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SHORT-TERM SENSORY LEARNING DOES NOT ALTER PARVALBUMIN NEURONS IN THE BARREL CORTEX OF ADULT MICE: A DOUBLE-LABELING STUDY

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Abstract—We have previously reported that a classical conditioning paradigm involving stimulation of a row of facial vibrissae produced expansion of the cortical representation of the activated vibrissae ("trained row"), this was demonstrated by labeling with 2-deoxyglucose in layer IV of the barrel cortex. We have also shown that functional reorganization of the primary somatosensory cortex is accompanied by an increase in the density of small GABAergic cells and glutamate decarboxylase 67-positive neurons in the hollows of barrels representing the "trained row." GABA neurons of the rat neocortex co-localize with calcium-binding proteins [parvalbumin, carletinin, calbindin D28k] and neuropeptides (vasoactive intestinal polypeptide, somatostatin). In the present study we have examined GABAergic parvalbumin-positive, interneurons in the cortical representation of "trained" facial vibrissae after short-term aversive training, in order to determine whether the observed changes in glutamate decarboxylase 67-positive neurons are accompanied by changes in parvalbumin-positive neurons. Using double immunofluorescent staining, it was found that (i) all parvalbumin-positive neurons in the barrel hollows were glutamate decarboxylase 67-positive, (ii) following aversive training density of glutamate decarboxylase 67-positive neurons in barrel hollows increased significantly compared with controls and (iii) density glutamate decarboxylase 67-positive/parvalbumin-positive neurons in "trained" barrel hollows did not change compared with controls. This study is the first to demonstrate that the density of double-labeled glutamate decarboxylase 67-positive/parvalbumin-positive neurons does not alter during cortical plasticity, thus suggesting that some other population (i.e. parvalbumin negative) of GABAergic interneurons is involved in learning-dependent changes in layer IV of the barrel cortex.

Key words: plasticity, S1, GAD+/PV+ staining, barrels, vibrissae.

In mice and rats, the projection from vibrissae (whiskers) to the contralateral somatosensory cortex produces a spatial representation of sensory receptors, termed barrels or the barrel field. In mice the barrels are located in layer IV of primary somatosensory cortex (S1) and each is composed of a wall (side) around a central hollow. Three classes of neurons can be identified: spiny stellate cells, star pyramidal cells located in barrels and pyramidal cells located in septa between barrels. The central hollow is less cellular and is rich in neuropil (Woolsey and Van der Loos, 1970). The vibrissae-to-barrels system provides a useful model for studying the mechanisms underlying different forms of neural plasticity (Hand, 1982; Simons and Land, 1987; Kossut, 1992; Diamond et al., 1993; Glazewski and Fox, 1996). The cerebral cortex can undergo considerable plastic changes as a result of sensory use or learning (monkeys: Jenkins et al., 1990; Recanzone et al., 1992; rats: Kilgard and Merzenich, 1998; mice: Siucinska and Kossut, 1996, 2004; guinea-pigs: Bakin and Weinberger, 1990). Learning can modify the functional cortical representation of mystacial vibrissae in the adult mouse. Using the 2-[^{14}C] deoxy-D-glucose (2DG) brain-mapping technique (Sokoloff et al., 1977), we have previously demonstrated that three daily sessions of training in a classical conditioning paradigm, pairing stimulation of one row of vibrissae with a tail-shock, evoked an enlargement of the cortical representation of whiskers used in the training. We found that the width of 2DG-labeled cortical representation of the "trained" row increased in layers IIIb and IV following short-lasting aversive classical conditioning training. The expansion was observed in conditioned but not in pseudoconditioned mice or in animals that received only conditioned stimulus. If the training was discontinued, the enlargement of vibrissal representation progressively faded. The reversal could be accelerated by a behavioral extinction procedure (Siucinska and Kossut, 1996).

During the whisker stimulation and tail-shock pairing we observed that the behavior of mice visibly changed. By the end of the first session the stroking of row B vibrissae evoked a rigid posture, signifying an expectation of an unpleasant stimulus. This behavior was not observed in pseudoconditioned mice. In a group of mice where the heart rate was monitored during whisker stimulation and tail-shock pairing, deceleration of the heart rate during the third training session was observed (Siucinska and Kossut, 1996).

We have also observed that inhibitory interneurons, located in the cortical region where the plastic changes take place, are modified. The learning-dependent changes in layer IV were accompanied by increases in the density of GABA cells (Siucinska et al., 1999) and in glutamate decarboxylase 67 (GAD67) mRNA expression and protein

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Abbreviations: ANOVA, analysis of variance; CS, conditioned stimulus; GAD, glutamate decarboxylase; GAD+, glutamate decarboxylase-positive neurons; GAD67, glutamate decarboxylase 67; PBS, phosphate-buffered saline; PSEUDO, pseudoconditioning random application of stimuli group; PV, parvalbumin Ca^{2+}-binding protein; PV+, parvalbumin-positive neurons; PV−, parvalbumin negative; S1, primary somatosensory cortex; UCS, unconditioned stimulus; 2DG, 2-[^{14}C] deoxy-D-glucose.

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level (Gierdalski et al., 2001) at the center of the barrels (barrel hollows) representing trained vibrissae. The GABAergic neurons in the barrel cortex are located mainly in the barrel sides (Lin et al., 1985; Chmielowska et al., 1986, 1988; Keller and White, 1986, 1987). The ratio of GABA cells located in barrel walls compared with barrel hollows in adult animals is 2.3 (Siucinska et al., 1999). In the rat cerebral cortex and hippocampus, GABA was found to co-localize with other markers including the calcium binding proteins calretinin and calbindin, and neuropeptides somatostatin and vasoactive intestinal polypeptide (Celio 1986, 1990; Rogers, 1992). Different subclasses of GABAergic neurons co-localized with one or more of the above factors. In the barrel cortex, co-localization of GABA neurons with vasoactive intestinal polypeptide (Staiger et al., 1995, 1996a, 2002), parvalbumin (Ren et al., 1992; Alcantara et al., 1996; Staiger et al., 1996b, 2002; Maier and McCasland, 1997) and calbindin D28k (Alcantara et al., 1996; Maier and McCasland, 1997; Staiger et al., 2002) has been described.

Interneurons containing parvalbumin are the dominant GABAergic subclass in layer IV of the barrel cortex (Ren et al., 1992; Alcantara et al., 1996; Staiger et al., 1996b; Porter et al., 2001). Studies of the barrel cortex imply that basket and chandelier cells, known to contain parvalbumin Ca^{2+}-binding protein (PV), are responsible for perisomatic inhibition. They are likely to be strongly excited by thalamic afferents and to participate in the early stages of cortical sensory information processing (Staiger et al., 1996b; Porter et al., 2001). These neurons are highly metabolically active and label heavily with 2DG (McCasland, 1996; Maier and McCasland, 1997; McCasland and Hibbard, 1997; McCasland et al., 1997). Parvalbumin in GABAergic cells acts as a buffer against elevation of intracellular Ca^{2+} during fast repetitive firing, thereby blocking the spike frequency adaptation due to the Ca^{2+}-activated potassium conductance (Kawaguchi et al., 1987). Recent data from knockout (PV−/−) mouse studies demonstrated that the elimination of PV changes depressing synapses into facilitating ones (Caillard et al., 2000) and confirmed the important role of PV in the stability of neuronal networks (Vreugdenhil et al., 2003; Schwaller et al., 2004). Many parvalbumin-positive neurons (PV+) interneurons are connected by gap junctions and can significantly regulate the input–output function of the barrels (Gibson et al., 1999; Galarreta and Hestrin, 1999, 2002; Priest et al., 2001; Amitai et al., 2002).

Parvalbumin-containing neurons were found to be affected by several treatments evoking neuronal plasticity: monocular deprivation in rats reduced PV immunoreactivity in the neuropil of the superior colliculus (Hada et al., 1999); exercise increased the number of PV+ cells in the hippocampus (Arida et al., 2004); cocaine sensitization by repeated administration resulted in transient increases in the number of PV+ neurons in the striatum, while subsequent withdrawal of cocaine decreased their number (Todtenkopf et al., 2004). Immediate-early genes thought to be indicators of plasticity were found expressed in PV-containing neurons of the barrel cortex (Filipkowski et al., 2000; Staiger et al., 2002). Brain dysfunctions can also affect this inhibitory subsystem; it was recently found that in the prefrontal cortex of schizophrenic patients, the expression of PV mRNA is decreased (Tamminga et al., 2004).

To understand S1 learning-dependent plasticity it is crucial to determine which subtype of GABAergic neurons is affected by changes triggered by sensory training. The focus of this study was the effect of learning-induced cortical plasticity upon co-localization of parvalbumin (PV+) and GAD (a limiting enzyme of GABA synthesis) in neurons of barrel hollows representing a "trained" row of whiskers. If an increase in the density of PV+ neurons accompanies increases in glutamate decarboxylase-positive neuron (GAD+) density, the observed GABAergic upregulation participates in the process of buffering the strong excitation induced by training. Some of these data have appeared previously in a preliminary form (Siucinska, 2004).

EXPERIMENTAL PROCEDURES

Animal treatment

The experiments were performed on 33 young adult (8 weeks old) Swiss-Webster mice (25–30 g) of both sexes, kept in a temperature-controlled room (20 °C) with a natural light/dark cycle. Mice had free access to water and food. All animals were accustomed to neck restraint by being placed in a restraining apparatus for 10 min a day for 3 weeks prior to the start of experiments. The mice were divided into five groups, one experimental group: conditioned stimulus+unconditioned stimulus (CS+UCS) classical conditioning training [whisker stimulation=CS, paired with tail shock=UCS], and four control groups: pseudoconditioning random application of stimuli (PSEUDO), application of whisker stimulation only (CS only), application of tail-shock stimuli only (UCS only), and naive controls. After the completion of the experiments, the animals were coded. Experimental procedures involving animals were approved by the Animal Care and Use Committee of the Polish Academy of Science and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, Rev. 1996). All efforts were made to minimize the number of animals used and their suffering.

Classical conditioning training (CS+UCS)

Mice were placed in a restraining apparatus and row B vibrissae were stimulated manually using a fine paintbrush. Stimulation (CS) comprised three strokes on one side of the snout. Each stroke lasted for 3 s and was applied in the posterior–anterior direction along row B of the mystacial vibrissae. Great care was taken not to touch adjacent rows of whiskers and fur growing between the rows. For the last second of the last stroke a single tail-shock representing UCS was applied (0.5 s, 0.5 mA). After a 6 s interval the stimuli were repeated (see for detailed description Siucinska and Kossut, 1996). These paired stimuli were applied four times/min, for 10 min/day for 3 days. Classical conditioning training group: CS+UCS, $n=8$.

Pseudoconditioning (PSEUDO)

In this group, animals received stimulation of row B vibrissae, which was applied for the same duration as in classical conditioning group, but a single tail-shock representing UCS was given at random relative to whisker stimulation. Mice in this pseudoconditioned application of tail-shock-unpaired application of stimuli, for 3 days received the same number of stimuli per session as the animals in the stimulus pairing groups. Pseudoconditioning group: PSEUDO, $n=6$.