs summed up over all subjects), shows that the most pronounced effects of *change over time* are generally observed at midline electrodes, on the one hand at *occipital* sites (*POz* and *Oz*) and on the other at *frontocentral* sites (*FC1* and *FCz*).

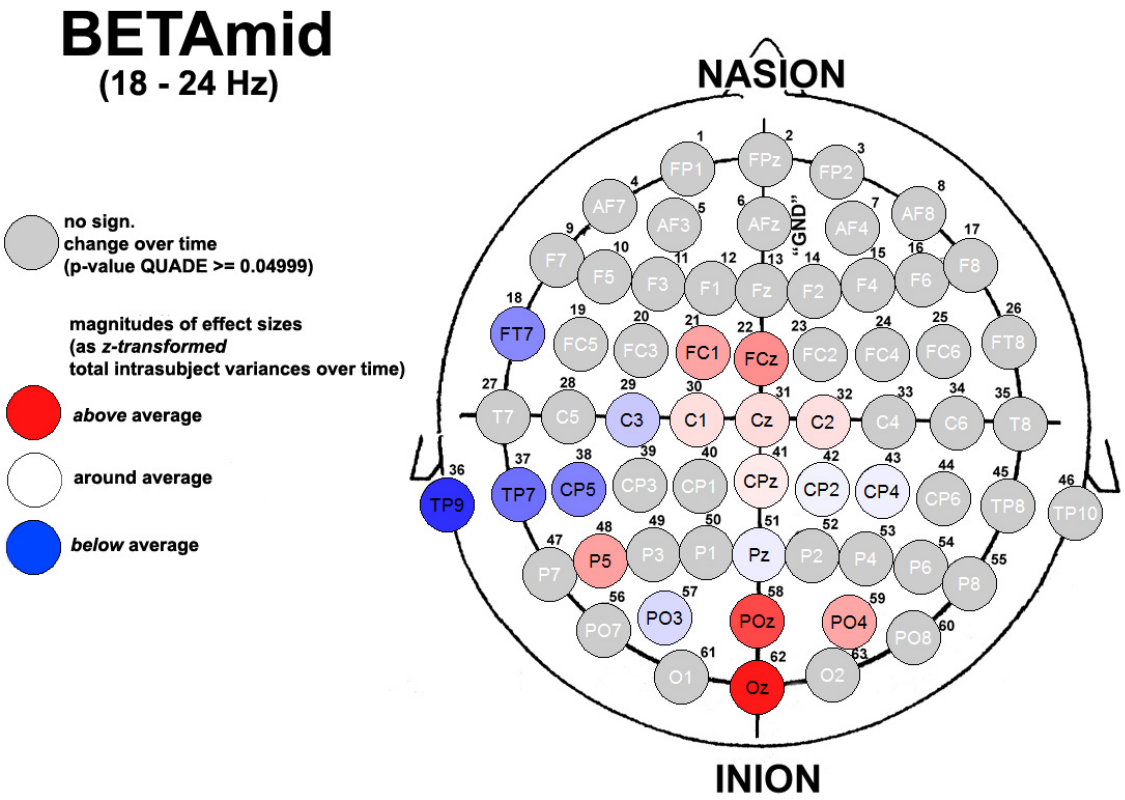
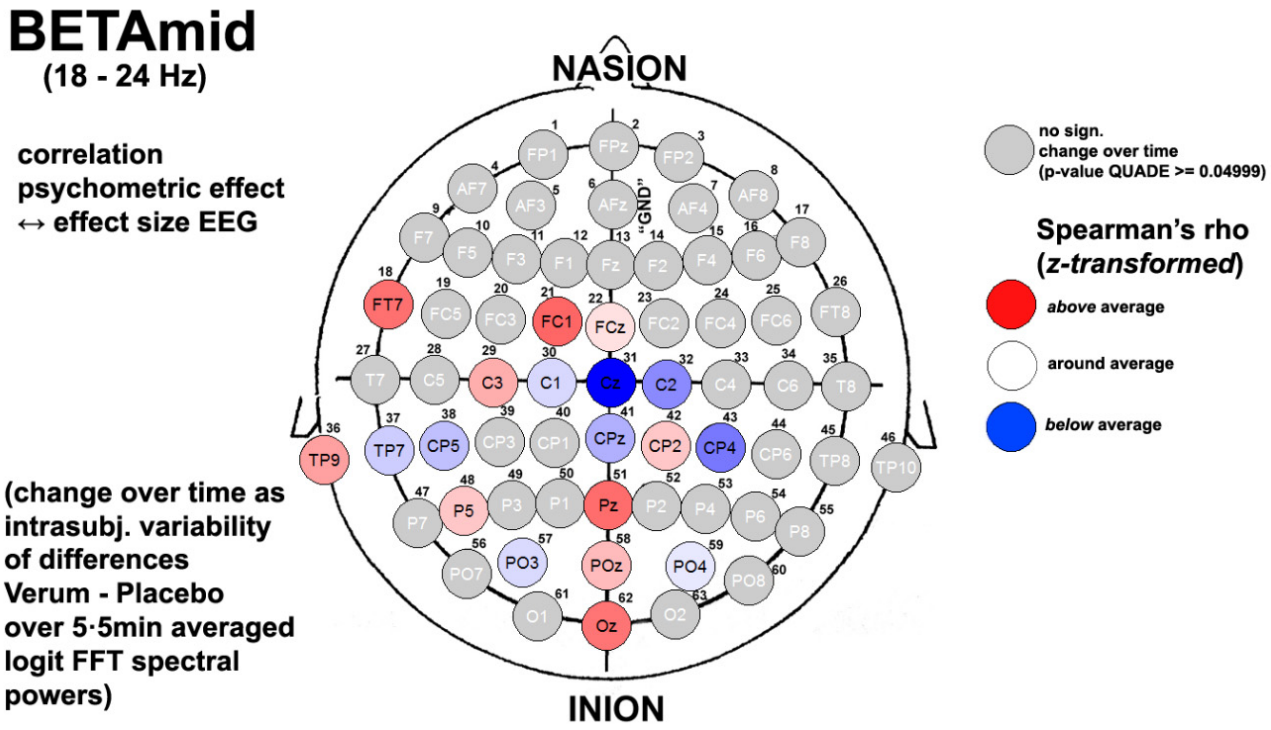


Figure 46. *Verum* topographic pattern of effect sizes of *change over time* as expressed as total intrasubject variance at each electrode for betaMid relative spectral powers (18 – 24 Hz). Values are *z* – transformed to facilitate visual inspection of the topographic pattern.



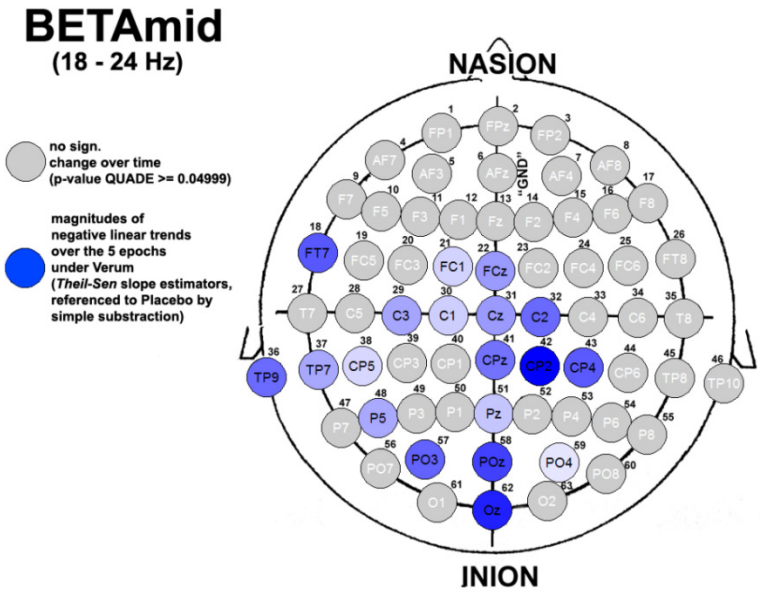
# electrode	Spearman's *rho*	p_{exact}
18	.665	.026
21	**.679**	**.022**
22	**.506**	**.113**
29	**.579**	**.062**
30	.396	.228
31	.027	.936
32	.264	.432
36	.597	.053
37	.378	.252
38	.355	.284
41	.328	.325
42	**.542**	**.085**
43	.232	.492
48	.542	.085
51	**.670**	**.024**
57	.401	.222
58	**.560**	**.073**
59	.424	.194
62	**.661**	**.027**

Figure 47. Topography of associations between magnitudes of *psychometric* effects (hynoidal state score *Verum* minus hynoidal state score *Placebo*) and magnitudes of effects of *change over time* (total intrasubject variance) of EEG betaMid (18 – 24 Hz) relative spectral powers investigated using *Spearman's* non–parametric correlation. A left–lateralized topographic pattern is observed. Relevant electrodes are highlighted in bold.

The topographic distribution of betaMid powers' (18 –24 Hz) magnitudes of *change over time* as measured as total intrasubject variances (figure 46) under *Verum* compared to the topographic pattern of *relationships* to the *psychometric effect* expressed as Spearman's *rho* (figure 47) reveals that indeed the mentioned electrodes with relatively largest *change over time* are those which show largest relationships with the psychometric effect. 4 among 19 *Quade* test significant electrodes show significant *Spearman* correlations with the psychometric effect within a *false discovery rate* of 0.95 electrodes, due to α-error accumulation because of multiple hypothesis testing. Interestingly, the electrodes showing smallest such relationships are mostly those at *central midline* sites.

Extracting only the information of the *linear trend* over the five epochs by the non-parametric *Theil-Sen regression slope estimator* (Wilcox, 1998), see figure 48, excluding all non-linear information, and referencing these linear trends to those of *Placebo* reveals that all *Quade*-test significant electrodes show a *negative* linear trend of the time course of betaMid relative spectral powers. Looking only at midline electrodes from anterior *FCz* to posterior *Oz*, posterior sites seem to show stronger negative linear trends in betaMid.

All Quade test significant electrodes show without exception only *negative* slopes for betaMid (18 – 24 Hz) as does also alpha4 (10.5 – 11.5 Hz), see above, and in contrast to delta (0.5 – 3.5 Hz) and thetaLow (3.5 – 6.5 Hz) which show only *positive* slopes, see above.



# electrode	difference *Theil-Sen* slopes Verum – Placebo
18	-.0676
21	-.0178
22	-.0405
29	-.0361
30	-.0196
31	-.0403
32	-.0603
36	-.0599
37	-.0350
38	-.0161
41	-.0568
42	-.1030
43	-.0669
48	-.0346
51	-.0228
57	-.0632
58	-.0769
59	-.0100
62	-.0897

Figure 48. Extracting only the information of the linear trend over the five epochs, excluding all non-linear information, using the non-parametric *Theil-Sen slope estimator* (Wilcox, 1998) and referencing it to those obtained under *Placebo*, all *Quade* test significant electrodes show without exception only negative slopes for betaMid (18 – 24 Hz) as does also alpha4 (10.5 – 11.5 Hz), see above, and in contrast to delta (0.5 – 3.5 Hz) and thetaLow (3.5 – 6.5 Hz) which show positive slopes, see above.

3.3.2.5. Results in gamma1 (30 – 40 Hz) *EEG relative spectral powers*

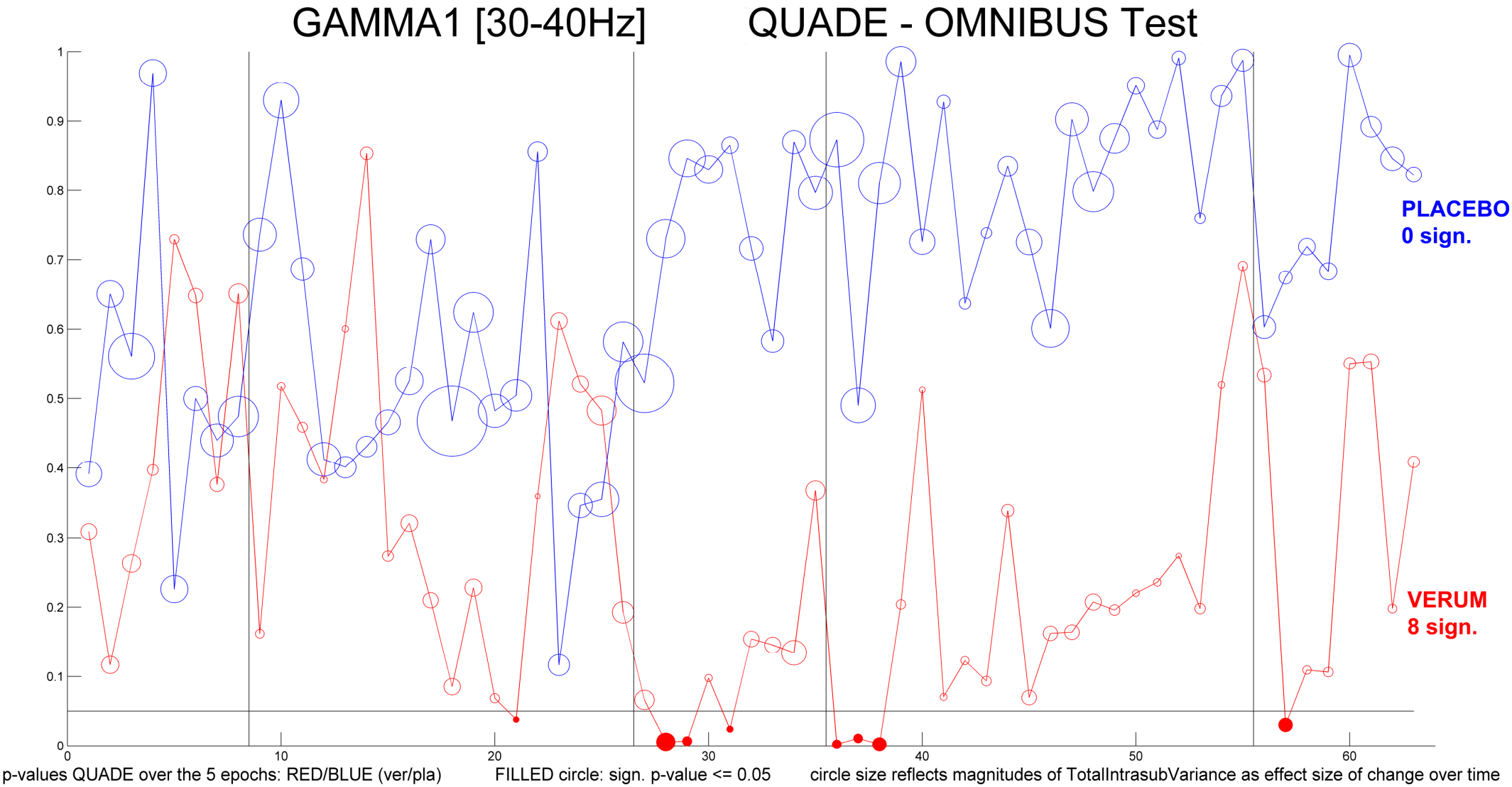


Figure 49. Comparing *Verum* (red) vs. *Placebo* (blue) with regard to significance of *change over time* for each 5 • 5min means of relative spectral EEG powers in gamma1 (30 – 40 Hz) by multiple *Quade* tests for each of the 63 electrodes (x – axis), *p* – values on y – axis. Due to false discovery rate, *three* false – positive findings are expected under *Verum* and under *Placebo.* Vertical black lines subdivide scalp regions to facilitate the understanding of electrodes' spatial organisation (frontopolar/anterior–frontal, frontal, central, parietal, occipital). Mind the symbology explained under the plot.

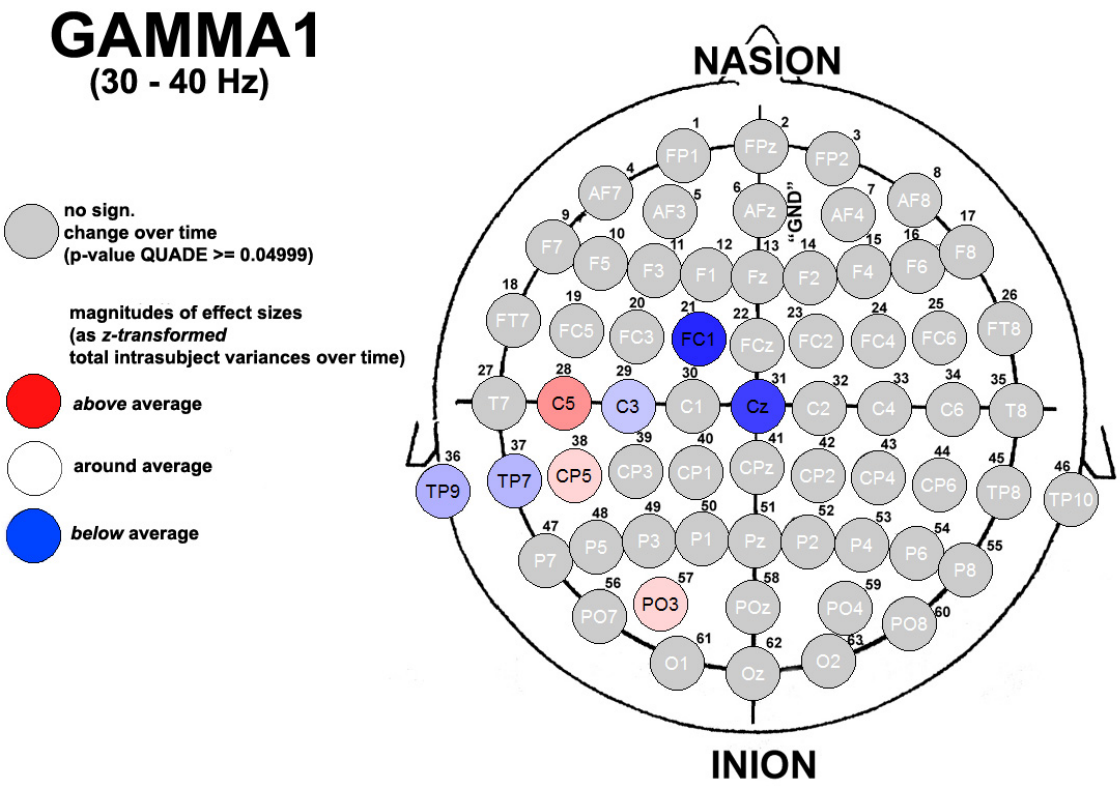
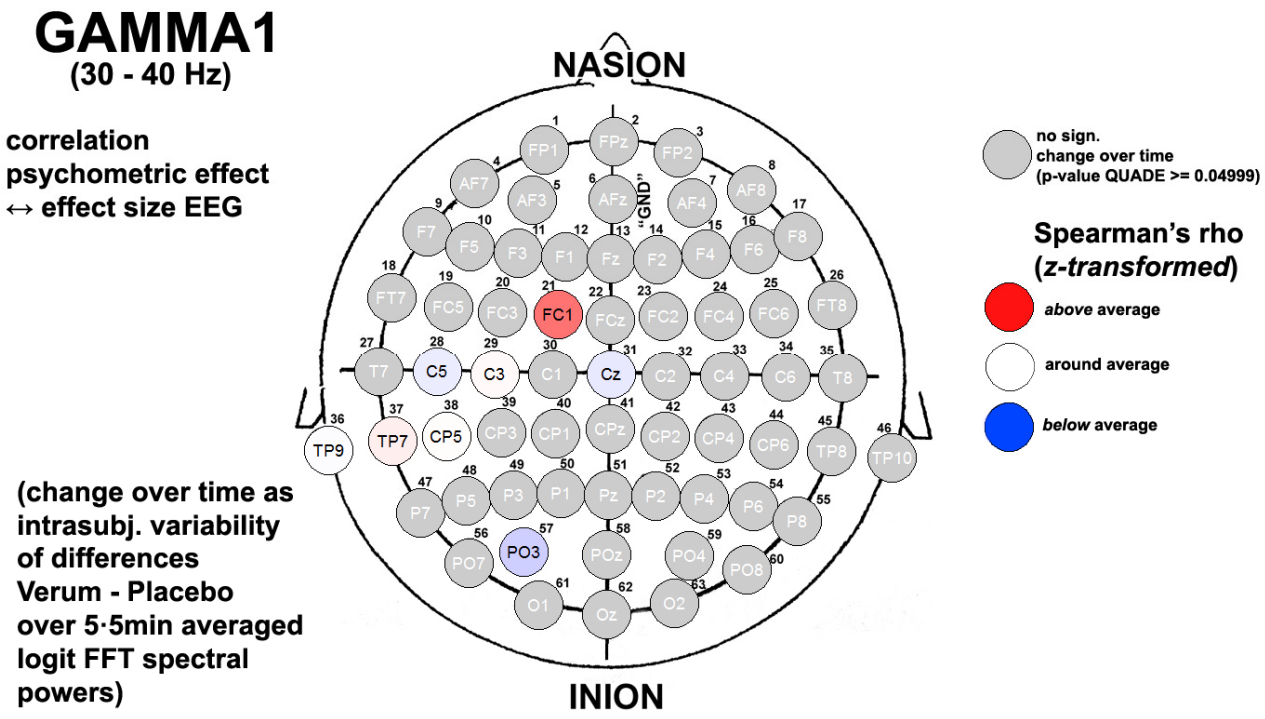


Figure 50. *Verum* topographic pattern of effect sizes of *change over time* as expressed as total intrasubject variance at each electrode for gamma1 relative spectral powers (30 – 40 Hz). Values are *z* – transformed to facilitate visual inspection of the topographic pattern.

The topographic distribution of *change over time* of **gamma1** relative spectral powers **(30 – 40 Hz)**, see figure 49, shows statistically significant *Quade* tests under *Verum* at 8 electrodes vs. at none electrodes under *Placebo*. These findings show that only under *Verum* at least one of the five epochs shows gamma1 powers which come from an underlying population which is significantly different from those corresponding to at least one or more other remaining four time points and that this happens at ≈ 13% of all 63 electrodes.

Analyzing the topography of effect sizes of *change over time,* as measured as total intrasubject variances (intrasubject variance over all 5 epochs summed up over all subjects), see figure 50, shows that the most pronounced effects of *change over time* are observed at *C5* and *CP5*, while relatively smallest effects are found at *FC1* and *Cz.*



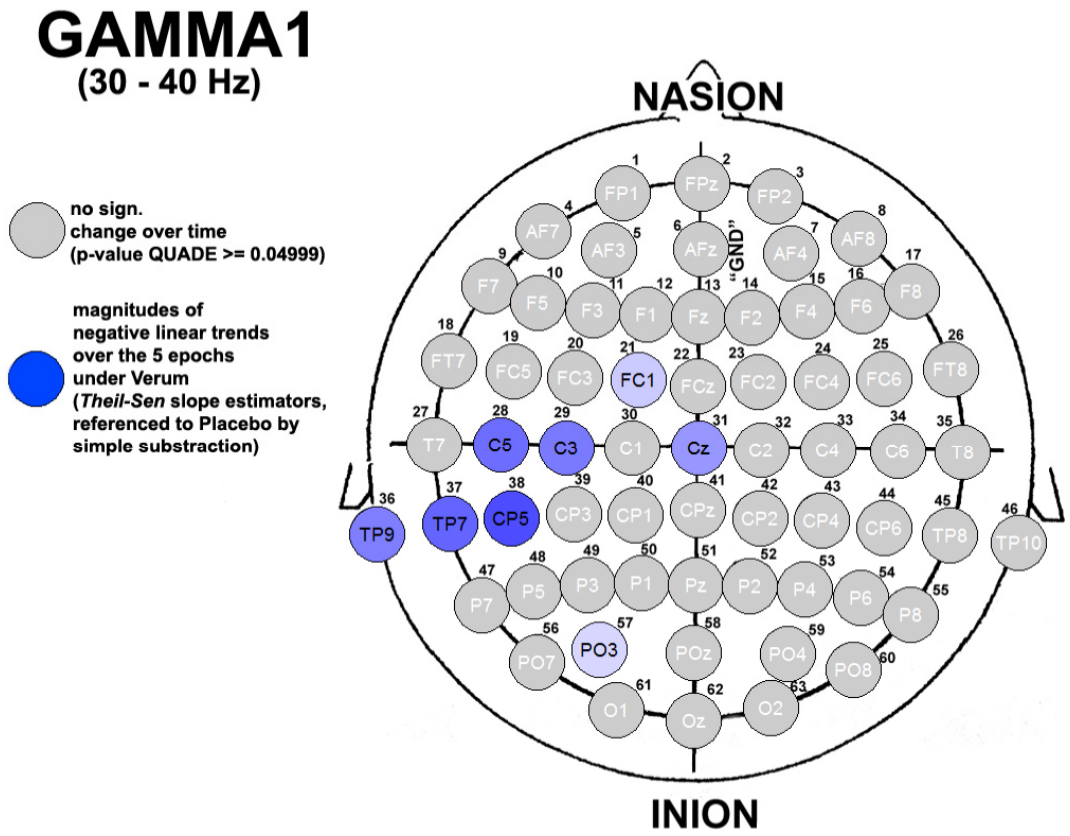
# electrode	Spearman's *rho*	p_{exact}
21	**.788**	**.004**
28	.569	.067
29	**.620**	**.042**
31	.560	.073
36	.606	.048
37	.633	.036
38	.615	.044
57	.501	.116

Figure 51. Topography of associations between magnitudes of *psychometric* effects (hynoidal state score *Verum* minus hynoidal state score *Placebo*) and magnitudes of effects of *change over time* (total intrasubject variance) of EEG gamma1 (30 – 40 Hz) relative spectral powers investigated using *Spearman's* non–parametric correlation. Relevant electrodes are highlighted in bold.

All electrodes with significant *change over time* as operationalized by the *Quade* test show relative large correlations between the psychometric effect and the EEG spectral power effects in gamma1, see figure 51, expressed as contrasts *Verum* – *Placebo* of intrasubject variances to quantify the effect size of *change over time*. Five among 8 *Quade* test significant electrodes show significant *Spearman* correlations with the psychometric effect within a *false discovery rate* of 0.4 electrodes, due to α-error accumulation because of multiple hypothesis testing. Although the largest association is found at *FC1*, no specific topographic pattern can be observed in gamma1 associations with the psychometric effect as compared to the other reported EEG frequency bands.

EEG effects found above 20 Hz, especially in the EEG gamma frequency band, have been criticized to be strongly interfered by non-cerebral bioelectric sources such as EMG and EOG of miniature saccades (e.g. Pope, Fitzgibbon, Lewis, Whitham, & Willoughby, 2009; Whitham et al., 2007, 2008), but it was demonstrated that the *surface Laplacian filtering* of EEG signals, as used in this present study, suppresses remaining EMG contamination (see Whitham et al., 2007), so the reported results in gamma1 can be considered as indeed predominantly reflecting *cerebral* EEG activity rather than EMG interferences. But even if they were exclusively reflecting EMG, signals still would not be meaningless, but could be interpreted as reflecting changes in *arousal* levels inducing changes in skeletal muscular tone. Magnitudes of *change over time* in gamma1 seem to be one of the best physiological correlates of magnitudes of the psychometric effects found in the present work, because almost all *Quade*– test sign. Electrodes show sign. correlations: Specific psychometric reactivity to the *Verum* Binaural Beat stimulus correlates well with specific physiological reactivity of *change over time* in EEG gamma1 relative spectral powers.

Extracting only the information of the *linear trend* over the five epochs by the non-parametric *Theil-Sen regression slope estimator* (Wilcox, 1998), see figure 52, excluding all non-linear information, and referencing these linear trends to those of *Placebo* reveals that all *Quade*-test significant electrodes show a *negative* linear trend of the time course of gamma1 under *Verum*.



# electrode	difference *Theil-Sen* slopes Verum – Placebo
21	-.0264
28	-.0741
29	-.0677
31	-.0527
36	-.0656
37	-.0789
38	-.0905
57	-.0210

Figure 52. Extracting only the information of the linear trend over the five epochs, excluding all non-linear information, using the non-parametric *Theil-Sen slope estimator* (Wilcox, 1998) and referencing it to those obtained under *Placebo*, all *Quade* test significant electrodes show without exception only negative slopes for gamma1 (30 – 40 Hz) as does also alpha4 (10.5 – 11.5 Hz) and betaMid (18 – 24 Hz), see above, and in contrast to delta (0.5 – 3.5 Hz) and thetaLow (3.5 – 6.5 Hz) which show positive slopes, see above.

3.3.2.6. Comparison/associations of topographic patterns of linear temporal trends over the five stimulus time points of EEG relative powers between the different EEG frequency bands which showed most relevant and sign. change over time

In order to compare the particular topographic patterns of *change over time* of the EEG relative spectral powers at the distinct EEG frequency bands over the five time points and over all *N* = 12 subjects, and this for the set of common electrodes which showed significant *change over time* in the *Quade* test, dimensionality was reduced to the linear trend using the non-parametric *Theil-Sen regression slope estimator* (see Dytham, 2011; Wilcox, 1998), as done above. As mentioned, this estimator offers the important advantage of robustness to outliers and superior precision in the presence of skewed and heteroskedastic data as compared to standard least squares estimators.

To compare the found togographic distributions of linear trends over time in the different EEG frequency bands with each other, i.e. how they topographically relate to each other, a pairwise comparison of the *Placebo* referenced *Theil-Sen regression slopes* is performed resulting in an intercorrelation matrix: For every pair of EEG frequency bands to be compared, first the set of those electrodes is selected which show significant *change over time* using the *Quade* test in *both* bands. The length of this set is reported as *N*, then association between slopes of linear trends of *change over time* of EEG relative spectral powers of each two EEG frequency bands *over all their common Quade test significant electrodes* is calculated using non-parametric rank–order *Spearman* (*rho*) and also linear *Pearson* (*r*) correlation coefficients. Gamma1 was excluded because common sets would have far too small *N* for the calculation of correlations. This described intercorrelation matrix is found in table 9.

Table 9. Associations between topographic patterns of linear trends over the five stimulus time points of EEG relative powers between the four most reactive EEG frequency bands using an intercorrelation matrix of *Placebo* referenced *Theil-Sen regression slopes* at *N* common *Quade test* significant electrodes which are shared by the compared pair of EEG frequency bands using non-parametric *Spearman* correlation coefficient (*rho*) and linear *Pearson* (*r*): Correlations are calculated for each pair over its common *Quade test* sign. electrodes.
Gamma1 was excluded because common sets would have far too small N for the calculation of correlations.

	alpha4		**betaMid**		**delta**		**thetaLow**
alpha4							
betaMid	*N* = 10 ***rho* =.605** *r* = .346	 *p* = .064 *p* = .331					
delta	*N* = 16 ***rho* = –.640** *r* = –.598	 *p* = .008 *p* = .015	*N* = 15 ***rho* = –.697** *r* = –.736	 *p* = .004 *p* = .003			
thetaLow	*N* = 16 ***rho* = –.023** *r* = .006	 *p* = .933 *p* = .983	*N* = 6 *(two small N)*		*N* = 15 ***rho* = –.200** *r* = –.221	 *p* = .475 *p* = .427	

As to be seen in table 9, topographic patterns for delta, alpha4 and betaMid show strong associations between each other, i.e. the variability of magnitudes of linear trends of *change over time* of EEG relative spectral powers are strongly related in these EEG frequency bands: The higher the positive linear trends/slopes over the common electrodes are in delta, the lower were those of alpha4 and betaMid; these correlations are sign. o almost sign. This means that at roughly the same scalp sites where higher linear *increases* of delta were observed, at the same time, larger linear *decreases* of alpha4 and betaMid happened. These findings could be understood as a shift from *faster* towards *slower* EEG frequency bands which is a clear sign of decreasing (cortical) arousal levels. Interestingly, the thetaLow topographic pattern of magnitudes of linear trends of *change over time* over the five stimulus epochs shows only low, non–significant relationships with those of the three other EEG frequency bands, thus the

topographic pattern of linear trends of thetaLow behaves clearly differently as compared to the remaining three other ones. Apart from this investigation by the described intercorrelation matrix, topographic patterns of linear temporal trends over the five stimulus time points can also be compared manually–visually comparing the figures for *thetaLow* (figure 40), *delta* (figure 36)*, alpha4* (figure 44) and *betaMid* (figure 48) with each other.

3.3.2.7. Summary EEG findings

Multiple evidence many times superior to *false discovery rates* is found for *change over time* of EEG relative spectral powers over the five stimulus epochs only under *Verum,* but not under *Placebo*: The employed Binaural Beat stimulus induces specific *change over time* in EEG relative spectral powers, this effect is most pronounced in delta, thetaLow, alpha4, betaMid and gamma1. Associations of this *change over time* in EEG relative powers clearly superior to random are found with the psychometric effects. Extracting linear trends of time courses of EEG relative spectral powers over the five stimulus epochs by the non–parametric *Theil–Sen slope estimator* (Wilcox, 1998) shows that the slower EEG frequency bands delta and thetaLow show, without excpetion, only *positive* slopes, i.e. they increase within the five stimulus epochs, while the faster EEG frequency bands alpha4, betaMid and gamma1 show only *negative* slopes, i.e. they decrease over the five stimulus epochs. These temporal linear trends are strongly intercorrelated for delta, alpha4 and betaMid, but not for thetaLow. Comparing slope–magnitudes, delta, thetaLow and alpha4 show roughly comparable levels, whereas slopes for betaMid and gamma1 are smaller.

3.3.3. Domain of stress–related salivary biomarkers: Cortisol, α -Amylase and Salivary Secretory Immunoglobulin A (SIgA)

Comparing pre vs. post saliva samples under *Verum* vs. under *Placebo* reveal that under *Verum* no significant changes are observed for cortisol,

α-Amylase and Salivary Secretory Immunoglobulin A (SIgA), while under *Placebo* all these three stress-related biomarkes show significant *increases*, see table 10. Baseline *pre* – levels under *Verum* vs. *Placebo* do not show any sign. differences, which makes the observed pre vs. post findings more attributable to the different treatment conditions.

Table 10. Results of stress – related salivary biomarkers, measured *pre* vs. *post* each time under *Verum* vs. *Placebo,* collected by passive drool technique while collection times were measured. As highlighted in blue, all three biomarkers show significant increases *pre* vs. *post* only under *Placebo,* but not under *Verum.* Assay results for α – Amylase and SIgA were normalized for saliva flow rates [ml / min] obtained by measuring total saliva volumina and dividing them by the measured times. Because *cortisol* saliva concentrations do not depend on saliva flow rates, this normalization is not necessary in this case.

Cortisol [µg / dl]

$M_{verum\text{-}pre}$ = **.0918**	$SD_{verum\text{-}pre}$ = .05996	$M_{placebo\text{-}pre}$ = **.1012**	$SD_{placebo\text{-}pre}$ = .04408
$M_{verum\text{-}post}$ = **.0847**	$SD_{verum\text{-}post}$ = .04321	$M_{placebo\text{-}post}$ = **.1197**	$SD_{placebo\text{-}post}$ = .05125
Wilcoxon signed-ranks Z = –.800 , **p_{exact} = .465**		Wilcoxon signed-ranks Z = – 2.040, **p_{exact} = .042**	
Spearman's rank corr *rho* = .881, p_{exact} = .0003		Spearman's rank corr *rho* = .860, p_{exact} = .001	

Wilcoxon signed-ranks *verum–pre* ⇔ *placebo–pre* Z = –.628, **p_{exact} = .569**
Spearman's rank corr *verum–pre* ⇔ *placebo–pre rho* = .490, p_{exact} = .110

α – Amylase [U / min]

$M_{verum\text{-}pre}$ = **84.06**	$SD_{verum\text{-}pre}$ = 99.4840	$M_{placebo\text{-}pre}$ = **76.67**	$SD_{placebo\text{-}pre}$ = 71.8080
$M_{verum\text{-}post}$ = **107.98**	$SD_{verum\text{-}post}$ = 84.4756	$M_{placebo\text{-}post}$ = **102.90**	$SD_{placebo\text{-}post}$ = 94.3575
Wilcoxon signed-ranks Z = – 1.334, **p_{exact} = .204**		Wilcoxon signed-ranks Z = – 2.275, **p_{exact} = .021**	
Spearman's rank corr *rho* = .692, p_{exact} = .016		Spearman's rank corr *rho* = .909, p_{exact} = .0001	

Wilcoxon signed-ranks *verum–pre* ⇔ *placebo–pre* Z = –.078, **p_{exact} = .970**
Spearman's rank corr *verum–pre* ⇔ *placebo–pre rho* = .776, p_{exact} = .004

Salivary Secretory Immunoglobulin A (SIgA) [µg / min]

$M_{verum\text{-}pre}$ = **272.09**	$SD_{verum\text{-}pre}$ = 152.0205	$M_{placebo\text{-}pre}$ = **304.83**	$SD_{placebo\text{-}pre}$ = 297.6298
$M_{verum\text{-}post}$ = **391.13**	$SD_{verum\text{-}post}$ = 250.0620	$M_{placebo\text{-}post}$ = **430.02**	$SD_{placebo\text{-}post}$ = 337.7117
Wilcoxon signed-ranks Z = – 1.490, **p_{exact} = .151**		Wilcoxon signed-ranks Z = – 2.197, **p_{exact} = .027**	
Spearman's rank corr *rho* = .517, p_{exact} = .089		Spearman's rank corr *rho* = .734, p_{exact} = .009	

Wilcoxon signed-ranks *verum–pre* ⇔ *placebo–pre* Z = –.157, **p_{exact} = .910**
Spearman's rank corr *verum–pre* ⇔ *placebo–pre rho* = .580, p_{exact} = .052

In order to compare pre/post response magnitudes under Placebo between the three distinct stress–related biomarkers, as calculated as the differences of post – pre, the intercorrelation matrix was computed using *Spearman's* rank order correlation: Cortisol ⇔ α-Amylase *rho* = –.070 with p_{exact} = .835, cortisol ⇔ SIgA *rho* = .147 with p_{exact} = .651, α-Amylase ⇔ SIgA *rho* = .399 with p_{exact} = .201. Obviously, cortisol response magnitudes under Placebo are almost independent from those of the other measured biomarkers, while α-Amylase and SIgA response magnitudes show certain dependency, although not reaching significance, probably due to small sample size.

The individual increases in stress-related biomarker actibities as observed under Placebo are not statistically associated with the psychometric response magnitudes (*hypnoidal state score*, HSS) to the Binaural Beat stimulus under *Verum*, because the correlation matrix does not show any significant nor relevant correlations: Cortisol magnitudes ⇔ psychom. magnitudes *rho* = –.119 with p_{exact} = .716, α-Amylase magnitudes ⇔ psychom. magnitudes *rho* = –.140 with p_{exact} =.667, SIgA magnitudes ⇔ psychom. magnitudes *rho* = –.049 with p_{exact} = .886. Nonetheless, because the same subjects were measured under *Verum* vs. *Placebo,* because effort was made to keep all other conditions more or less constant and because of the double-blind methodology, it seems no to be due to pure random that an increase of stress-related biomarkers was systematically observed under *Placebo* while not under *Verum,* e.g. because naturally occurring activating processes under *Placebo* may be inhibited under *Verum.*

3.4. Excluding possible presentation order effects

The concept of statistical *interaction* is generally based on comparing *magnitudes* of effects in a dependent variable in function of different levels of an independent factor. Because most non-parametric methods use *rank transformation* of the raw data, i.e. they work with the only information that something is higher or lower while rejecting the information of *magnitudes* of these differences, investigating interaction effects with non-parametric methodology is problematic. On the other hand, parametric statistics rely on assumptions such as *(multi)normal distribution, homoscedasticity, sphericity* (e.g. in the case of repeated measures with k > 2) etc. which are frequently violated by real-world data. With respect to the investigation of interaction effects in the *ANOVA* context, a trade-off between non-parametric vs. parametric methodology, a "middle ground" or "hybrid technology", was proposed and investigated in Monte Carlo simulations by Leys and Schumann (2010): the adjusted rank transform test (ART). In the following, main and interaction effects are investigated by classical *ANOVA*, but then compared with ART results.

Investigating possible effects of the presentation order influencing psychometric effects, a classical *mixed-design repeated measures ANOVA* was computed with *verum_vs_placebo_stimulus_condition* as repeated *within*-subjects factor, with the two levels *Verum* vs. *Placebo,* and *presentationorder* as *between*-subjects factor, with two levels *Verum-Placebo* vs. *Placebo-Verum*. As expected, the main effect in repeated *within*-subjects factor confirmed the existence of the psychometric effect showing significance with $F_{(1, 12)} = 10.307$, $p = .009$, $\eta_p^2 = .508$; comparison with the ART approach gives similar results with $F_{(1, 12)} = 11.427$, $p = .007$, $\eta_p^2 = .533$. There was no significant *between*-subjects main effect of *presentationorder* with $F_{(1, 12)} = .153$, $p = .704$, $\eta_p^2 = .015$; using ART makes no relevant difference with in $F_{(1, 12)} = .067$, $p = .802$, $\eta_p^2 = .007$. There was also no significant interaction effect between *presentationorder* × *verum_vs_placebo_ stimulus_condition* with $F_{(1, 12)} = .039$, $p = .848$, $\eta_p^2 = .004$. Using the ART, similar results were found for this interaction with $F_{(1, 12)} = .066$, $p = .803$, $\eta_p^2 = .007$.

In conclusion, neither significant main effects nor significant interactions could be found for *presentationorder*, neither with classical *ANOVA* nor with the advanced ART over the two employed presentation orders. Hence, the found psychometric effects can be considered as independent from possible presentation order effects and thus have indeed to be attributed to be caused by the different auditory stimulation conditions and not by unspecific presentation order biases.

Investigating possible presentation order effects on the heart rate variability (HRV) effects in *logit_HFpower_percents,* the application of the ART is problematic, because two intercrossed *repeated within-subject factors* have to be used at the same time: first *verum_vs_placebo_stimulus_condition* and second *fiveTimepoints*. Thus, only the classical *mixed-design repeated measures ANOVA* was computed with the mentioned factors and *presentationorder* as *between*-subjects factor. Because classical *ANOVA* is sensitive to outliers, data were checked by the two-sided robust test for multiple outliers proposed by Iglewicz and Hoaglin (1993) setting the threshold for the modified *Z* score ≥ 2.4 which resulted in one outlier detection for subject number 11 in epoch 1 under Placebo. This outlier was replaced by the corresponding linear least square interpolation of the other 4 remaining data points of this subject. Neither the main effect of *between*-subject factor *presentationorder* is significant with $F_{(1,\ 12)} = 1.938$, $p = .194$, $\eta_p^2 = .162$, nor the interaction effects *presentationorder* × *verum_vs_placebo_stimulus_condition* with $F_{(1,\ 12)} = .795$, $p = .394$, $\eta_p^2 = .074$ nor *presentationorder* × *verum_vs_placebo_stimulus_ condition* × *fiveTimepoints* with $F_{(2.969,\ 12)} = 1.723$, $p = .184$, $\eta_p^2 = .147$ (Greenhouse-Geisser correction with $\varepsilon = .742$). But as expected, the HRV effect previously found by non-parametric methodology can also be seen in the significant interaction effect of *verum_vs_placebo_stimulus_condition* × *fiveTimepoints* with $F_{(2.969,\ 12)} = 3.000$, $p = .047$, $\eta_p^2 = .231$ (Greenhouse-Geisser correction with $\varepsilon = .729$). While as expected the *within*-subjects factor *fiveTimepoints* shows significance with $F_{(2.914,\ 12)} = 4.843$, $p = .008$, $\eta_p^2 = .326$, its interaction *fiveTimepoints* ×

presentationorder does not show significance with $F_{(2.914,\ 12)} = 1.751$, $p = .180$, $\eta_p^2 = .149$ (both with Greenhouse-Geisser correction with $\varepsilon = .729$). In conclusion, the found HRV effects can be considered as independent from possible presentation order effects and thus unspecific presentation order biases can be excluded.

In the case of EEG findings, investigating possible presentation order effects is very problematic: Because first, a *MANOVA* etc. would have to be performed which would imply that the variability of all relevant EEG frequency bands would have to be pooled which in itself is highly problematic. Second, the found EEG effects do not appear over the same electrodes, so meaningful comparison over spatially different patterns in the distinct EEG frequency bands is not easy to operationalize. Given that psychometric and HRV effects seem independent from possible presentation order and given that significant and meaningful correlations were found between psychometric effect vs. effects in the distinct EEG frequency bands, it seems not very probable that the found EEG effects are significantly contaminated by possible presentation order effects.

Investigating possible effects of the presentation order on the three saliva analytes, a classical *mixed-design repeated measures ANOVA* was computed with *verum_vs_placebo_stimulus_condition* as the first repeated *within*-subjects factor, with the two levels *Verum* vs. *Placebo,* and *pre_vs_post* as the second repeated *within*-subjects factor, with the two levels *pre* vs. *post* and *presentationorder* as *between*-subjects factor, with the two levels *Verum-Placebo* vs. *Placebo-Verum*. There was neither a significant *between*-subjects main effect of *presentationorder* for cortisol concentrations [µg/dl] with $F_{(1,\ 12)} = .281$, $p = .608$, $\eta_p^2 = .027$, nor for saliva flow rate normalized α–Amylase secretion [U/min] with $F_{(1,\ 12)} = .000\ 005$, $p = .998$, $\eta_p^2 < .000\ 000\ 1$, nor for SIgA secretion [µg/min] with $F_{(1,\ 12)} = .047$, $p = .832$, $\eta_p^2 = .005$. Corresponding interaction effects showed values far from significance. Thus, the found effects in saliva analytes can be considered as free from possible presentation order effects and thus unspecific presentation order biases can be excluded.

4. Discussion

The present work aimed to find evidence superior to random and superior to unspecific Placebo effects whether there is or there is not *psychophysiological reactivity* to auditory stimulation by Binaural Beats in the EEG alpha and theta brain wave frequency bands, especially in terms of modulating levels of arousal. The present work is, to the author's best knowledge, the first study which found for the first time *multimodal* evidence (psychometric–self reports, parasympathetic outflow by respiration–controlled heart rate variability, EEG cortical activities and neuroendocrinological stress biomarkers cortisol, α-Amylase and Salivary Secretory Immunoglobulin A, SIgA) for the specific efficacy of auditory Binaural Beat stimulation contra Placebo satisfying the standards of *Evidence-based Medicine* (Sackett et al., 1996; Timmermans & Mauck, 2005): randomized placebo-controlled double-blind trails and rigorous selection of bio–psycho–socially healthy participants for a highly controlled laboratory study. Until today, previous works offer contradictory results, and there are only few results available which satisfy the standards of Evidence-based Medicine. Most remarkably, those previous studies did neither exhaustively ask for volunteers' medical conditions or medication/substance (ab)use, nor operationalized nor test *bio-psycho-social* health of the subjects by extended test batteries and most of them neither used sufficient sample sizes nor discussed the problem of statistical test power of the employed research designs at all (see e.g. Abeln, Kleinert, Strüder, & Schneider, 2013; Carter, 2008; Kasprzak, 2011; Lavallee, Koren, & Persinger, 2011; Le Scouarnec et al., 2001; Wahbeh, Calabrese, Zwickey, & Zajdel, 2007; Waldkoetter & Sanders, 1997). Under the contradictory studies are on the one hand works which report *confirmation* of Binaural Beat effects (Brady & Stevens, 2000; Ioannou & Bhattacharya, 2012; Lai et al., 2010; Lane et al., 1998; Padmanabhan et al., 2005; Reedijk et al., 2013), while on the other hand others found *no specific effects* using their specific employed research designs (e.g. Goodin et al., 2012; Stevens et al., 2003; Vernon, Peryer, Louch, & Shaw, 2012; Wahbeh et al., 2007; Weiland et al., 2011).

That the present work required that possible subjects had to answer a two–step online test battery of both 45 min and had to interact via eMail after the first one to ask for access to the second one, filtered out less motivated persons and reinforced *compliance* of the included persons. A further advantage of the present work is that subjects' age has rather small variability with *M* = 23.93 years and *SD* = 3.965, min = 19 and max = 31, so the sample was kept more or less homogenous in the variable *age*, reducing age biases of the found results. All participants were university students which means that subjects' *educational level* was kept invariant.

What gives special validity to the results of the present work is the use of a *within*–subjects design in combination with a randomized double–blind placebo–controlled methodology aimed to reduce confounding biases and to control *unspecific* triggers. Moreover, the employed design of auditory stimuli makes it impossible for the subjects to hear any perceivable differences between Placebo vs. Verum stimuli which strongly underpins the validity of the employed Placebo stimulus. This methodology, taken altogether, strongly supports the causal attribution that indeed the used auditory Binaural Beat stimulation *specifically* provoked the observed effects. This causal attribution has to be taken even more seriously because specific efficacy could be confirmed not only in one single domain, but simultaneously in different physiological systems and these effects moreover showed significant and meaningful associations to the effects found in the psychometric domain. Although the present work still lacks further confirmatory replications by other researchers, the author of the present work found sufficient evidence to answer the unclear question over decades in this rather small research field whether there is or there is not specific psychophysiological reactivity to auditory Binaural Beat stimulation with a clear: *yes there is.* Despite the impacts in this special research domain, the success of the present work, as compared to previous works, should be taken as an example that the key to successful science stays and falls with the level of methodological rigor invested in all aspects of the scientific endeavour.

Apart from fulfilling the requirements of a highly controlled laboratory study following the standards of Evidence–based Medicine, the present work operationalized the efficacy of auditory Binaural Beat stimulation under question for the first time as *change over time* in combination with non–stationary stimuli designed as a *sweep,* i.e. with perpetually changing carrier (f_1 and f_2) and Binaural Beat frequencies (Δf), i.e. a stimulus with slow, but perpetual *change over time.* The basic idea was to use a *Verum* stimulus with perpetual change over time versus a Placebo stimulus without such change: If significant *change over time* is observed in the dependent variables only under *Verum* and not under Placebo, and given that subjects cannot hear perceivable differences between both stimuli, then the causal attribution is admissible that the *Verum* stimulus with its frequency sweep has causally and specifically induced such *change over time.* Because it is probable that subjects' sensitivity to different stimulation frequencies is not completely identical, which means here that they would show different levels of reactivity at different time points of the sweep, operationalizing efficacy as significant *change over time* by a statistical *overall* or *omnibus* test partly compensates for those possible intersubject dissimilarities. For the concept of *change over time* it is not so important *when* exactly*,* i.e. at which frequencies of the sweep, reactivity occurs, but *how much* change happens *over all time points taken altogether*: If at least at one time point data over all subjects imply an underlying population with a *central tendency* of its distribution which is significantly different from those underlying the remaining time points, then reactivity is detected. Moreover, the use of non–parametric statistics with adequate statistical test power makes these inferences more robust against outliers, non–normal population distributions etc. Furthermore, the *within*–subject design, i.e. that every subject serves as its own control, reduces the influence of confounding trait third variables and thus makes (together with the randomization and post–hoc exclusion of presentation order effects) causal attribution of the found effects to the treatment much stronger as compared to *between*–subject designs, as unfortunately used by e.g. Crespo et al. (2013). Moreover, *within*–subject designs offer a decisively higher statistical test power as compared to *between*–subject designs, in the case of identical sample size *N.* Taken altogether, all the described specialties employed in

the present work seem to be responsible for the clear findings as compared to previous works. Which of the taken methodological decisions, or which subset of them is in the end to be considered as the most relevant for the achieved confirmation of Binaural Beat efficacy, cannot be answered by the present work and has to be studied in further investigations. It might be considered as most probable that all these decisions aiming to implement a high level of methodological rigor enhanced each other altogether in a synergistic way.

The found significant increases in salivary stress – related biomarkers (cortisol, α – Amlyase, salivary secretory Immunoglobulin A) under *Placebo,* which are absent under *Verum,* can be interpreted as neuroendocrinologic signs for activation triggered by internal processes under Placebo which are absent or inhibited under *Verum:* Because the same subjects were investigated both under *Placebo* and under *Verum*, context conditions were kept very similar, all instructions came from the same auditory recordings in eyes-closed condition, so sensorial input other than the auditory stimuli was absent and presentation order effects could be excluded, the found difference in pre vs. post salivary stress – related biomarkers under *Placebo* and not under *Verum* can be *causally* attributed to the differences in auditory stimulation, i.e. the presence vs. absence of Binaural Beats embedded into the dynamical noise mask. Although salivary stress – related biomarkers suggest activating processes under *Placebo,* no corresponding signs of arousal have been found in *Placebo* EEGs in term of significant change over time. Although it is very unlikely that the increases in salivary stress – related biomarkers under *Placebo* are just measurement errors or are due to biases or processes which randomly occurred under *Placebo*, especially this interesting finding of discrepancy between biomarkers vs. EEG findings needs to be confirmed in future replications of the experiments, as all the other findings of the present work also call for replications. It could be suspected that the Binaural Beats under *Verum* perhaps *inhibit* processes which naturally occur under *Placebo* and which are of activating nature. Future studies combining EEG and fMRI could moreover try to identify and locate brain regions associated with these possible processes, which could help to generate hypotheses of what

exactly happens under *Placebo*, but not under *Verum*. Moreover, no hints could be derived from the interviews, so extended psychometric measurements could generate hypotheses of these possible e.g. cognitive or affective processes – if they would be accessible to subjects' conscious introspection.

Arousal levels

Apart from the demonstrated *general* confirmation of the specific multimodal efficacy of Binaural Beat stimulation, more in detail, linear projections of the time courses of EEG relative spectral powers by the robust *Theil–Sen slope estimator* (Wilcox, 1998) clearly show a specific *decrease* of cortical arousal over the treatment time which was not observed under Placebo: *Slower* EEG frequency bands showed a linear *increase* over the time of 25 min, while the *faster* bands showed a linear *decrease* over time and moreover these temporal developments showed strong associations between each other over topographical scalp sites (see table 9). Thus, the employed *Verum* Binaural Beat stimulus has demonstrated its power to specifically reduce levels of arousal. Apart from manifold therapeutic implications for clinical contexts, as mentioned and outlined in the introduction, further basic research using the proposed paradigm could try to better understand the involved underlying cerebral neurocircuitry: Further basic research could try to *target* these structures by other stimuli than auditory Binaural Beat stimulation, i.e. by e.g. (topographical 3D) neurofeedback or *transcranial magnetic stimulation* (TMS) etc., all with the aim to reduce levels of arousal. A very interesting application for basic research might be the use of the *culture-free* stimuli of the present work in the emerging field of psychophysiology of modulation of gene expression and epigenetics, as outlined in the very recent and higly interesting publication of Kaliman et al. (2014). In the context of future studies trying to target brain structures which underly the effects found in the present work, the author of the present work feels obliged to warn researchers that this possible new knowledge one day could be unfortunately *abused* in e.g. military contexts, as humanity yet has done with all new knowledge: *Sensory deprivation* e.g. was and is intentionally used in the illegal *Guantanamo* prison by extraordenarly criminal US –

military psychiatrists (see e.g. Allhoff, 2008; Bloche & Marks, 2005; Marks & Bloche, 2008) to provoke psychotic episodes in prison inmates, practices which remind the native German author of the present work of the *nazi* doctors' terrible and unprecedented crimes in concentration camps during the *Holocaust* under the horrible *Hitler* dictature (see the miletone publication of Lifton, 2000). But the fact that obviously on large time scales constructive discourses in the end have mostly won the perpetual fights between constructiveness and destructiveness all over the time course of humankind, hope is given that this possible new knowledge could rather contribute to promote peoples' salutogeneses and maintenance of health in the hands of responsible professionals being able to love and to respect the never-ending secrets of nature than it might do harm in hands of protagonists who lack these fundaments.

Possible clinical applications

Apart from basic research perspectives, the employed *Verum* Binaural Beat stimulus could be further investigated for its *effectiveness* in applied–practical clinical contexts by *field studies* aiming to reduce states of hyperarousal in clinical states or clinical populations as described in the introduction: Further research should investigate *effectiveness* in e.g. chronic psychophysiological insomniacs or as a *booster* before psychotherapeutical sessions, i.e. that patients could relax approx. 30 min with the Verum Binaural Beat stimulus before psychotherapeutical interactions and thus, hopefully, the impact of these interventions could be enhanced. It is imaginable that this pre-treatment could help patients to get better access to suppressed material and to facilitate *regression to primary object relations* in the context of psychoanalytical or psychodynamical inspired psychotherapies. *Problem actualization* was identified in large meta-analyses as the second most important factor for efficacy of all psychotherapeutical interventions (Grawe, 2005), i.e. gaining access to the problems to be treated following the motto *talking is silver, real experience is gold* which means that problems can be better solved when the patient can *feel* and *experience* them in contrast to "just talking about it". What is to be changed has to be experienced in reality in the psychotherapeutic setting. *Problem actualization* for interpersonal problems can be described

in psychoanalytical terms as *transference* and *countertransference* (see e.g. Etchegoyen, 2005). Gaining more emotional access to the problems or traumata may be facilitated by the proposed pre-treatment by Binaural Beats and thus the struggle for integration might be intensified or even shortened. Certainly, as always, caution is given that every kind of weakening of defence systems can over–challenge the possibilities of patients' integration resources, in the worst case, implying possible life–threatening episodes. This is why first field studies with clinical population would need special precautions for patients' security. But, remembering the problematic pharmacological options to treat states of hyperarousal, as outlined in the introduction, i.e. their lack of sufficient specificity and their dangerous side effects (at least for long term treatment), the proposed *Verum* Binaural Beat stimulus is likely to offer better risks–benefits trade-offs, once its *effectiveness* would be proven in *field studies*. Apart from clinical populations, the *Verum* Binaural Beat stimulus of the present work could be investigated as an intervention in the context of occupational psychology or preventive medicine targeting the *restorative processes* outlined in figure 1 aiming to reduce morbidity and even mortality. As outlined in the introduction, the *Verum* Binaural Beat stimulus offers the advantage that it does not require any active learning processes for its application, as it do other relaxation techniques as e.g. meditation practices, thus in the future it might be considered as a *low–threshold* intervention to reduce arousal and support restorative self–regulation processes.

Relations to hypnotizability

Psychometrically, observations in *hypnoidal state scores* (HSS) suggest that just the Placebo stimulus itself provoked yet a *moderate* hypnoidal state following the definitions of Pekala (2009), but that the *Verum* stimulus specifically *deepened* it (while still in the "moderate" level) with an remarkable increase as expressed with an effect size of r = .513 which is, following the classification of effect size magnitudes proposed by Cohen (1988), to be considered as a *large* effect.

First, the *hypnoidal state score* (HSS) measured under Placebo, a score derived from the self–report *Phenomenology of Consciousness Inventory* (PCI) (Pekala, 1991) by several regression analyses with the coadministered behavioral *Harvard Group Scale of Hypnotic Susceptibility* (HGSHS:A) (Shor & Orne, 1962) over various stimuli and populations, predicted well (Spearman's *rho* = .671 and p_{exact} = .020) the magnitude of difference between HSS_Verum and HSS_Placebo, i.e. predicted well the *specific* psychometric effect of the Binaural Beat stimulus (see figure 32). Second, the two non-responders could be predicted because they showed the lowest Placebo HSS among all subjects. Thus, it has to be concluded that the magnitude of HSS psychometric reactivity to the employed Binaural Beat stimulus can be reliably predicted by subjects' trait *hypnotizability*: They more a subject is habitually or trait–like *hypnotizable* as observed under the unspecific Placebo stimulus operationalized as HSS, the larger will be the effect of the Binaural Beat stimulation on the intraindividual *increase* of HSS. How large HSS will be under Verum seems for ≈ 45% of the intersubject variability predictable by the *trait* hypnotizability of the subject ($D = .671^2 \approx .450$). Seen psychometrically, the Binaural Beats in *Verum* stimulus thus *enhanced* or *boosted* subjects' trait propensity/ability to enter hypnoidal states, or in more colloquial terms, the Binaural Beats in *Verum* stimulus could be named a *trance booster.* Given this finding of an interaction between *trait* hypnotizablity and magnitudes of psychometric Binaural Beat effects, rigorous selection of subjects is crucial for any further works about Binaural Beat effects on human subjects: If mainly subjects with low *trait* hypnotizability would enter the sample without the investigators knowing it, the probability that many of them would show small effects or even would behave as *non–responders* would be large. Almost no studies on Binaural Beats controlled for *trait* hypnotizability, which might partly explain the accumulated contradicting results in this small research field with regard to Binaural Beats in alpha, theta or delta EEG frequency bands.

This is why *hypnotizability* should be discussed a bit further. Following what the authors of the consensus statement of the British Society of Medical and Dental Hypnosis (BSMDH) and the British Society of Experimental and

Clinical Hypnosis (BSECH) addressed as the *narrow definition* of hypnosis*, hypnotizability* refers to the differing propensity of individuals to enter *trance states*, induced by any procedure which can induce such states, not only by means of verbal suggestions in the context of social interaction between a "hypnotist" and subject (Kirsch et al., 2011). Although the term *trance* might seem at a first sight related to rather modern or even postmodern discourses, one of the earliest uses of this word in English language can be found in manuscripts of one of the most impacting English poets of the Middle Ages, from the early year 1386, who was the first poet to be buried in *Poet's Corner* of *Westminster Abbey* (London, England): Geoffrey Chausser (cited following Pekala & Kumar, 2000). Defining *trance* by general verbal descriptions is difficult, but most definitions describe *trance* as an altered state of consciousness which differs from normal waking states in two main aspects: decreased sensitivity or responsiveness to external stimuli (or total lack of it) and a shift from voluntary to automatic activity (Pekala & Kumar, 2000). Other definitions include furthermore decrease of executive functioning, redistribution of attention, availability of memories and heightened ability for fantasy production, reduction of reality testing and tolerance from reality distortion, increased suggestibility, role behaviour, and posthypnotic amnesia (Pekala & Kumar, 2000). Apart from these problematic verbal–descriptive and general conceptual definitions, most relevant for empirical research is the *operational* definition of *trance* by the *hypnoidal state score* (HSS) as described by Pekala and Kumar (2000): Integrating observational data of manifest *behavior* to hypnotic inductions as quantified by the observational *Harvard Group Scale of Hypnotic Susceptibility* (Shor & Orne, 1962) and retrospective self–report data from the *Phenomenology of Consciousness Inventory* (PCI) (Pekala, 1991) by regression modelling using several stimuli and several populations projects the multidimensional phenomenology of *trance* onto one higher latent variable: *trance depths* or HSS. Several weighted subscales of the PCI project onto this higher latent variable. Apart from confirmed sufficient *reliability,* the operational definition of *trance* by the HSS as developed by Pekala and Kumar (2000) offers the advantage with regard to *validity* that it was derived by integration of both a behavioral measure and an introspective–self report

measure, over several distinct stimuli and populations. Although a verbal "*expresis verbis"* general definition of *trance* is problematic, the HSS offers a reliable and valid *operational* definition which in the present work has proven its utility another time.

Although *hypnosis, trance* and its related phenomenological domains still suffer from some connotation of *pseudo–science* among uniformed persons*,* high–quality neuroscience has taken these domains as an emerging field under study, mainly in the context of neurocientific basic research of *attention* (e.g. publication in *Nature Reviews Neuroscience*: Raz & Buhle, 2006)*, resting state networks, consciousness* research etc. A literature search in the data base *ISI Web of Knowledge* by the search strings *TOPIC: (hypnosis) Refined by: [excluding] RESEARCH AREAS= (ANESTHESIOLOGY OR PHARMACOLOGY PHARMACY OR PEDIATRICS OR GENERAL INTERNAL MEDICINE) Timespan=All years* results in 13918 references found which indicates a quite large scientific interest, searching by *TOPIC: (hypnotizab*) Timespan=All years* results in 1128 found publications (retrieved 25/03/2014).

The neurocientific interest in *hypnosis* or *trance* phenomenology triggered by the many observations which discovered high heritability of *trait* hypnotizability and its correlations with properties of several specific neuronal circuits, namely *dopaminergic* and *oxytocinergic* systems (see e.g. Bryant, Hung, Guastella, & Mitchell, 2012; Ott, Reuter, Hennig, & Vaitl, 2005; A. Raz, Fan, & Posner, 2006; A. Raz, 2005).

In the context of these emerging fields of basic neuroscience, the Binaural Beat stimulus employed in the present work having shown its remarkable interaction with *trait* hypnotizability could offer a new paradigm with inperceivable differences between *Verum* and *Placebo,* thus a new so–called *culture-free* hypnotic induction method which does not require verbal suggestions. A replication of the present work with combined fMRI and EEG could try to identify brain regions which are specifically stimulated by the *Verum* Binaural Beat stimulus, substracting brain activity under *Placebo* from that under *Verum.* Further studies should try to investigate the role of the *ascending reticular activation systems* (ARAS) as theorized in the introduction of the present work, e.g. by fMRI and PET/SPECT. Because the

magnitudes of these specific effects on specific brain regions are hypothesized (following the findings of the present work) to be correlated with *trait* hypnotizability, correlational statistical 3D maps could be computed in order to localize those brain regions whose specific reactivity to the *Verum* Binaural Beat is moderated/mediated by *trait* hypnotizability. Doing the same with *PET* or *in vivo magnetic resonance spectroscopy* (MRS) could help to further elucidate the role of different neurotransmitter systems in *trait* hypnotizability. *Diffusion tensor imaging* (DTI) together with *Voxel-based morphometry* (VBM) could further discover anatomical correlates of *trait* hypnotizability. Those proposed possible further developments of the present work could contribute to elucidate the field of *consciousness neuroscience* (see e.g. the peer–reviewed journal *Frontiers in Psychology – Consciousness Research*) by offering a new experimental paradigm, i.e. to better understand the neuroscience of hypnoidal phenomenology, i.e. how the human brain creates *altered states of consciousness* vs. normal wake states. These fields are related to neurocience's remarkably emerging mainstream interest of how exactly attentional neurocircuitry works, so this new paradigm could offer fruitful contributions also in this context.

Experts in psychometry could argue that a fundamental limitation of the present work is that up to now, to the author's best knowledge, no validation of the Spanish version of the *Phenomenology of Consciousness Inventory* (PCI) (Pekala, 1991) has been published, thus the validity of the psychometric findings of the present work could be legitimately questioned. Hence, in order to give some empirical evidence for the validity of the derived *hypnoidal state score* (HSS), subjects were invited after the last experiments via eMail to answer the Spanish version of the *Tellegen Absorption Scale* (TAS34) (Tellegen & Atkinson, 1974), a 34-item scale with five–point Likert answer format which measures the personality trait *absorption,* which is in the words of its authors to be understood as "openness to absorbing and self-altering experiences". The TAS34 scores have been identified to represent one of the best predictors of *hypnotizability* (see e.g. Glisky & Kihlstrom, 1993; Glisky, Tataryn, Tobias, Kihlstrom, & McConkey, 1991). *Absorption* moreover has shown to be statistically related to *Openness to Experience* of the *Big Five*

personality traits (McCrae & Costa, 1999), but only to its subfacet *imaginative involvement* (i.e. Fantasy, Aesthetics, and Feelings), but not to its subfacet *sociopolitical liberalism* (i.e. Actions, Ideas, and Values). There is some evidence that intersubject variability of absorption and response magnitudes to hallucinogenic drugs seem both associated with the binding potential of serotonin *5-HT$_{2A}$* receptors (Ott et al., 2005; Studerus, Gamma, Kometer, & Vollenweider, 2012), which are stimulated by hallucinogenic drugs such as lysergic acid diethylamide (LSD), psilocin (the pharmacologically active metabolite of psilocybin) or mescaline while being potently antagonized by so–called *atypical antipsychotic drugs* such as clozapine, risperidone, quetiapine, olanzapine, asenapine etc. Activation of hypothalamic *5-HT$_{2A}$* receptors increases concentrations of ACTH, oxytocin, prolactin and renin. Psilocin causes effects which are typical for excitation of the sympathetic nervous system, such as tachycardia, mydriasis, sweating etc. Interestingly, the *absorption* trait has been shown to be positively associated to dream recall frequency (Watson, 2003). High vs. low dream recall frequency has been very recently demonstrated to be related to differences in regional cerebral blood flow (rCBF) as measured by [^{15}O]H_2O–PET in resting states during wakefulness and REM sleep in the temporoparietal junction (TPJ) and medial prefrontal cortex (MPFC), where high dream recall frequency was associated with increased activity, hypothesized to promote mental imagery and/or memory encoding of dreams (Eichenlaub et al., 2014). The same author found one year before that the event-related potential (ERP) *P3a* during both sleep and wakefulness*,* which reflects attention-orienting processes, was larger in subjects with high vs. low dream recall frequencies (Eichenlaub, Bertrand, Morlet, & Ruby, 2013). Interestingly, treatment with *selective serotonin reuptake inhibitors* (SSRI) in healthy subjects suppresses dream call frequency (Pace-Schott et al., 2001). Dream recall frequency has also been shown to be related to the psychoanalytically inspired concept of *thick vs. thin boundaries,* where subjects with higher dream recall frequency show significant thinner boundaries (Hartmann, 1989; Schredl, Kleinferchner, & Gell, 1996)*.* Probably interesting in the context of the EEG findings of the present work with *right*-lateralized decreasing linear trends in 10.5 – 11.5 Hz alpha4 (see figure 44) is that awakenings from stage 2 NREM sleep with

successful dream recall seem to be associated with lower 8-12 Hz alpha activity of *right* temporal areas (Marzano et al., 2011). Interestingly, among N = 50 subjects, remarkable temporal stability for hypnotizability was found over a 25 year period with test–retest r = .71, for 15 year even r = .82, values which are as large as those which have been found for intelligence and other personality traits which strongly implies important underlying genetic factors of hypnotizability (Piccione, Hilgard, & Zimbardo, 1989). Indeed, remarkable influence of genetics has been found for hypnotizability in twin and genotyping studies (Bauman & Bul, 1980; Morgan, 1973; Raz et al., 2006; Raz, 2005, 2007), but also for absorption (Ott et al., 2005) and the *Big Five* personality trait *openness to experience* (Jang, Livesly, & Vemon, 1996; Stoltenberg et al., 2002).

In order to obtain possible evidence for the validity of the *hypnoidal state score* (HSS), given that the *absorption* as measured by the TAS34 was identified as a good predictor of *hypnotizability,* as mentioned above, and because to the author's best knowledge a Spanish validation of the TAS34 was not published up to now, the author of the present work gave the English TAS34 version to two bilingual (Spanish–English) psychologists separately for translation, afterwards these two persons discussed possible differences in their opinions. Later on, two other bilingual psychologists back–translated this preliminary Spanish version which resulted in literally almost the same English items. This final version was then presented in an online questionnaire to until now N = 153 participants of Spanish university population, advertised in digital social networks, posters and flyers. Resulting M = 57.68 and SD = 22.049 are in line with the findings for the English and also for the German TAS34 version (Ritz & Dahme, 1995). Comparing the found arithmetic means of the new Spanish vs. those of the German version (M = 60.05, SD = 19.98) using Welch's t test, an adaptation of the Student's two-sample t-test for independent samples to the possibility that the two variances are not equal, gives $t(296.681) = -1.0861$ with p = .221. Thus, given that the employed sample sizes are more than sufficient and thus adequate statistical test power is to be assumed, no significant mean difference between the new Spanish vs. the German version was found, which is to be understood as

one empirical hint that these two TAS34 adaptations behave comparably, at least when applied to university populations. The *Shapiro–Wilk* test (Royston, 1982; Shapiro & Francia, 1972; Shapiro & Wilk, 1965) confirmed that the TAS34 sum scores of the new Spanish TAS34 adaptation are excellently compatible with populational Gaussian normality distribution with $W = .994$, $p = .908$. Moreover, exploratory factor analyses (EFAs) were computed. The *Kaiser-Meyer-Olkin* (KMO) measure of factorizability with range between 1 and 0 which indicates whether the strengths of relationships among all variables of the matrix are large enough to allow adequate EFAs showed with KMO = .881 a value classified as *meritorious* (≥ .8), while *marvelous* would be ≥ .9, *middling* ≥ .7, *mediocre* ≥ .6, *miserable* ≥ .5 and *unacceptable* < .5 (Cureton & D'Agostino, 1983). Thus the found KMO indicates that EFAs are fully feasible with the given data matrix. Now, the program *Factor 9.2.* (Lorenzo-Seva & Ferrando, 2011) for EFAs was used which confirmed *unidimensionality* of the Spanish TAS34 version: As method for estimation of the factor model, i.e. dimension extraction, *unweighted least squares* (ULS) was used, because it is distribution–free (Krijnen, 1996) and moreover because simulation studies have found that it is the method of choice in situations in which few factors are retained (Jung, 2013). *Parallel Analyses* (PA) based on *Minimum Rank Order Factor Analysis* (Timmerman & Lorenzo-Seva, 2011) were computed using *Pearson* correlation vs. *Polychoric* correlation matrices with 5000 permutations of the raw data (Buja & Eyuboglu, 1992) as method to obtain the needed random correlation matrices. Both options resulted in an advised number of dimensions to be retained of *one*. Moreover, also the new *HULL* method (Lorenzo-Seva, Timmerman, & Kiers, 2011) which aims to find common factors searching for an optimal balance between model fit and number of retained factors/dimensions based upon *Pearson* correlation vs. *Polychoric* correlation matrices (and as model–fit parameters *Comparative Fit Index,* CFI, vs. *Root Mean Square Error of Approximation*, RMSEA) lead for both options to an advised number of common factors to be retained of *one*. Thus, *unidimensionality* of the new Spanish TAS34 version and hence *factorial validity* is definitively proven. Using the obsolete *Kaiser–Guttman* criterion (Guttman, 1954; Kaiser & Dickman, 1959) which advises to retain as many factors as their *eigenvalues* are ≥ 1 and which

regularly *over*estimates dimensionality, 6 factors are obtained which is in line with the results for the English version (Tellegen, 1992). It is to be assumed that the use of actual methodology for determining dimensionality on these former data would also result in confirmation of *unidimensionality:* Absorption seems to be a unidimensional trait, but seems to have several subfacets. Checking *psychometric reliability* by the parameter for *internal consistency,* Cronbach's α, results in a more than excellent α = .941 (Kline, 2000), with no item which would relevantly increase α of the remaining scale if deleted. This finding is almost identical as compared to the German version of Ritz and Dahme (1995) who found Cronbach's α = .95 for the TAS34. Taken altogether, this new Spanish TAS34 version has proven excellent *psychometric reliability* and has shown almost identical *M* and *SD* as compared to its original English and validated German versions. With regard to *convergent validity,* this new Spanish TAS34 version showed significant correlations in the *N* = 12 subjects of the present work only with the scale *Openness to Experience* of the coadministered and very well established Spanish version of the *Big Five personality factors* instrument *NEO–FFI* (Manga et al., 2004) with *Spearman's rho* = .641 and p_{exact} = .037, while all remaining four *Big Five personality factors* demonstrated correlations around zero, far away from significance. This is completely in line with the findings of Glisky et al. (1991) that the original English TAS34 version showed significant and large associations to *Openness to Experience*. Thus, quite strong evidence for *convergent validity* of the new Spanish TAS34 version could be found, even more important, it was found for the *N* = 12 subjects of the present work. Trait *hypnotizability* was operationalized in the present work as the *hypnoidal state score* (HSS) under *Placebo* derived from the unvalidated Spanish version of the *Phenomenology of Consciousness Inventory* (PCI) of Pekala (1991). A strong evidence in terms of validity that *HSS_Placebo* in deed measures *hypnotizability* is the fact that within the *N* = 12 subjects of the present work a significant and large association with the new Spanish TAS34 of *Spearman's rho* = .600 and p_{exact} = .043 was found, which is just in line with the findings of Glisky and Kihlstrom (1993) and Glisky et al. (1991) who found the TAS34 score to be one of the best predictors of *hypnotizability.* Taken altogether, although the entire Spanish PCI version still calls for

psychometric validation studies, sufficient empirical evidence could be found to trust the validity of HSS scores in the present work.

Importantly, the absorption trait *TAS34* did not show any statistical associations for the *N* = 12 volunteer sample of the present work with dimensions defined as *pathological*, measured previously in the inclusion vs. exclusion online test batteries (references of the employed psychometric instruments are cited above, see chapter *2.1 Subjects*), although caution is advised because of relatively small sample size: TAS34 scores did not show neither significant nor relevant *Spearman's* rank order correlations with *Schizotypy* total scores (*rho* = .016, p_{exact} = .962), nor with the *SCL-90* subscale *psychoticism* (*rho* = –.144, p_{exact} = .652), nor with Big Five Personality trait *neuroticism* (*rho* = .035, p_{exact} = .913), nor with the BDI-II, *Beck's Depression Inventory* (*rho* = –.004, p_{exact} = .993), nor relevantly with the *Epworth Sleepiness Scale* (*rho* = –.200, p_{exact} = .529), nor with TAS20 *Alexithymia* (*rho* = .083 , p_{exact} = .796), nor positively with *Dickman's dysfunctional impulsivity* (*rho* = –.307, p_{exact} = .093), nor negatively with *Self Directedness* (*rho* = .044, p_{exact} = .892), a known common psychometric negative correlate of many personality disorders (see e.g. Laidlaw, Dwivedi, Naito, & Gruzelier, 2005). Although those subjects who exceeded thresholds in pathological dimensions were rigorously excluded from participation, variability of the included subjects in pathological dimensions within subclinical magnitudes theoretically might have been associated with TAS34 scores. The reported null results imply that the variability captured by the TAS34 reflects interindividual fluctuations of a normal personality trait, related to the domain of the Big Five *Opennes to experience*. Thus, the TAS34 scores have nothing to do with pathological dimensions, at least for the population represented by the employed *N = 12* volunteers. The same conclusion is obtained when the mentioned pathological dimensions are investigated for possible associations with the psychometric effect of the present work, i.e. the differences between HSS_*Verum* minus HSS_*Placebo* and also with the HSS_Placebo itself, interpreted as a measure of trait hypnotizability.

Interestingly, TAS34 scores show a significant negative correlation with *Dickman's functional impulsivity* (*rho* = –.601, p_{exact} = .042) and surprisingly a significant positive correlation with *Edinburgh Handedness Inventory* scores (*rho* = .617, p_{exact} = .036): Although left–handers were excluded from participation applying strict thresholds, the remaining interindividual variability in the dimension *handedness* was associated with TAS34 scores. The psychometric effect of the present work demonstrated an association with *Edinburgh Handedness Inventory* scores of *rho* = .359, p_{exact} = .251, while HSS_*Placebo* showed *rho* = .211, p_{exact} = .507. This means that although that reported interesting correlation of remaining variability of *handedness* was found with TAS34 scores, the psychometric effect of the present work and also hypnotizability (which itself correlates significantly with the TAS34 scores, *rho* = .600, p_{exact} = .043) show only weak associations with *handedness,* which moreover cannot be investigated properly given the relatively small sample size of *N* = 12. Thus, the psychometric effect found in the present work can be considered as relatively independent from remaining variability of *handedness.* Moreover, *handedness* did not show associations with the *Big Five personality trait* openness to experience (*rho* = .125, p_{exact} = .695).

Topographic patterns/lateralizations

Although it is difficult to interpret the found EEG topographic patterns, calling for replications with simultaneous EEG and fMRI recordings, Graffin, Ray and Lundy (1995) reported an increase of theta power at posterior sites during a standard verbal hypnotic induction, as it was also clearly observed in the linear trends for thetaLow (3.5-6.5 Hz) under the *Verum* Binaural Beat stimulus of the present work, see figure 40. Moreover, in the present work lateralizations to the *right* hemisphere were found in central and parietal regions for delta and alpha4: For delta *logit*–transformed EEG

relative spectral powers, the topographical pattern of *change over time* as expressed as *total intrasubject variance* (see figure 34) and also the linear trend computed using the *Theil–Sen slope estimator* (Wilcox, 1998), see figure 36, show under *Verum increases* over the 25min treatment time lateralized to *right* scalp sites: C2, C4, C6, Cp2, Cp4 and CP6, most pronounced at C4 and C6. Linear trends of alpha4 (see figure 44) show a *decrease* over the 25min treatment also at *right* scalp sites: P2, P4 and P6. Note that operationalizing *change over time* not as Placebo–referenced *linear trends*, but as *total intrasubjects variances* gives for alpha4 a *left*–lateralized topographic pattern (see figure 42) with effects most pronounced at C3, C1, CP3 and CP1. This is not to be understood as contradictory, but as the consequence of the two distinct ways to operationalize the concept *change over time,* i.e. two different manners to represent the phenomena. Studies using simultaneous EEG and fMRI recordings have shown that central alpha powers correlate *positively* with local cerebral blood perfusion in brain areas associated with modulation of attention and preparedness for external input and that delta powers correlated *negatively* with cerebral blood flow (CBF), see e.g. O'Gorman et al. (2013). Given these evidences of coupling between CBF and EEG spectral powers, a rough comparison of the reported EEG findings of the present work with an interesting fMRI study is valid: At the *Bender Institute of Neuroimaging*, Justus–Liebig-University, Gießen/Germany, the psychologist Hannes Hempel studied in his doctoral dissertation using fMRI the effects of trance induction by treatment with 12min African drum music and its visualization by the common computer program *winamp* vs. 3min control condition (white fixation cross on a black background) in N = 28 human healthy adults (Hempel, 2009). The contrast between control vs. treatment condition searching for structures with *higher* activation under control as compared to treatment shows a clear lateralization towards the *right* hemisphere, see figure 53.

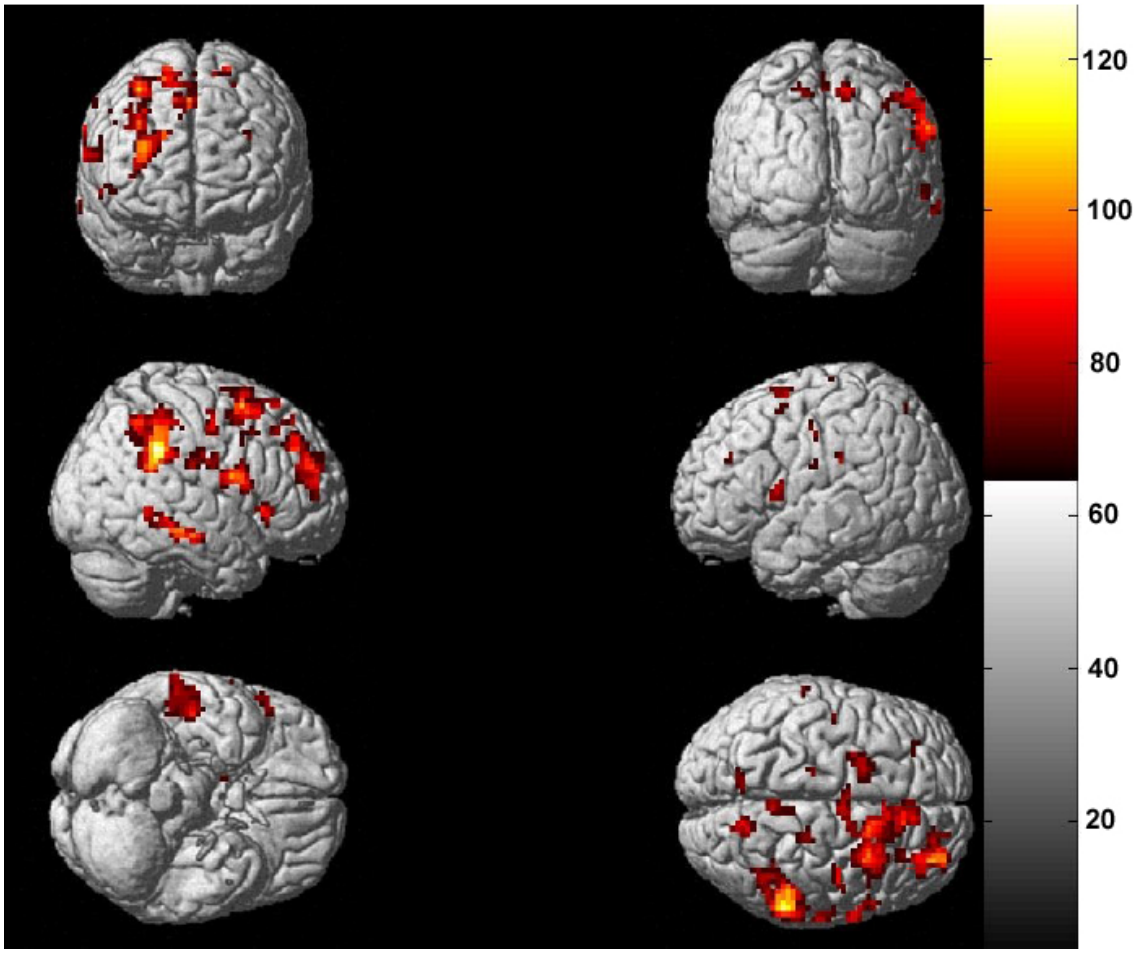


Figure 53. Contrast of 3min control (white fixation cross on black background) vs. 12min trance induction by African drum music and its computerized visualization searching for brain regions with *higher* activations under control as compared to treatment in an fMRI study with *N* = 28 human healthy young adults at *Bender Institute of Neuroimaging* (BION), Justus–Liebig University Gießen/Germany by Hempel (2009)[5]. A clear lateralization to the *right* hemisphere was observed. Reproduced with kind permission of the author.

Not only Hempel (2009) found that the effects caused by his trance induction method showed a *lateralized* pattern in fMRI measures, but also an early neuroimaging study measuring regional cerebral blood flow (rCBF) with inhalation of the radioactive gas ^{133}Xenon found strong rCBF–increases in the *right* hemisphere triggered by hypnotic induction (Crawford, Crawford, & Koperski, 1983). In a very recent fMRI study, electronically published at 26th of March 2014 in *PloS ONE* (Huber, Lui, Duzzi, Pagnoni, & Porro, 2014), using voxel-based morphometry and fMRI resting-state functional connectivity, found that

[5] Downloadable in German language at http://geb.uni-giessen.de/geb/volltexte/2010/7421/pdf/HempelHannes_2010_01_26.pdf

structural and functional correlates of interindividual differences in hypnotizability among 34 healthy adult women showed patterns of clear lateralizations: As the authors summarize in their abstract, hypnotizability was *positively* correlated with *gray matter volume* in portions of the left superior and medial frontal gyri, while a *negative* correlation was observed with gray matter volume in the left superior temporal gyrus and insula. For the domain of *functional connectivity*, *positive* correlations were found (i) with connectivity between medial posterior areas, including bilateral posterior cingulate cortex and precuneus, both lateral visual networks, but interestingly also the *left* fronto-parietal network and (ii) with connectivity between the executive-control network and a right postcentral/parietal area. Hypnotizability moreover demonstrated *negative* correlations with functional connectivity between the right fronto-parietal network and the right lateral thalamus which seems interesting in the framework of hypnotizability theories concerning interindividual differences in thalamo–cortical *gating.*

Given that *increase* of delta EEG powers has been shown to be correlated to *decreased* CBF (O'Gorman et al., 2013) and given the *right*–lateralized findings in hemodynamic responses to trance induction found by Hempel (2009) by comparing control condition with treatment, the observed *right*–lateralization in the present work with regard to delta EEG relative spectral powers might be interpreted as a neurophysiological correlate of *trance*: Taking into account the findings of Hempel (2009), the EEG findings of the present work at least in delta could be interpreted as an evidence that the employed *Verum* Binaural Beat stimulus *specifically* induced, or at least deepened *trance.* In contrast to Hempel (2009), the present work with its specific methodology could not find effects in frontal brain regions as hypothesized by the *transient hypofrontality hypothesis* of altered states of consciousness (ASCs) proposed by Dietrich (2003).

Concerning previous psychophysiological investigations on hemispheric lateralizations under hypnosis, Kihlstrom (2013) reports in a remarkable recent review on the role of hypnosis for neuroscience that results have been conflicting so far: Some found no signs for lateralizations, others

report evidences primarily for the *right* hemisphere, but again other works report results concerning the *left* hemisphere (see e.g. Jasiukaitis, Nouriani, Hugdahl, & Spiegel, 1997). With regard to brain asymmetries, especially in the context of hypnosis, the interested reader is strongly advised to read the vigorous warning of Raz, Schwartzman and Guindi (2008) in order to prevent that the EEG findings of the present work might contribute to what the authors denounced as *urban legends,* the *dual–brain tale:* that the left hemisphere would be "the analyzing rationalist", whereas the right hemisphere would be the "holistic poet" or "the rocking rake" in us. Besides these urgent warnings of simplistic exaggerations, the authors state that "[…] brain laterality is very much a vibrant theme in hypnosis research". Reports of lateralized EEG findings and other evidences for brain asymmetries need to be digested soberly and an umbrella of elevated scientific scrutiny might prevent researchers' heads from getting wet.

Taken altogether, although associations of the present work's results with those of Hempel (2009) concerning a trance *state* and the very recent findings of Huber et al. (2014) concerning magnitudes of *trait* hypnotizability can be seen, we should keep in mind the final conclusion of the excellent and broad review of neuroscientific studies on *meditation* by EEG, ERP and neuroimaging (i.e. fMRI, PET, SPECT, etc.) methods by Cahn and Polich (2006): "[…] However, none of the approaches has yet isolated or characterized the neurophysiology that makes explicit how meditation induces altered experience of self. […]" In contrast to the wide and rather diffuse spectrum of different *meditation* and *hypnotic induction* practices and associated related domains of self–experience, which include entire worlds of complex higher cognitive and executive functioning, the *Verum* Binaural Beat stimulus of the present work might offer a reductionistic, standardized and moreover inaudible paradigm (resulting in more valid *Placebo* contrasts and moreover offering *culture–free* stimulation). The proposed paradigm could help further multimodal research to better identify especially the searched neurophysiology of altered experience of self, but thus in general to identify the neurophysiology of normal human consciousness and genesis of normal experience of self.

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6. APPENDIX

6.1. Translation of Spanish text on page 76 into English:

After we have fixed and tested all sensors, now we will start with the experiment. From now on, everything which matters you will hear from the headphones. The sensors are quite sensitive, so you have to help us to avoid interferences.

Please, during the experiment, **do not talk**, only focus on my voice. – Close your eyes now, **your eyes have to remain closed** almost for all the time in the experiment, this is very important: keep your eyes closed. Although your eyes are closed, try not to move them a lot. Eye movements can cause interferences with the sensors. But you don't have to focus on that especially, just **keep your eyes *closed and relaxed.***

Now let's do a test on something which *later* you must **NOT** do: Move your eyes NOW so fast as you can, from right to left, from left to right, back and forth, go on … go on … GOOD, that's it. Now, let's do the same, but from bottom to top, move your eyes now as fast as you can down and up, go on ... go on VERY GOOD. Now you know what eye movements are. From now on, **keep your eyes closed and quiet;** quieeet; relaxed.

Another thing that you have to try to avoid is ... *clenching your teeth.* Let's test this now, although *later* you must **NOT** do it: Clench your teeth now, first slightly…and ok, ready, stop it! [3 sec pause] Repeat it, clench your teeth slightly…and ok, ready, stop it! [3 sec pause]. And for the last time, clench your teeth strooooongly…and ok, ready, stop it! ... In the experiment, try **NOT** to clench your teeth, to move a lot your mandible, your tongue or to swallow a lot… all this can interfere with the sensors. If you have to do it, do it fast, but smoothly. But don't focus too much on this, just keep your mandible caaaalm and relaaaaxed.

Finally, the most important thing is that you must not move your head, your body, your arms or your legs; try to keep yourself *calm,* find a comfortable position now and try **NOT** to move. If you cannot avoid it, do it fast, but smoothly. If, for example, you feel itchy somehow, it would be worse to fight against this itch than to scratch fast, but smoothly. From now on, try to keep yourself quiet.

From now on, the experiment lasts 30 minutes and has three phases. Please, do not talk until I will tell you that the experiment has ended.

Now, we start with phase ***one:*** For the last time in the experiment, open your eyes NOW. We will record your normal state, which we call *baseline* in science. Look, there is a white point fixed to the door, do you see it? Well, now we do a thing: fix your eyes to this point and try not to move your eyes...try not to do a lot of eye blinks, only when you feel uncomfortable and do it fast, but smoothly. It will last one minute and a half, here we go **NOW**: FIX THE POINT…go on…30 seconds, very good… one minute, very good… **ready.** Relax and close your eyes, close them, don't move them a lot, try to keep quiet [3 sec pause].

Very good, NOW, I want you to imagine a thing as real as you can, when I say "NOW", but only for one minute and a half...Imagine now your daily way to *work* or *university* or just a way which you know very well. Imagine now, in detail, the colours, the sounds, the smells, how you leave your home and how you start your way. Here we go...**NOW**... [3 sec pause]. Very good, we recorded your normal state with all sensors, the *baselines*…Leave your eyes closed and keep quiet…We also have to analyze changes in your hormones, the *baseline* of your hormones. We use a modern technology, it works with a simple saliva sample. It's easy, we just need one milliliter saliva. Now, I explain how we will do this: Later on, when I will say "NOW", you will open your eyes and you will lean forward in the lounger, and you will put your head down a bit. The

experimenter will give you a test tube into one hand and a plastic tube into the other. When the experimenter will say “NOW”, you will have to swallow all the saliva which was in your mouth, to eliminate it, put quickly the plastic tube into your mouth and the other end into the test tube. While the experimenter measures the time with a chronometer, your task is…continuously collect some saliva in your mouth, without doing anything else than waiting a bit, spit it out into the test tube using the plastic tube, collect a bit more saliva, until you are told “THAT’S IT”. This way we will collect one milliliter of saliva: The time you will need to collect it will not be longer than three minutes. After we have this milliliter, you will give the test tube to the experimenter and simply make yourself comfortable again in the lounger searching for a comfortable position. You will close again your eyes and you will keep quiet as before. The experimenter will go out of the room for some minutes with your saliva sample to store it into the freezer. While you are alone, imagine one more time the way from your home towards your work, university or whatever you had imagined before. You will hear that the experimenter will return to the room and, after a while, you will listen again to my voice by the headphones and the experiment will go on.

Now, when I tell you “NOW”, you will open your eyes and you will lean forward in the lounger with all your body, putting also your head down a bit, and the experimenter will give you the test tube and the plastic tube…Be prepared, here we go…”NOW!” [The experimenter manually changes the experimental phase in the recording by keystroke and one more time when the voice starts again].

6.2. Translation of Spanish text on page 77 into English:

You hear me here again. Just focus on my voice. We go on with the experiment. Make yourself comfortable, keep your eyes closed, try not to move them a lot, keep quiet, you already know this. Phase ***one*** of the experiment now has finished, and now, we start with phase ***two*** of the experiment:
Now your task is to relax yourself as deeply as you can. Enjoy the tranquility here, feel the lounger, feel how comfortable it is to be in it. Try not to think of anything special, simply let yourself go a while with the music, don’t think of anything special, try to let your mind a complete blank. Let yourself go a while with the music…

Simply listen to the music and to my voice. The music will change later to a special noise. We will work with your imagination: I will ask you to imagine some things mentally; your task will be to imagine them as real as you can, just like a movie and you being in it. – Maybe my voice and what you will imagine could seem a bit strange in the beginning just wait a bit: – You will get used to it bit by bit. The important thing is that you try to imagine everything as real as you can. You don’t have to do anything, you are simply here on the lounger. Now, the important thing is my voice. You don’t have to do anything, just listen: Enjoy the tranquility here and relax.

Your consciousness leaves now bit by bit the reality of the exterior – your attention focuses more and more onto your inner world… your consciousness submerges more and more into your interior.

Everything which comes from the outside, now has no meaning at all: All the noise from outside, all the lights from outside, – everything from outside, now has no importance at all: You are simply here, caaaaalm, quieeeet and comfortably relaaaaxed. – Let yourself go – more and more submerge yourself into your interior, more and more profoundly. Let yourself go. Time goes by now more and more slowly, more and more slowly; time has no importance.

Your body is relaxed and will go to sleep now, but your mind will stay awake – a state of being caaaaalm, quieeeet and comfortably relaaaaxed.
I will count now backwards – from six to one, every time you will be more relaxed:

Six – The forehead, the muscles and nerves of the forehead relax, they let go.

Five – The cheeks, the muscles and nerves of the cheeks relax.

Four – The scalp, your muscles and nerves relax. – The head is quiet, light and relaxed.

Three – Now, relax the neck, the shoulders, the arms and the hands – all the muscles and nerves relax.

Two – the relaxation extends from your neck, goes down your chest, the abdomen, to your legs towards the feet – all the muscles and nerves relax.

One – the relaxation goes up your back, the back relaxes, the spinal column relaxes – all the muscles and nerves relax.

All your body is relaxed, it relaxes totally. But your mind is awake – a state of being caaaaalm, quieeeet and comfortably relaaaaxed.

Now, let all your thoughts and feelings go – while you are here on the lounger. Thoughts and feelings go away like clouds, they come and they go – coooome … and go – they do not have any importance at all now. If an image or a thought stays, say mentally "I leave this behind, I let myself go". Thoughts and feelings don't have any importance now, they are just clouds, which come and go, let yourself go. – Now, time goes by more and more slowly, time has no importance now. Thoughts and feelings go away like clouds, they coooome and they goooo, they have no importance, let yourself go.

Now, imagine vividly that you are on a meadow, – you are on a meadow – look around: It's a meadow with smooth grass, with flowers of all kinds of colours – listen how the butterflies flutter between the flowers, – it's a very smooth sound, listen how they flutter and search for nectar, look how smooth are these butterflies, smooth and sensitive – smell how the flowers exude their aroma: There is only peace and tranquility… It's a marvellous afternoon in spring. The sun shines, shines smoothly and the temperature is very comfortable for you.
Now you are walking around on the meadow, slowly. The meadow ends into a huge lake out of pure and crystalline water which shines bluish. Look how beautiful the lake is. Far away you see mountains which extend up to the sky. The meadow, the lake and the mountains are very old …
On top of the mountains you see now, quite far away, a huge screen. Although it is far away you can see it well. – All images, thoughts and memories, which come up to you, appear on this screen on top of the mountains – far away from you. Whatsoever happens on the screen, you are absolutely safe on the meadow in front of the lake. It's like to be in the cinema: Although many things happen on the screen, you are safe on your cinema seat – the meadow is your cinema seat. On the meadow you are completely free. The meadow is your interior, here you are safe, here is peace.

There is always something to see on the screen, there is a sequence of images. These images are not real, they are images, constructions which the brain produces permanently. Sometimes we are conscious about them, sometimes not – but the sequence of imagery never stops, they are produced automatically. These images are not real, they are only projections onto the screen – far away on top of the mountains. This imagery cascade is like the blood which flows permanently through your body.
Although there is always something to see on the screen, you have the power to decide whether to see it or not. It's always you who has the control, and on the meadow in front of the lake you are safe and secure. If something appears on the screen which you don't like – you don't *have* to see it, just wait until the image has gone or changes.

Leave all your thoughts and feelings behind – for the time that you are here on the lounger. Thoughts and feelings come and go like clouds, coooome and goooo, cooooome and goooo. If an image, if a thought stays, say mentally: “I leave this behind, I let myself go”. Time goes by more and more slowly, it has no importance at all. Thoughts and feelings come and go like clouds, let yourself go. Nothings matters now, let yourself go.

Now, the music will change into a noise, let yourself go until my voice will wake you up. Now, the music will change into a noise which moves slowly around your head … let yourself go with this noise. I won’t talk while you are listening. If an image, if a thought stays, say mentally: “I leave this behind, I let myself go”. Thoughts and feelings come and go like clouds, come and go, none of them has any importance now. Let yourself go! Let go! Let yourself go! Let go! Let yourself go! Let go! Let yourself go with this noise!

[25 min Binaural Beats stimulus *Verum* vs. *Placebo*]

[with low voice] Here I am again with you...Eyes closed, keep quiet. [Now with normal voice] Now phase three of the experiment begins. Eyes closed, keep quiet.

Now, we will go on with the experiment. We will have to take another saliva sample; it’s just the same as we did before. When I say “NOW”, you will lean forward and we will give you a plastic tube and another test tube… Here we go... “NOW”.

6.3. Translation of Spanish text on page 80 into English:

[*with low voice*] You hear me again. Simply focus on my voice. We go on with the experiment. Eyes closed, keep quiet. [*now with normal voice*] Now, phase ***four*** of the experiment begins. Eyes closed, keep quiet.

[Stimulating music, three minutes: *Junior Senior - Move Your Feet* mixed with techno music, cyclically changing *stereo–panning* to evoke stress/activation]

The experiment now has finished, but stay on the lounger, later we will remove the sensors. Open your eyes NOW, breathe in deeply and breathe out the air strongly … once again … breathe in, breathe out … breathe in, breathe … **You are completely back in reality,** you are on a lounger in an experiment at university. Thank you for your participation, stay on the lounger. You are completely back in reality! The experiment now has finished.