GABAergic mechanisms gate tactile discrimination learning

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In contrast to mechanisms mediating synaptic plasticity, the pharmacological basis of perceptual learning remains to be clarified. Here we report that a specific form of perceptual learning is influenced by GABAergic mechanisms. We induced perceptual learning by Hebbian co-activation of the skin of the tip of the right index fingers in human subjects. Under placebo conditions, tactile 2-point discrimination was improved on the co-activated, but not on the left, index finger. This augmentation was completely eliminated by lorazepam, a $GABA_A$ receptor agonist. No drug effects were found on the left index finger indicating that the drugs had no effect per se on performance. The results demonstrate that perceptual learning is subject to pharmacological gating by basic mechanisms known to mediate and modulate synaptic plasticity. *NeuroReport* 14:1747–1751 © 2003 Lippincott Williams & Wilkins.

Key words: $GABA_A$; Cortical plasticity; Learning; Neuromodulation; Neurotransmitters; Psychophysics; Synaptic plasticity; Tactile performance; Two-point discrimination

INTRODUCTION

Extensive practice and training improves perceptual or motor performance and is associated with specific changes of cortical representations [1–3]. However, the pharmacological basis of perceptual learning processes remain to be clarified. To study perceptual learning in parallel to cortical reorganization, we recently introduced a co-activation protocol that follows closely the idea of Hebbian learning: Synchronous neural activity, necessary to drive plastic changes, was evoked by tactile co-activation of the skin of the index finger [4–7]. In fact, from a number of animal studies, the importance of temporally correlated inputs has been assumed to play a key role in mediating plastic changes [8,9].

Co-activation is a task-free, passive stimulation protocol. Many studies have demonstrated that learning and plastic cortical changes can be evoked by variation of input statistics alone, provided the statistics are sufficiently altered [10–12]. For example, perceptual learning occurs even without awareness by repetitive exposure to stimuli that are below the threshold of visibility and that are irrelevant to the central task [13]. Our results provide further evidence that perceptual performance is subject to improvement by solely manipulating the input statistics without invoking attention or reinforcement. In previous studies we demonstrated that plastic changes induced by co-activation results in a particular form of perceptual learning. Spatial tactile discrimination performance as an indirect marker of learning processes and associated cortical reorganizations was measured in human subjects before and after co-activation of a small skin region on the tip of the index finger. After 3 h of co-activation, we found a lowering of thresholds, an effect that was reversible within 24 h [4–7].

Cellular studies on synaptic plasticity suggest that there might be only few, but very basic mechanisms that control regulation of synaptic transmission. In particular, the NMDA receptor, a specific subtype of the glutamatergic receptors, has been implicated in synaptic plasticity [14,15]. To investigate the involvement of NMDA receptors in perceptual learning, we have recently shown that blocking NMDA receptors with a single dose of memantine blocks perceptual learning of discrimination induced by co-activation [16]. To be operative, NMDA receptors require sufficient depolarization, thus, the balance of excitation and inhibition plays an important role in controlling plasticity. To further corroborate the role of NMDA receptors in perceptual learning, here we used the co-activation protocol to demonstrate that the enhanced inhibition mediated by lorazepam, a $GABA_A$ receptor agonist, blocks co-activation-induced perceptual learning.

MATERIALS AND METHODS

Experimental groups: We tested 24 right-handed subjects of both sexes aged 20–46 years: 16 subjects provided a placebo-controlled baseline, and eight were tested under

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lorazepam, a benzodiazepine that enhances $GABA_A$ receptor function [17,18]. The study was performed in accordance with the Declaration of Helsinki. The subjects gave their written informed consent, and the protocol was approved by the local ethical committee of the Ruhr-University Bochum.

Drugs and drug application: Lorazepam was administered perorally (2.5 mg) 1.5 h before onset of co-activation. After 3 h, blood plasma level concentration was assessed. The mean ($\pm$ s.e.m.) level of 49.00 ± 7.86 ng/ml is within the effective range [19]. The placebo was administered 2 h before co-activation.

Psychophysical tests: Subjects were tested in a 2-alternative forced-choice simultaneous spatial 2-point discrimination task [4–7]. As a rule, the index finger of the right hand was used for co-activation, and the index finger of the left hand served as control of the selectivity of co-activation (placebo group), and for assessment of unspecific side effects of the drug. Seven pairs of needles (diameter 200 μm) were mounted on a rotatable disc that allowed us to switch rapidly between distances. We used 0.7, 1.0, 1.3, 1.6, 1.9, 2.2 and 2.5 mm separations, zero distance was tested with a single needle. To accomplish a rather uniform and standardized type of stimulation the disc was installed in front of a plate which could be moved up and down. The arm and fingers of the subjects were fixed on the plate and the subjects were then asked to move the arm down. The down-movement was arrested by a stopper at a fixed position above the needles. The test finger was held in a hollow containing a small hole through which the finger touched the needles at approximately the same indentations in each trial [5,6]. Each distance was tested 10 times in randomized order resulting in 80 trials per session. The subject had to decide immediately if he had the sensation of one or two tips. The summed responses were plotted against distance as a psychometric function for absolute threshold, fitted by a binary logistic regression (SPSS). Threshold was taken from the fit at that distance for which 50% correct responses was reached. To obtain a stable level of discrimination, subjects were tested on 5 sessions over several days (s1–s5) and statistically analyzed for stability (ANOVA). At the 5th session, after assessment of thresholds of both the test and the control finger (pre-condition = s5), drugs were administered. Then the co-activation protocol was applied to the right index finger. Discrimination performance for the index fingers of each hand was re-tested immediately after termination of the co-activation (post-condition = s6). Assessment of discrimination performance of the test finger was repeated after 24 and 48 h (s7 and s8).

Co-activation: The co-activation protocol was the same as that described previously [4–7]. Interstimulus intervals of stimuli for co-activation were 100–3000 m s in pseudo-randomized order (average frequency 1 Hz, pulse duration 10 ms). Pulses were recorded on tape and were played back via portable tape recorders. Subjects were instructed not to attend stimulation. In fact, all subjects resumed their normal day work. To transmit the co-activation stimuli to the skin, a small solenoid (diameter 8 mm) was mounted to the tip of the right index finger (Fig. 1). The basic idea is to co-activate in a Hebbian manner receptive fields to strengthen their mutual interconnectedness. The solenoid stimulated simultaneously (co-activated) the skin portions of the index finger under the solenoid (for a discussion of the estimate of receptive field sizes of the human fingertip see [6]). According to these data, receptive fields within 8 mm overlap partially or are non-overlapping. Stimuli were applied at supra-threshold intensities. Duration of co-activation was 3 h.

All psychophysical and electrophysiological data were statistically analyzed using ANOVA or Student's paired *t*-test.

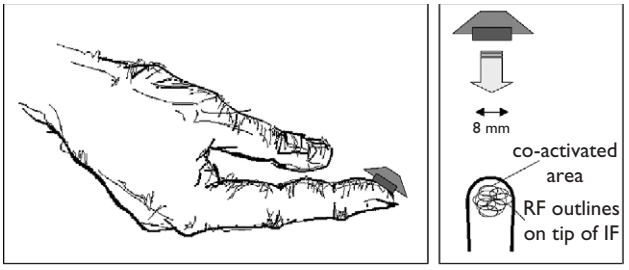

Fig. 1. Application of co-activation. A small solenoid with a diameter of 8 mm was mounted on the tip of the right index finger to co-activate the receptive fields representing the skin portion under the solenoid.

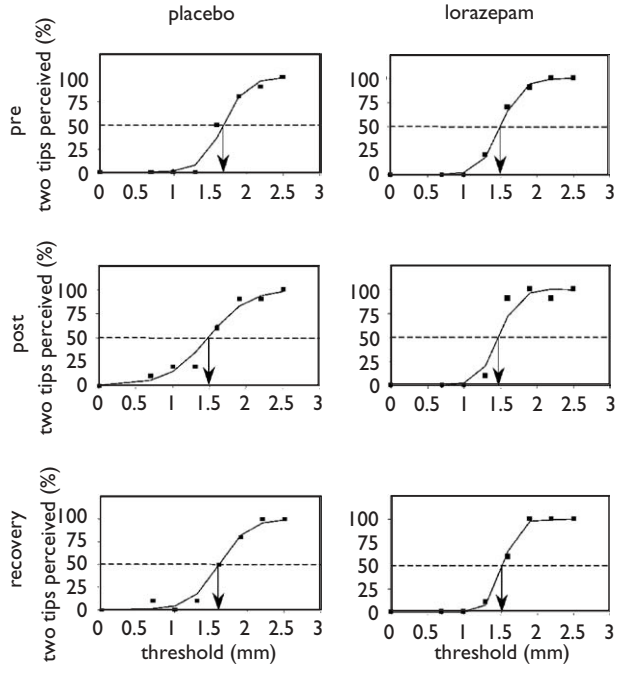

Fig. 2. Psychometric functions illustrating the co-activation-induced effects on discrimination threshold for an individual subject from each drug group (placebo controlled, lorazepam ($GABA_A$ agonist). Correct responses (percentages; black squares) are plotted as a function of separation distance together with the results of a logistic regression line. Top row: pre-condition before co-activation; middle row: post-condition, immediately after co-activation; bottom row: recovery assessed 24 h after termination of co-activation. 50% level of correct responses is indicated (dashed line) together with resulting thresholds (arrows).

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RESULTS

As a first step, we assessed tactile 2-point discrimination thresholds under placebo conditions. During the initial five sessions pre-co-activation, all subjects in this group fulfilled the criterion for stable performance (ANOVA sessions s1–s5-pre: $F = 0.311$; $p = 0.869$, $n = 18$). Three hours of co-activation on the tip of the right index finger lowered discrimination thresholds for the right index finger (1.55 ± 0.20 mm pre *vs* 1.35 ± 0.23 mm post co-activation, ANOVA: $F = 8.887$; $p = 0.009$, pre–post difference *post hoc* $p < 0.005$, $n = 16$). Psychometric functions show a distinct shift towards smaller separation distances after co-activation (Fig. 2). Assessment of thresholds 24 and 48 h after co-activation revealed normal, pre-co-activation thresholds, confirming reversibility of changes (Figs 2 and 3). We found no correlation between individual pre-thresholds and amount of improvement (Pearson, $r = -0.173$; $p > 0.5$, $n = 16$). As a control, and to demonstrate the specificity of the co-activation-induced changes, we measured thresholds of the index finger of the left hand, which was not-co-activated. Thresholds remained unchanged ($p = 0.234$, Fig. 3) confirming the lack of generalization of co-activation across hands [4–8].

All subjects in the lorazepam group reached a stable level of performance during the initial sessions (ANOVA sessions s1–s5-pre: $F = 1.633$; $p = 0.194$, $n = 8$). Application of a single dose of lorazepam abolished the co-activation-induced improvement of spatial discrimination performance in all subjects. Mean thresholds pre-co-activation were 1.62 ± 0.11 mm and 1.62 ± 0.13 mm after co-activation under lorazepam (ANOVA: $F = 1.101$; $p = 0{,}377$, pre–post difference *post hoc* $p = 0.449$, $n = 8$; Figs 2 and 3). Given that lorazepam might exert non-specific side effects it was important to show that the drug did not affect spatial

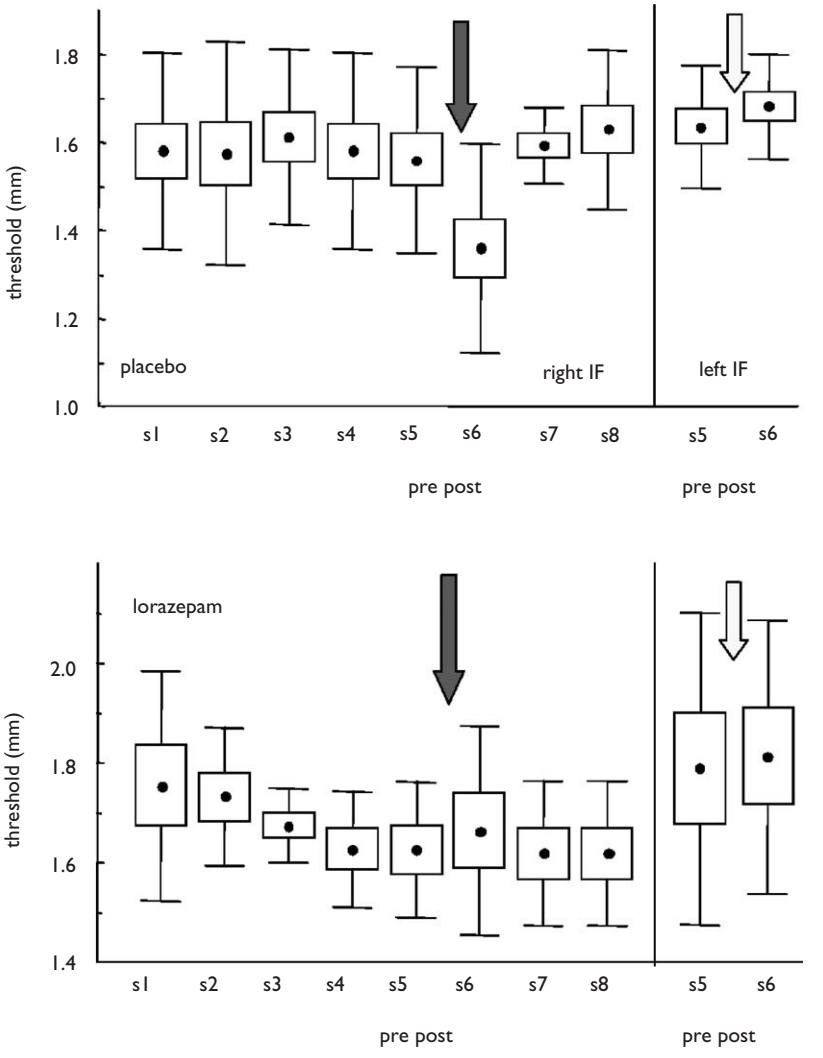

Fig. 3. Average effects of co-activation on discrimination thresholds for each group. Dots represent mean thresholds, boxes show the standard errors and whiskers correspond to s.d. Co-activation period on right index finger (3 h) is indicated by arrow. For each group, discrimination thresholds obtained for the test finger (right index finger) are shown during initial testing (s1 to s4) and pre-(s5) and post-(s6) co-activation. Recovery is shown for 24 h (s7) and 48 h (s8) after co-activation. For the control finger (index finger of the left hand that was not co-activated), thresholds are shown for the pre- and post-condition (s5 and s6). The lack of effects for the control finger indicates finger-specificity of the co-activation protocol (placebo group), and lack of non-specific side effects in the lorazepam group.

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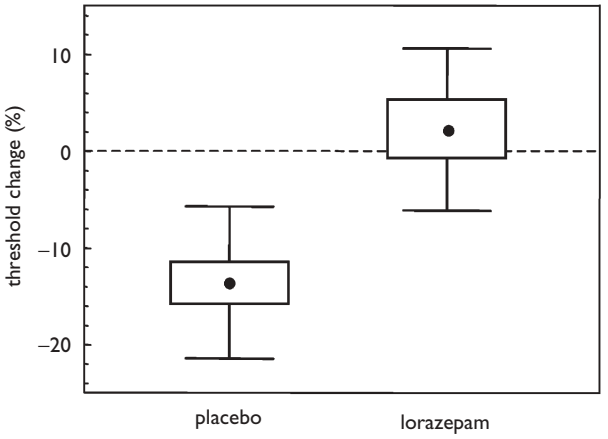

Fig. 4. Mean individual changes in discrimination threshold (percentages) for the placebo and the lorazepam group expressed as post-co-activation relative to pre-co-activation.

discrimination *per se*, which was indicated by the lack of effects on the left non-co-activated index finger (*t*-test, $p = 0.499$, $n = 8$, Fig. 3). Figure 4 summarizes the percentage changes found in the placebo and in the lorazepam group.

DISCUSSION

Our results indicate that the degree of perceptual improvements evoked by a short-term learning paradigm can be manipulated and controlled by GABAergic mechanisms. Generally, substances such as those used in our study might evoke severe side effects due to global excitability changes, disturbance of the excitation–inhibition balance, or attentional differences. Previous studies of the co-activation effects had shown that the co-activation-induced improvement in discrimination performance is highly specific to the co-activated finger with no transfer to the fingers of the other hand [4–7]. We therefore used the performance of the index finger of the left hand to measure the specificity of the observed drug effects. Lorazepam had no effect on spatial discrimination performance *per se*, which together with the consistency of the effects across subjects supports the specific nature of the drug influence observed for the right index finger.

We suggest that the complete abolition of perceptual learning by lorazepam that enhances $GABA_A$ receptor-mediated inhibition is due to the hyperpolarizing effect of lorazepam. Conceivably, this action makes it more difficult to reach threshold for opening of the Mg^{2+} block of the NMDA receptors. This view is consistent with previous cellular studies. In slice preparations it has been shown that benzodiazepines are very effective in blocking long-term potentiation (LTP) [17,18,20]. Combining psychophysical testing with mapping somatosensory evoked potentials, we have recently shown that the co-activation effects can be blocked by application of memantine, a NMDA receptor blocker [16]. Single cell recordings in rat somatosensory cortex after co-activation revealed persistent LTP-like changes in responsiveness [21]. Combined, our data are compatible with the view that NMDA receptor activation is involved in the manifestation of this particular type of fast perceptual learning.

We have previously shown that motor training leads to cortical reorganization in primary somatosensory cortex (SI) in parallel to improved motor performance. Both effects on motor performance and SI reorganization could be blocked by lorazepam [22]. For the motor system it was shown that practicing movements results in improvement in performance and parallel changes of motor cortex. These use-dependent changes could be reduced by application of a NMDA receptor blocker as well as by lorazepam, indicating that NMDA receptor activation and GABAergic inhibition operates in use-dependent plasticity in intact human motor cortex [23,24]. Similarly, short-term visual deprivation leading to excitability changes in the human visual cortex has been reported to be modifiable by GABA receptors [25].

To obtain information about cortical sites involved in mediating the observed learning processes we have previously combined the assessment of discrimination thresholds with recording of somatosensory evoked potentials or with fMRI imaging in human subjects before and after co-activation. These data revealed that the co-activation-induced gain of perceptual performance was linearly correlated with the amount of cortical reorganization of the finger representation in primary somatosensory cortex [6]. Accordingly, perceptual learning processes induced by Hebbian mechanisms, which are localized in primary somatosensory cortex, are subject to pharmacological gating by GABAergic mechanisms known to mediate and modulate synaptic plasticity.

CONCLUSION

To obtain insight into pharmacological mechanisms governing perceptual learning in humans, we made use of a particular form of perceptual learning of tactile spatial discrimination abilities: Motivated by Hebbian learning protocols, we stimulated a larger skin portion of the tip of the right index finger for a couple of hours in a synchronous manner. The resulting learning processes induced by this type of co-activation led to a lowering of the 2-point discrimination performance of the right, but not of the left index finger. Oral application of lorazepam, a benzodiazepine that enhances $GABA_A$ receptor-mediated inhibition, completely eliminated the perceptual learning observed for the co-activated index finger. In contrast, the left, non-co-activated index finger that served as control remained unaffected, demonstrating that lorazepam treatment had no unspecific side effects on discrimination. We suggest that the complete abolition of perceptual learning by lorazepam is due to its hyperpolarizing effect, which makes it more difficult to reach threshold for opening of the Mg^{2+} block of the NMDA receptors. This view is consistent with cellular studies. We conclude that perceptual learning processes, which are induced by Hebbian mechanisms, are gated by GABAergic mechanisms known to mediate and modulate synaptic plasticity at a cellular level.

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