Mechanisms of inhibition in the somatosensory system and perceptual threshold calculation

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Allgemeine Zusammenfassung

Die Verarbeitung sensorischer Informationen wird aufsteigend durch die Eigenschaften der Reize und absteigend durch individuelle Erfahrungen und Erwartungen gesteuert. Neuronale Schaltkreise regulieren die reiz- und aufgabenabhängige Informationsverarbeitung durch Hemmung neuronaler Verarbeitungsprozesse. Die Hemmung kann die auf- und absteigende Kontrolle kortikaler Regionen verändern und die Integration oder Segregation neuronaler Aktivität beeinflussen.

In dieser Arbeit werde ich eine neuropharmakologisch-neurobildgebende Studie vorstellen, in der die Wahrnehmungsprozesse im somatosensorischen Kortex untersucht werden und die sich speziell mit den inhibitorischen Mechanismen auf der Ebene von SI (primärer somatosensorischer Kortex) und SII (sekundärer somatosensorischer Kortex) befasst. Durch die Verabreichung verschiedener GABA-Agonisten (Gamma-Aminobuttersäure) - dem wichtigsten inhibitorischen Neurotransmitter im Gehirn - konnte in der vorliegenden Arbeit die primäre Bedeutung eines schnellen GAB_AA-Agonisten gezeigt und ein Modell der Hemmungsausbreitung von SI zum SII erstellt werden. Das Paradigma implizierte die taktile Stimulation auf dem Niveau der individuellen Wahrnehmungsschwelle, wobei das Reizniveau an die sensorischen Wahrnehmungsleistung der Teilnehmer kontinuierlich angepasst wurde. Diese adaptive Strategie wirft verschiedene Fragen hinsichtlich der Optimierung der Methode zur Schwellenschätzung auf. Der Schwellenwert wird indirekt aus den Reaktionen des Probanden auf die einzelnen Stimuli berechnet. Er hängt daher nicht nur von der Empfindlichkeit des sensorischen Systems, sondern auch von den Entscheidungsvorgängen und von dem Reaktionsverhalten der Versuchsperson ab. Insbesondere subjektive Tendenzen (englisch Bias) der Ebene der Entscheidungsfindung oder auf der Ebene des Antwortverhaltens könnte den Schwellwert beeinflussen. In Anbetracht der Bedeutung einer präzisen Schätzung des Schwellwertes bei Experimenten, in denen im Bereich der Schwelle stimuliert wird, konzentrierte sich die zweite Studie auf die Entwicklung einer Schwellenbestimmungsmethode die eventuelle Entscheidungs- und Antworttendenzen korrigiert. In der vorliegenden Arbeit wurde ein neues adaptives Verfahren

entwickelt und evaluiert. Die Implementierung, Vorteile und Grenzen dieses Verfahrens wurden auf der Grundlage von Simulationen untersucht und beschrieben.

Table of Contents

Abbreviations

AM	Adaptive method
GABA	y-aminobutyric acid
$GABA_AR$	GABA Receptor A
$GABA_B$ R	GABA Receptor B
MCS	Method of constant stimuli
MEG	Magnetoencephalography
PEST	Parameter estimation by sequential testing
QUEST	Quick Estimate of Threshold
SEF	Somatosensory evoked field
SI	Primary Somatosensory Cortex
SII	Secondary Somatosensory Cortex
ZEST	Zippy Estimation by sequential Test

1. General abstract

The elaboration of sensory information is controlled bottom-up by stimulus characteristics and top-down by individual experience and expectations. Neural circuitries regulate information processing through inhibition. Inhibition can alter up- and down-regulation of cortical regions and allowing integration of neural activity.

In this work I will present a neuropharmacological-neuroimaging study explores the perception processes in the somatosensory cortex, specifically addressing inhibitory mechanism at the level of SI (primary somatosensory cortex) and SII (secondary somatosensory cortex). Administering different agonists of GABA (gamma-aminobutyric acid) – the main inhibitory neurotransmitter in the brain - it was possible to show the primary importance of fast $GABA_A$ agonist as well as build a model of inhibition propagation from SI to SII. The paradigm implied the tactile stimulation at the level of individual perceptual threshold, adapting the stimulus level on the basis of participant performance. This adaptive strategy arises different questions about which method would be optimal to estimate the threshold. The threshold value is indirectly calculated from the responses of a subject to a stimulus and therefore depends not only on the sensitivity of the sensory system but also on decision making and motor response. In particular, a bias at the level of decision making or at the level of response answer could affect the threshold value. Considering the importance of a precise estimation of the threshold, the second study focused on the methods for its calculation. A new adaptive procedure, that enables stimulation at the sensory threshold, and at the same time includes an on-line bias correction, was developed. The implementation, advantages and limits of this method are described on the basis of the results obtained through different simulations.

Dedicated to every teacher

2. General introduction

2.1. Excitation and Inhibition balance

In the central nervous systems, the activity of brain regions and the processing of information involve the balance between neuronal excitation and inhibition. By altering this balance, the priority in the brain activation are established (Heider et al., 2006; Marino et al., 2005; Oklun & Lamp, 2008; Shouesboe & Waagepetersen, 2007). In order to shed light on how the mode of processing is modulated by inhibition in distinct brain regions, in the first study presented here, inhibitory mechanisms were investigated in the somatosensory system.

In general, the excitation-inhibition mechanism is guaranteed by the neuronal connection structure between excitatory and inhibitory cells. In detail, the interneurons, that are inhibitory, can either have short axons and form circuits with nearby neurons or work as projection cells, that send their axons to distant brain targets (Kandel et. al. 2000; Rothand & Draguhn, 2012; Tepper et al. 2008). Since excitatory neurons innervate the largest part of the brain, it is predictable that an unrestricted activity of these neurons, would result in overexcited chaotic brain activity, with similar patterns to those observed in epilepsy. However, excitatory neurons are specifically innervated by inhibitory interneurons that release gamma amino butyric acid (GABA) onto the synapses, inhibiting the activity of the postsynaptic neuron (Andrews, 1979; Roth et al., 2003; Li & Xu, 2008). Therefore, the modulation of excitatory brain cells by inhibition avoids overactivation and provides a functionally defined activation threshold, allowing the functioning of the brain in specific task-dependent contest, and suppressing neural activities that could interfere.

The modulation between inhibitory interneurons and excitatory cells are reciprocal. Clearly interneurons inhibit the principal cells, but it should be underlined that principal cells are in turn exciting the interneurons. This is explained by the high connectivity among excitatory and inhibitory neurons. A single interneuron can inhibit > 50 % of principal cells located within ~100 μm and receive their excitatory input (Isacsoon & Scanziani 2011). Therefore, when inhibitory interneurons are activated by excitatory neurons local network, and in turn influence neural circuits with inhibition,

this phenomenon is called feedback or recurrent inhibition. Moreover, even subcortical nuclei innervate the cortical cells by excitatory synapses that are both principal cells and interneurons, thus generating feedforward inhibitory circuits (Buzsaki, 1984). Both feedback and feedforward mechanism are explanatory of the fact that excitation and inhibition are inseparable (Isacsoon & Scanziani 2011). Furthermore, GABAergic interneurons innervate each other by highly reciprocal connections (Galarreta and Hestrin, 2002).

2.2. Inhibition and oscillatory activity

In general, inhibitory feedback connection tend to generate oscillatory activity. These rhythmic and synchronous oscillations of neuronal membrane potential characterize cortical activity and can be distinguished depending on their frequency. Specifically, inhibition seems to affect neurons' synchronization in the range of the fastest oscillations (20-80 Hz) (Atallah and Scanziani, 2009). These fast frequencies are related to the transmission of information across cortical areas, enabling neurons to cooperate in the depolarization of common targets, and consequently in the propagation of neuronal signals.

The characteristic features of interneurons explain their involvement in the generation of synchronized oscillations. Since the interneurons are electrically coupled via gap junctions, a large number of interneurons can be synchronized (Hestrin and Galarreta, 2005; Bennet & Zukin, 2004). Gap junctions not only synchronize electrical activity, but also allow metabolic coupling and chemical communication. Even the strength of oscillation seems to be due to the fact that feedback connections are guaranteed by synaptic junctions (Bartos et al., 2007; Vida et al., 2006; Galarreta and Hestrin, 2002).

One approach to investigate the role of GABA in the processing of sensory information is to study the oscillatory activity. Since oscillatory changes might characterize different sustained processing states, the study of how sensory processing is modulated by inhibitory mechanisms should focus on the dynamics of brain responses to sensory stimuli. Therefore, in my study I will investigate the

effects of inhibitory agonists on the processing of somatosensory evoked brain responses.

2.3. Inhibition Receptors and Agonists

In the central nervous system, as previously introduced, inhibition is mediated by the transmitter GABA. Presynaptic terminals release GABA that binds with three different types of GABA receptors (GABARs), such as type A, B, and C. $GABA_C$Rs are ligand-gated Cl^- channels comprised of a prokaryotic protein (rho) subunits. These receptors are primarily expressed in the retina, therefore these will not be addressed in my study (Chebib & Johnston, 1999; Chua & Chebib, 2017).

$GABA_A$Rs, are ligand-gated ion channels permeable to Cl^-, composed by three subunits alpha1, beta2, and gamma2. These subunits modulate receptor responses to GABA and its agonists such as benzodiazepines, barbiturates, picrotoxin, anaesthetic steroids, volatile anaesthetics, ethanol and penicillin (Harris et al., 1995; Macdonald & Olsen, 1994). The GABA-binding site is directly responsible for opening the Cl^- channel. The increased chloride conductance drives the membrane potential towards the reversal potential of the Cl^- ion which is about –75 mV in neurons, inhibiting the firing of new action potentials. This mechanism is responsible for the sedative effects of $GABA_A$ agonists.

$GABA_B$Rs are heptahelical receptors coupled to K^+ channels via guanosine triphosphate (GTP)-binding proteins (G proteins) composed by two subunits $GABA_{B1}$ and $GABA_{B2}$. $GABA_B$ receptors are located both pre- and postsynaptically. Presynaptically, they mediate inhibition by activating K^+ conductance and diminishing Ca^{2+} conductance. In addition, $GABA_B$ receptors may affect K^+ channels through a direct physical coupling to the K^+ channel, not mediated through a G-protein intermediate. Postsynaptically, $GABA_B$ receptor activation produces a characteristic slow hyperpolarization through the activation of the K^+ conductance. This effect appears to be mediated through a pertussis-toxin-sensitive G protein that inhibits adenylyl cyclase. GABA binds to the $GABA_{B1}$ subunit to activate the receptor and the $GABA_{B2}$ subunit is responsible for coupling to G-proteins (De-Pei, Hui-Lin, 2010).

2.3.1. GABA and Alprazolam

Making use of their enhancing GABAergic inhibition, benzodiazepines are mainly used as anxiolytics, sedative-hypnotics, muscle relaxants and anticonvulsants. Almost 10% of the population use benzodiazepine, such as Alprazolam, Chloridiazepoxide, Diazepam, Furazepam and Triazolam. The first study (Schmidt et al., 1967) about benzodiazepines and their role in the GABAergic system has shown that benzodiazepines are capable of enhancing presynaptic inhibition via $GABA_A$ receptors. Several other researches replicated these findings, and nowadays there is a general consensus that the benzodiazepines enhance the actions of GABA. In our study, Alprazolam, that is commonly used as treatment for anxiety disorders, was used as $GABA_AR$ agonist.

2.3.2 GABA and Baclofen

Baclofen is a medication used to treat muscle spasticity due to spinal cord injury or multiple sclerosis as well as hiccups and muscle spasm. Common side effects include sleepiness, weakness, and dizziness. The main effect of this $GABA_BRs$ agonist, consists in the reduction of excitatory transmitter release. The importance of the pre-synaptic GABA was investigated considering the clinical application of this drug. The Baclofen involvement in the transmitter release seems to be linked to three different mechanisms that include an increase in K^+ conductance, a reduction of Ca^{2+} conductance and a reduction of transmitter release (Lambert et al., 1991).

2.3.3. GABA and Ethanol

The effect of some drugs that act on the inhibitory mechanisms of the central nervous system depends on their doses (Brailowsky & García, 1999). High doses of Ethanol (greater than 100mM) have an anesthetic effect, whereas lower concentrations (10 to 100 mM) have intoxicating effect. Furthermore, Ethanol induces modification of sleep, locomotor activity and body temperature with a similar profile as the benzodiazepines. All these modifications are dependent from the doses and the timing of the Ethanol intake, but also from the state of nutrition of the subjects (Liljequist & Engel,

1982). Due to their similarities, several authors proposed that Ethanol and Benzodiazepines interact with $GABA_A$Rs channel with analog mechanisms. Studies with mice and human have shown synergistic effects of Ethanol and GABA (Cott et al., 1976; Banna, 1969; Nestoros, 1980). Furthermore, it was shown that a chronic consumption of Ethanol alters the properties of central GABARs (Tran et al. 1981). The acute administration of Ethanol seems to facilitate GABAergic transmission (increasing conductance of Cl- by the $GABA_A$Rs) and inhibit glutamatergic function (decreasing the cationic conductance associated with the NMDA receptor). Differently, the development of tolerance linked to the chronic consumption of Ethanol is associated with a decrease in the GABAergic function and an increase in the glutamatergic one (Brailowsky & García, 1999). Recent studies suggest that the increase in the $GABA_A$Rs function by Ethanol is linked to the inhibition of the $GABA_B$Rs (Brailowsky & García, 1999).

The evidences about the inhibitory effects of Alprazolam, Baclofen and Ethanol motivated our choice to use these GABA agonists in our study. Since they act on different receptors subtypes, differential results were expected.

2.4. Phasic and Tonic inhibition

Inhibition mechanisms could be described as "phasic" and "tonic". The term “phasic” indicates a short-lasting inhibition caused by the activation of $GABA_A$Rs as consequence of action potentials in a presynaptic interneuron (Jasper, 2012). A more long-lasting form of inhibition occurs when $GABA_B$Rs are activated by the spillover of GABA. The spillover indicates the high quantity of GABA, due to the simultaneous release of GABA by several interneurons or by the inhibition of GABA uptake. When this is the case, even GABA released by a single interneuron is enough to activate $GABA_B$Rs (Scanziani, 2000). This can occur also on cells not directly contacted by that interneuron, therefore GABA overcoming the uptake leaves the synapses and reaches $GABA_B$Rs (Scanziani, 2000).

Tonic $GABA_A$ inhibition is activated by GABA in the extracellular space that binds with molecularly

and functionally specialized $GABA_A$Rs consisting in alpha-6 or delta subunits (Farrant and Nusser, 2005). Moreover, asynchronous or spontaneous release of GABA can cause a long-lasting form of inhibition (Manseau et al., 2010).

As described above, tonic and phasic inhibition are mediated by different GABAR subtypes, respectively $GABA_B$Rs and $GABA_A$Rs. The role of these different inhibitory mechanisms, involved in the process of information elaboration in the somatosensory system, will be investigated in this study. To this aim, $GABA_A$ agonists such as Alprazolam, Ethanol, and $GABA_B$ agonist, Baclofen, were administered to investigate whether phasic (fast) inhibition, mediated by $GABA_A$, or tonic (slow) inhibition controlled by $GABA_B$ are involved in the process of information elaboration in the somatosensory system.

2.5. Inhibition in the somatosensory cortices

Cortical processing of sensory input is mediated by various contextual factors, such as attention, expectation, previously or simultaneously presented competing sensory input, and motor-dependent gating (Dietz et al., 1987; Sarter et al., 2001; Gordon et al., 2019). The study of early sensory evoked brain responses, originating from primary sensory cortices, showed that the influence of these factors manifests in a reduction of response amplitudes as compared to well-attended stimuli not involving any elaborate processing. For instance, response decrements have been described for uni- and cross-modal interactions of multiple stimuli (Meredith & Stein 1986; Huttunen et al. 1996; Giard & Peronnet 1999; Foxe et al. 2000; Calvert et al. 2004; Kayser, Logothetis 2007; Lee et al. 2013). Effects of attention and sensori-motor gating, i. e. the response decrement of somatosensory evoked responses during the execution of motor actions, appear to originate from GABA-ergic modulations on the level of the thalamus (Cheng et al. 2017, Park et al. 2017, Urbain, Deschênes 2007). However, context-dependent reductions of sensory responses also suggest a possible involvement of GABA-mediated inhibitory mechanisms that modulate the neuronal circuits of primary somatosensory cortex.

Neuroanatomical and histological studies have demonstrated a high concentration of GABARs in primary somatosensory cortex (Zilles et al., 2002) suggesting their important role in the modulation of somatosensory processing. Evidence for the involvement of GABA as a mediator of sensory processing comes from studies on the modulation of the receptive field size and reorganization in SI (Garraghty et al. 1991; Desgent & Ptito 2012; Mittmann & Imbrosci 2014; Alloway &Burton 1991; Dykes & Landry 1984). In those studies, it has been shown that changes in the functional organization of SI are associated with inhibition of SI neurons that are normally under GABAergic control (Griffen & Maffei, 2014). Despite these findings, the picture of how GABA modulates early sensory processing in primary and

secondary somatosensory cortices is rather incomplete. In particular, our knowledge about the involvement, the functional role and the dynamics of different GABARs in the processing of tactile input is still poor.

The presented study firstly focuses on the role of specific GABARs in the processing of sensory stimulation. In detail, it is aimed to identify the GABA agonists that modulate sensory process. Depending on the involvement of $GABA_AR$ or $GABA_BR$ in primary (SI) and secondary somatosensory cortex (SII) the type of the underlying inhibitory mechanisms whether tonic or phasic inhibition will be inferred. Secondly, the dynamics of GABAergic modulation in these cortical areas and their temporal interactions are investigated. Specifically, three alternative scenarios on how inhibition could affect SI and SII were compared. The first hypothesis is that the inhibition on SI is forwarded to SII. The second is that inhibition is directly acting at the level of SII, and the third is the case of a combination of both mechanisms.

In this experiment, participants were involved in eight different sessions in which they received GABA agonists (each in two randomly assigned sessions). Each session included a baseline resting-state magnetoencephalography (MEG) measurement, followed by the administration of GABA agonists and after 90 minutes, a post-drug resting-state MEG measurement. Afterwards, the somatosensory evoked fields (SEFs) were measured for tactile stimuli presented to the index (D2) or middle finger (D3) of the left hand, using a piezo-electric tactile stimulator (in-house constructed stimulator: Li Hegner et al., 2007-2010; Wühle et al., 2010-2011; Weisz et al., 2014). Both single and double stimulation trials were performed. However, in this thesis, I refer only to the single stimulation trials results. In the double stimulation trials, the first stimulus was presented either to D2 or to D3, with near threshold or maximal intensities, differently the second stimulus was always presented to D2 with maximal intensity. In single stimulation trials, in which either D2 or D3 was stimulated, the intensity was either at maximal intensity or at threshold intensities.

Near-threshold intensities were determined during the experiment through an adaptive procedure. There are different methods to realize stimulation at the perceptual threshold. The threshold can be either be determined beforehand and then the threshold stimulation intensity is applied afterwards, during the main experiment investigating the processing of near-threshold stimuli. Alternatively, the threshold intensity is adapted continuously during the experiment to stay around the threshold throughout the whole examination. The adaptive approach is considered more efficient since firstly it compensates any sensory adaptation, that is reflected in a reduced sensibility to a stimulus when presented several times at the same intensity. Importantly, threshold can also be affected by changes in bias, a tendency toward a specific answer. While bias correction is possible for some procedures, it is still not commonly used in adaptive procedures. Secondly, the stimuli intensities are always slightly above or below the finally estimated threshold. This means that less trials should be needed for an accurate calculation of the threshold (Watson and Fitzhugh, 1990). This issue will be the target of the second article here presented.

In the current study, depending on whether the participant's response to a first near-threshold stimulus was correct or incorrect, the intensity in the next near-threshold stimulation was decreased or increased, according to a one-down-one-up rule. Therefore, incorrect responses resulted in an increase of the stimulation intensity by 20 μm (the maximal stimulation is fixed to 1mm), while the intensity was decreased of the same amount in case of a correct response.

In order to find out whether GABAergic inhibition acts on the level of SI and SII, it was estimated the relation between SI and SII activation before GABA agonists administration and therefore could determine the activity-dependent transfer function between SI and SII. The baseline SI-SII activation is compared to the situation resulting from the administration of GABA agonists. If SI activation is reduced also a reduction might be found on the level of SII. However, if the activity in SII is not further diminished by additional inhibitory processes, then

the relation between SI and SII activation found without any GABA agonist should not be affected by the administration of a GABA agonist. Any change of the ratio will demonstrate that additional inhibitory processes take place between the propagation of activity from SI to SII or on the level of SII. In order to get a rough estimate of the transfer function, three points were determined: zero, near-treshhold and above threshold.

In our analysis, the effect of each GABA agonist was compared with a Placebo condition. The findings of our experiment suggest firstly, that GABAergic modulation occurs predominantly at the level of SI, involving the fast reacting $GABA_A$Rs, that specifically binds to Alprazolam. Secondly, data show that the inhibitory effects on the level of SII seem to be due to the propagated inhibition from SI to SII.

Analyzing the effects of inhibition on the perceptual task, our behavioural data did not reveal any change of participants' sensory thresholds, although the neurophysiological effects of Alprazolam on information processing at the level of SI and SII were shown. Furthermore, only a weak effect on reaction times towards a prolongation for Alprazolam as compared to Placebo could be demonstrated. The effect of GABA on the modulation of information processing in SI and SII was evidenced by the somatosensory evoked magnetic field data. It was shown that Alprazolam, the fast GABA agonist is the main responsible for inhibition in the sensory cortex and also in the transmission of the inhibitory effect from the SI to SII in the proposed model. These results significantly underline the importance of combining neuropharmacological and neuroimaging data while investigating psychophysiological measures. Indeed, thanks to this approach, it was revealed that there could be a discrepancy between behavioural and brain data. Specifically, behavioural results - that are affected by sensory processes, decision making and motor reactions – appear not to be sensitive enough to capture the specific neural mechanisms taking place.

2.6. Perceptual threshold calculation

Sensation and perception refer to different processes linked to the continuum of activation and elaboration of information. Sensation is related to the stimulus' activation of sensory receptors, it can therefore be described as a bottom-up process. Differently, since perception implies the selection, categorization and finally interpretation of this sensory input, it can be classified as a top-down process. In other words, sensation is a physical process related to the mere activation of sensory cells. Implying the elaboration of the sensory input, connecting it with memories, emotions, expectations, the perception is a psychological process (Mechelli et al., 2004; Pourtois et al., 2012; Zhang et al., 2014; Kveraga et al., 2007; Gilbert & Sigman, 2007; Gilbert & Wu, 2013; Gilbert & Li, 2013; Gazzaley & Nobre, 2012; Deseilles et al., 2011; Bar et al., 2006). Moreover, perception presupposes sensation, but sensations might also not result in perception. The last is also the case of sensory adaptation, that occurs when a stimulus is presented with a constant intensity for a long period of time and cause a decrease in sensitivity of the receptors, making the stimulus less noticeable (Mc Burney & Balaban 2009; Warks et al., 2007). Sensory adaptation has been reported for loud noise, high temperatures or strong scents (Binder et al., 2008; Webster, 2012). Sensory adaptation also happens when the intensity of stimuli is decreased and receptors then increase their sensitivity, this is the case of pupils that dilate to capture more light (Laughlin, 1989).

However, neural adaptation is strongly linked to stimulus intensity, e.g. the intensity of a light increases, senses will adapt more strongly to it (Groves & Thompson, 1970; Rankin et al., 2009, Cevik, 2014).

In order to study the processing of near-threshold stimuli, the stimuli should be at the threshold throughout the whole experiment. In order to avoid that the processing is altered by adaptation or learning the threshold should be tracked and the stimulation should be adjusted accordingly. In the second study presented here, I developed a new adaptive procedure which I applied in a

backward masking paradigm with emotional faces as stimuli. In this paradigm, emotional faces (prime) are presented and then masked by a neutral face (mask). In order to investigate the difference of perceived and non-perceived facial expressions of emotions, the delay between the prime and mask is presented at the threshold. As a result, the emotional valence of the faces is perceived in a well-defined percentage of trials and then it is compared to trials in which the valence could not be correctly identified.

The minimum and sufficient level at which a stimulus can be detected by the sensory system, is defined as an absolute threshold (Okawa & Sampath, 2007, Galanter, 1962). Nevertheless, although stimuli are not consciously perceived when presented below the absolute threshold, they might still be processed subliminally and can elicit a behavioural response (Kunst-Wilson & Zajonc, 1980; Rensink, 2004; Radel, Sarrazin, Legrain, & Gobancé, 2009; Loersch, Durso, & Petty, 2013).

To estimate the threshold of perception, different methods have been developed, such as method of limits, method of constant stimuli, adaptive methods (maximum-likelihood procedures and staircase procedures). It is important to distinguish between threshold estimation procedures to determine the thresholds and situations in which the aim is to stimulate always at the threshold. In the second application, only the adaptive methods can be used, since the stimulus level is constantly adapted to the threshold value.

In the Method of Limits (Gescheider, 1997; Herrick, 1967; 1972; 1979), firstly the subject is stimulated by easily detectable stimuli. The level of the stimuli is decreased in a stepwise fashion (descending sequence) until the subject cannot detect them anymore. Then, another sequence of stimuli is presented, this time starting from non-detectable stimuli (ascending sequence) towards the detectable level of stimulation. Both sequences are repeated several times. The procedure yields several momentary threshold values. In a following step, mean values are calculated for ascending and descending sequences separately. In the final step,

averages of the previously calculated means will result in the absolute threshold.

Another classical procedure is the method of constant stimuli (MCS) (Laming & Laming, 1992; McKee, et al., 1985; Treutwein, 1995), in which a set of preselected stimuli is presented with parameters that are in an interval that ranges from 0 % to 100 % of the possibility to be perceived. A stimulus at a specific level of the interval is randomly presented a defined number of times per session. After the stimulus presentation, the participant of the experiment will indicate if the stimulus was perceived or not. The psychometric function is sampled by evaluating the percentage of detected stimuli for each level of the stimulation. The function usually shows a steady increase in performance correlated to the increased stimulus intensity. For estimating the threshold in the method of constant stimuli, a sigmoid function is fitted to the performance values of the entire range of stimulation levels. Since the range of stimulus levels leading to 0 and 100 % perception, a high number of trials will be needed to make sure that the entire range of stimulus levels is sufficiently covered. A high number of trials implies that the investigation is time demanding. In this article, the method of constant stimuli will be considered as reference to which compare the results of our proposed method.

In order to reduce the time for estimating the threshold, adaptive methods have been developed (Leek, 2001). Typically, an adaptive method implies that the stimulus level for each trial is chosen according to the performance of stimulus perception in the previous trial or trials (Treutwein, 1995; Watson and Pelli, 1983). In this way, the stimuli are mostly presented at a level that is around the individual´s threshold value (Levitt, 1971) and less time is wasted exploring stimulus perception at levels distant from the threshold.

Lately, different adaptive methods have been proposed (Watson & Pelli, 1983; Treutwein, 1995; Leek, 2001). They can differ in various parameters, such as the step size between the stimuli (the amount of difference between consecutive stimulus values), the initial stimulus level used in the first trial, the rule chosen to determine the stimulus parameters administered

in the subsequent trial, and the stopping rule (the decision for ending the process) (Leek, 2001). Since the rule of stimulus selection in the next trial is most critical, it is used to categorize the different procedures.

The maximum-likelihood procedures (Rossi, 2018), are characterized by stimulus placement on each trial, driven by consulting the current best estimate of the entire underlying psychometric function after every stimulus–response trial. As the adaptive track grows in length, the estimated function becomes better defined by the collection of data points generated from previous trials. After each trial, the set of stimulus levels and the proportion of correct responses associated with each level are combined to form a psychometric function. The individual points are fitted with an ogival function and a current estimated threshold level is extracted. A new psychometric function is generated after each trial or set of trials, and subsequent trials are placed at a targeted performance level on the most up-to-date function.

Among the most used maximum-likelihood procedures there are PEST (parameter estimation by sequential testing) (Taylor & Creelman, 1967), QUEST (Quick Estimate of Threshold) (Watson and Pelli, 1983; King- Smith et al., 1994), ZEST (Zippy Estimation by sequential Test) (Treutwein, 1995).

Finally, staircase procedures (Treutwein, 1995; Garcia-Perey, 1998) use one or more previous responses to select the next trial placement. In a simple up–down staircase procedure, the stimulus level is reduced when in the previous trial the subject 's response perceived while it is increased when the response is not perceived.

In the calculation of the sensory threshold, among the factors that can affect the result, there are the biases (Macmillan & Creelman, 1990; Higgins & Green, 2011). There are several types of biases that can occur at different stages of perceptual processing: at the sensory level (e.g. due to sensory adaptation), at the decision making level (e.g. due to a preference of one stimulation condition over another), the response selection level (e.g. a general preference to

rather respond with the right than with the left hand in bimanual response tasks). Accordingly, any of the aforementioned internal processing biases is able to significantly distort threshold estimates. Consequently, I was wondering whether bias correction could be implemented in adaptive threshold detection procedures. The observer's bias can be reduced by an appropriate design of the threshold detection experiment or it can be corrected during subsequent data analysis. In the experiments where only the sensitivity and the biases are of interest, they can be studied offline. Differently, when the experimental procedure requires to stimulate at the threshold throughout the whole experiment then only an online procedure is meaningful. Therefore, I designed an adaptive procedure for online bias correction. The introduced threshold procedure will thus account for the subject's processing bias and provide a stimulation at the subject's sensitivity.

The bias correction procedure will be illustrated through different simulations. Simulations are based on the sensory decisions of a virtual observer with defined sensory capabilities and biases. Results of the simulations obtained by the proposed AM procedure were compared to the results of the well-known method of constant stimuli, used here as a model of reference. The methodological comparison was done either with or without bias correction. The simulations gave us the possibility to test the effects of different biases using a high number of trials. By systematically exploring the effects of bias it was possible to determine the conditions under which the new method could be advantageous and determine its limits.

3. Study I: Inhibition in the somatosensory system: an integrative neuropharmacological and neuroimaging approach

Abbreviated title: **GABA receptor-dependent activation of SI and SII**

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3.1. Abstract

The presented study investigates the functional role of GABA in somatosensory processing, using a combined neuropharmacological-neuroimaging approach. Three different GABA agonists ($GABA_A$: alprazolam, ethanol; $GABA_B$: baclofen) were investigated in a double blind cross-over design in 16 male participants, accomplishing a tactile perception task. Somatosensory evoked magnetic fields modulated by GABAR-agonists and placebo were recorded using whole-head magnetoencephalography. Peak latencies and amplitudes of primary (SI) and secondary (SII) somatosensory cortex source activities confirmed the previously reported role of GABA as a modulator of somatosensory processing. Significant inhibitory effects on the latency of SII and on the amplitude of SI and SII were found exclusively for alprazolam, a positive allosteric modulator at $GABA_A$ receptors. The $GABA_B$ agonist baclofen did not have any modulatory effect.

Moreover, we investigated whether the observed effects of alprazolam on the level of SII were explainable by the mere propagation of activity from SI to SII modulated by $GABA_A$ receptors, independently from any further $GABA_A$-mediated inhibition in SII. By estimating the transfer function between SI and SII activation under placebo conditions, we were able to predict SII activity for the administration of GABA receptors agonists under the assumption that GABA exclusively acts at the level of SI. By comparing measured and predicted data, we propose a model in which the initial activation of SI is modulated through $GABA_A$ receptors and subsequently propagated to SII, without any significant further inhibition. In addition, initial $GABA_A$ effects in SI appear to be strongly potentiated with time, selectively in SI but not in SII.

Keywords: GABA, MEG, alprazolam, baclofen, ethanol, cortical inhibition, tactile stimulation, somatosensory cortex

3.2. Declaration of contributions

1) Chiara Fioravanti, Christoph Braun and Ulf Ziemann conceptualized and designed the experiment.

2) Chiara Fioravanti, Margherita Carboni and Cecilia Mazzetti conducted the experiment.

3) Margherita Carboni and Diljit Singh Kajal performed the analysis.

4) Chiara Fioravanti and Christoph Braun wrote the manuscript.

6) This work was revised by Christoph Braun and Ulf Ziemann.

3.3. Acknowledgements

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3.4. Introduction

Cortical processing of sensory input has repeatedly been shown to be mediated by the inhibitory transmitter GABA (Dykes et al., 1984; Edden et al., 2012; Dehner et al., 2004; Cheng et al., 2017). Indeed, neuroanatomical and histological studies have demonstrated a high concentration of GABA receptors (GABAR) in SI (Zilles et al., 2002) advocating an important role of GABA receptors for the modulation of somatosensory processing. Despite abundant research showing an involvement of GABAR in the processing of somatosensory information (Cheng et al., 2017; Park et al., 2017; Urbain and Deschênes, 2007; Garraghty et al., 1991; Desgent and Ptito, 2012; Mittmann and Imbrosci, 2014; Alloway and Burton 1991; Dykes et al., 1984; Griffen and Maffei, 2014), the picture of how GABA modulates early sensory processing in primary (SI) and secondary (SII) somatosensory cortex is still incomplete. In particular, our knowledge about the involvement, the functional role and the dynamics of the different types of GABARs in the processing of tactile input is rather limited.

In the cortex, there exist two types of GABA receptors: $GABA_AR$ and $GABA_BR$. In this study, we assessed the role of the $GABA_AR$ modulators alprazolam and ethanol and the $GABA_BR$ agonist baclofen for the processing of somatosensory information in SI and SII. Besides the somatosensory and many other systems, $GABA_ARs$ are involved in the control of saccadic eye movements. Thus, the slowing of the visually-guided saccadic peak velocity (SPV) has been suggested as a behavioral proxy of drug-enhanced neurotransmission through the $GABA_AR$ (Blom et al., 1990; De Haas et al., 2010; Roche and King, 2010).

The second receptor type, $GABA_BR$, has modulatory binding sites for baclofen, a derivative of GABA used for the treatment of spasticity (Nayeem et al., 1994; Sieghart, 1995; Ducic et al., 1993). $GABA_ARs$ and $GABA_BRs$ not only differ in their molecular structure, but also in their mechanisms of operation and their inhibitory kinetics (Connors et al., 1988; Deisz, 1999). A

fast inhibitory postsynaptic potential (IPSP) is related to the activation of $GABA_AR$s. Conversely, slow IPSPs are mediate by $GABA_BR$s.

The current study investigates the functional involvement of these two GABAR types in modulating perceptual processing in somatosensory cortices, through a combined neuroimaging and pharmacological approach. To this end, we administered $GABA_AR$ modulators, alprazolam and ethanol, the $GABA_B$-receptor agonist baclofen, and a placebo in different sessions in order to study participants' performance in a tactile detection task.

We administered two different $GABA_AR$ modulators since while alprazolam is a positive allosteric modulator for subunits α1, α2, α3 and α5, ethanol is an agonistic modulator for $GABA_AR$ with subunit type α4 and α6 (Rudolph & Möhler, 2014).

Processing of tactile stimulus information in SI and SII was inferred by concurrent recordings of neuromagnetic brain signals. Based on the time constants of sensory processing in SI (below 150 ms: Wühle et al., 2011) and SII (ranging from 150 ms to more than 1 s: Wühle et al., 2010) and the pharmacokinetics of the different GABAR agonists we hypothesize the involvement of $GABA_AR$ for the fast and early processing in SI and $GABA_BR$ for the slower, subsequent processing in SII. Efficacy of our $GABA_A$-ergic intervention was verified by measuring saccadic peak velocity (SPV) (Holmqvist et al., 2011), a sensitive indicator of $GABA_A$.

In the GABA-mediated inhibition, the contribution of direct inhibitory mechanisms, on high levels of the somatosensory afferent pathway, cannot easily be distinguished from indirect mechanism that are only propagated from lower to the higher processing levels. Any GABAergic mediated suppression of SII activity could be due to a) inhibitory effects directly acting at the level of SII, b) a response decrement occurring at the level of SI or earlier that is only propagated to SII, c) by a combination of both mechanisms. To disentangle potential mechanisms modulating SII activities, we firstly investigated the propagation of activity from SI to SII for the placebo condition. Assuming no additional inhibition occurring after processing in

SI, the resulting *transfer function* allowed us to predict the activity in SII based on the activity in SI also in case of administering GABAR agonists. Comparing the predicted and measured activities, we delineated a model describing the modulation dynamics of SI activity and its propagation to SII.

3.5. Methods

3.5.1. Participants

Sixteen right-handed male volunteers (mean age ± sd: 27.4±4.3 years) participated in the study. Female participants were excluded to avoid menstrual cycle-related effects on cortical activity (Smith et al., 1999, Premoli et al., 2014a). Excluding criteria were: I) neurological and psychiatric diseases, II) history of drug abuse (including nicotine and alcohol), III) previous prescription of alprazolam or other benzodiazepines, or baclofen within one year prior to the study, IV) previous prescription of anxiolytics or hypnotics, medicine to lower high blood pressure or diuretics, levodopa, lithium, non-steroidal anti-inflammatory drugs, tricyclic antidepressants, memantine, and anesthetics, V) muscular weakness, lung or liver disease. The study was approved by the Ethics Committee of the Medical Faculty of the University of Tübingen. All participants gave written informed consent prior to study enrolment.

3.5.2. Experimental Procedures

The investigation was designed as a double-blind, double-dummy randomized placebo-controlled study. The double-dummy design was necessary because the participants were tested on off-the-shelf formulations of alprazolam and baclofen given as tablets, and ethanol given as drink, in a placebo-controlled randomized design.

Subjects participated in eight different sessions. All subjects received GABAR agonists alprazolam, baclofen, ethanol, and placebo in a within subject design. Each of the four conditions was repeated once in a subsequent session resulting in 4 x 2 sessions. To prevent carryover

effects from one session to the next, a minimum gap of 3 days was scheduled between two successive sessions. Carryover drug effects between sessions can be safely excluded if 5 half-lives are exceeded, which is the case with all tested drugs (see Table 1 for information on pharmacokinetics) (see Table 3) (see Table 2).

The sequence of conditions was balanced across subjects. Alprazolam, baclofen, and placebo were provided in form of tablets. A total of three tablets was given in each session. At least one of the tablets was a placebo tablet. In detail, one tablet of 1 mg of alprazolam was administered together with two placebo tablets. Two tablets of 25 mg of baclofen were given together with one tablet of placebo. In the ethanol and placebo sessions, 3 tablets of placebo were distributed (Table 1 and Table 2). The ethanol dose leading to a calculated blood alcohol concentration of 0.8 ‰ was administered as a drink, diluted with lemon soda. The amount of soda was adjusted according to the participants' body weight ((0.55 *W)/(ρ *c/100)) where W is the body weight in kg, ρ is the density of alcohol (0.79 g/ml), and c is concentration of alcohol in percent (90 %) (Widmark, 1981). A beverage of 350 ml lemon soda without alcohol served as control for the ethanol intervention and was offered in all sessions in which ethanol was not the tested drug. Although the sugar (8.1g/100 mL) contained in the 350 ml of the lemon soda might have compensated inhibitory effects of GABAR agonists, it cannot account for the differential effects found for Alprazolam in the current study.

At the end of each session, the participants compiled a questionnaire. It was asked which type of medicament they thought to have taken in each session. The results showed that the subjects' guessing of the undergone intervention was not better than chance. Drug dosages were taken from our previous studies that demonstrated effects on motor cortex excitability in transcranial magnetic stimulation induced motor evoked potential and EEG potential recordings (Ziemann et al., 1995; Lücke et al., 2014; Fuhl et al., 2015; Premoli et al., 2014a; Premoli et al., 2017).

After a baseline resting-state magnetoencephalography (MEG) measurement, alprazolam, baclofen or placebo were given to the participants. A post-drug resting-state MEG measurement after drug intake was performed 90 min later when the alprazolam and baclofen reached their peaks of blood concentrations (Premoli et al., 2014a; Premoli et al., 2017). Due to its faster pharmacokinetics, the ethanol and thus also the placebo drink was administrated 60 min prior to the post drug measurement, i.e. 30 min after intake of the tablets (Fig. 1).

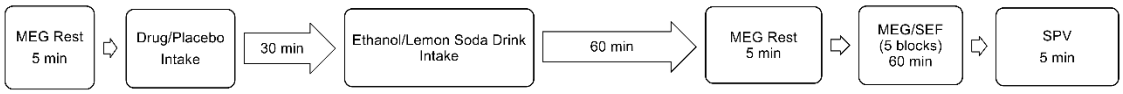

Fig.1: Study design: prior to and after drug/placebo intake brain resting-state activity was recorded by MEG (baseline and post-drug measurement, respectively). Thereafter, changes in the processing of somatosensory stimuli were investigated by stimulating index and middle finger of the left hand. In the present study only somatosensory evoked magnetic fields (SEF) to single finger stimulation are reported. Finally, visually-guided saccadic peak velocity (SPV) was measured by electrooculography as a marker of drug-induced $GABA_AR$-mediated sedation (Blom et al., 1990; De Haas et al., 2010).

Table 1: Drug characteristics. While baclofen was administered in form of 2 tablets, alprazolam was provided as a single pill. In total, there were 6 different placebo tablets differing in size, shape and color. Depending on the condition, one or two different types of placebo were given to participants. Ethanol was dissolved in lemon soda in order to cover the smell and taste of alcohol. A drink of 350 ml of pure lemon soda served as placebo for the ethanol cocktail.

Drug	Code	Brand name and mode of action	Latency of peak	Half-life
	Pla1 Pla2 Pla3 Pla4 Pla5 Pla6 Soda	Lichtenstein, pill, round, 8 mm, white, kerf Lichtenstein, pill, round, 7 mm, white, kerf Winthrop, dragee, round, blue, flat Winthrop, pill, round, 8 mm, blue, kerf Fagon, pill, oval, white, flat Caelo, pill, oval, 17x8mm, white, flat 350 ml lemon soda		
Baclofen	Bac	Lioresal® Agonist at the $GABA_BR$	1 h	3-4 h
Ethanol	Eth	90 % alcohol Agonist at the α4- and α6-subtypes of the $GABA_AR$	½ h	120 mg/kg/h
Alprazolam	Alp	Alprazolam-ratiopharm® Positive allosteric modulator at the α1-, α2-, α3-, and α5-subtypes of the $GABA_AR$	1 h	11.2 h (6.3–26.9 h)

Table 2: Administration of drugs and placebos. In all conditions participants received 3 tablets and a drink of 350 ml lemon soda drink. While baclofen was administered in form of 2 tablets and 1 additional placebo, alprazolam was provided as a single pill together with two placebo tablets. In the placebo and in the ethanol condition 3 placebo tablets were administered, two of one type and one of another type. Thus, in all conditions participants received a 350 ml drink and 3 pills of two different types.

Condition	Administration	
	Dosage	Additional Placebo
Placebo	3 tablets: 2 x Pla5 and 1 x Pla6 350 ml of lemon soda	
Baclofen	2 x 25 mg tablet	1 tablet: Pla2 350 ml lemon soda
Ethanol	350 ml drink: 0.55 g/kg ethanol dissolved in lemon soda	3 tablets: 2 x Pla3 and1 x Pla4
Alprazolam	1 mg tablet	2 tablets: Pla1 350 ml lemon soda

After the administration of the drugs or placebo, somatosensory evoked fields (SEFs) were measured for tactile mechanical stimuli that were presented to the index (D2) or middle finger (D3) of the left hand, using a piezo-electric tactile stimulator (in-house constructed stimulator: Li Hegner et al., 2007-2010; Wühle et al., 2010-2011; Weisz et al., 2014). The piezo-electric stimulator consists of a control unit and two stimulation modules that were attached to the fingertips of the index (D2) or the middle finger (D3). Each module housed eight rods which were arranged in a 2 x 4 matrix and which can be protruded individually in a graded fashion. In the experiment only the four inner rods of the matrix were used (Fig. 2). The stimulus duration (σ)

was 0.05 s. The rotation of the fixation cross (x) appearing 0.85 s (single stimulation) after the end of the stimulation indicated that the subjects could report the number of stimuli perceived by button press (response time: up to 1.6 s). The sound created by the piezo-electric stimulator was attenuated by placing the hand together with the stimulator under a thick cotton blanket and by masking the sound with white noise presented via plastic tubes and foam ear plugs. The intensity of the white noise was adjusted such that the sound of the stimulator could not be recognized.

One stimulation session consisted of 5 blocks with 320 trials each. There were single (timing depicted in Fig. 2) and double stimulation trials (timing not shown). In this paper, we refer only to the single stimulation trials. In 60 % of the 1600 trials per session a paired pulse stimulus was provided. In the remaining 40 % of trials, single stimuli were delivered. While in the paired pulse stimulation, the first stimulus was presented either to D2 or to D3 of the left hand with near threshold or maximal intensities the second stimulus was always presented to D2 with maximal intensity. In single pulse trials, in which either D2 or D3 was stimulated, stimulation intensity was either at maximal intensity or at threshold intensities. For each condition 160 stimuli were applied except for double stimulation trials at threshold intensities for which 320 stimuli were presented (see Table 3).

The maximal stimulation intensity resulted in a skin indentation of 1 mm. Near-threshold intensities were determined during the experiment through an adaptive procedure, separately for D2 and D3 stimulation. Depending on whether in a paired pulse trial the response to a first, near-threshold stimulus was correct or incorrect the intensity in the next near-threshold stimulation of the same finger was decreased or increased according to a one-down-one-up regime. Incorrect responses resulted in an increase of the stimulation intensity by 20 µm. In contrast, the intensity was decreased by 20 µm in case of a correct response. Following this procedure, the threshold intensity asymptotically reached a performance level corresponding to 50 % cor-

rect responses. Participants were asked to indicate the number of tactile stimuli they had perceived via button presses with their right hand at the end of each trial. Since the potential number of perceived stimuli ranged from 0 to 2, there were three response buttons. The response option 2 was only relevant in double stimulation. The option 0 was given since it is possible that the participants do not perceive any stimuli if their intensity is below their subjective perceptual threshold (Fig. 2). Depending on the current stimulation condition and the participants' response, it was decided whether the response was correct or incorrect.

Table 3: Number of stimuli for the different stimulation conditions. In the current study, only results for the single pulse stimulation are presented.

	Single pulse stimulation				Paired pulse stimulation				Total
Intensity	Maximum		Threshold		Maximum		Threshold		
Stimulation site	D2	D3	D2	D3	D2	D3	D2	D3	
Number	160	160	320	320	160	160	160	160	1600
Percentage	10%	10%	20%	20%	10%	10%	10%	10%	100%

In the present study, it was of interest to what extent the involvement of GABAR-mediated inhibition can be demonstrated differentially in SI and SII using a neuroimaging approach. In particular, the study aimed at the detection of differential effects of $GABA_AR$- and $GABA_BR$-agonists in primary and secondary somatosensory cortex. For this purpose, we focused on the stimulation of D2 and D3 with maximal and near-threshold intensities, using exclusively data from single pulse stimulation trials. Brain responses to tactile paired pulse stimulation will be reported elsewhere.

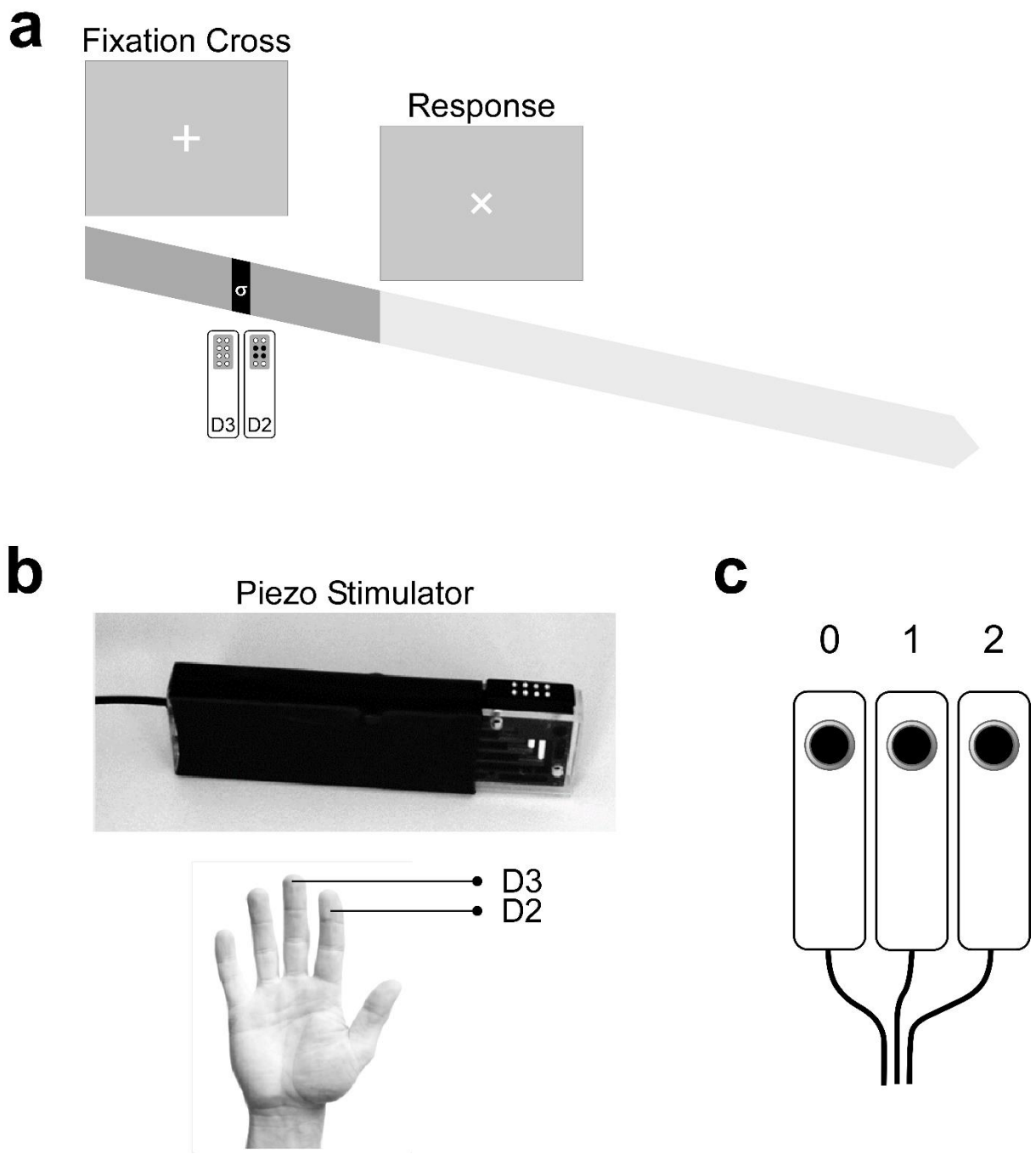

Fig. 2: In single stimulation trials, either index (D2) or middle finger (D3) of the left hand were stimulated at near-threshold or maximum intensity, using a piezo-electric tactile stimulator (b). The stimulus duration (σ) was 0.05 s. The rotation of the fixation cross (x) appearing at $\tau = 0.85$ s (single stimulation) after the end of the stimulation indicated that the subjects could report the number of stimuli perceived by button press (response time: up to 1.6 s). Response options were 0, 1 and 2 (c).

3.5.3. MEG acquisition

Neuromagnetic activity was recorded while participants were seated in an upright position, using a 275-sensor whole-head MEG system (CTF MEG, Coquitlam, BC, Canada) recording at a sampling frequency of 1172 Hz and a bandwidth of 0-293 Hz. Prior to the MEG recording, three fiducial coils were placed at the nasion, and the left and right pre-auricular points to determine the participant's head position during the MEG recording. Coil positions were continuously recorded to identify and discard measurements with head movements larger than 5 mm. Moreover, locations of fiducials were stored to allow for offline co-registration with individual T1-weighted MR images obtained for each participant. During MEG recordings, participants were asked to minimize head and body movements, to avoid movement artifacts and eye blinks (Weisz et al., 2014). Prior to each neuromagnetic measurement participants repositioned their head to the position of their first session. To this end, the participant's current head position was presented on the screen in front of them together with the position in the first session. Matching both positions enabled us to keep the same head positions across all sessions with an error of less than 5 mm.

3.6. Analysis

3.6.1. MEG data analysis

Data preprocessing included bandpass filtering (1.5 - 40 Hz), and segmentation of data into trials time-locked to somatosensory stimulus presentation. Trials contaminated with eye blinks and eye movements were discarded from further analyses. Preprocessed data were averaged across trials (Wühle et al., 2011). Effects of GABA on the somatosensory evoked responses were studied for stimuli applied to D2 and D3 at the maximal intensity. Data obtained from near-threshold stimulation of D2 and D3 were only used in the second part of the study, modeling the propagation of the inhibition from SI to SII. Data of the same drug recorded on repeated sessions were averaged to increase their signal-to-noise ratio.

3.6.2. Source analysis

Source analysis was performed to disentangle the activities originating from SI and SII. Since the extent of cortical tissue in SI and SII being activated by stimulating single fingers is focal, sources were modelled by equivalent current dipoles (Wühle et al., 2010). Source analyses for each participant were based on individual evoked magnetic brain responses, averaged across all drugs, separately for stimulation of D2 and D3. To obtain a good signal-to-noise ratio for the source localization procedure, only trials with maximal stimulation intensity were included. Individual spherical head models were constructed by marking ninety points on the head surface of each participants' MRs and fitting a sphere to them (Wühle et al., 2010).

For source analysis, we used a three-dipole model (Wühle et al., 2010), with one dipole representing SI activity contralateral to the stimulated left hand, and a pair of dipoles for SII of each hemisphere (contralateral to stimulation: SIIc; and ipsilateral to stimulation: SIIi). Since somatosensory stimulation was administered to the left hand, SIIc is in the right and SIIi in the left hemisphere. For dipole fitting, a sequential procedure was applied. First, the SI source was fitted. For this purpose, the MEG-data were filtered using a highpass of 6 Hz. The filter suppressed slow activities originating from SII. Using the original bandpass filter data between 1 and 40 Hz the SI dipole was then kept fixed and SII activities were fitted with a pair of mirror-symmetric dipoles. In order to optimize the fitting results SIIc- and SIIi-dipoles were finally adjusted individually. In order to sort out ambiguities in dipole orientation and thus polarity in the source activities across all subjects, the orientation of SI dipoles was set in anterior-posterior direction and the one of SII dipoles to inferior-superior direction.

To get an estimation of the time course of the dipole activities we calculated the leadfield L for the three different sources. The leadfield describes the contribution of each source to the sensors and depends on the location of the sources, the location of the sensors, and on the geometrical and electrical properties of the head. The measured magnetic field is the product of the leadfield and the source activity S plus residual activity ε not explained by the model (Formula 1). With

$B(m,n)$ being the magnetic field measured at m sensors and n samples, $S(k,n)$ the source activity for k sources, $L(m,k)$ the lead field and $\varepsilon(m,n)$ the model error, B can be written as (Wühle et al., 2010):

$$B = LS + \varepsilon \qquad (1)$$

An estimate of the source activity can be obtained by regression analysis. Assuming the error in equation (1) to be Gaussian, the source activity results from multiplying the measured magnetic by the pseudo-inverse of the lead field (Formula 2).

$$S = (L'L)^{-1}L'B \qquad (2)$$

To differentiate the GABAergic effects on the processing of tactile stimuli in SI and SII, we investigated latency and amplitude for all three sources in the different drug conditions. Since individual source activities might not have a clear peak structure, a cross-correlation approach was used to determine the latency of the source activity peaks. In this approach, a template waveform is compared to the individual wave shapes. The shift between template and individual waveform leading to maximal correlation is used to correct the peak latency that had been determined for the template. As templates the grand average (group average) waveforms for SI, SIIc and SIIi were used (Walser et al., 1986).

To assess the drug-induced modulation of the SI and SII source activities, we selected four fixed time points (SI:75 ms, 100 ms; SIIc: 117 ms; SIIi: 123 ms), representing the peak maxima in the grand average of the source activities averaged across all participants and all conditions (Fingers, Drugs). Amplitudes of source activities were determined and compared at these four time points for individual participants and conditions. Since the source activity for SI revealed a bimodal wave shape, two time points were defined for the SI activity. In contrast, for SIIc and SIIi only one peak latency was selected (Fig. 3). Amplitudes of source activities for all experimental conditions were determined for the selected latencies and subjected to statistical analyses.

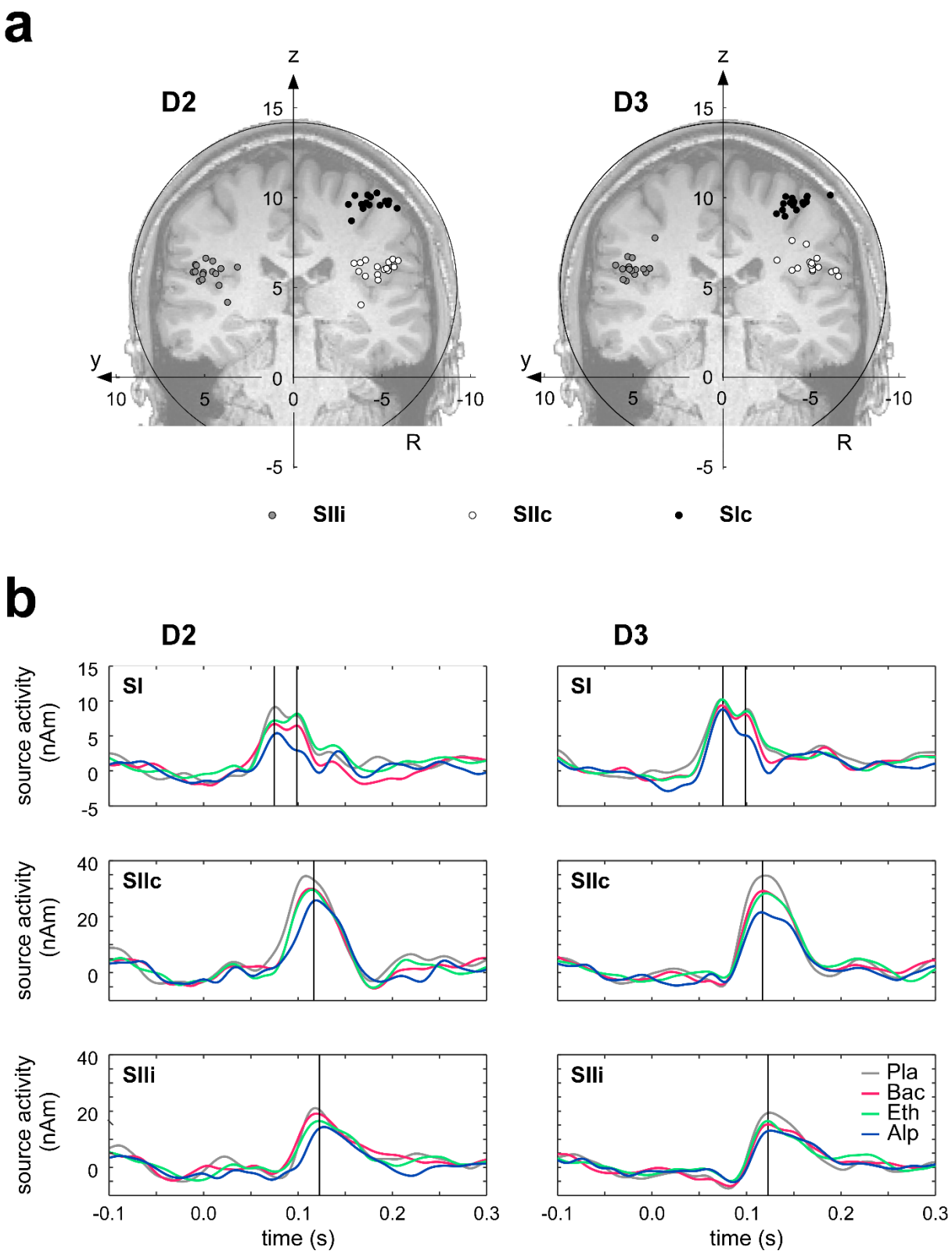
a
D2
D3
z
y
R
15
10
5
0
-5
-10
SIIi
SIIc
SIc
b
D2
D3
SI
SIIc
SIIi
source activity (nAm)
time (s)
Pla
Bac
Eth
Alp

Fig. 3: (a) Individual dipole source locations for contralateral SI (black dots), contralateral SIIc (white dots) and ipsilateral SIIi (grey dots): source localization results were warped into a common coordinate system. Dipoles for SI were verified to yield a fronto-parietal orientation in order to have the same polarity of the source activity across all subjects. For the same reason, the orientation of dipoles representing secondary somatosensory cortices pointed in inferior-superior direction. (b) Drug-induced modulations of source activity elicited by single-pulse tactile stimulation of the index finger (D2) or middle finger (D3) with maximal intensities: SI activity is depicted in the upper row. Activities of contralateral and ipsilateral SII are depicted in the middle and the bottom row, respectively. Activity waveforms for the different drugs are superimposed: *Placebo* (PLA, grey); *Baclofen* (BAC, red); *Ethanol* (ETH, green) and *Alprazolam* (ALP, blue).

3.6.3. Statistical analyses

The Statistical Package for Social Science (SPSS version 25) and StatView sas. Institute inc. version 5.0.1. were used for statistical analyses. We performed repeated measures analyses of variance (rmANOVA) with a level of significance established at $p < 0.05$ to study the effects of *Drug* (levels: Alprazolam, Baclofen, Ethanol, Placebo) on peak velocity of saccades, and the effects of *Drug* and *Finger* (levels: D2, D3) on latencies of SI and SII activations. In case of factores with more than 2 levels Greenhouse-Geisser correction was applied (Greenhouse and Geisser, 1959). The correction factor for degrees of freedom is presented as factor □ □ Prior to all rmANOVAs, we verified that variables were normally distributed using the Shapiro-Wilk test (Shapiro & Wilk, 1965).

In case of variables not being normally distributed, the non-parametric pairwise Wilcoxon signed-rank test for dependent samples was applied with a significance criterion of 5 %. In particular non-parametric testing was applied for the analyses of the mean and the variance of

reactions times across trials. Furthermore, non-parametric statistics was used to study the effects of *Drug* on SI and SII evoked brain activity.

Activity in SII: inhibition or propagated inhibition?

In the second part of the data analysis, we investigated whether the activity in SII under the influence of GABAR agonists can be explained by mere propagation of the drug-induced inhibited activity in SI forwarded to SII (Fig. 4b), or alternatively, by additional GABA-driven inhibitory modulation between SI and SII or at the level of SII (Fig. 4c). Assuming GABAR agonist induced inhibition to occur only in SI but not in SII, the propagation of inhibited SI activity from SI to SII can be delineated from the relation between SI and SII activities in the placebo condition (Fig. 4a).

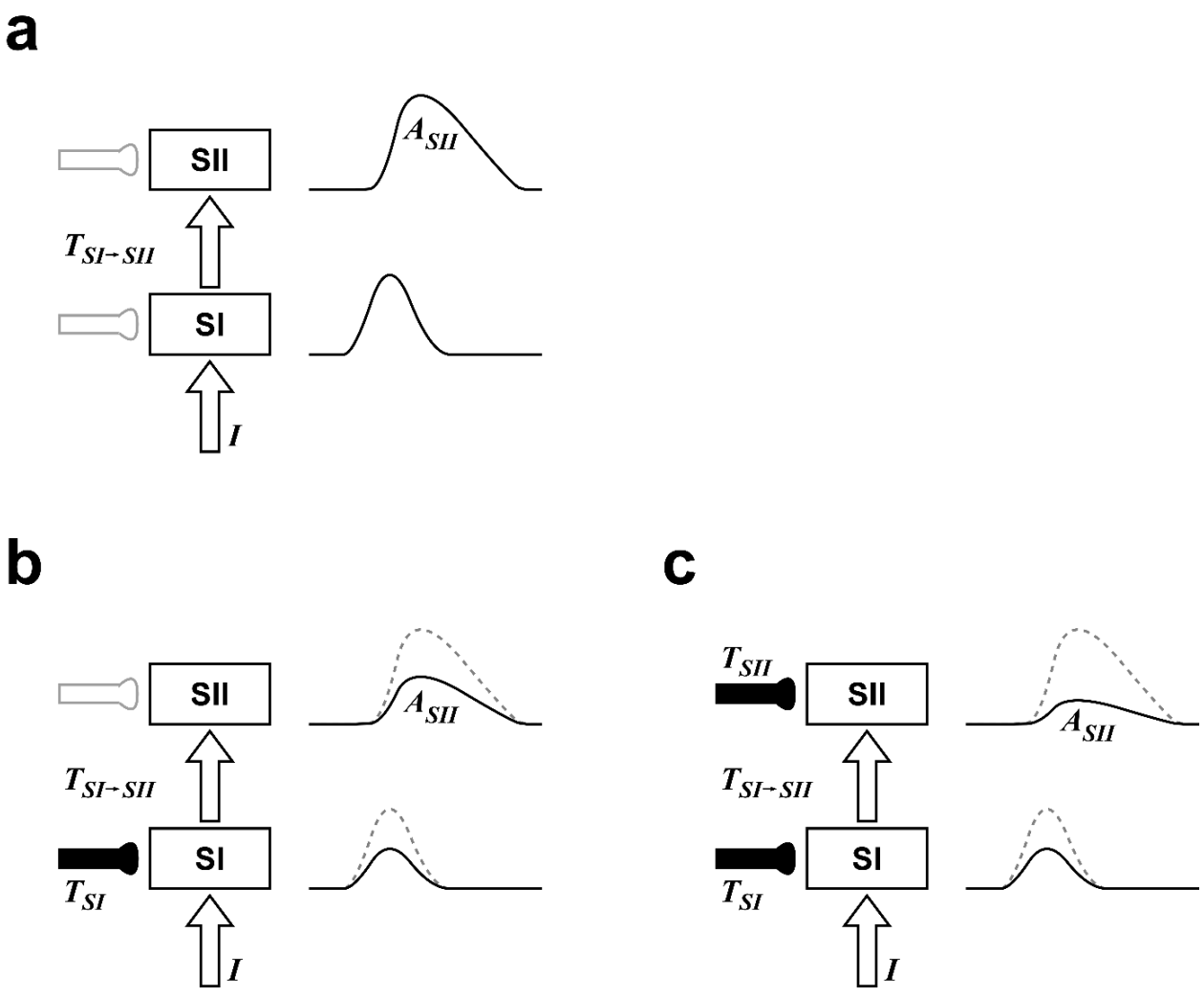

Fig. 4: Schematic sketch explaining the reduced activity in SII under the effect of GABAR-agonists. (a) Placebo-condition: no inhibition takes place at the levels of SI or SII. Input *I* to SI is propagated to SII. The relation between SI and SII activation is given by a characteristic transfer function $T_{SI \to SII}$ (Activity in SII: $A_{SII} = T_{SI \to SII} \otimes I$) (b) No additional effect of GABAR-agonists at the level of SII: sensory input I to SI is inhibited according to T_{SI} (inhibitory neuron to SI indicated in black) and propagated with the characteristic transfer function as defined in (a) to SII (Activity in SII: $A_{SII} = T_{SI \to SII} \otimes T_{SI} \otimes I$). (c) Effects of GABAR-agonists both at the level of SI and SII (inhibitory input to SI and SII indicated by solid black neurons). In addition to the propagated reduced activity of SI, the activity in SII is further suppressed by additional inhibitory input (Activity in SII: $A_{SII} = T_{SII} \otimes T_{SI \to SII} \otimes T_{SI} \otimes I$). In case of additional inhibition on the level of SII, activity in SII is less than expected by the characteristic transfer function defined in (b). Dashed source activities in b and c represent the activities under placebo. Solid wave shapes in (b-c) represent activities inhibited by GABAR agonists, selectively at the level of SI (b) or at the level of both SI and SII (c).

To test whether the SII activity induced by GABAR agonists can be explained by the propagation of the suppressed activity in SI, we determined the *transfer function* expressing SII-activity as a function of SI-activity for different stimulation intensities, independent of any administered GABAR-agonist, i.e., during the *Placebo* condition (Fig. 5). We delineated the transfer function for three stimulation intensities: zero intensity, near-threshold intensity and maximal intensity. While the relation for near-threshold and maximal intensities was determined experimentally, the selection of zero activities for both, SI and SII was based on the plausible assumption that without any stimulation no evoked responses can be recorded. Given the relation between SII and SI activities for these three stimulation intensities, the SII activity for any arbitrary SI activity was obtained by piecewise linear interpolation.

Individual transfer functions were determined for D2 and D3 using a jackknife procedure (Efron, 1981) because transfer functions of each subject were too noisy to obtain good estimates (Fig. 5a and 5b). To assess whether SII activity reflects the propagated activity of SI even in case of GABA-agonist administration or whether GABA-agonists exert an additional inhibitory influence on SII, SII activity was predicted based on SI activation and compared with the experimentally determined SII activity. Fig. 5c shows the prediction error as difference between the predicted and the measured SII activities for subject 1. Significant differences between predicted and measured data indicate that the activity in SII cannot be explained by the propagation of the activity in SI.

If x_i are the raw data for the subject i, the jackknife resampled data $\hat{x}_i$ will be $\hat{x}_i = \frac{1}{n-1}\sum_{k\neq i} x_k$. Since the jackknife procedure leads to a reduction of the within condition variance by a factor of 1/(n-1)^2, with n being the number of subjects, the jackknife resampled data need to be corrected in case of parametric statistical testing (see Appendix). Variance corrected jackknife resampled data $\tilde{x}_i$ can be obtained by transforming the jackknife resampled data $\hat{x}_i$ as follows: $\tilde{x}_i = (n-1)(\hat{x}_i - \bar{x}_i) + \bar{x}_i$, with $\bar{x}_i$ being the mean of the jackknife resampled data (see supplement).

Prediction errors were analyzed for SII activities predicted from either peak of SI source activity. The impact of the different GABAR agonists and the influence of the latency of SI on the predictability of SII activity from SI were assessed, using rmANOVA with factors *Peak* (level: first and second peak of SI source activity), *Drug* (*Alprazolam, Baclofen and Ethanol*) and *Hemisphere* (levels: left and right). Deviations of the prediction error from zero were tested individually for each drug with one sample t-tests.

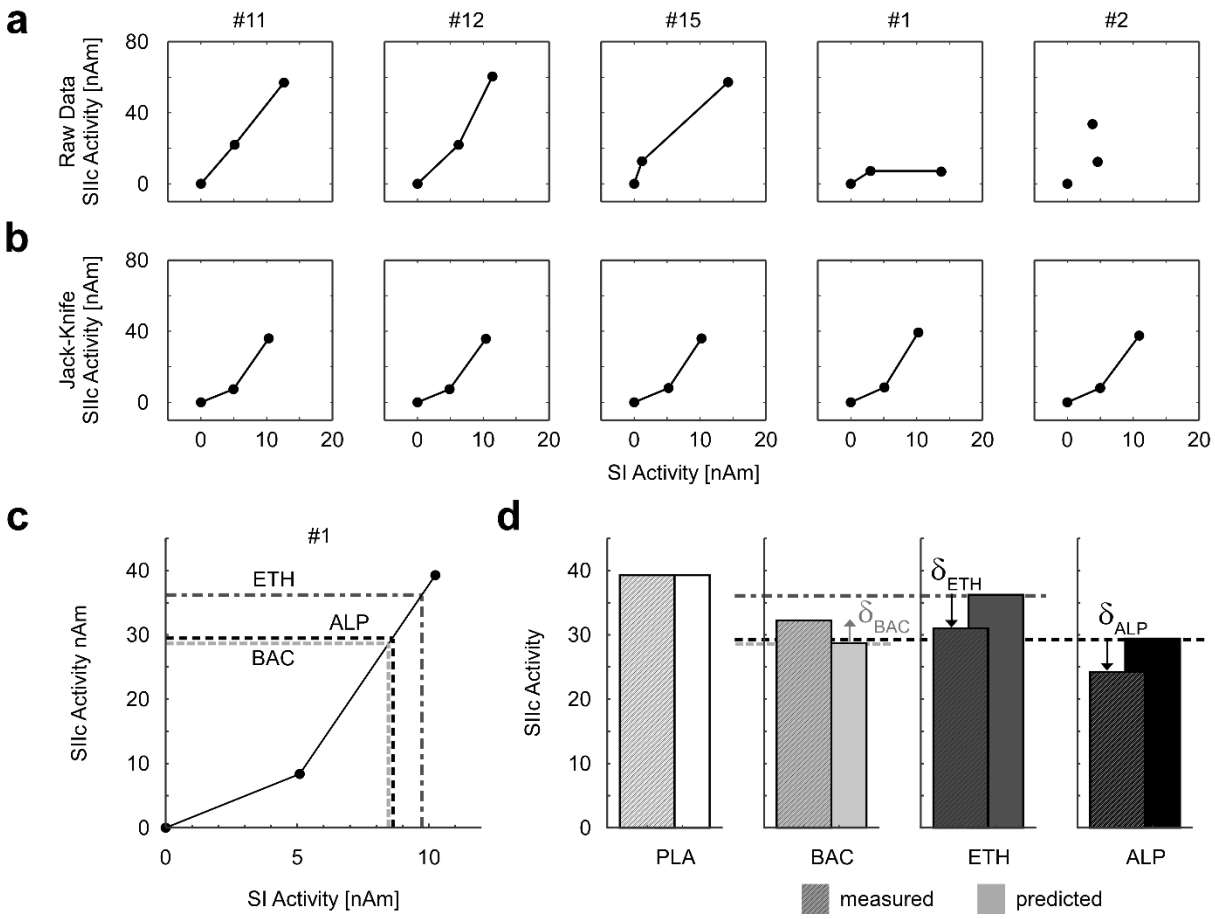

Fig. 5: (a) The figure shows examples (subjects 11, 12, 15, 1, 2) of the transfer functions between the initial SI and the SIIc peak activity for tactile stimulation of finger D3. While subjects 11 and 12 showed a steady transfer function, it was slightly distorted for subject 15 and even more distorted for subjects 1 and 2. To get a better estimate of the transfer function, data were resampled using a jackknife approach (b). In (c) the resampled transfer function (solid line) relating the SII activity to the activity of SI in case of placebo is shown for subject 1. In case of no further inhibition between SI and SII and on the level of SII, the SII activity can be predicted from the activity in SI for different GABA-agonists (dashed lines). (d) The measured (hatched) and predicted (plain) activity of SIIc for SI activities observed after administering the different drugs are shown. The predicted SII activities result from entering the GABA-agonist dependent SI activities in the transfer function in (c). The prediction error (□) the difference between the

measured and predicted SIIc activity, is indicated by the arrow (grey: measured activity > predicted activity; black: measured activity < predicted activity). In the presented example the measured SII activity for *Baclofen* (light grey and dashed line) is larger than predicted. In contrast, for *Ethanol* (dark grey and dash dotted line) and *Alprazolam* (black and dashed line) the measured SII activity is smaller than predicted.

3.6.4. Method for saccadic peak velocity analyses

Saccadic peak velocity (SPV), as a well-established measure of $GABA_AR$ activation, was derived from SPV data, recorded in a visually guided saccade task. The task was performed in the magnetically shielded room after having finished the tactile stimulation part of the study (Fig. 1). Participants sat in front of a display with an eye-to-screen distance of 40 cm. They were instructed to make saccades to a black dot jumping between three positions on the grey screen (left, middle and right) subtending a view angle of ± 11.3 degrees. Participants were asked to maintain their head in a straight position.

SPV data were lowpass filtered at a frequency of 100 Hz and segmented in windows of ± 45 ms around the saccade onset. Computing the derivative of the eye position signal resulted in the eye movement velocity. Subsequently, the peak of eye movement speed for both eyes was chosen in the period of the saccade. Peak values were averaged across trials resulting in the SPV parameter. An rmANOVA with the factor *Drug* was performed. Post-hoc comparisons between drugs and placebo identified the drugs resulting in significant slowing of SPV.

3.6.5. Behavioral data

To verify the effectiveness of the GABAR agonists, we calculated the subjects' reaction times before and after the drug intake, while they rated how many stimuli they perceived. We considered a longer reaction time as behavioral inhibition. In addition to the mean of reaction times, we also studied the variance and skewness of reaction times across trials. Furthermore, in order

to assess effects of the GABAR agonists on somatosensory perception, we studied mean, variance and skewness of stimulus intensities across trials, for near-threshold stimulation, and compared the threshold parameters between the different drug conditions.

3.7. Results

In the result section, we firstly report the effects of GABAergic modulation on somatosensory perception and response behavior. Secondly, we illustrate the effects of different GABAR agonists on the neurophysiological processing of tactile information in SI and SII. Thirdly, we demonstrate the effectiveness of alprazolam as a $GABA_AR$ agonist by presenting the results for the eye-movement task. In the last part of this section we provide evidence for the hypothesis that GABA plays a role in modulating sensory processing in SI rather than in SII. Throughout the result section means and standard errors of the means are presented unless indicated differently.

3.7.1. Reaction time results and perceptual thresholds

Although mean reaction times across trials were normally distributed, we used non-parametric pairwise Wilcoxon singed-rank tests for statistical testing in order to be consistent with the analysis of other behavioral parameters that were not normally distributed. For all drugs *(Placebo, Baclofen, Ethanol, Alprazolam)* reaction times were significantly longer for near-threshold stimulation as compared to maximal stimulation intensities even when tested two-sided (*Placebo*: p=.0038, *Baclofen*: p=.0004, *Ethanol*: p=.0023, *Alprazolam*: p=.0005, Fig. 6a). Comparing mean reaction times between Placebo and GABAR agonists for which an increase in response times was expected due to the inhibitory effects of the GABAR agonists a significant increase of the mean reaction time was only observed for *Alprazolam* at near threshold stimulation intensities (one-sided test: p=.035) but not for any other drug (Fig. 6a). For maximum stimulation intensities, only a trend towards prolonged reaction times for *Alprazolam* compared

to *Placebo* was observed (one-sided: p=.055). Variances of the reaction times across trials were not normally distributed and therefore a non-parametric Wilcoxon signed-rank test was applied. For all drugs, the variance of reaction times was significantly larger for near-threshold intensities than for maximum intensities when tested two-sided (*Placebo*: p=.0027, *Baclofen*: p=.0009, *Ethanol*: p=.0013, *Alprazolam*: p=.0004, Fig. 6b). Comparing the variance of reaction times between *Placebo* and the GABAR agonists there was only a significant difference for *Alprazolam* (low intensity: p =.0004, high intensity: p = .0004), but not for any other GABAR agonists (p>.352). Comparing the skewness of reaction times for each GABAR agonist and placebo using a non-parametric Wilcoxon signed-rank test no significant effects were found (all p >.16). Furthermore skewness of reaction times did not differ significantly for near threshold and maximal stimulation intensity (all p> .12).

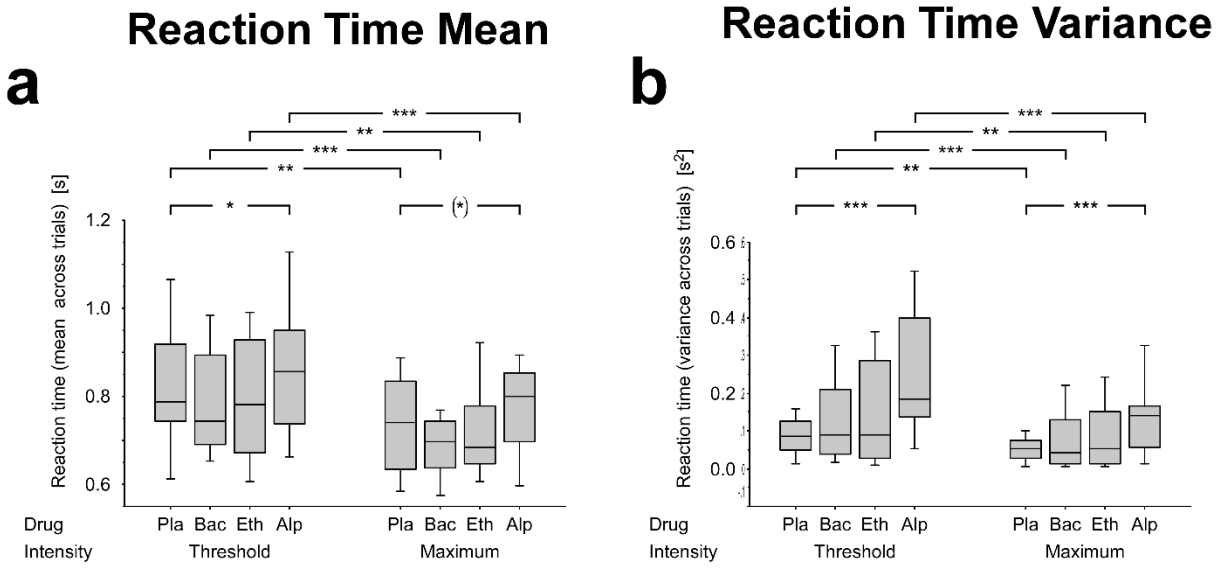

Fig. 6: To study effects of GABAR agonists, Baclofen (Bac), Ethanol (Eth) and Alprazolam (Alp) on behavior, mean (a) and variance (b) of reaction times across trials were investigated. Reaction time parameters for d2 and d3 were collapsed. Parameters are presented as box-whisker plots, with median, 1st and 3rd quartile and range. Using the non-parametric Wilcoxon

signed-rank test, effects of GABAR agonists were compared to placebo. Furthermore differences in reaction time parameters for near-threshold and maximum intensities were analyzed. *** indicates a significance level of $p < .001$, ** $p < 0.01$, * $p < .05$, (*) trend with $p<.075$.

To infer whether the GABAR agonists affected the mean, variance and skewness of the sensory threshold the stimulation intensity for near threshold stimuli across trials were statistically analyzed using a non-parametric Wilcoxon signed-rank test. No significant differences between GABAR agonists and Placebo were found for none of the parameters.

3.7.2. Effects of drugs on the saccadic peak velocity (SPV)

rmANOVA showed a significant effect of *Drug* ($F(2.66, 39.92)=3.71$; $\varepsilon=0.887$; $p=0.023$) on SPV. In the post-hoc t-test SPV was lower under *Alprazolam (*349.7 ± 13 °/s) *than Placebo* (394.5 ± 13.27 °/s, $t(15)=2.79$; $p=0.014$). In agreement with our previous findings (Premoli et al., 2014a; Lücke et al., 2014; Fuhl et al., 2015), *Ethanol* (391.3 ± 13.68 °/s) and *Baclofen* (366.9 ± 17.28 °/s) did not differ from the *Placebo* condition ($p > 0.05$).

3.7.3. Effects of drugs on the latency of SI and SII source activity

Effects of GABAR agonists on peak latencies of SI and SII source activity were studied using a rmANOVA with factor *Drug* (Levels: Placebo, Baclofen, Ethanol and Alprazolam) and the factor *Finge*r (Levels: d2 and d3). For the analysis of SII the additional factor *Hemisphere* (Level: contralateral and ipsilateral with respect to stimulation) was considered. No significant impact of factors *Drug* and *Finger* on SI latencies was found. On the level of SII, we could confirm the well-known shorter latency for contralateral than for ipsilateral SII (*Hemisphere* ($F(1, 15)=19.28$; $p<.001$), (SIIc: 119 ± 1 ms; SIIi: 126 ± 1 ms). Furthermore, a main effect of *Drug* ($F(2.49, 37.40)=3.361$; $\varepsilon=0.8311$; $p=0.035$) was found. Pairwise post-hoc t-tests of SII-latencies between GABAR agonists and *Placebo* revealed a significant latency prolongation for

Alprazolam as compared with *Placebo* (t(15)=2.799; p=0.014; SIIc: *Placebo* 117 ± 2 ms*; Ethanol* 119 ± 2 ms; *Baclofen* 118 ± 2 ms; *Alprazolam* 121 ± 2 ms; SIIi: *Placebo* 125 ± 2 ms; *Ethanol* 125 ± 2 ms; *Baclofen* 124 ± 2 ms, *Alprazolam* M±SE: 130 ± 2 ms) (Fig. 7).

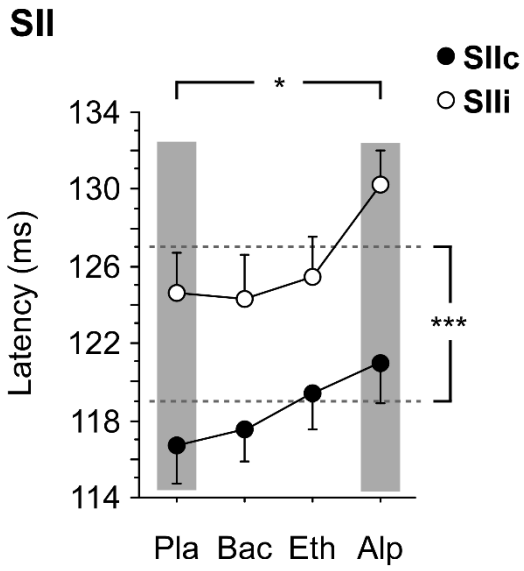

Fig. 7: Effects of drugs and placebo on the latencies of ipsi- and contralateral SII source activities. The black dots refer to latencies found for contralateral secondary somatosensory cortex (SIIc). White dots refer to ipsilateral secondary somatosensory cortex (SIIi). The bars indicate standard errors of the mean, for clarity they are only shown in one direction. The grey column indicates the significant latency difference comparing Alprazolam to Placebo. The dashed lines indicate the means of the latencies across drugs for the contra- and the ipsilateral SII. *** indicates a significance level of p< 0.001; * indicates a significance level of p < 0.05. The graphic shows a significant latency prolongation for Alprazolam, compared with Placebo.

3.7.4. Effects of drugs on the amplitude of SI and SII source activity

SI amplitude

Since not all conditions of the SI source activity were normally distributed, non-parametric pairwise Wilcoxon signed-rank tests were used for statistical comparisons. Firstly, we tested for each drug whether it had any inhibitory effect on the amplitude of the first or second peak of the SI response (Fig. 8a). If a significant reduction was observed, it was tested whether the reduction differed significantly between the two peaks. Results showed a significant decrease of SI amplitude for Alprazolam as compared to Placebo for both peaks (early peak Alprazolam [1st quartile, median, 3rd quartile]: [4.080 nAm, 6.919 nAm, 10.070 nAm], Placebo: [7.285 nAm, 9.682 nAm, 11.738 nAm], $p = 0.0131$; late peak Alprazolam: [2.426 nAm, 5.195 nAm, 9.143 nAm], Placebo: [4.005 nAm, 8.463 nAm, 14.168 nAm], $p = 0.0038$). The reduction in amplitude for Alprazolam was stronger for the second than for the first peak ($p = 0.039$). All other drugs had no significant impact on any peak of the SI amplitude.

SII amplitude

Pairwise non-parametric testing, using the Wilcoxon signed-rank test, revealed significantly stronger contralateral SII activations as compared to ipsilateral activations for all drug interventions. Comparing SIIc amplitude for GABAR agonists to Placebo revealed a significantly lower amplitude for Alprazolam ($p=0.0032$) and for Ethanol ($p=0.0072$). Source activity of SIIc for Baclofen compared to Placebo did not differ significantly (Fig. 8b). SIIi amplitude was not modified by any drug (Fig. 8b).

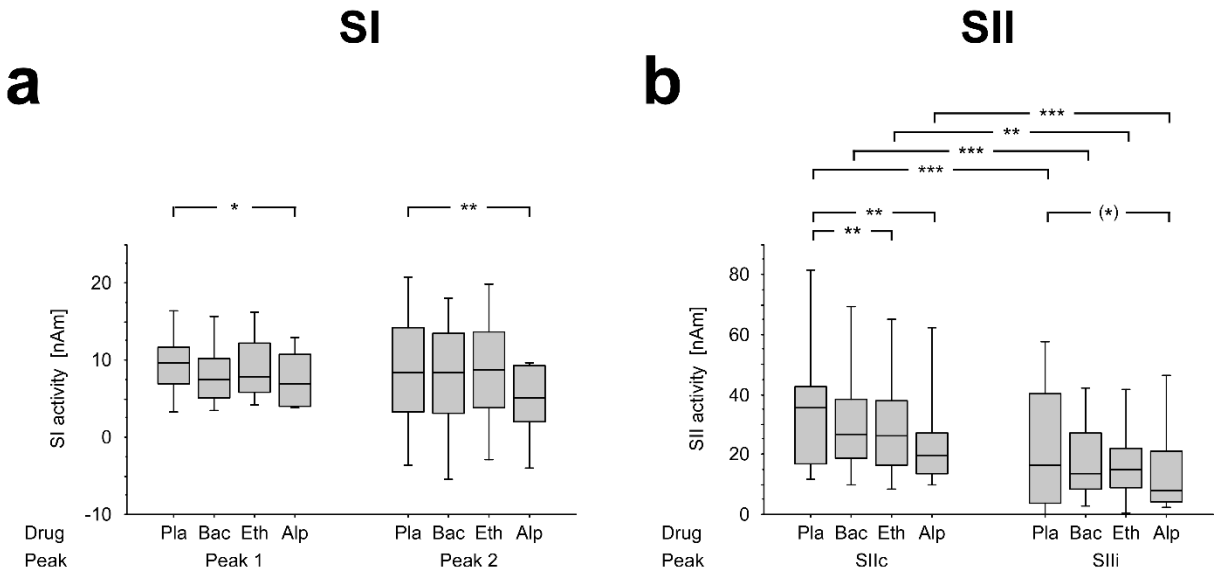

Fig. 8: Amplitudes of source activities evoked by single tactile stimuli with maximal intensity for the different GABA-agonists alprazolam (Alp), baclofen (Bac), ethanol (Eth), and placebo (Pla). Activities are presented in box-whisker plots with the median indicated as solid black horizontal line within the box, representing first and third quartile. (a) In SI, *Alprazolam* reduced the activity as compared to *Placebo* for both peaks, yet stronger in the second peak. (b) In SII, a lower activity for SIIi was found compared to SIIc for all drug interventions. Suppressive effects on SIIc source activity could be shown for Alprazolam and Ethanol when compared to Placebo. For SIIi only a trend for reduction in amplitude could be shown for Alprazolam. *** indicates a significance level of $p < 0.001$; ** $p < 0.01$; * $p < 0.05$, and (*) indicates a trend $p < 0.075$.

3.7.5. Mechanism of inhibition on the level of SII: propagation versus inhibition

Mechanisms of GABA-induced inhibition of SII were studied by comparing the observed peak activities with activities that would be predicted from SI activity when propagated without any further inhibition on the level of SII. A significant prediction error, i.e. the difference between the predicted and the observed activity (in Fig. 5c), would thus be indicative for a GABA-

driven SII-specific modulation of sensory information. Due to the bimodal wave shape of the SI source activity, prediction errors were determined for transfer functions derived from each SI peak separately.

Results of the rmANOVA comparing prediction errors for the within subject factors Drug (levels: Alprazolam, Baclofen, and Ethanol), Hemisphere (levels: contra- and ipsilateral hemisphere with respect to the stimulated hand) and *Peak* (levels: transfer function based on peak 1 of SI activity at latency 75 ms and peak 2 at 100 ms) evidenced a significant interaction between factors *Drug*Peak* ($F(1.68, 25.27)=3.714$; $\varepsilon=0.943$; $p=0.045$). To study which drug generates a significant difference between prediction errors based on peak 1 and 2, pairwise comparisons of prediction errors between first and second peaks were performed separately for contra- and ipsilateral SII. While in SIIc, the prediction error for *Alprazolam* was slightly stronger for the second than for the first peak ($t(15)=2.234$, $p=0.0412$), the errors did not differ between first and second peak for SIIi ($t(15)=1.842$, $p=0.0853$). One sample t-tests, exploring whether prediction errors for the peaks of SI activity, SII hemispheres and the three GABAR agonists deviated significantly from zero, revealed a significant effect for *Alprazolam* for the second peak of the SI and SIIc activity ($t(15)=2.464$, $p=0.026$).

In summary, for the first peak of SI, results did not show any significant difference between the predicted and the measured SII source activity, supporting the model of a mere propagation of the GABAergic effect evident at the level of SI to SII (Fig. 9a). With regard to the second peak of SI, the prediction error differed significantly from zero for *Alprazolam* and the contralateral SII activation. Astonishingly, the measured activation of SII was stronger than the SII activity predicted by applying the transfer function to the second peak of the SI activity (Fig. 9b). Thus, a model assuming the propagation of the inhibited SI activity of the second peak to SII is, in any case, - without or with further additional inhibitory modulation on the level of SII - incompatible with the observed SII activation.

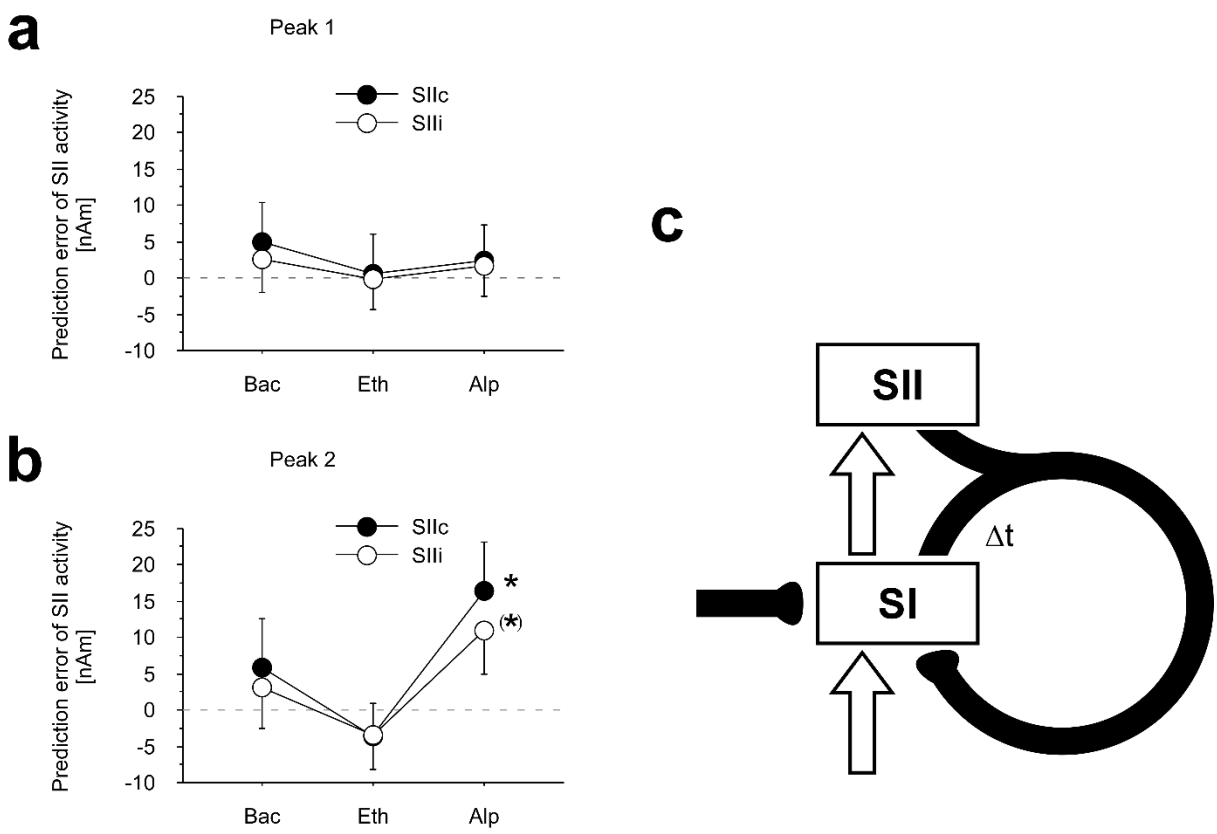

Fig. 9 (a): Prediction error results and model of propagation: For the first peak of SI, no significant difference was found between the predicted SII GABAergic effect and the measured activity. (b): for the second peak of SI, a significant difference was found between the predicted SII GABAergic effect and the measured activity for the *Alprazolam* condition. The experimentally obtained SII activity is larger than what is predicted based on mere propagation of the inhibited SI activity. (c): Based on our data we propose the following model: The initial sensory input to SI experiences $GABA_A$ergic modulation (inhibitory neuron to SI indicated in black). The inhibited SI activity is propagated to SII without any further GABA-dependent response decrement. In parallel with the propagation of the activation from SI to SII, the activity in SI is further inhibited either within SI, or by recurrent inhibitory fibers from SII leading to a further $GABA_A$-dependent reduction in SI activity (peak 2) by *Alprazolam* (black feedback loops). For clarity we show error bar only in one direction.

3.8. Discussion

The current paper investigated the role of GABA and the specific involvement of different types of GABARs in modulating somatosensory processing. Several animal and human studies on cortical plasticity demonstrated that GABA plays a modulatory role in somatosensory cortex (Dykes et al., 1984; Mittmann and Imbrosci, 2014; Garraghty et al., 1991; Alloway and Burton, 1991). However, the involvement of different GABAR types at the various levels of the somatosensory system is still unclear. Using median nerve stimulation in MEG, Huttunen et al. (2008) studied the effects of lorazepam, a benzodiazepine that binds to $GABA_AR$, on sensory processing in a paired-pulse stimulation design, very similar to the one used in our experiment. For single stimuli, a decrease of the P35m, P60m and N140m components generated in contralateral SI and a response decrement of the SII source were observed. The lorazepam-induced decrements of these components were taken as evidence for a $GABA_AR$-mediated inhibitory modulation of the postsynaptic potentials. No changes in SI or SII latencies due to lorazepam administration were observed.

Also in our study, we found no significant effect of *Alprazolam* on latencies of brain activation in SI. In contrast, besides the well-known shorter latency for SIIc as compared to SIIi (Simões and Hari, 1999), a significant latency prolongation for SII activity could be demonstrated for A*lprazolam* relative to *Placebo*. Concerning the activation of SI and SII, significantly smaller amplitudes were found for *Alprazolam* than for *Placebo* in SI and bilateral SII. Since components of evoked magnetic fields to tactile stimulation are less clearly separable and less accurately time-locked to the stimulation than in median nerve stimulation, the peaks of source activity obtained in our study are reasonably well corresponding with the SI and SII activities described by Huttunen et al. (2008). Given the inhibitory effects of *Alprazolam* and *Ethanol* (for SIIc only), inhibition in SI and SII seems to be selectively mediated by $GABA_ARs$ and most effectively by alprazolam, but not by $GABA_BRs$.

3.8.1. Methodological considerations

The effectiveness of alprazolam on the central nervous system was confirmed by slower eye movement velocities in the SPV examination. For the same dose of alprazolam, similar results had been found by Premoli et al. (2014a) and for other $GABA_AR$ agonists of the benzodiazepine family such us propofol (Gao et al., 1991), midazolam (Ball et al., 1991; Paut et al., 1993), and diazepam (Hommer et al., 1986).

Differently to alprazolam, no effects of ethanol (with the exception on SIIc amplitude) and baclofen on somatosensory processing could be demonstrated. In neuropharmacological studies, not finding any effect for a substance, triggers the question, whether its dosage is sufficiently high or whether the substance is just ineffective. In our study, instead of concluding that the α4- and α6-subtypes of the $GABA_AR$ for ethanol and the $GABA_BR$ for baclofen play a less important role in sensory processing, it could be argued that the dosage of these GABA-agonists was too low to become effective.

Regarding baclofen, studies using identical doses of 50 mg revealed significant effects, e.g. on TMS-induced plasticity: McDonnell et al. 2007; long-interval intracortical inhibition: McDonnell et al. 2006; TMS-evoked EEG potentials (50 mg of baclofen reduced the N100 amplitude at the site of stimulation): Premoli et al. 2014a; Premoli et al. 2014b. Furthermore, the administered dose of baclofen corresponds to clinically prescribed and effective doses. We therefore regarded the dosage of baclofen as sufficiently high to probe the existence of functionally relevant $GABA_BR$. Since SPV reduction is specific for α-1 subtype $GABA_AR$ activation, SPV is not expected to be modulated by the $GABA_BR$ agonist baclofen.

For ethanol, the dose was adjusted to participants' body weight, resulting in an approximate blood alcohol level of 0.8 ‰. Although ethanol is a $GABA_AR$ agonist, it showed no reduction in SPV. While eye-tracking studies by Lücke et al. (2014) and Fuhl et al. (2015) reported comparable results, the work of Roche and King (2010) showed an increase of SPV after alcohol

intake. Similarly to baclofen, the used dosage of ethanol (0.8 ‰) had significant effects in studies on TMS-induced plasticity (Lücke et al. 2014; Fuhl et al. 2015). Low-dose ethanol was shown to enhance relatively selectively tonic inhibition mediated by the extrasynaptic α4- and α6-subunit containing $GABA_AR$ (e.g. Wallner et a. 2003). Therefore, previous evidence suggest that dosages were not too low for producing significant effects in the central nervous system. Anyway, dose-response curves would have been capable of solving the dosage effect issue. Not having tested dose-response relationships is a limitation of the present study.

Given these considerations, we conclude that receptors specific for low-dose ethanol and baclofen play a less important role in the processing of passively perceived tactile stimuli. Although alprazolam and ethanol are both $GABA_A$ agonists, their differential inhibitory effects could be explained by their differential affinity to different subunits of the $GABA_AR$ (Sieghart, 1995, Table 1).

Another argument questioning the interpretation of our findings is that impairments of attentional processes by alprazolam may have contributed to the presented behavioral and electrophysiological findings (Michael et al. 2007; Buffett-Jerrott and Stewart 2002). However, it is unlikely that our findings are fully explained by modulation of attentional processes. Thought as acute exposure to ethanol at doses similar or even less to the one tested in our experiments also had detrimental effects on attention in previous studies (Rohrbaugh et al. 1987; Jääskeläinen et al. 1999) but no effects on reaction time of SEFs in our experiments. Furthermore, a significant modulation of attentional processes (by alprazolam) should also have affected somatosensory perception threshold and should have produced a significant interaction between Drug and Intensity on reaction time, both of which was not the case (Fig. 6).

A potential limitation of the study consists in the selection of the participants. Since only males were included, the findings cannot be generalized to females. However, motivated by previous

studies (Smith et al., 1999, Premoli et al., 2014a), we wanted to reduce intersubjects variance as much as possible and therefore studied only males.

3.8.2. Behavioral effects

Despite the effects of alprazolam on information processing at the level of SI and SII, no change in somatosensory perception threshold and only an effect on the mean and the variance of reaction times towards a prolongation and a larger variability of the response times for *Alprazolam* as compared to *Placebo* was found in the present study. Similar results were found in an animal study (Eto et al., 2012). Since somatosensory perception threshold and reaction time depend on the motor response that is generated at the end of the information processing, probably compensatory mechanisms that cover the correspondence between GABA administration and threshold estimates, are involved. In contrast to psychophysical measures, evoked magnetic fields provide a more direct and probably more sensitive readout of the GABAergic modulation of information processing in SI and SII. This is a crucial aspect advocating combined neuropharmacological and neuroimaging approaches studying the direct cortical effects of altered receptors.

For baclofen, the work of Durant et al. (2018) did not provide evidence for a behavioral effect (zig-zag motor test) up to a single oral dose of 60 mg. This is consistent with our present findings. Not having included subjective scales, as was done by Durant et al. (2018), to test behavioural effects of baclofen, is admittedly a limitation of our study.

3.8.3. Histological and neurophysiological effects

3.8.3.1. GABA receptor types

Zilles et al. (2002) investigated the distribution of GABAR types in the human cerebral cortex comparing data of different imaging modalities. While a high density of $GABA_A$Rs was docu-

mented for SI, no data were reported for SII. In the same study, $GABA_AR$ densities were reported for lower and higher cortical sensory regions only for visual cortex, with higher $GABA_AR$ densities in V1 compared to V2. Considering the results of Zilles et al. (2002), and of the present study, it can be hypothesized that $GABA_ARs$ are involved in the first steps of information processing taking place in SI.

Using a combined neuropharmacological-neuroimaging approach, it was possible to confirm the role of GABA as a modulator of somatosensory processing. Moreover, the latency of SII and the amplitude of SI and SII were mediated exclusively by the positive $GABA_AR$ modulator alprazolam. Considering the almost exclusive modulation of SI and SII latency and activity by alprazolam in our study, $GABA_ARs$ seem to be the functionally most relevant mediators of inhibition in the first steps of cortical processing of tactile information.

$GABA_ARs$ have fast kinetics and are therefore suitable to mediate fast and early processing in the sensory pathway (Connors et al., 1988; Deisz, 1999). In particular, fast processing is needed in primary sensory cortex that is characterized by a high information throughput rate. In contrast, secondary sensory regions integrate information on a much longer time scale and are thus assumed to be modulated more slowly. In fact, typical time constants of sensory processing range below 150 ms for SI (Wühle et al., 2011, Huttunen et al. 2008), and between 150 ms and 1000 ms for SII (Wühle et al., 2010). Therefore, effects of $GABA_AR$ agonists are expected to occur predominantly on the level of SI. However, in contrast to this expectation, inhibitory effects of alprazolam were found on the level of both, SI and SII. As further discussed in the following section, inhibitory effects of SII most likely reflect the propagation of the inhibited activation of SI. Our results, reporting SI as the main locus of action for the positive $GABA_AR$ modulator, alprazolam, are therefore in line with the hypothesis that $GABA_ARs$ control early stages of cortical processing.

3.8.3.2. Propagation model

The simulation (Fig. 9) suggested that the GABAergic suppression of SII activity is due to a response decrement on the level of SI that is propagated to SII. No further substantial inhibition seems to occur at the level of SII. Results furthermore suggest that the activation of SII is selectively driven by the initial activity of SI. (Fig. 9c). Regarding the strong $GABA_A$-dependent inhibition of the second component of the SI the current data unfortunately cannot disclose whether the reduced activity is due to the dynamics within SI or results from recurrent inhibitory input from SII. To disentangle inhibitory effects within SI and from SII to SI, experimental designs that involve differently complex stimuli and tasks requiring differential interaction between SI and SII might be a promising strategy.

3.9. Conclusion

The current study aimed to investigate the role of GABA as a potent mechanism controlling the processing of sensory information in SI and SII. Previous research has shown that GABAergic receptors play a crucial role in functional reorganization and top-down control of somatosensory processing. Our results suggest that GABAergic modulation occurs predominantly at the level of SI involving the fast reacting $GABA_A$ receptors sensitive to alprazolam. Inhibitory effects on the level of SII appear to be the consequence of propagated inhibited activity of SI to SII. Moreover a combined neuropharmacological and neuroimaging approach allowed us to disclose the temporal dynamics of inhibitory processes within SI and between SI and SII. Thus, it is possible to shed light on the neuronal mechanisms supporting GABAergic modulation of processing in SI and SII.

3.10. References

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4. Study II: A new adaptive procedure for estimating perceptual thresholds: the effects of observer bias and its correction

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4.1. Abstract

Adaptive threshold estimation procedures sample close to a subject's perceptual threshold by dynamically adapting the procedure based on the subject's performance. Yet, perceptual thresholds not only depend on the observers' sensory capabilities but also on any bias in their expectations and responses, that distorts threshold estimates. Using the framework of signal detection theory (SDT), independent estimates of both, an observer's sensory sensitivity and internal processing bias can be obtained and allow for bias correction. While this approach is commonly available for estimation procedures engaging the method of constant stimuli (MCS), correction procedures for AM are only scarcely applied. In this article, we introduce a new AM that takes individual biases into account and that allows a bias-corrected assessment of sensitivity. This novel AM is validated with simulations and compared to a typical MCS-procedure, for which the implementation of bias correction has been previously demonstrated.

Comparing AM and MCS demonstrates the viability of the presented AM. Besides its feasibility, the results of the simulation reveal both, advantages and limitations of the proposed AM. The procedure has considerable practical implications, in particular for the design of shaping procedures in sensory training experiments, in which task difficulty has to be constantly adapted to an observer's performance, in order to improve training efficiency.

Keywords:

Adaptive procedure, method of constant stimuli, perception, signal detection theory, threshold estimation

4.2. Declaration of contribution

1) Ranganatha Sitaram conceptualized and designed the backward masking experiment.

2) Chiara Fioravanti and Diljit Singh Kajal conceived the idea for the bias corrected AM procedure and for performing a comparative study between the AM and the MCS. They conducted the experiment.

3) Christoph Braun and Diljit Singh Kajal performed the analysis.

4) Chiara Fioravanti and Christoph Braun wrote the manuscript.

5) Christoph Braun, Ranganatha Sitaram, Sergio Ruiz, Axel Lindner revised the manuscript.

6) This work was revised by Christoph Braun and Ranganatha Sitaram.

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4.4. Introduction

Perceptual thresholds might vary due to different variables such as fatigue, fluctuations of attention or learning (Gorea and Sagi, 2000). Adaptive threshold estimation procedures are the most effective approaches to provide quasi-instantanious estimates of fluctuating sensory thresholds. Instantanious estimates are needed, for example, in experiments in which the sensory stimulation should be kept close to an individual's threshold, like in sensory learning experiments. A common problem related to all threshold estimation procedures is that the thresholds reflect not only the individual's sensitivity but also their internal processing biases. There are several types of biases that can occur at different stages of perceptual processing: at the sensory level (e.g. due to sensory adaptation), at the decision making level (e.g. due to a preference of one stimulation condition over another), the response selection level (e.g. a general preference to rather respond with the right than with the left hand in bimanual response tasks). Accordingly, any of the aforementioned internal processing biases is able to significantly distort sensory threshold estimates. The observer's bias can be reduced by an appropriate design of the threshold detection experiment or it can be corrected during subsequent data analysis. Neverthless, there are occasions where an online bias corrections is requested. Signal detection theory (SDT) has been chosen as a tool to independently assess an individual's sensitivity and bias by modeling perception as a decision-making process (Green and Birdsall, 1978; Harvey, 1992; Macmillan and Creelman, 1990; Macmillan and Creelman, 2004; Macmillan, et al., 2004; Swets, 1961; Wickens, 2001; Gorea and Sagi, 2000) [for details see supplementary material 1]. In SDT, d' (the distance between the peaks of the two probability density functions, describing response behavior for individual stimuli) is increasing with stimulus discriminability and thus stimulus strength. Based on SDT, the criterion upon a sensory decision is a function of the individual's bias and defines the probabilities to select alternative responses for each stimulus (Gorea and Sagi, 2000) (Fig. 2).

In fact, for some threshold estimation procedures, such as the method of constant stimuli (MCS), bias correction procedures provided by SDT are readily established. However, this is not the case for the adaptive procedures, which – as compared to the mentioned procedures – have the advantage of providing quasi-instantaneous threshold estimates. Therefore, the presented study, introduces a new adaptive procedure that is able to correct the subject's bias (Kajal, 2018).

The rationale behind the proposed approach is explained and investigated through several simulations, which also demonstrate the feasibility of the procedure. Prerequisites, advantages, and limitations of the approach are discussed. In order to validate the new method, it is compared to a standard non-adaptive procedure, the MCS. Amongst the variants of MCS application, the "classic" version – which is clearly distinct from AM – was chosen, as it is not involving any adaptation based on subjects' responses.

To illustrate practical applications of the new bias-corrected adaptive threshold estimation procedure, it is simulated and discussed in the context of a visual backward masking paradigm ((Del Cul, et al., 2006); also see (Di Lollo, et al., 2000; Enns and Di Lollo, 2000; Vorberg, et al., 2003; Breitmeyer & Ogmen, 2000).

4.5. Methods

In order to validate the new adaptive procedure – introduced in detail below – various simulations were carried out. Specifically, a virtual observer was defined in the framework of signal detection theory, which had experimentally controlled sensory capabilities and biases. In a next step, the estimation of the virtual observer's threshold was simulated, using either the MCS or the chosen AM procedure, either with or without bias correction. Moreover, results obtained by AM for the simulation with time-varying sensitivities and the simulation of linearly changing biases are presented.

4.5.1. Threshold Estimation Procedures

In the following paragraph, we briefly describe the method of constant stimuli and the adaptive method:

1. Method of constant stimuli (MCS) (McKee, et al., 1985; Treutwein, 1995) refers to a procedure in which a set of preselected stimuli are presented with stimulus parameters that could cover the whole perceptual range (i.e., 0% to 100% correct responses). Thresholds are then usually calculated offline in a separate step, namely from a fitted psychometric function that relates stimulus parameters to an observer's response pattern.

2. Adaptive methods (AMs) approximate the stimulus parameters that lead to a predefined performance level (e.g. 70.7%, or 66.7% correct responses). This is obtained by varying stimulus parameters across trials, on the base of the observer's responses to preceding stimuli (Treutwein, 1995; Watson and Pelli, 1983). In AMs, stimulus parameters are more densely sampled around the individual´s threshold value (Levitt, 1971). Whereas the MCS can explore the whole range of stimulus parameters for which the threshold is to be determined, AMs are regarded as being more efficient than MCS, insofar as a smaller number of trials is needed for threshold estimation (Watson and Fitzhugh, 1990). As a consequence, AM can provide quasi-instantaneous threshold estimates.

4.5.2. Virtual Experiment

For the simulations of the threshold estimation procedures a virtual experiment was conducted. In each trial (Fig.1) of the virtual experiment, a prime stimulus, which was either an emotionally positive (happy) or negative (sad) face, was presented for 16 ms. After a given delay, the prime stimulus was masked by an emotionally neutral face of the same identity, presented for 250 ms. In such a paradigm, the emotion of the facial expression cannot be determined for a delay of zero and the probability to correctly identify the emotion increases with the duration of the

delay. To determine the delay corresponding to the perceptual threshold, at which the emotional expression of the prime is reliably perceived, a black screen of varying duration is displayed between the prime and the mask stimuli. The respective durations could correspond to one of ten different values Δt (16.7 ms * k, with k ranging from 0 to 9), based on the 60 Hz frame rate of the projector. After the presentation of the mask, a black blank screen was shown. To indicate the emotional valence of the prime (negative or positive), one of the two virtual response buttons was selected.

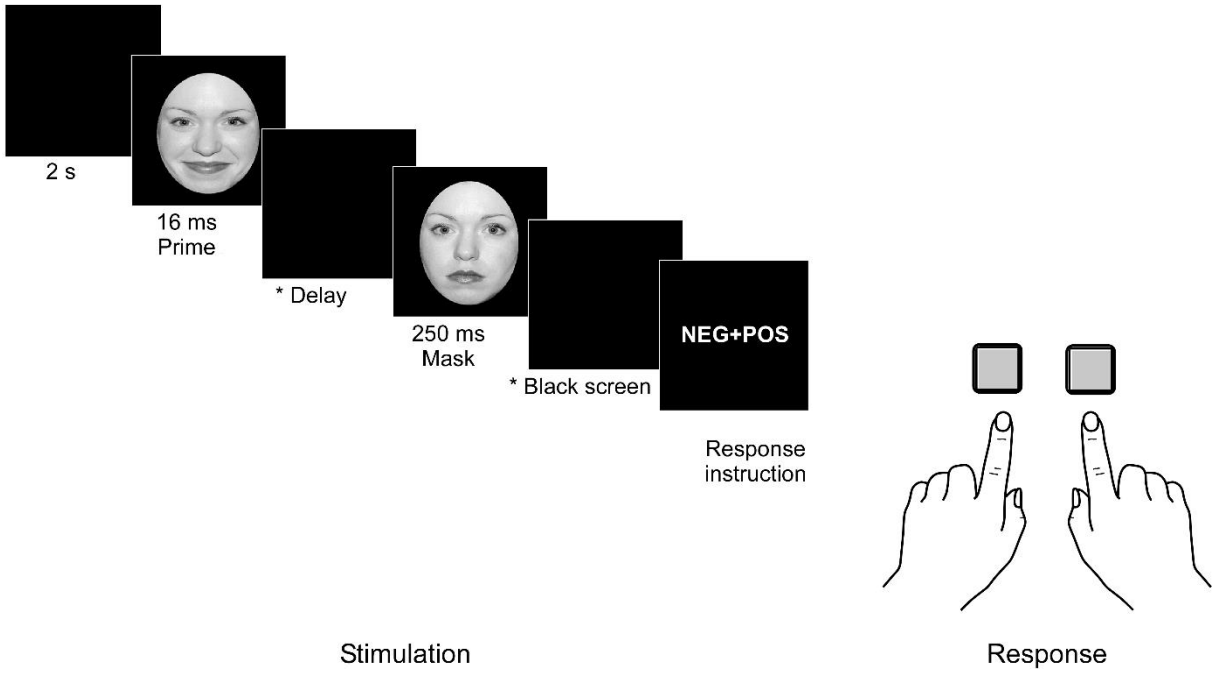

Fig. 1: Backward masking task. In the original setup to which the simulation refers, the assignment of the response buttons was randomly altered on a trial by trial basis. The instruction "Neg + Pos" informed the subject that the left button should be pressed if the emotion of the prime was negative and the right button should be pressed if the emotion was positive. The "Pos + Neg" indicated the reverse assignment.

In the simulation of the MCS approach, different 10 predefined delays were presented across trials in a pseudo-randomized order (Leek, 2001). To determine the threshold, a sigmoid psychometric function (logistic regression) was fitted to the probability of correct responses as a function of the predefined delays. The threshold delay was determined for a level of correct identification of the emotional expression of 66.7%.

For the simulation of the AM, the 'two-down one-up rule' (Leek, 2001) was applied to select the stimulus delay in the upcoming trial. In this procedure, the delay between the prime and the mask decreases by one step (16.7 ms: 1 frame) after two correct responses and increases by one step with each incorrect response. Assuming a stationary threshold, the delay can be expected to asymptotically approach the threshold. In case of the two-down one-up procedure not requiring that the correct stimuli occur in a sequence, the threshold will converge to performance level of 66.7 % of correct responses. Differently, when correct answers are requested to appear consecutively, to decrease the delay by one step, the performance level would converge to 70.7 % correct responses (Leek, 2001) (see supplementary material [2]).

4.6. A new adaptive method with bias correction procedure

The newly proposed threshold estimation procedure combines an adaptive method with bias correction based on SDT. According to SDT, the response of an observer depends on the positions of the criterion in the probability density functions that describes stimulus discriminability. In this study the probability density functions are centered at $\pm d'(\Delta t)/2$, a fixed criterion γ for all delays Δt implies comparable changes in discriminability for sad and happy faces with changes in mask delays (Gorea and Sagi, 2000). Since a differential change of the detectability of sad and happy faces across mask delays can be considered as a sensory bias, the estimation

of individual criteria for each mask delay was implemented. In the context of the SDT, bias corrections correspond to a shift of the criterion to the common center of both Gaussian distributions. In Fig. 2, the unbiased criterion corresponds to $g_c = 0$ (solid vertical line) and the bias-dependent criterion is g (dashed vertical line). Given an estimated bias of γ, the bias correction determined observer's response, if the criterion was at the cutting point of both probability density functions.

Like in the standard AM procedures, the novel approach estimates the threshold using the two-down one-up method. The procedure considers an initial estimate of the observer's bias. Along with the adaptive procedure, the bias estimate will be continuously updated during each trial and will be subsequently used to determine an observer's bias-corrected response. Biases were estimated for each delay from all previous trials using the SDT procedure (see next paragraph). The delay chosen for the subsequent trial involved firstly the estimation of a bias-corrected response (Fig.2), and secondly followed the two-up-one-down rules on the basis of the corrected response. Depending on the estimated location of the criterion, it could happen that an observer's response is reversed, i.e. a "negative emotion" response might be turned into "positive emotion" response, and conversely a "positive emotion" judgement might be turned into a "negative emotion" response. Using SDT, the bias-corrected response can be characterized only by a certain probability for being correct. Thus, in the algorithm of the bias-corrected two-down one-up rule, the selection of the masking delay of the following trial will be based on probabilistic considerations.

The rules for selecting the stimulus parameters for the next trial in the AM are summarized in the flowchart (see appendix I). To define the mask delay of the following trial, four different conditions need to be considered in the approach (Fig. 2)

a) Assuming that in a certain trial a 'happy face' (HF) is presented and the observer's criterion to classify the stimulus as 'happy' or 'sad' is at a level of $g < 0$ *(this situation*

describes the case of a bias towards happy faces) (Fig. 2a), the probability to choose 'sad face' as a response corresponds to the rate of $p_{IS}(t)$. In contrast, the probability to select HF as a response is referred to as $p_{CH}(t)$. The probability $p_{CH}(t)$ can be thought of as being composed of $p_{CH} = p_{CHa} + p_{CHb}$, where $p_{CHa} = \int_{x=\gamma_c}^{x=+\infty} P(x)dx$ is the cumulative probability for $x > g_c$, and $p_{CHb} = \int_{x=g}^{x=g_c} P(x)\,dx$ is the probability for $g < x \leq g_c$. $g < 0$ represents an observer's perception criterion and $g_c = 0$ the bias-free perception criterion. In other words, p_{CHa} refers to the cumulative probability to identify the happy face in case of no bias, and p_{CHb} to the part of the probability that is due to the bias. $P(x)$ is assumed to be normally distributed. In the proposed method, the selection of the next stimulus is based on an observer's bias-corrected response. If the virtual observer responded with 'sad face' (SF), the response was wrong, regardless of any potential bias (Fig. 2). However, given the response bias towards HF, whenever the virtual observer answers HF, only a proportion $\frac{p_{CHa}}{p_{CHa} + p_{CHb}}$ of these responses can be accepted as HF. For the remaining trials, the bias corrected response will be converted to SF. In an individual trial, depending on the estimated proportion, the response will be kept or changed, based on random selection. In detail, a random number r will be drawn from a uniform distribution between 0 and 1. If $r > \frac{p_{CHa}}{p_{CHa} + p_{CHb}}$, the observer's response will be changed from HF to SF, i.e., from correct to incorrect.

b) Likewise, for HF stimuli, a HF response will remain unchanged when the criterion is set at a level of $g > 0$ *(this situation describes the case of a bias towards sad faces)* (Fig. 2b). The probability of incorrectly perceiving a happy face $p_{IH}(t)$, i.e. responding with SF to the HF stimulus, can be split into a proportion depending on the bias-free criterion

p_{IHa} and a proportion that corresponds to the observer's bias p_{IHb}. If it holds for the random variable $r > \frac{p_{IHa}}{p_{IHa} + p_{IHb}}$, the SF response will be changed to HF.

c) For SF stimuli, a SF response will remain unchanged when the criterion is set at a level of $g < 0$ *(this situation describes the case of a bias towards happy faces in the presence of a SF stimulus)* (Fig. 2c). The probability of p_{IS} (responding with HF to the SF stimulus), can be split into a proportion depending on the bias-free criterion p_{ISa} and a proportion corresponding to the observer's bias p_{ISb}. In the case of $r > \frac{p_{ISa}}{p_{ISa} + p_{ISb}}$ the HF response will be changed to SF.

d) Finally, for SF stimuli a HF response will remain unchanged if the criterion is set at a level of $g > 0$ *(describing a bias towards sad faces)* (Fig. 2d). The probability of correctly perceiving a sad face p_{CS}, i.e. responding with SF to the SF stimulus, can be split into a component depending on the bias-free criterion p_{CSa} and a proportion that corresponds to the observer's bias p_{CSb}. If it holds for the random variable r, drawn from a uniform distribution between 0 and 1, $r > \frac{p_{CSa}}{p_{CSa} + p_{CSb}}$, the SF response will be changed to HF. In all four cases the bias-corrected response is used for the selection of the next stimulus according to the two-down one-up rule in the subsequent step of the algorithm.

In the presented simulation, corrections of the responses were carried out only after acquiring a first estimate of the bias for each individual delay. A minimum of 25 trials, and a minimum of at least 3 trials in each of the signal detection theory response categories for the current delay

t ($p_{CH}(t), p_{CS}(t), p_{IH}(t), p_{IS}(t)$) was required prior to the application of the correction procedure.

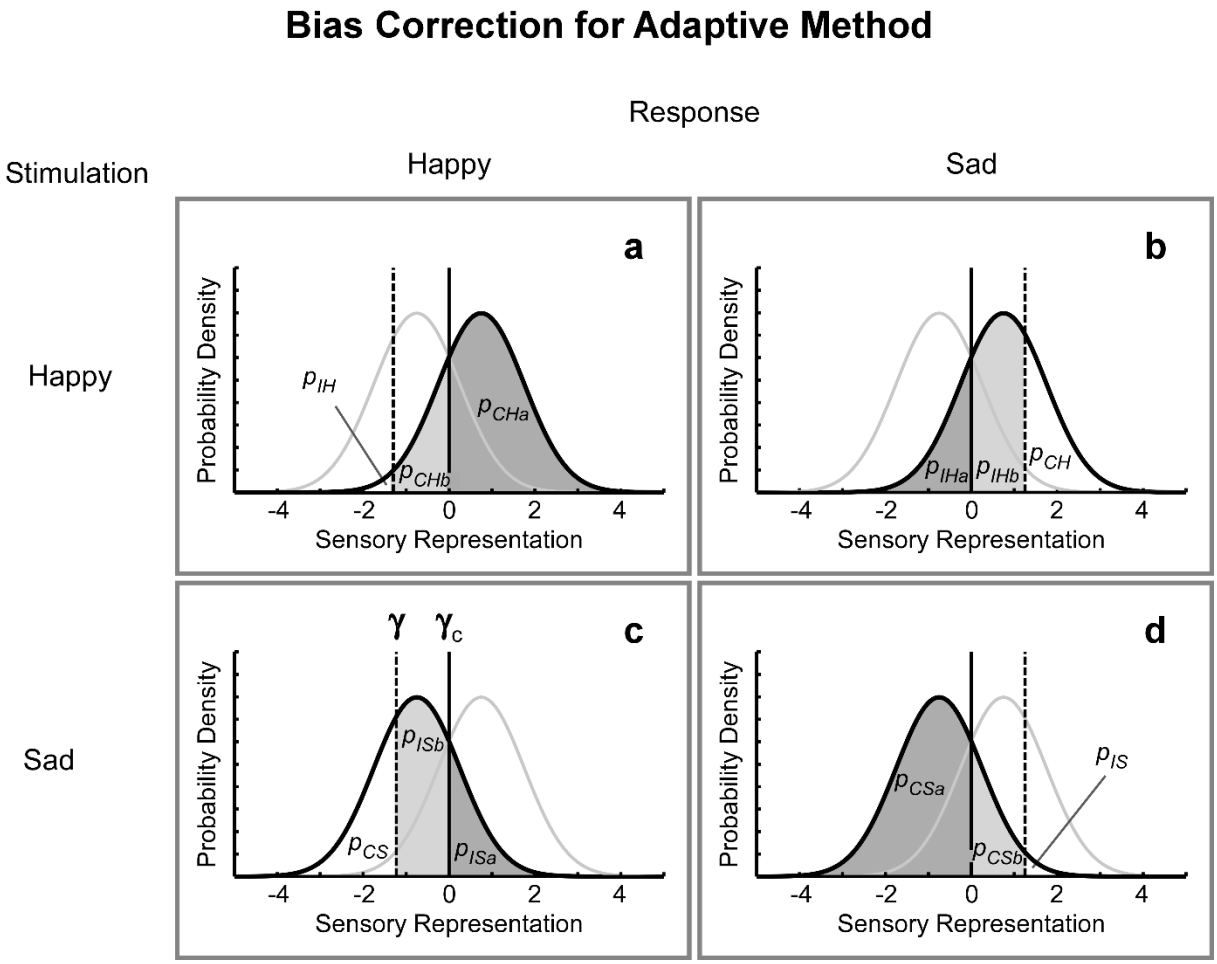

Fig. 2**:** In the adaptive method with bias correction, the observer's bias is estimated and the stimulus is chosen depending on the two-down one-up procedure and the bias-corrected response. Since in a single trial it is unknown how much the decision reflects the bias, a probabilistic correction needs to be applied. For this purpose, probabilities for correctly identified happy faces ($p_{CH}(t)$), for correctly identified sad faces ($p_{CS}(t)$), for incorrectly identified happy faces ($p_{IH}(t)$), and for incorrectly identified sad faces ($p_{IS}(t)$) will be

split into a part that describes the probability for the unbiased criterion $g_c = 0$ (solid vertical line) and the proportion due to the bias-dependent criterion g (dashed vertical line). The unbiased proportion of the probabilities refers to $p_{\cdot a}$ and the component that is due to the response bias to $p_{\cdot b}$. Depending on the stimulation and the response, the '•'symbol represent correctly or incorrectly identified happy faces or sad faces (CH, IH, CS, IS), in a) and b) HF stimuli and in c) and d) SF stimuli are presented. In a) and c) the correction needs to be considered for the response HF, and in b) and d) for the response SF.

The flowchart (appendix I) displays rules for the selection of a delay for the next trial, implemented through an algorithm that is based on corrected responses. After reaching a valid bias estimate, it is possible to correct an observer's response by taking the initial bias value into account and using a new bias-free decision criterion set to $\gamma = 0$.

4.6.1. Simulation of the virtual observer

In order to study the performance of the AM and MCS in a simulation, the responses of a virtual observer to the presented stimuli are needed. The decision of how a stimulus was perceived by the virtual observer was simulated on the basis of SDT. The virtual observer's detection skills were arbitrarily defined for ten delays Δt (16.7 ms * k with k ranging from 0 to 9). Each delay was assigned a different d' according to $d' = 12(\Delta t)$.

The probabilistic decisions of how HF and SF were perceived, were based on Gaussian normal distributions centered at $\pm d'/2$ and with standard deviations σ of 1.

Since the d' of 0.861 corresponds to a percentage of correct answers of 66.7%, i.e. correctly identified happy and sad faces, the virtual observer's preset threshold is 72 ms. For the simulations, different observer's biases (g) were considered (0.0, 0.2, 0.5 standard deviations (σ) of the Gaussian distribution. Once the virtual observer's detection skills, i.e. perceptual parameters, were defined, their performance for four different numbers of trials (100, 200, 500, and

1000 trials) were evaluated by simulations. To simulate the virtual observer's decision, a random number r_s was chosen from the normal distribution with standard deviation of 1 and $m = d'/2$ for HF and $m = -d'/2$ for SF. Depending on whether r_S exceeded the predefined decision criterion g: $r_S \geq g$, the virtual observer's response was HF. Conversely, in case of $r_S < g$, the response was SF.

To evaluate the new method, a virtual observer's performance for different numbers of trials and several levels of biases was simulated based on SDT and its threshold detection capabilities were estimated with and without bias correction, for both, the AM and MCS.

4.6.2. Simulation of AM sensitivity and bias

In the simulations of the threshold estimation procedure, the virtual observer's responses were fed into the AM and the stimulus in the next trial was selected accordingly. The sequence of stimulation was assumed to converge towards the preset threshold of 72 ms. The results of the simulation for each set of parameters (level of biases and number of trials) were iterated for 1000 times and the corresponding thresholds were inferred. (Fig. 3) (Note that the observer's responses are based on a probabilistic selection of responses. Therefore, the simulated thresholds vary for the same stimulus conditions.)

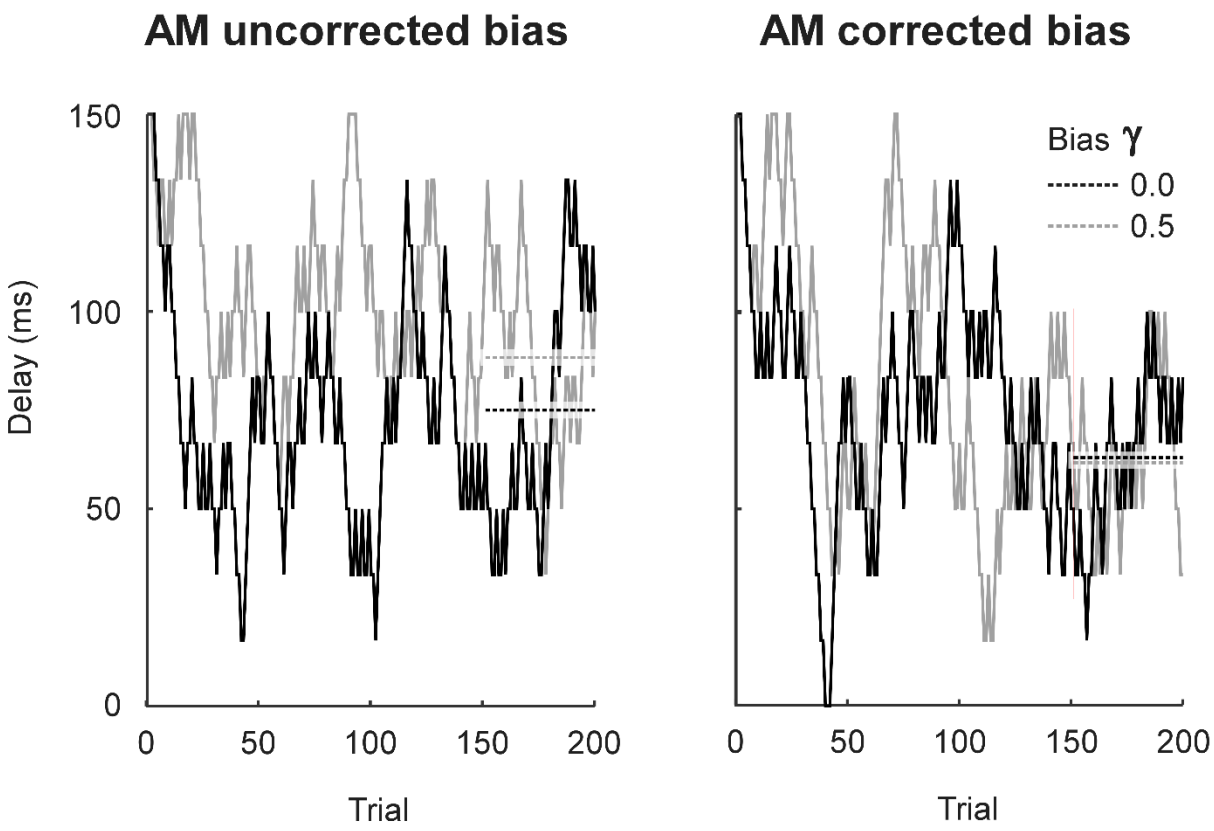

Fig. 3: Simulation of the AM for a bias of 0.0 (black) and a bias of 0.5 (grey) with (right diagram) and without correction of biases (left diagram). Threshold estimates were based on the average of mask delays across the last 50 trials. Averages are represented as horizontal lines extending from trial 151 to 200.

4.6.3. MCS Simulation

In order to contrast the performance of the AM to a method for which bias correction had been already established, AM was compared with MCS by simulations. In the MCS approach parameter settings for the virtual observer were identical to those used in the AM (see above). In the MCS, a psychometric function was fitted to the frequency of correct responses as a function of the ten different delays. Considering Fechner's law (Fechner, 1860) of logarithmic relation between perceived and physical magnitudes of sensory input (Dehaene, 2003), the logarithms of all delays were calculated. To avoid the problem of obtaining a value of minus infinity for a

delay of zero, 1 ms was added to all delays prior to the transformation. Afterwards, a Weibull psychometric function was fitted to the frequencies of correctly identified emotional face expressions:

$$f(x) = 1 - (1 - g)e^{-\left(\frac{kx}{t}\right)^b}$$

$$k = -log\left(\frac{1-a}{1-g}\right)^{\frac{1}{b}}$$

x = logarithmic transformation of delays

g = performance at chance level: in our example set to 0.5

t = threshold

a = performance level defined as threshold (0.667)

b = slope index of the psychometric function

The Weibull function asymptotically converges towards 50% for a delay of <1.0 ms and towards 100% for increasing delays. The Weibull function fitted to the psychophysical data resulted in a threshold estimate for each observer, i.e. a delay for which a performance level of 66.7% was reached. The MCS threshold of 66.7% correct responses was chosen to comply with the two-down one-up procedure dependent threshold level of the AM.

4.6.4. Simulation of AM for time-varying sensitivities

Two scenarios were studied to simulate how efficiently the adaptive procedure can track changes of a virtual observer's sensitivity. In scenario one, a successful perceptual training was

simulated, in which it was assumed that the observer improves the sensitivity for detecting the emotion of the face stimuli linearly across 1000 trials. The threshold started at 100.0 ms and decreased by 0.033 ms/trial. After 500 trials a sudden improvement of the threshold by 16.7 ms was introduced in order to explore the behavior of the algorithm for sudden changes. Thereafter, the threshold decreased at the same rate as at the beginning until it reached a value of 50.0 ms after 1000 trials. In scenario two, which was inspired by slow variations of participants' attention in threshold experiments, the performance of the virtual observer was simulated to vary randomly. The random changes of the observer's sensitivity were simulated by lowpass filtering white noise sampled at 1/trial. The low-pass cut-off was set such that fluctuations of threshold changes lasting less than 100 trials were suppressed. The virtual observers reactions to the delays presented in different trials were computed on the basis of SDT (see paragraph 'Simulation of the virtual observer' above). Observer's biases of 0.0, 0.2 and 0.5 were simulated. For estimating the threshold the AM procedure was run with and without bias correction. To investigate difference between a two-down one-up procedure and a three-down one-up procedure converging at thresholds with a performance of 66.7 % and 75.0 % correct responses, respectively (see supplementary material [2]), both approaches were simulated.

4.6.5. Simulation of linearly changing bias

To investigate how the bias correction works in biases varying across trials, a linear change of the bias from 0.0 to +1.0 and from 0.0 to -1.0 across 1000 trials, was simulated. The threshold estimation used a two-down one-up and a three-down one-up procedure resulting in 66.7 % and 75.0 % threshold performance, respectively. Both simulations were run with and without bias correction.

4.7. Results

In Fig. 4, the mean and standard deviation values across the 1000 repetitions of the simulations are presented for corrected and uncorrected thresholds, different bias values and different trial numbers. Fig. 5 summarizes the estimated bias values that were inferred from the virtual observer's response, as a function of trials.

The simulation shows that in the case of a low number of trials (≤ 200), both, for corrected and uncorrected thresholds, the MCS fails to reliably fit a Weibull function. The AM, on the other hand, is not affected by this problem. As expected, in the uncorrected AM procedure, the estimated thresholds are independent of the number of trials. In the corrected AM, the estimated threshold values approached the threshold that was preset in the simulation. Since the threshold of a single individual obtained with one method, falls within the 95 % confidence interval (± 2 standard deviations) of the other method, individual thresholds values do not differ significantly between methods. However, comparing the mean of a group of N subjects would reduce the confidence interval by a factor of $\sqrt{\frac{1}{N}}$ of the standard deviation. Threshold values are consistently lower in MCS than in the AM if a large enough group size is studied. As expected, the reliability of the threshold, indicated by the standard deviation, is independent from the number of trials. In both cases, bias uncorrected and bias corrected AMs. In contrast, the standard deviation for MCS decreases with increasing number of trials (Fig. 4). In Fig. 5, the estimated biases are presented for different trial numbers and at different simulated bias levels. The accuracy of the bias, i.e. its deviation from the simulated preset bias value, and its precision reflected by the standard deviation (the lower the standard deviation, the higher the precision), grew with increasing trial numbers. The performance of the bias estimates is comparable for both, the AM and the MCS smaller errors for the AM (Fig. 5).

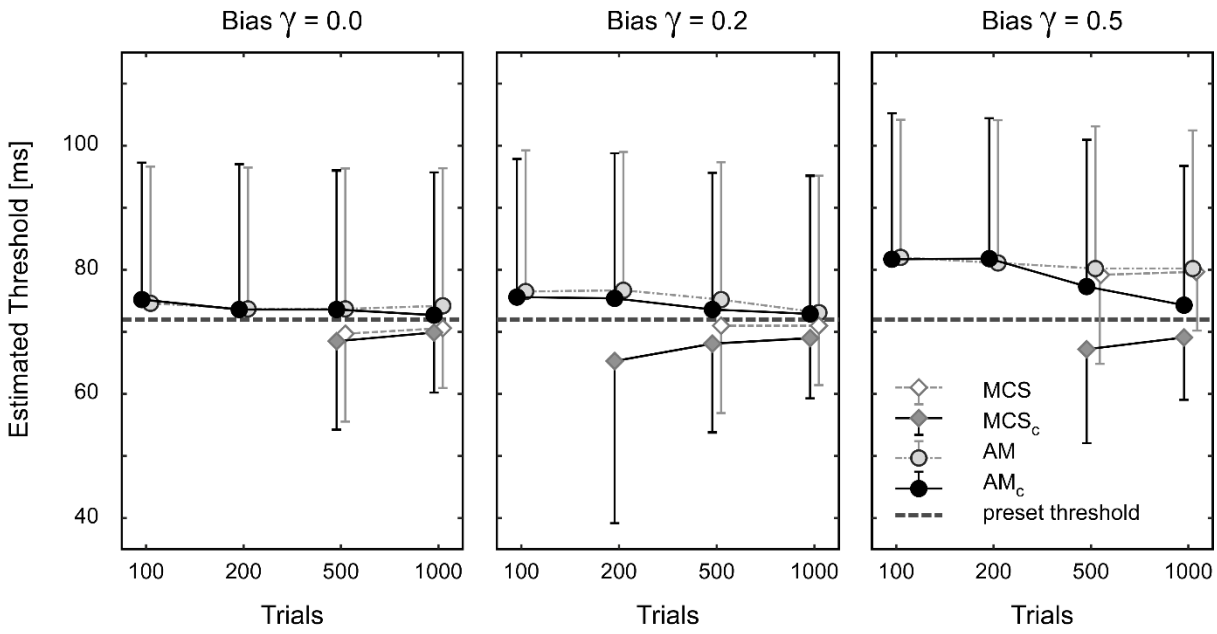

Fig. 4: threshold estimates of the AM and MCS with and without bias correction for a bias of 0.0, 0.2 and 0.5 (left, middle and right part) and for trial numbers of 100, 200, 500, 1000 (abscissa). For each condition, mean and standard deviations across 1000 simulations are presented. Missing results for 100 and 200 trials are due to the failure of reliably fitting a sigmoid Weibull function and thus being unable to estimate a threshold value. Bias correction is based on the bias estimate, derived from the observer's previous responses. The bias values are related to the width of the normal distribution, describing the variability of the stimulus perception.

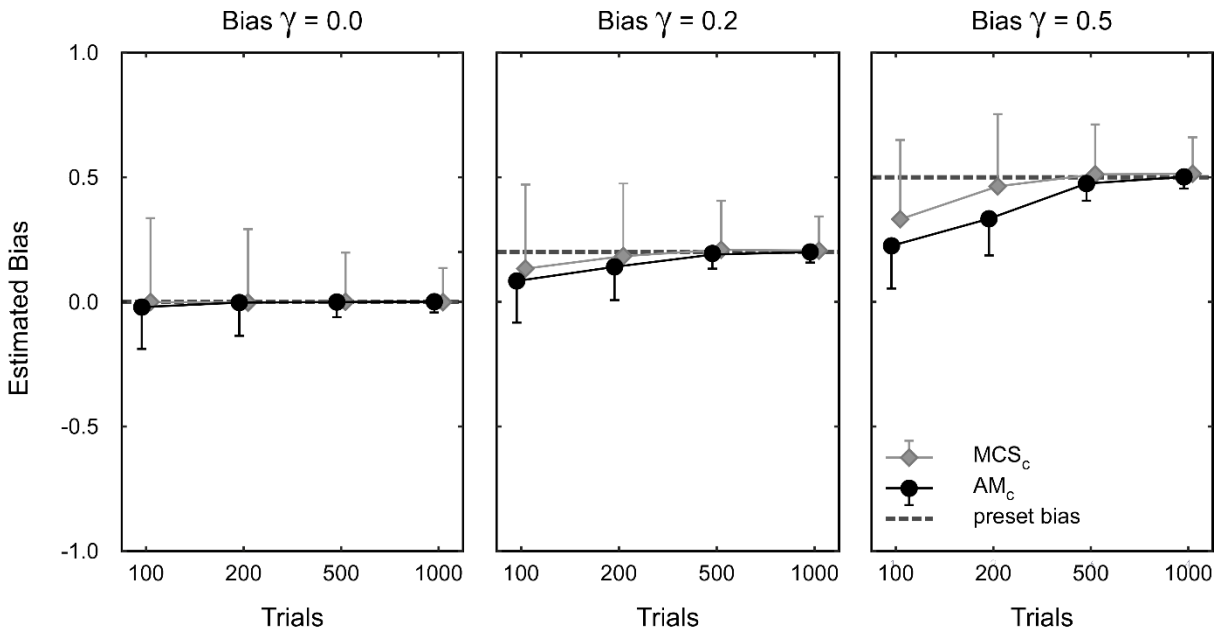

Fig. 5: shows the estimated biases values determined by the AM and the MCS with bias correction. Mean bias estimates $\tilde{\gamma}$ and standard deviations $\sigma_{\tilde{\gamma}}$ across 1000 simulations are presented for simulated decision criteria g of 0.0, 0.2, and 0.5 and for trial numbers of 100, 200, 500, and 1000 (abscissa).

Simulation results in figure 6 demonstrate that the AM is capable of tracking the participant's threshold continuously, yet with a delay of about 50 trials. Also the AM estimated threshold follows the preset threshold of the virtual observer in a smoothened way, due to the hysteresis of the AM procedure. Due to the steady change of virtual observer's threshold and the delay of the estimation procedure, it is clear that the threshold estimate cannot converge to the virtual observer's threshold. For a randomly varying sensitivity, the AM threshold procedure tracks the fluctuation of the preset sensitivity as long as their time constants are well above the time interval across which the threshold parameters are averaged. In contrast to the two-down one-up procedure, the three-down one-up approach yields less variable threshold estimates. Interestingly, for the variable sensitivity of the virtual observer the latter approach yielded also more

precise threshold estimates than the two-down one-up approach. In the simulations without any bias correction, the accuracy of the threshold was best for zero bias and worst for a bias of 0.5. The bias correction showed a good outcome especially for the randomly varying sensitivity of the virtual observer. Also the bias correction mechanism is switched on relatively early for intensities around the threshold (step-like lines for the different biases in figure 6 panel e), f), g) and h), it takes some more time for the procedure to become effective for mask delays further away from the threshold.

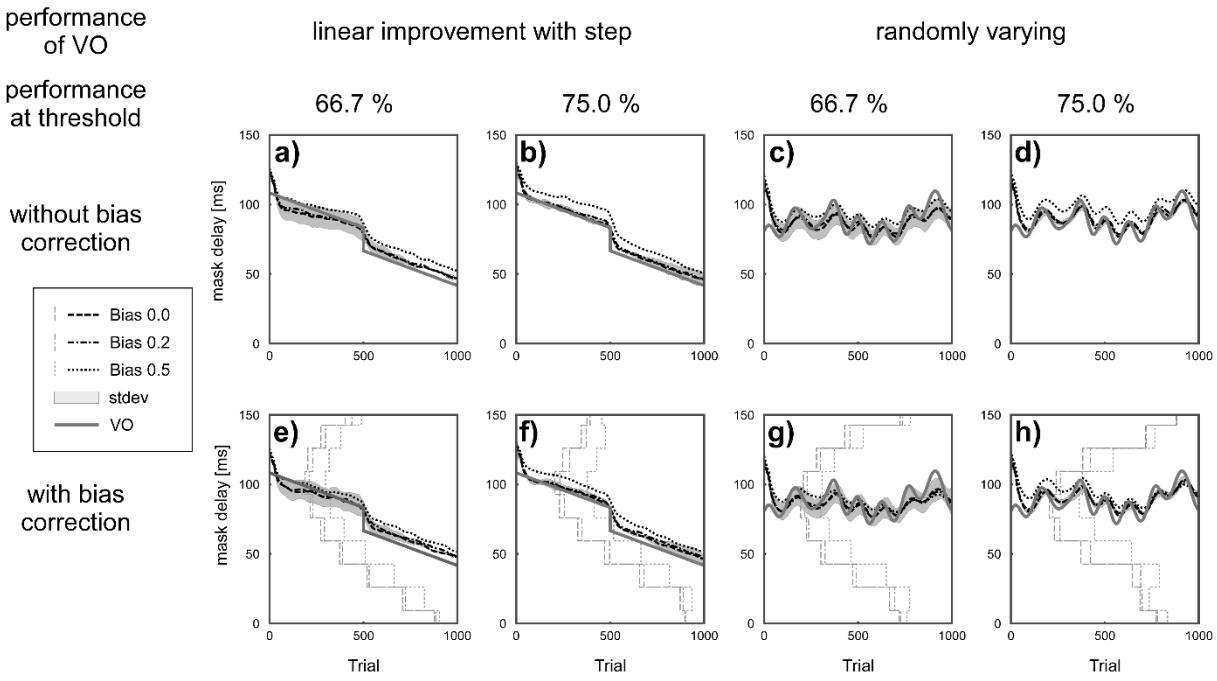

Fig 6: Simulation of the performance of the AM for a virtual observer's changing sensitivity across trials. In scenario 1 (panel a), b), e) and f)) the virtual observer's sensitivity, i.e. threshold, improves linearly from 100.0 ms to 83.3 ms from trial 1 to trial 500. At trial 501 a sudden change of the improvement (16.7 ms) was simulated in order to demonstrate the behavior of the AM to fast changes. From trial 501 onwards to trial 1000 the virtual observer's threshold further decreased again linearly resulting in a final threshold of 50.0 ms in trial 1000. In scenario 2 (panel c), d), g) and h)) a randomly changing virtual observer's threshold was simulated. In

both scenarios biases of 0.0 (dashed lines), 0.2 (dashed-dotted lines) and 0.5 (dotted lines) were simulated. The AM procedure was run with a two-down one-up procedure (a), c), e) and g)) and a three-down one-up procedure (b), d), f) and h)) resulting in a threshold performance of 66.7 % and 75.0 % correct responses, respectively. The threshold estimation was done without (a) – d)) and with bias correction (e) – h)). The standard deviation of the threshold estimate for 1000 repetitions for the zero bias simulation is shown as grey area. The step-like grey lines indicate the average trial number across the 1000 repetitions at which the bias correction was switched on. The trial at which the correction becomes active (steplike grey lines) varies for the different delays that are presented to the subject and for the bias (0.0: solid lines, 0.2: dashed-dotted lines, and 0.5: dotted lines). Since the estimated threshold follows the simulated threshold only after a delay, the estimated threshold is shifted by a minimum number of trials to the right. The standard deviation is smaller if the estimated threshold is defined by 75 % correct responses. In case of no bias correction there is a constant offset of the estimated threshold with respect to the preset threshold of the virtual observer. The bias correction works well for randomly varying thresholds of the virtual observer, yet, fails for the steady improvement of the virtual observer's sensitivity.

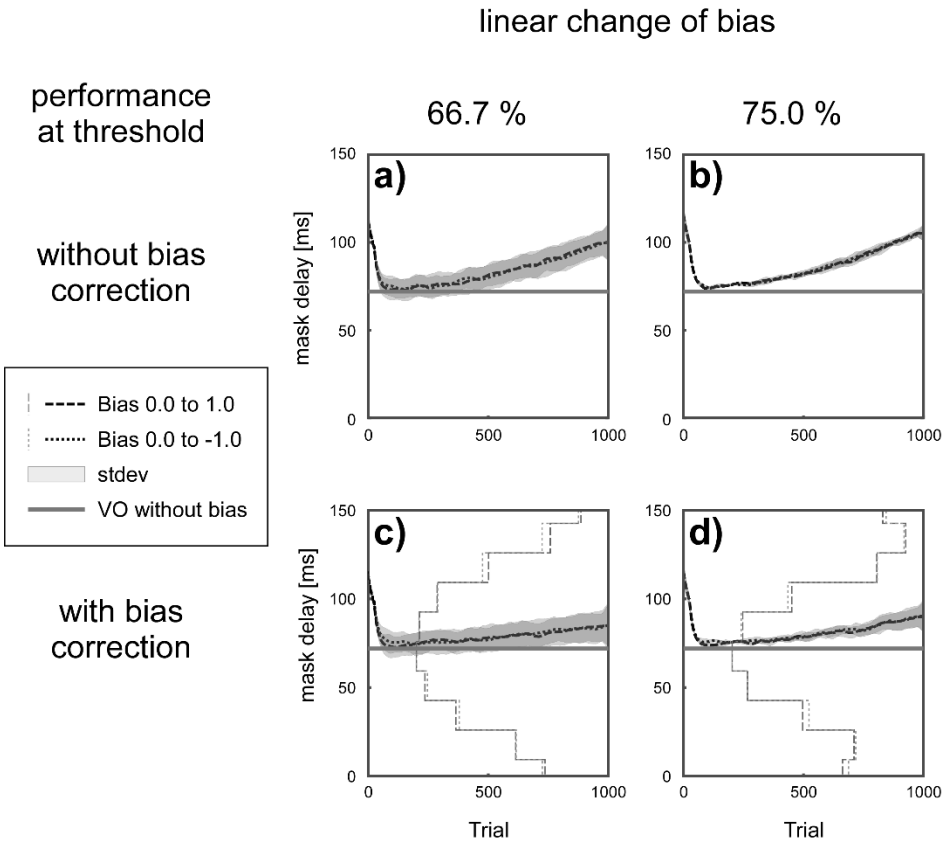

Fig. 7. Simulation of a linearly changing bias across trials. The solid grey horizontal line represents the preset performance of the virtual observer (VO) without any bias. Thresholds estimates for a steady bias increase from 0.0 to 1.0 and a decrease from 0.0 to -1.0 are depicted in dashed and dotted lines, yet, they are indistinguishable. Grey areas indicate the standard deviation of the threshold. Steplike lines represent the trials at which the bias correction became active (grey dashed lines for the increasing bias and grey dotted lines of the decreasing bias). In contrast to panel a) and b) bias correction was activated in panel c) and d). While in subplots a) and c) the two-down one-up rule was applied, in panels b) and d) the three-down one-up was chosen, resulting theoretically in a threshold performance of 66.7 and 75.0 %, respectively.

In order to investigate how the bias correction procedure deals with systematically changing biases over trials, linearly changing biases were simulated for a preset threshold delay of 72 ms. While in condition one the bias was increasing linearly from 0 to 1 across trials 0 to 1000, it

was decreasing from 0 to -1 in condition two. Effects of decreasing and increasing biases on the virtual observer's threshold did not differ (Fig 7). Comparing the estimated thresholds with and without bias correction, the threshold deviated less from the preset threshold of 72 ms if the correction was activated. However, the bias correction was unable to properly eliminate the simulated linear bias (Fig. 7).

4.8. Discussion

In this paper, a new approach for assessing and correcting the observer's bias in an adaptive threshold procedure (AM) is proposed. Previously suggested threshold estimation procedures that consider sensitivity and bias, have used Bayes' theory –that starts from an apriori distribution of probabilities for threshold parameters, including sensitivity and bias – and estimates their aposteriori probabilities based on the sampled data (Lesmes et al., 2015). In the method proposed by van Dam and Ernst (2015), the observer's bias is assessed through a set of Kalman Filters. In contrast to this class of methods, the presented procedure is assumption-free and does not depend on any apriori knowledge.

Using simulations, the performance of the new adaptive method is compared to the method of constant stimuli. In order to show whether the limitations we encounter are specific to the procedure or are a general issue of threshold estimation procedures, employing bias correction, we used a standard MCS method as a reference that excluded any response-dependent sampling near the threshold. Furthermore, for both methods, bias-uncorrected and bias-corrected simulated threshold estimates, were presented. Results indicate that both methods were able to estimate sensitivity and bias correctly, in case of a sufficiently large number of trials (>200 or > 500). In case of fewer trials, ranging between 100 and 200 ms, the MCS fails to accurately fit a sigmoid psychometric function to the experimentally acquired data. In contrast, the AM procedure is less dependent on number of trial, and still allows for

an estimate of the threshold with and without bias correction, for trial numbers ranging between 100 and 200 trials (Watson, 1990; Treutwein, 1995; Leek, 2001).

In MCS a psychometric function is fitted to the percentage of correct responses for each delay. If responses for single delays are too noisy as in the case of few trials, then the fitting of the psychometric function might be corrupted and will eventually fail. The failure of the MCS for low trial numbers, in the chosen example of the backward masking paradigm, results from the necessity of this approach to distribute the total number of trials to all different steps of delays, leaving only a fraction of trials for a single delay. In contrast, in AMs, the delays in individual trials are mostly concentrated around the threshold delays, and thus a larger number of trials is available for calculating the threshold estimates. MCS versions that sample the observer's responses close to the threshold with higher numbers of trials are certainly less affected by this problem, yet, involve the response-dependent selection of the sampling region – a characteristic feature of AM.

In the simulations of the AM, the average mask-delay of the last 50 trials was used as threshold estimate after the preset number of trials had been reached. In the simulations, the average across 50 trials resulted in robust threshold estimates. Using the two-down one-up rule, the probability to obtain a stable threshold by chance is 0.01 % for averaging 30 trials, and 0.0025 % for 50 trials. Therefore, in an empirical threshold estimations, an approach that uses an average of the last 50 trials (that applies the AM procedure until the threshold converges and no further systematic changes of the threshold can be observed) appears to be an appropriate criterion to terminate the threshold procedure.

Results of the bias-uncorrected AM reveal that the threshold estimate does not vary much with the number of trials. Assuming that the delays used for stimulation in the AM converge towards the threshold rather quickly, and using the averaged delay of the last 50 trials in each run, threshold delays should in fact be independent of the number of trials. This is especially true for trial numbers larger than 100. Since in the presented example there were only 10 steps of delays, the lowest delay (0.0

ms) could be reached within 20 trials using the two-down one-up rule, even if the procedure starts at the maximum delay (150 ms).

With increasing numbers of trials, the bias corrected AM yielded threshold estimates approaching the value preset in the simulation. In the AM method, bias-corrected estimates of the threshold rely on the bias-corrected responses of the observer, that determine the stimulus delay for the next trial according to an *m*-down *n*-up rule requiring *m* correct trials and *n* incorrect trials prior to decreasing or increasing the delay, respectively. A first, estimate of the bias is generated during the initial trials. Afterwards, when the bias estimate is available, the algorithm for bias correction becomes effective. Along with the threshold procedure, the bias estimate is constantly updated and thus becomes increasingly more reliable. However, this strategy implies that a sufficiently high number of trials had been sampled in order to get a good estimate of the bias. The simulations show that it takes around 200-500 trials in the MCS and the AM, respectively.

The convergence to the bias-corrected threshold becomes slower with increasing biases. The reason for this relation is the fact that the tails of the probability density functions, defined in the SDT, become smaller with more extreme bias values and thus increasingly difficult to estimate. For instance, in case of a decision criterion shifted towards ‘sad’ favoring more ‘happy face’ responses (see Fig. 2), the slow convergence is due to the low probability of obtaining an incorrect response when presenting a happy face (Fig. 2a). Similarly, in case of a shift of the decision criterion towards ‘happy’, the probability for obtaining an incorrect judgment when presenting a sad face will be low (Fig. 2d). With the low probabilities of responses, the time that is needed to reach a sufficiently high number of trials to reliably estimate the bias, increases drastically. However – as shown in the simulations of time varying thresholds of the virtual observer for stimulus parameters close to the threshold – more trials are available and thus the procedure for bias correction is switched on earlier than for stimuli further away from the threshold.

In summary, for both the AM and the MCS, bias correction improves threshold accuracy, in particular for strong biases. However, an effective bias correction algorithm requires a relatively large number of trials (see: S1a-b). Since for stronger biases, the required number of trials to obtain a stable estimate is large for both methods, and therefore certain advantages of the AM over the MCS (fewer trials needed to estimate the threshold) might get lost.

If the mean threshold of a larger group of individuals is studied, differences between methods will become evident. Since the standard error of the mean will decrease by the square root of N, with N being the number of individuals, the minimum group size, for which the application of a bias correction is beneficial, can be inferred.

With a sufficient number of subjects or repetitions, thresholds could be consistently higher in the AM as compared to the MCS in settings similar to our virtual experiment: The systematically higher threshold for the AM might result from the lower limit of the mask delay. In case of two correctly perceived emotional face expressions presented with a mask delay of 0 ms, the delay should be further decreased according to the two-down one-up rule. As a matter of fact, however, reducing the delay below 0 ms is not possible, and thus, delays for low thresholds in the AM will be slightly overestimated. The reliability of threshold estimates that are indexed by the standard deviation across simulation repetitions, is constant for the AM, independently of the number of trials and bias levels. In contrast to MCS, the standard deviations of different threshold levels are rather high, rendering the AM less reliable. With the two-down one-up rule applied here, only a few of the most recent trials determine the variation of the stimulus delay in the upcoming trials, making the approach very sensitive to noise. An alternative rule that considers responses of a higher number of "most recent" trials, reduces the effects of noise and as has been shown in our simulation, will enable the estimation of threshold levels for performance levels higher than 66.7%.

From the standard deviation of the threshold (Fig. 6), it is evident that a three-down one-up threshold, resulting in a performance level of 75.0 %, provides more precise threshold estimates than the two-

down one-up rule. Although the former procedure needs possibly more time to reach the threshold, the higher precision outweighs this drawback by far.

Results for the simulation of a linear changing bias showed similar effects on the virtual observer's threshold for decreasing and increasing biases (Fig. 7). This result is to be expected because flipping the sign of the decision criterion doesn't affect the overall correct responses, which is the sum of the numbers for hits and correct rejections. The bias-corrected estimated thresholds deviated less from the preset threshold of 72 ms compared with the uncorrected one. However, the correction procedure was unable to fully correct the simulated bias. Most likely, this is due to the fact that the bias was estimated using all previous trials, before to apply its correction in the current trial. With this strategy, the bias in a current trial is always underestimated and thus the correction is incomplete.

In general, a better threshold and bias estimate was initially expected for the adaptive method because there were more trials around the threshold. However, assuming a systematic relation between mask delay and discrimination performance in the psychometric function, like we did in our simulations, fitting a steady psychometric function to all data points in the MCS, might compensate for the higher error near the threshold.

While in this article bias was regarded as nuisance parameter that masks subjects' sensory capacities (Witte et al., 2013), it is important to underline that in other studies, changes in bias are the parameters of interest. For instance, studying perception of emotional stimuli in psychiatric diseases, such as depression (Bourne & Vladeanu, 2013), schizophrenia (Gooding & Tallent, 2002) or autism (Taylor et al., 2012; Ashwin et al., 2005), the observed higher or lower thresholds might result from a shift in the sensory bias rather than from altered sensitivity. Since the proposed method differentiates sensitivity and bias, deriving estimates for both of them, the method might have a wide range of applications in psychotherapy, in which would be interesting to modulate these parameters independently.

4.9. Conclusion

In this study, a new adaptive threshold estimation procedure was introduced, which is capable of correcting an observer's bias. The performance of the new procedure was simulated and compared to other approaches. The conducted simulations provide insights into the performance of classical threshold estimation procedures with and without bias correction under the presence of an observer's bias and disclose limitations of the procedures in this context.

Comparing signal detection theory-based bias correction for the MCS and the AM, many trials are needed in both approaches. Experiments engaging many trials are, however, not only time consuming, but also bear the risk of being inaccurate due to loss of subjects' compliance, variations in attention and effects of learning. To avoid these problems, observers' bias should be reduced as much as possible by choosing appropriate experimental designs. For instance, to minimize the effects of an observer's response selection bias, i.e. the preference of a participant to respond more frequently with his/her dominant hand, the assignment of response buttons – like for indicating positive and negative emotions in our study – could be balanced across trials.

The lower standard deviations for MCS across simulations indicate a better reliability of this method and argue for the application of this approach at least for higher trial numbers. In contrast, combining the AM with bias correction is beneficial for low biases when there is a need for keeping trial numbers low. The AM with bias correction becomes the method of choice, if during an experiment the stimuli should constantly be applied at an observer's dynamically changing perceptual threshold. In experiments in which sensory performance is trained and thus observers' sensory performance is assumed to change, the continuous adaptation of sensory stimulus parameters to the current perceptual threshold allows maintaining tasks demands constant across the whole experiment. Although the methodological framework presented in this study leaves space for further improvements, the new approach reveals a promising potential with a relevant impact on psychophysics, behavioral learning, and neurofeedback training.

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4.11. Supplementary material

[1] Estimation of threshold in the framework of signal detection theory

The fundamental assumption of signal detection theory is that sensory inputs, such as the masked sad or happy face, are represented as continuous variables, referred to as sensory representations. Given a decision criterion γ the probability for categorizing a happy face as happy can be inferred from the integral of the probability density function rightwards to the criterion. The probability density function is thereby most commonly assumed to be normally distributed. Likewise, the probability for judging the happy face as sad corresponds to the integral of the probability function leftwards to the criterion (Fig.S1). An alternative probability density function characterizes the perception of a sad face (Fig.S1). A large overlap of the two probability density functions indicates that the observer has difficulties to discriminate the corresponding stimuli. In contrast, a large distance d', between the peaks of the probability density functions indicates high discrimination performance. Given the sensory representation and the probability density functions, signal detection theory assumes that a criterion g is used to come to a sensory decision. If the sensory representation exceeds the decision criterion g the stimulus will be perceived as a happy face. Inversely, if the sensory representation is below the criterion, the stimulus will be reported to be a sad face. The criterion for sensory decisions might vary according to previous information and decision biases. Both, sensitivity d' and criterion g can be estimated based on subjects' response behavior, i.e. the number of hits (the percentage of correctly identified happy faces), correct rejections (the percentage of correctly identified sad faces), misses (incorrectly perceiving the happy face as sad) and false alarms (incorrectly perceiving the sad face as happy) (Table S1).

For a normal distribution for happy and sad faces with mean and standard deviation m_H and s_H, m_S and s_S, respectively, d' is defined as:

$$d' = \frac{\mu_H - \mu_S}{\sqrt{\frac{1}{2}\left(\sigma_H^2 + \sigma_S^2\right)}}$$

Assuming standard deviations of 1 for happy and sad face stimuli, d' can be inferred from measurements of the hit rate and false alarm rate (Fig.S1):

$d' = Z\left(p_H\right) - Z\left(p_F\right)$, where $Z\left(p\right),\ p \in [0,1]$ is the inverse of the cumulative distribution function of the Gaussian distribution.

The criterion g equals to:

$$g = \frac{1}{2}\left(Z\left(p_H\right) + Z\left(p_F\right)\right)$$

Plotting hit rates as a function of false alarms will result in the 'Receiver Operating Characteristic' (ROC) (Egan, 1975; Macmillan & Creelman, 2004; Swets, 2014). The ROC describes the sensitivity (d') independent of any bias. Therefore, ROC curves are iso-sensitive functions with variable levels of bias (Macmillan & Creelman, 1990). The Fig.S1e shows the perceptual representations described in Fig.S1a, b, c, d plotted in a ROC. It is noticeable that each curve in the graph is representing the same sensitivity value, Fig.S1a and Fig.S1b as well Fig.S1c and Fig.S1d are part of the same curve despite the different bias levels appear as different points in the same curves. The data presented in Fig.S1a, b, c, d can be fitted on the psychometric function to calculate the threshold. The different bias values evidently influence the threshold value, for values lower than $g = 0$.

		Perception	
		Happy face	Sad face
Stimulus	Happy face	Hit (H)	Miss (M)
	Sad face	False Alarm (F)	Correct Rejection (C)

Table S1: Representation of the subject's responses in dependence of the presence or absence of the stimulus.

Typically, when studying the threshold for the perception of emotional face stimuli, the bias that might be introduced by focusing on features that are related to either to the happy or the sad face is ignored, and only the percentage of corrected trials, i.e. $\frac{n_{hit} + n_{correct\,rejection}}{n_{hit} + n_{correct\,rejection} + n_{misses} + n_{false\,alarms}}$, is considered. Thus, sensory thresholds might be distorted and do not reflect the observer's' real performance.

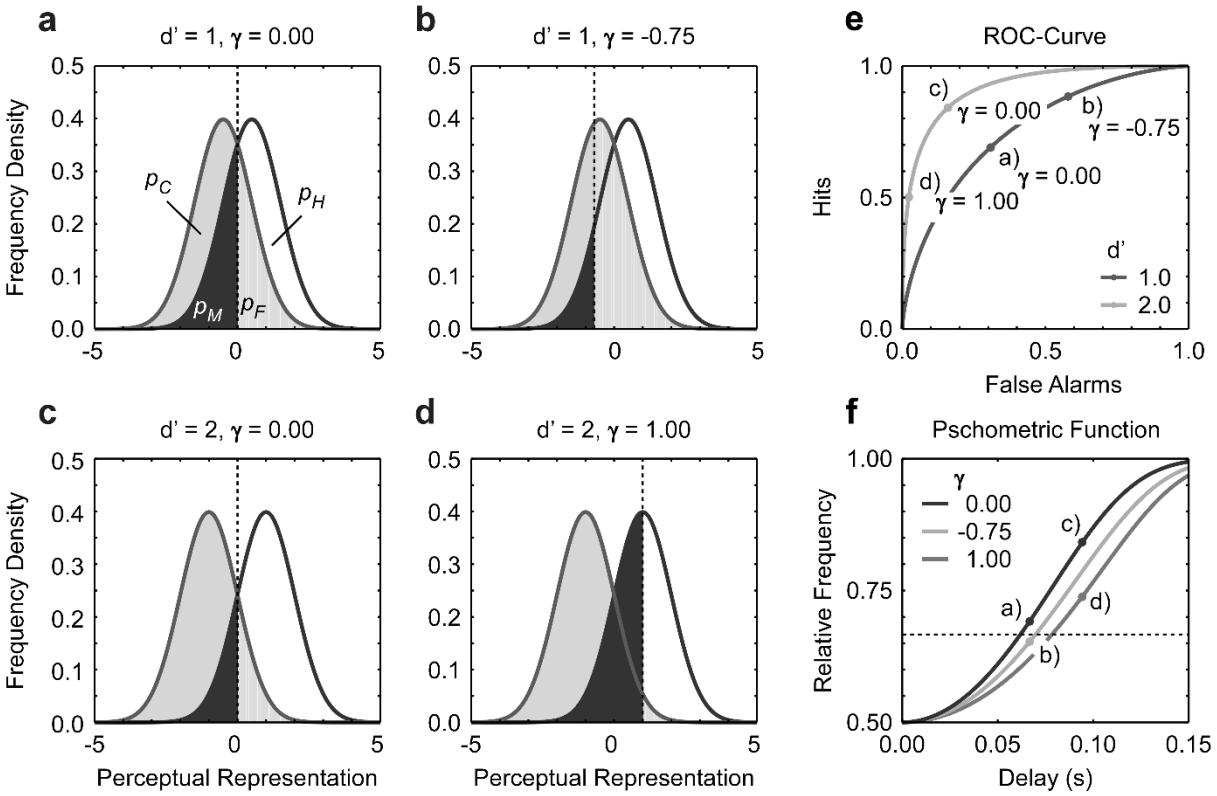

Fig.S1 a-f: signal detection theory probability distributions for perceiving happy and sad faces depending on the perceptual representation of the stimulus based on d' and the criterion position g. In figures a) and b), d' is smaller than in c) and d), and thus stimuli are more difficult to discriminate. While in b) a shift of the criterion towards sad faces increases a bias towards happy faces, in c) a shift of the discrimination criterion towards happy faces causes a bias for choosing sad face responses. e) The ROC curve represents the previously described distributions. f) shows the data of the distributions fitted to the psychometric function to calculate the respective threshold values.

Fig.S1a and Fig.S1c illustrate the situation in which the criterion is set at 0 ($g=0$) with a different degree of sensitivity with $d'=1$ (Fig.S1a) and $d'=2$ (Fig.S1c). The perceptual representation of the observer improves in the second case (Fig.S1c), represented by the greater area under the curves (hit rate and correct rejection areas) compared to the areas displayed in the Fig.S1a. Furthermore, the influence of the criterion position is shown in Fig.S1b and Fig.S1d. In the first case, with the criterion set at $g<0$, the hit rate has a higher value than in case of a bias $g=0$ but, at the same time, the

percentage of false alarm has also increased. When the criterion $g > 0$, the hit rate as well as the false alarm are lower, whereas there is a higher percentage of correct rejections and misses.

[2] AM two-down one-up rule and threshold performance level

The AM method in the manuscript uses mostly the 'two-down one-up'-rule to determine the mask delay of the following trial. After two correct responses, that do not necessarily have to occur in sequence, the delay becomes shorter and thus the task more difficult. The mask delay will be increased (and the task difficulty decreased) after each incorrect response. The reason why this procedure converges at a performance level of 66.67% is explained in the following paragraph.

In case of the basic 'one up-one down' method, the delay increases with each incorrect answer and decreases with each correct response. With this rule, the series will converge towards a threshold at which 50% of correct answers will be achieved (Leek, 2001). However, since a 50% performance may be reached by answering randomly, it is common practice to select thresholds for higher performance levels. At the convergence of the adaptive procedure with any rule for increasing or decreasing stimulus parameters, the probability of increasing the delay, p_{up}, and decreasing the delay, p_{down}, is 50% (Leek, 2001). If p_c is the probability to obtain a correct response and n is the number of trials which are taken into account, the number of trials in which the delay will be decreased is $\frac{1}{2}np_c$ in case of the two-down one-up rule. Therefore, $n(1\text{-}p_c)$ will be the number of trials in which the delay will be increased. Thus, the probability of a decreasing value will be $p_{down} = \frac{\frac{p_c}{2}}{\frac{p_c}{2}+(1\text{-}p_c)} = \frac{p_c}{2\text{-}p_c}$. At the convergence of the two probabilities, $p_{down} = p_{up} = \frac{1}{2}$, p_c will be 0.667. Note, that the rule does not require two correct responses in sequence to decrease the delay. In case of a three-down one-up rule, the number of trials in which the delay is decreased is $\frac{1}{3}np_c$, and the number of trials in which the delay is increased is again $n(1\text{-}p_c)$. p_{down} will be $\frac{\frac{p_c}{3}}{\frac{p_c}{3}+(1\text{-}p_c)} = \frac{p_c}{3\text{-}2p_c}$ and thus for $p_{down} = p_{up} = \frac{1}{2}$, p_c yields 0.75.

The performance level for a two-down one-up -rule in which the delay is only decreased if responses of two *successive* trials are correct, is derived considering pairs of trials. For n trials there are $\frac{n}{2}$ pairs. The pairs could consist of either two incorrect responses (incorrect-incorrect), one correct and one incorrect response (correct-incorrect or incorrect-correct), or two correct responses (correct-correct). In the first place, the probability to decrease the delay results from trials with two correct responses with a probability of $\frac{n}{2}p_c^2$. Furthermore, the delay will also be decreased if the pair of incorrect-correct responses is followed by a pair of correct-incorrect responses and by any such sequence with any number of correct-correct pairs in between. Thus the total number of trials in which a decrease of the delay will occur results in $\frac{n}{2}p_c^2 + \frac{n}{2}p_c^2(1-p_c)^2\sum_{i=0}^{\infty}p_c^{2i}$. The sum can be replaced by $\frac{1}{(1-p_c^2)}$ and thus the probabilities for increase and decrease at the equilibrium will be

$$p_{down} = p_{up} = \frac{n(1-p_c)}{n(1-p_c)+\frac{n}{2}p_c^2+\frac{n}{2}p_c^2(1-p_c)^2\frac{1}{(1-p_c^2)}} = \frac{1}{2}.$$

Simplification leads to $\frac{(1-p_c)}{(1-p_c)+\frac{p_c^2}{(1+p_c)}} = \frac{1}{2}$ and finally to a performance level of the two-down one-up method with two subsequent correct responses of $p_c = \sqrt{\frac{1}{2}}$.

4.12. References

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5. General Discussion

Perception is both a bottom-up process driven by stimulus input's characteristic and a top-down process driven by expectations. Different sensory cortex circuits dynamically interact to assure the elaboration of sensory information. This imply a coherent merging of the bottom-up and top-down process' outputs.

In the first study of my thesis, I addressed the potential role of GABA in modulating sensory processing on the level of primary and secondary somatosensory cortex (SI-SII) using a neuropharmacological-neuroimaging paradigm. In order to infer the role of GABA in the propagation of tactile information from primary to secondary somatosensory cortex, both, neuromagnetic brain responses were studied for supra- and near-threshold stimuli. In order to investigate the brain activity at near-threshold, stimulus intensities needed to be updated throughout the experiment. Since sensory threshold might change due to sensory adaptation, attention variations or biases affecting the subjects' responses, a new method was developed that allows for a constant stimulation at the sensory threshold by applying an ongoing bias correction. The second study thus focused on the implementation and the evaluation of the new method.

Multidisciplinar studies have shown the fundamental homeostatic role of the inhibitory transmitter GABA. In the human brain, its high concentration is indeed only exceeded by the excitatory neurotransmitter glutamate. The inhibitory interneurons and excitatory cells innervations are reported to be organized in a complex structure that includes feedback and feedforward connections. The balance between the inhibitory transmitter GABA and the excitatory transmitter glutamate assure a specific activation of brain areas, avoiding an overexcited chaotic brain activity. Three types of receptors bind for GABA, such as $GABA_A$ and $GABA_B$ in the central nervous system and $GABA_C$ in the retina. GABAR are also characterized by different subunits, binding with fast/tonic

or slow/phasic GABA agonists. The presented study focused on the role of different GABAR in sensory information processing. To this aim, three different kinds of GABA agonists, Alprazolam, Baclofen and Ethanol, were administered during a tactile stimulation experiment and their effects on somatosensory evoked fields (SEFs) were investigated.

Results of the study showed that fast $GABA_A$ agonist Alprazolam has a significant inhibitory effect both at the levels of SI and SII. However, it was still unclear whether the GABA is active only on the level of SI or also on SII, or whether any inhibition on the level of SII is the mere propagation from SI to SII. To answer this question, I have studied the propagation of information processing from SI to SII. Referring to a previously calculated transfer function, a model was proposed to explain inhibitory effects on the level of SI and SII. The results showed that GABA is acting only on the level of SI and propagating the reduced activity to SII, without any additional inhibition at the level of SII.

This experiment was able to highlight the different role of the GABAR subtypes and the dynamic of inhibitory propagation in the somatosensory area. It would be interesting to investigate the data from the double stimulation to verify if the inhibition of the second stimulus is also mediated by GABA and in particular by the receptors for $GABA_A$. Further experiments could explore the role of inhibition in the interaction between multiple sensory stimuli. In studies on the functional organization of somatosensory cortex it has been shown that the homuncular organization varies in a task-dependent manner (Braun et al., 2001; Braun et al., 2002). There are good reasons to assume that the task-dependent modulation is mediated by GABA. Investigating task-dependent chances of the functional organization in SI in combination with a neuropharmacological approach could disclose the underlying neurochemical and neurophysiological mechanisms.

To integrate the advantages of the adaptive methods, such as a high number of trials with near-threshold stimuli, with an on-line bias correction, I developed a new method for the calculation of the perceptual threshold. This method was described and simulated in the second study presented here.

In the proposed method, the algorithm for the calculation of the stimulus level assigned to each trial follows a two-down one-up procedure. A backward masking paradigm, with emotional faces as stimuli to discriminate and different delays as the level of perception difficulty assigned to each trial, was used for the simulations. To compare our results, the simulation was used also with the MCS serving as a refeence. The simulation results showed that for higher number of trials, the MCS seems to be more reliable, while the new adaptive method with on-line bias correction is more indicated in the case of low biases and when a low number of trials number is inevitable. The AM with bias correction is strongly suggested when the stimuli should be presented at the threshold level, that as known is not a constant value but it is also influenced by sensory adaptation, learning, and bias changes. This method allows to keep the stimulation at a constant level when from the other side the perceptual threshold is changing.

Simulations also disclosed limitations of the new procedure. One limitation is the high number of trials that are needed to estimate the bias in case of a strong bias. In this situation, the advantage of the adaptive method of requiring less trials than the Staircase method is lost. Therefore, designing threshold estimation procedures or stimulation close to the sensory threshold, it should be made sure that the bias is reduced. In order to avoid a bias introduced for example by the preference of subjects to respond with one of several buttons when they judge the presented stimuli, the assignment of the buttons to a certain response can be assigned on a trial by trial basis. In this situation the response bias would affect all response options similarly.

In conclusion, the here presented studies demonstrate that the combination of methods and the precise control of stimulation conditions provide means to get a more detailed insight in the cerebral information processing.

6. General introduction and discussion references

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