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Effects of sensory learning on intracortical synaptic transmission in the barrel cortex of mice

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Short communication

Abstract. Pairing tactile stimulation of a row of whiskers with a tail shock results in an expansion of the functional representation of the stimulated whiskers within the primary somatosensory cortex of mice. Using the same paradigm, the present study examined field potentials evoked in *ex vivo* slices of the barrel cortex. The amplitude of responses, evoked by single and repetitive stimuli in layer IV–layer II/III pathway contained within the barrel column corresponding to the whisker stimulated during training, was unchanged. In contrast, in a transcolumnar pathway from the "trained" barrel to layer II/III of the neighboring, "untrained" column, the amplitude of responses was reduced and responses to trains of stimuli applied at 40 Hz, but not at lower frequencies, depressed faster. These data are suggestive of a selective weakening of excitatory transmission and/or enhancement of inhibitory transmission in transcolumnar pathways, which accompany associative learning-induced cortical plasticity.

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Peripheral receptive zones are represented in the neocortex as topographically organized maps. In rodents, the representation of the whisker pad within the primary somatosensory cortex, termed the barrel field, contains distinct, barrel-like cytoarchitectonic structures within layer IV (Woolsey and Van der Loos 1970). The arrangement of barrel columns, spreading through the depth of the cortex, preserves the one to one correspondence to the spatial arrangement of the whiskers. Layer IV excitatory neurons, which receive thalamocortical input, project mainly to layer II/III (Feldmeyer et al. 2002). Layer II/III neurons of one-barrel column respond strongly to deflections of the corresponding (principal) whisker and more weakly to adjacent whiskers, thus forming the central and surround receptive fields (Armstrong-James and Fox 1987, Simons 1985).

Altered sensory experience may induce long-lasting reorganizations of cortical maps (For a review see Buonomano and Merzenich 1998). In adult rodents, whisker deprivation, pairing inputs from two whiskers and long-lasting stimulation modify receptive field properties of barrel cortical neurons and alter cortical body maps (Armstrong-James et al. 1994, Glazewski and Fox 1996, Glazewski et al. 1998, Hand 1982, Kossut et al. 1988, Wallace et al. 2001, Welker et al. 1992). Clipping a subset of whiskers results in a weakened activation of neurons to the input from deprived principal whiskers and in strengthening of responses to stimulation of spared neighboring whiskers, thus expanding the functional representation of spared whiskers (Glazewski and Fox 1996, Lebedev et al. 2000). Experimental evidence points to involvement of long-term potentiation (LTP)-like mechanism in this phenomenon (For a review see Fox 2002). Sensory deprivation-induced changes in neuronal reactivity are accompanied by a decrease in markers of synaptic inhibition: glutamate decarboxylase (GAD) immunoreactivity and GABA receptor binding (Fuchs and Salazar 1998, Welker et al. 1989a).

Expansion of cortical whisker representation has also been demonstrated to occur as a result of associative learning in a classical conditioning paradigm involving pairing tactile stimulation of a row of whiskers with a tail shock (Siucinska and Kossut 1996). This conditioning paradigm resulted in an increased density of GABA-immunoreactive neurons (Siucinska et al. 1999) as well as mRNA and protein level of GAD67 (Gierdalski et al. 2001). Thus, expansion of representations within the barrel cortex could be accompanied either by a decrease of GABAergic markers, as in the case of sensory deprivation, or by an increase of those, as in the case of the conditioning-related associative learning.

To investigate intracortical synaptic interactions in classical conditioning-modified whisker representations we recorded field potentials in *ex vivo* slices of the barrel cortex. Experiments were performed on adult female Swiss mice (7–11 weeks old during conditioning, 25–30 g) in accordance with the European Communities Council Directive (86/609/EEC) and approved by the Ethics Committee at the Nencki Institute. Mice were habituated to a neck restraint by being placed in a restraining apparatus for 10–12 min a day for 3–4 weeks prior to training (Siucinska and Kossut 1996). Training consisted of stroking the whiskers of the row B on one side of the muzzle (conditioned stimulus, CS) with a hand-held fine brush in the posterior to anterior direction. The CS lasted 9 s and comprised three strokes, 3 s each. During the last second of the last stroke an electrical tail shock (unconditioned stimulus, UCS; 0.5 mA, 0.5 s) was applied. After a 6 s interval the trial was repeated. The training of this group (termed CS + UCS) lasted for 10 min/day for 3 days. The second group of mice (termed CS) received only the CS delivered at the same schedule and the third, naive group received no stimulation.

One day after the end of training mice were anesthetized with sodium pentobarbital and decapitated. Their brains were rapidly removed and immersed in cold artificial cerebrospinal fluid (ACSF) consisting of (in mM): NaCl (126), KCl (3), NaH_2PO_4 (1.25), $NaHCO_3$ (26), $MgSO_4$ (2), $CaCl_2$ (2), D-glucose (10), bubbled with a mixture of 95% O_2–5% CO_2. Slices (thickness of 400 μm) were cut through the barrel field across all five-barrel rows in an oblique coronal plane (55 degrees from the sagittal plane) on a vibrating microtome (Chmielowska et al. 1989, Kossut and Juliano 1999). Slices were incubated in a fluid-gas interface chamber perfused with ACSF (33 ± 1°C) at a rate of 1.5–2 ml/min. The humidified atmosphere over the slices was saturated with 95% O_2–5% CO_2. A bipolar concentric stimulating electrode (diameter of 125 μm) was placed in visually identified barrel B, which corresponded to the whisker stroked during training. Field potentials were recorded by two glass micropipettes filled with

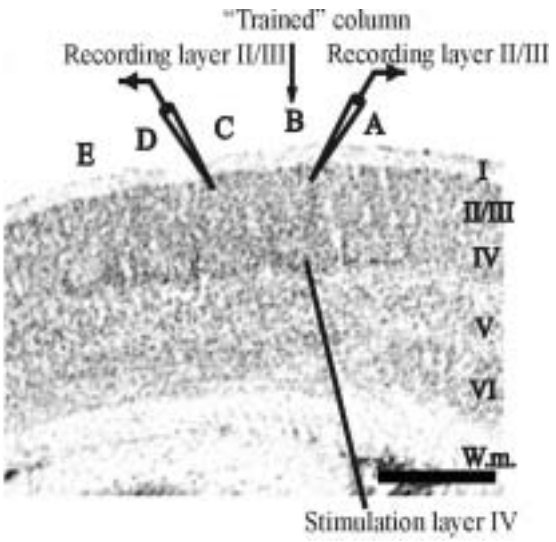

Fig. 1. A schematic drawing of the placement of stimulating and recording electrodes in barrel cortical slice. Nissl-stained section at which five barrels (A–E) are visible in layer IV. Roman numerals denote cortical layers. Scale bar: 600 μm.

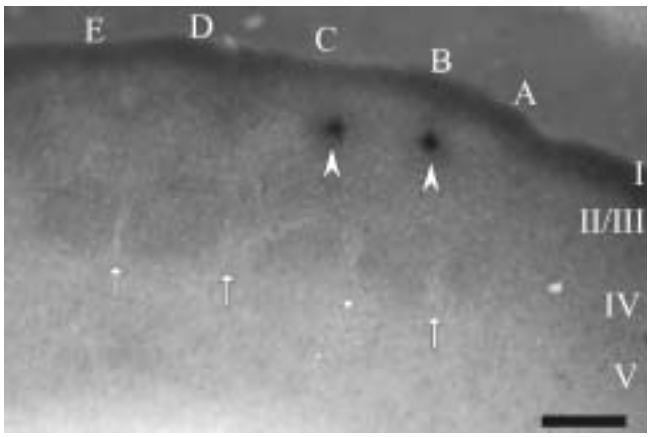

Fig. 2. Verification of the placement of recording microelectrodes within slice. Bright field image of a slice in which dye deposits (arrowheads) from recording micropipettes were made within layer II/III of barrel columns B and C and then histochemical reaction for succinyl dehydrogenase was performed. Arrows point to septa between barrels, which are visible as darker "clouds". Scale bar: 300 μm.

a mixture of 5% NaCl and 2% Pontamine sky blue (1–4 MΩ). One of the recording microelectrodes was placed within layer II/III of barrel column B and the other one within the same layer of barrel column C (see Fig. 1). Thus, two layer IV–layer II/III pathways were investigated in each slice: (i) the intracolumnar, vertical pathway (within barrel column B); and (ii) the transcolumnar, "oblique" pathway from column B to column C.

Slices were stimulated (pulse duration of 0.2 ms) either at low frequency (0.05 Hz) or using trains of eleven pulses applied at 2 Hz, 5 Hz, 10 Hz, 20 Hz, and 40 Hz with 30 s separation between trains. Stimulus intensity was adjusted to evoke responses of maximum amplitude in the investigated pathway. Field potentials were amplified, filtered (0.1–500 Hz) and acquired at a 10 kHz sampling rate using the 1401 interface and Signal 2 software (CED, UK). Three successive responses were averaged. The amplitude of responses was measured as a difference between largest negativity of the field potential and the baseline, represented by an average value of the record over 1 ms preceding the stimulus artifact. At the end of an experiment the localization of recording microelectrodes was marked by dye deposits (5 μA DC, 5 s, cathode connected to the electrode) and then histochemical reaction for succinyl dehydrogenase was performed (Fig. 2). One-way analysis of variance was used for statistical analysis. Data are presented as means ± SE.

In the first set of experiments field potentials were evoked in the vertical and the oblique pathways by single stimuli. Responses consisted of an early negative-going component, which was sometimes followed by a smaller positive wave (Fig. 3B). As illustrated in Fig. 3A, maximum responses evoked in the vertical, intracolumnar pathway were of greater amplitude and shorter latency to peak than responses in the oblique, transcolumnar pathway. The mean maximum amplitude of responses in the transcolumnar pathway was significantly smaller in the CS + UCS group of mice (1.9 ± 0.1 mV, n=22) than in the CS (2.5 ± 0.2 mV, n=9) and in the naive (2.4 ± 0.1 mV, n=19) groups (P<0.01). In contrast, no differences between three examined groups of mice were found in the intracolumnar pathway (Fig. 3A).

In the second set of experiments, short-term synaptic dynamics of responses to trains of eleven pulses at 2 Hz, 5 Hz, 10 Hz, 20 Hz, and 40 Hz was investigated in CS + UCS (n=6) and naive (n=6) mice. Depression of the amplitude of successive responses occurred at all tested stimulation frequencies and the magnitude of depression correlated with the frequency (not shown). For comparison, the amplitude of field potentials was normalized with respect to the amplitude of the response to the first pulse of a train. In the vertical

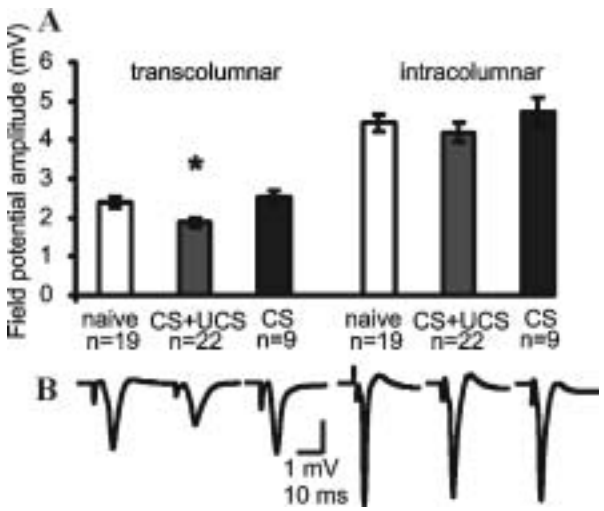

Fig. 3. (A) Comparison of mean maximum field potential amplitude in slices obtained from naive, trained (CS + UCS) and mice with stimulated whiskers without a tail shock (CS) in the "oblique", transcolumnar pathway as well as in the vertical, intracolumnar pathway. *P<0.01. (B) Representative examples of field potentials obtained in slices from naive, trained (CS + UCS) and CS mice, in trans- and intracolumnar pathways.

pathway no differences in short-term dynamics were found between slices obtained from CS + UCS and naive mice. In the oblique pathway, however, stronger depression of responses was evident at 40 Hz in slices obtained from CS + UCS mice in comparison to naive mice (Fig. 4). Remarkably, no significant differences between the two groups were found at remaining frequencies.

These results indicate that sensory-learning induced barrel cortical plasticity (Siucinska and Kossut 1996) is accompanied by selective modifications of transcolumnar, layer IV–layer II/III synaptic interactions. The observed reduction of field potential in slices prepared form CS + UCS mice most likely results from depression of excitatory transmission in synapses formed on layer II/III pyramidal neurons of barrel column C by afferents from barrel B. Long-term depression (LTD) of excitatory transmission has been found to occur in intracolumnar layer IV–layer II/III connections after deprivation of principal whiskers (Allen et al. 2003). Synaptic transmission between excitatory neurons in the barrel cortex displays mainly short-term depression during trains of stimuli (Feldmeyer et al. 2002). Intracellular studies have shown that whisker deprivation produced greater changes in short-term synaptic dynamics in layer II intralaminar excitatory connections compared to vertical layer IV–layer II connections (Finnerty and Connors 2000, Finnerty et al. 1999). These authors reported that whisker deprivation induced an increase in the synaptic strength of excitatory connections between the column having an intact, spared vibrissal input and deprived barrel columns. This effect was accompanied by a more pronounced short-term depression of responses during trains of stimuli of different frequencies. However, field potentials evoked by stimuli of a relatively high intensity, as used in the present study, are a product of a combination of excitatory and inhibitory synaptic transmission within a population of neurons, since stimulation of excitatory afferents activates feedback GABAergic inhibition disynaptically (For a review see Xu-Friedman and Regehr 2004). Thus, the observed stronger depression of successive responses during 40 Hz stimulation in slices obtained from CS + UCS mice

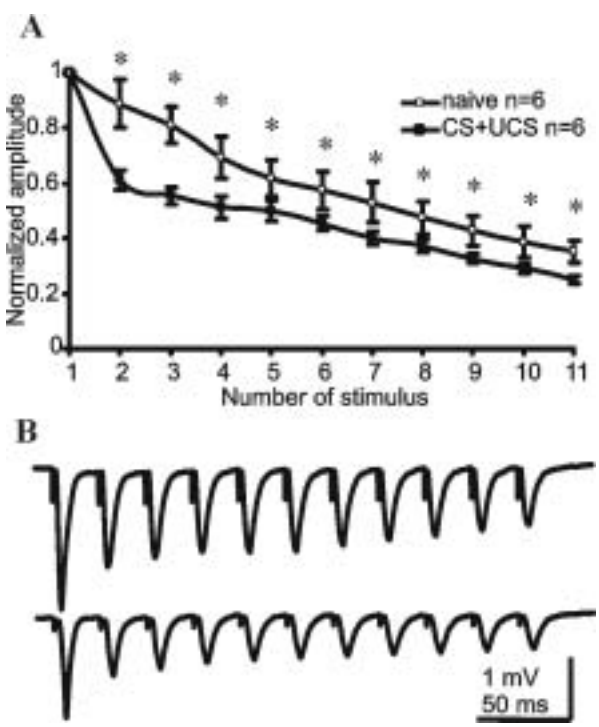

Fig. 4. Short-term synaptic dynamics in the "oblique", transcolumnar pathway. (A) Comparison of normalized amplitude of responses to 11 pulses applied at 40 Hz in slices prepared from trained (CS + UCS) and naive mice. The difference between curves is statistically significant (P<0.05). (B) Representative examples of responses obtained in slices from a naive mouse (upper trace) and a trained mouse (lower trace).

could, conceivably, result from enhanced GABAergic inhibition at the site of the recording. This conclusion is supported by the results of Finnerty and coauthors (1999) who reported that more pronounced depression of excitatory responses to trains of stimuli accompanied a general potentiation of the responses. In the present study more pronounced depression of field potentials to trains of stimuli in CS + UCS mice was connected with a reduction of response to a single stimulus. Moreover, stronger depression to successive stimuli was evident only for 40 Hz trains but not for lower frequencies, consistent with the timecourse of fast IPSCs, since neocortical $GABA_A$-dependent postsynaptic currents decay to baseline within 40–50 ms. Earlier work demonstrated classical conditioning-related increase of GABA immunoreactivity (Siucinska et al. 1999) mRNA and GAD67 protein level (Gierdalski et al. 2001). An increase in GAD immunoreactivity (Welker et al. 1989b), accompanied by an increase in the number of GABAergic synapses and enhanced long-latency responses to whisker deflections (Knott et al. 2002) has been found to occur after prolonged whisker stimulation.

In conclusion, the obtained data suggest that transcolumnar transfer of neuronal activity remains weaker for at least 24 hours after sensory associative learning. This effect might enable sharpening of columnar segregation of processed information.

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