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Inhibition in the somatosensory system: An integrative neuropharmacological and neuroimaging approach

C. Fioravanti [a,b,*], S.D. Kajal [b,c], M. Carboni [d,e], C. Mazzetti [f], U. Ziemann [g,1], C. Braun [b,h,1]

[a] Institute of Medical Psychology and Behavioral Neurobiology, Medical Faculty, University of Tübingen, Silcherstraße 5, 72074, Tübingen, Germany
[b] MEG Center, University of Tübingen, Otfried-Müller-Straße 47, 72076, Tübingen, Germany
[c] Graduate School of Neural and Behavioral Sciences, International Max Planck Research School, Österbergstraße 3, 72074, Tübingen, Germany
[d] EEG and Epilepsy Unit, University Hospital of Geneva, Rue Gabrielle-Perret-Gentil 4, 1205, Genève, Switzerland
[e] Functional Brain Mapping Lab, Department of Fundamental Neurosciences, University of Geneva, Chemin des Mines 9, 1202, Genève, Switzerland
[f] Department of Cognitive Neuroimaging, Donders Institute, Radboud University, Kapittelweg 29, 6525EN, Nijmegen, Netherlands
[g] Department of Neurology & Stroke, and Hertie-Institute for Clinical Brain Research, Hoppe-Seyler-Str. 3, 72076, Tübingen, Germany
[h] CIMeC, Center for Mind/Brain Sciences Cognitive Neuroscience, University of Trento, Corso Bettini 31, 38068, Rovereto, Italy

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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.

1. 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

* Corresponding author. MEG Center, University of Tübingen, Otfried-Müller-Straße 47, 72076, Tübingen, Germany.
E-mail address: chiarafioravant@gmail.com (C. Fioravanti).
[1] Shared senior authorship.

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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 $GABA_ARs$. Conversely, slow IPSPs are mediated by $GABA_BRs$.

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 and 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.

2. Methods

2.1. 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.

2.2. 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 × 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 (8.1 g/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

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, 17 × 8mm, 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 × 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

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, 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, 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).

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 × 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

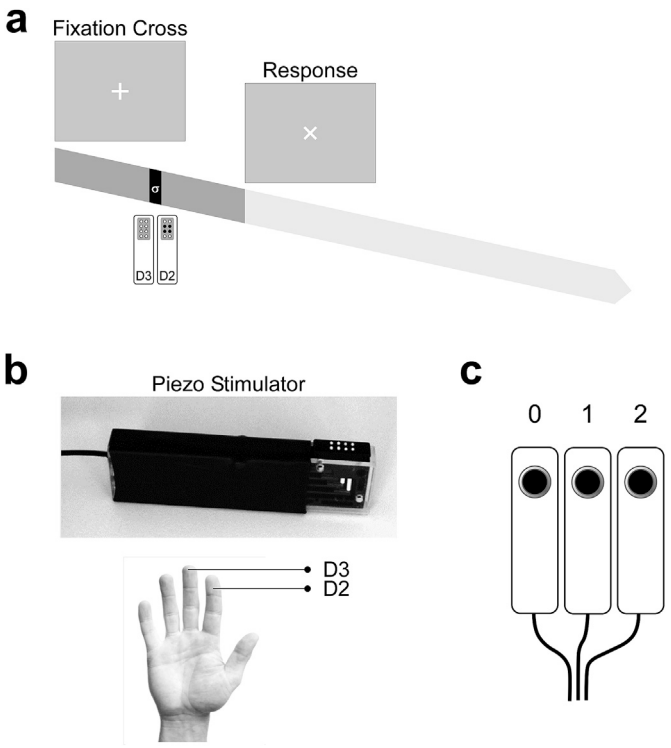


Fig. 2. In single stimulation trials, either index (D2) or middle finger (D3) of the left hand were stimulated at near-threshold or maximum intensity, using a piezo-electric tactile stimulator (b). The stimulus duration (σ) was 0.05 s. The rotation of the fixation cross (x) appearing at $\tau =$ 0.85 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).

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% correct 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

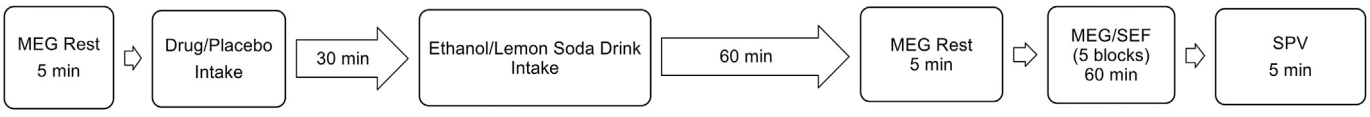


Fig. 1. Study design: prior to and after drug/placebo intake brain resting-state activity was recorded by MEG (baseline and post-drug measurement, respectively). Thereafter, changes in the processing of somatosensory stimuli were investigated by stimulating index and middle finger of the left hand. In 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 3
Number of stimuli for the different stimulation conditions. In the current study, only results for the single pulse stimulation are presented.

Intensity	Single pulse stimulation				Paired pulse stimulation				Total
	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%

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.

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.

2.3. 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.

2.4. 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.

2.5. 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):

$$B = LS + \varepsilon \tag{1}$$

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)).

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

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 m s, 100 m s; SIIc: 117 m s; SIIi: 123 m s), 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.

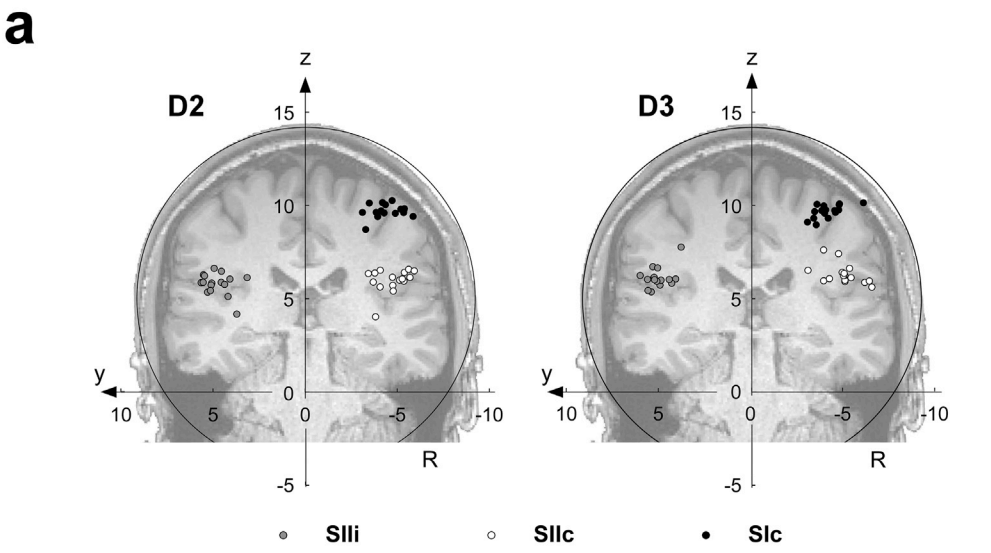
2.6. Statistical analyses

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 ε. Prior to all rmANOVAs, we verified that variables were normally distributed using the Shapiro-Wilk test (Shapiro and 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.

2.6.1. 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



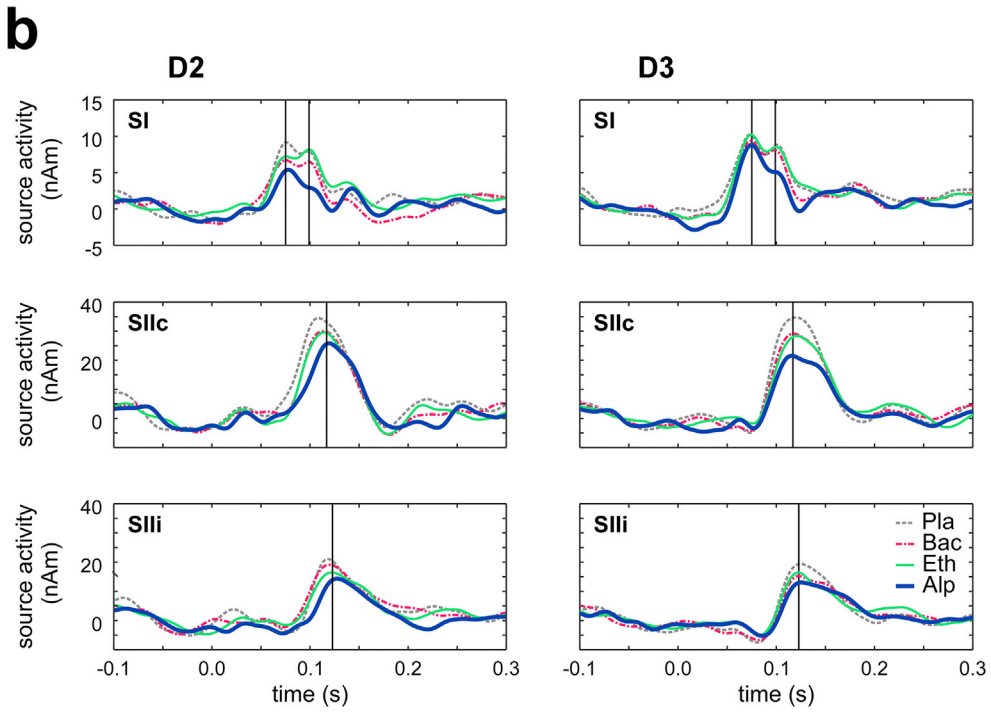


Fig. 3. (a) Individual dipole source locations for contralateral SI (black dots), contralateral SIIc (white dots) and ipsilateral SIIi (grey dots): Source localization results were warped into a common coordinate system. Dipoles for SI were verified to yield a fronto-parietal orientation in order to 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, dashed); *Baclofen* (BAC, red, dashed-dotted); *Ethanol* (ETH, green, solid light) and *Alprazolam* (ALP, blue, solid dark).

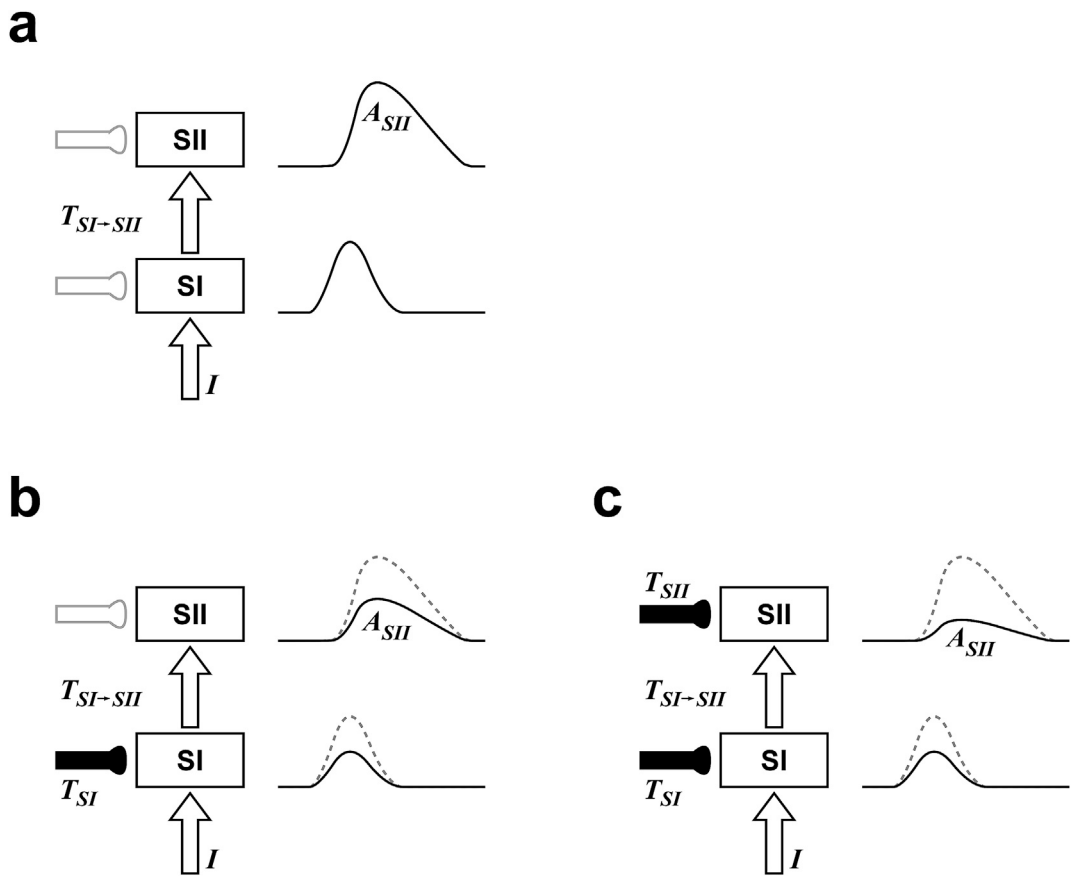


Fig. 4. Schematic sketch explaining the reduced activity in SII under the effect of GABAR-agonists. (a) Placebo-condition: no inhibition takes place at the levels of SI or SII. Input I to SI is propagated to SII. The relation between SI and SII activation is given by a characteristic transfer function $T_{SI\rightarrow SII}$ (Activity in SII: $A_{SII} = T_{SI\rightarrow SII} \otimes I$) (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: $A_{SII} = T_{SI\rightarrow SII} \otimes T_{SI} \otimes I$). (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: $A_{SII} = T_{SII} \otimes T_{SI\rightarrow SII} \otimes T_{SI} \otimes I$). 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).

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).

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–b). 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 $1/(n\text{-}1)^2$, 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 Appendix).

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.

2.7. Method for saccadic peak velocity analyses

Saccadic peak velocity (SPV), as a well-established measure of

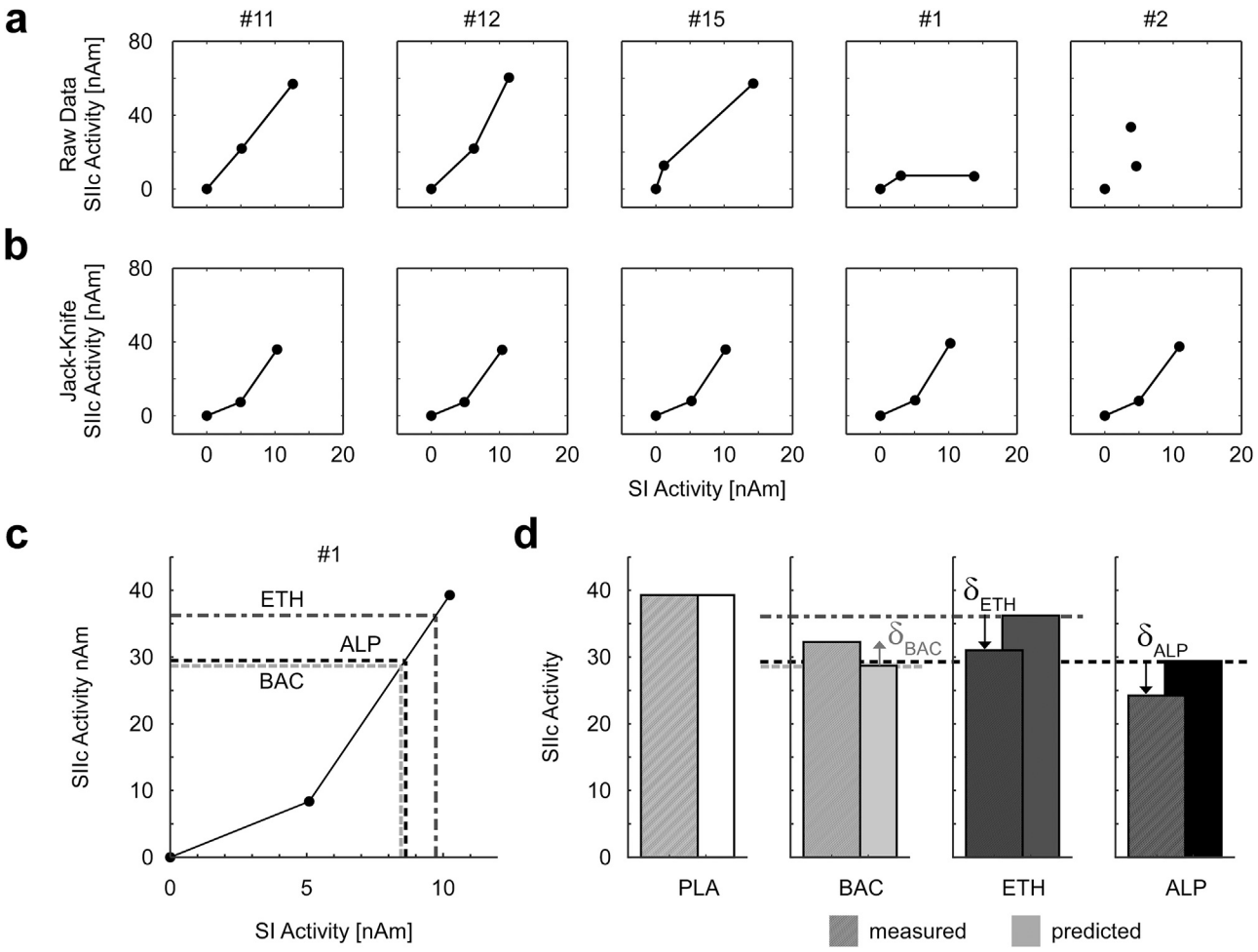


Fig. 5. (a) The figure shows examples (subjects 11, 12, 15, 1, 2) of the transfer functions between the initial SI and the SIIc peak activity for tactile stimulation of finger D3. While subjects 11 and 12 showed a steady transfer function, it was slightly distorted for subject 15 and even more distorted 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 (δ) 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.

$GABA_AR$ 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°. 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.

2.8. 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. 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.

3.1. 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*: $p = 0.0038$, *Baclofen*: $p = 0.0004$, *Ethanol*: $p = 0.0023$, *Alprazolam*: $p = 0.0005$, 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: $p = 0.035$) 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: $p = 0.055$). 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*: $p = 0.0027$, *Baclofen*: $p = 0.0009$, *Ethanol*: $p = 0.0013$, *Alprazolam*: $p = 0.0004$, Fig. 6b). Comparing the variance of reaction times between *Placebo* and the GABAR agonists there was only a significant difference for *Alprazolam* (low intensity: $p = 0.0004$, high intensity: $p = 0.0004$), but not for any other GABAR agonists ($p > 0.352$). 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 $p > .12$).

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.

3.2. 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* ($349.7 \pm 13\,^{\circ}/s$) *than Placebo* ($394.5 \pm 13.27\,^{\circ}/s$, $t(15) = 2.79$; $p = 0.014$). In agreement with our previous findings (Premoli et al., 2014a; Lücke et al., 2014; Fuhl et al., 2015), *Ethanol* ($391.3 \pm 13.68\,^{\circ}/s$) and *Baclofen* ($366.9 \pm 17.28\,^{\circ}/s$) did not differ from the *Placebo* condition ($p > 0.05$).

3.3. 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 *Finger* (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* $F(1, 15) = 19.28$; $p < 0.001$), (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).

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

3.4.1. 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

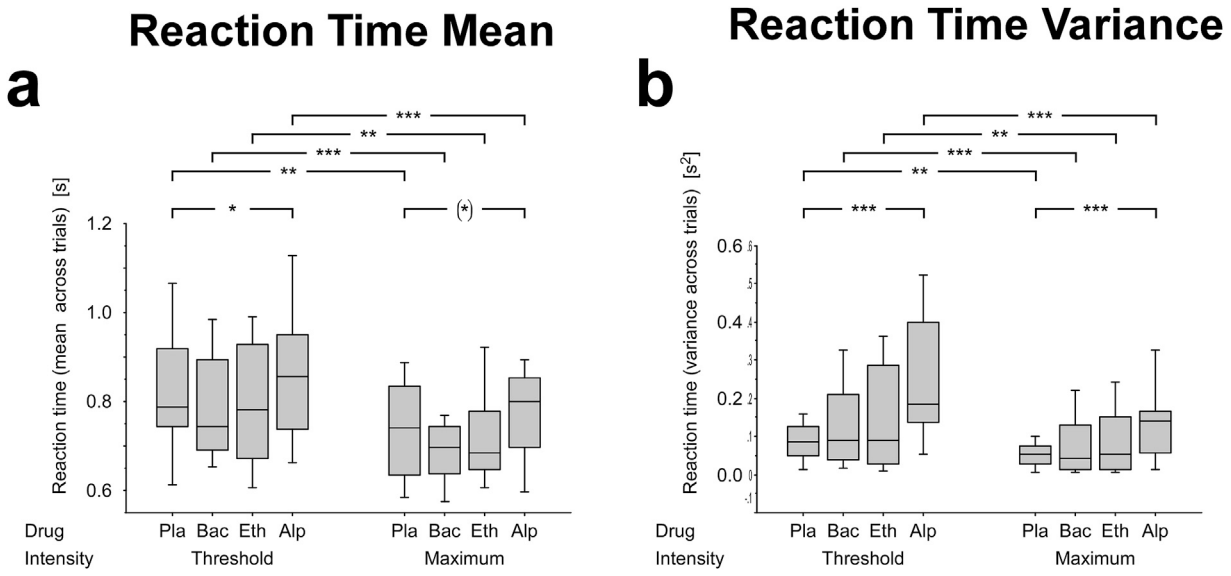


Fig. 6. To study effects of GABAR agonists, Baclofen (Bac), Ethanol (Eth) and Alprazolam (Alp) on behavior, mean (a) and variance (b) of reaction times across trials were investigated. Reaction time parameters for d2 and d3 were collapsed. Parameters are presented as box-whisker plots, with median, 1st 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 $p < 0.075$.

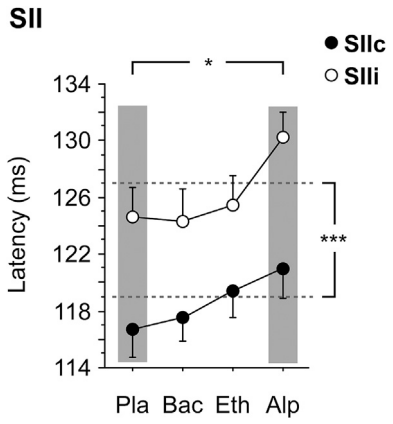


Fig. 7. Effects of drugs and placebo on the latencies of ipsi- and contralateral SII source activities. The black dots refer to latencies found for contralateral secondary somatosensory cortex (SIIc). White dots refer to ipsilateral secondary somatosensory cortex (SIIi). The bars indicate standard errors 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*.

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.

3.4.2. 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).

3.5. 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 (δ 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 m s and peak 2 at 100 m s) 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

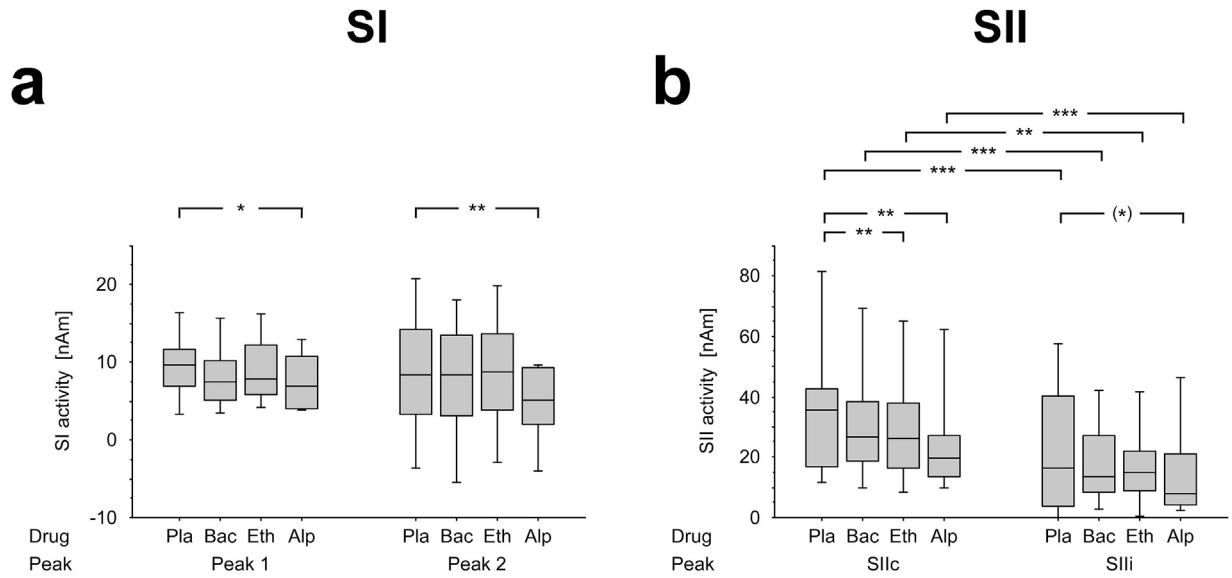


Fig. 8. Amplitudes of source activities evoked by single tactile stimuli with maximal intensity for the different GABA-agonists alprazolam (Alp), baclofen (Bac), ethanol (Eth), and placebo (Pla). Activities are presented in box-whisker plots with the median indicated as solid black horizontal line within 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 $p < 0.075$.

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.

4. 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 $GABA_BRs$.

4.1. 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

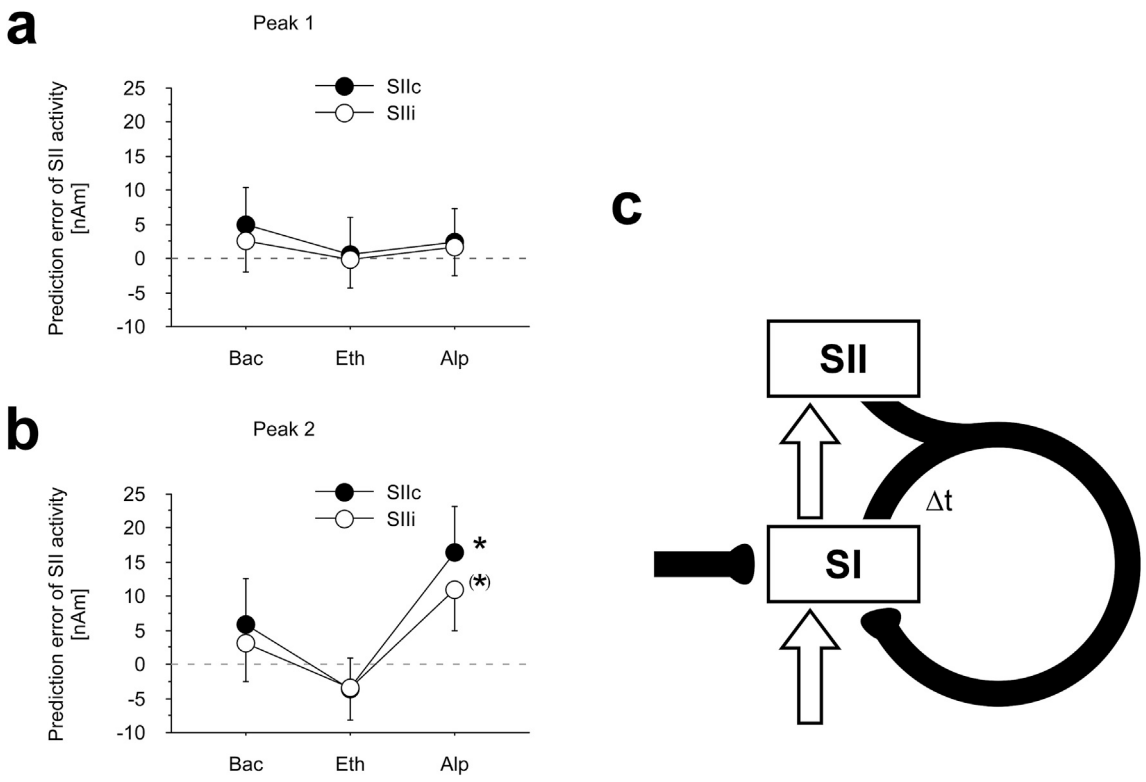


Fig. 9. (a): Prediction error results and model of propagation: For the first peak of SI, no significant difference was found between the predicted SII GABAergic effect and the measured activity. (b): for the second peak of SI, a significant difference was found between the predicted SII GABAergic effect 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.

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 α1-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 al., 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. Though as acute exposure to ethanol at doses similar or even less to the one tested in our experiments had detrimental effects on attention in previous studies (Rohrbaugh et al., 1987; Jääskeläinen et al., 1999), no effects on attention dependent parameters such as reaction times were found in our experiment. 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.

4.2. 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.

4.3. Histological and neurophysiological effects

4.3.1. 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 $GABA_ARs$ was documented 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.

4.3.2. 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.

5. 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.

Conflicts of interest

The authors declare no competing financial interests.

Statement of significance

The current combined neuropharmacological-neuroimaging study investigated the role of GABA as a mechanism controlling the processing of sensory information in SI, and SII. Our results evidence GABA-ergic modulation predominantly on 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 activation of SI to SII. The current study allowed us to disclose the temporal dynamics of inhibitory processes within SI and between SI and SII.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2019.116139.

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