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INTERNEURONS CONTAINING SOMATOSTATIN ARE AFFECTED BY LEARNING-INDUCED CORTICAL PLASTICITY

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Abstract—The maintenance of neural circuit stability is a dynamic process that requires the plasticity of many cellular and synaptic components. By changing the excitatory/inhibitory balance, inhibitory GABAergic plasticity can regulate excitability, and contribute to neural circuit function and refinement in learning and memory. Increased inhibitory GABAergic neurotransmission has been shown in brain structures involved in the learning process. Previously, we showed that classical conditioning in which tactile stimulation of one row of vibrissae (conditioned stimulus, CS) was paired with a tail shock (unconditioned stimulus, UCS) in adult mice results in the increased density of GABAergic interneurons and increased expression of glutamic acid decarboxylase (GAD)-67 in barrels of the ''trained'' row cortical representation. In inhibitory neurons of the rat cortex GAD co-localizes with several proteins and peptides. We found previously that the density of the parvalbumin (GAD+/Prv+)-containing subpopulation is not changed after conditioning. In the present study, we examined GABAergic somatostatin (Som)-, calbindin (CB)- and calretinin (CR)-positive interneurons in the cortical representation of ''trained'' vibrissae after training. Cells showing double immunostaining for GAD/Som, GAD/CR and GAD/CB were counted in the barrels representing vibrissae activated during the training and in control, untouched rows. We found a substantial increase of GAD/Som-containing cells in the trained row representation. No changes in the density of GAD/CR or GAD/CB neurons were observed. These results suggest that Som-containing interneurons are involved in learning-induced changes in the inhibitory cortical network.

Key words: GABAergic system, classical conditioning, somatosensory cortex, mice.

INTRODUCTION

Learning-induced reorganization of cortical activation involves changes in inhibitory interactions (Froemke et al., 2007; Galindo-Leon et al., 2009; Brosh and Barkai, 2009; Saar et al., 2012). We have previously described them in the layer IV of the somatosensory cortex of mice, namely the barrel cortex, where simple associative learning induces plasticity of the cortical representation of vibrissae. Classical conditioning, in which the stimulation of a row of vibrissae is paired with a tail shock, results in an increase in the area of the barrel field activated by the vibrissae stimulated during the conditioning (Siucinska and Kossut, 1996). This effect is paralleled by a rapid increase of GAD-67 mRNA and increased density of GAD and GABA immunoreactive cells in the hollows of barrels of the row receiving input from the stimulated whiskers (Siucinska et al., 1999; Gierdalski et al., 2001). In this row of barrels, the density of GAD immunoreactive puncta increased and more inhibitory synapses appeared on spines (Siucinska, 2006; Jasinska et al., 2010). An increased concentration of GABA was found in the presynaptic terminals of these synapses (Jasinska et al., 2010). Some changes were also found in the GABAa receptors, where upregulation of alpha1 subunit was observed (Lech et al., 2001). The physiological effect of GABAergic upregulation was examined by intracellular recordings, which revealed that the frequency of spontaneous inhibitory postsynaptic currents increased in excitatory neurons located in barrels receiving the conditioned stimulus (CS) (Tokarski et al., 2007). Investigations of GABAergic tonic currents revealed that after conditioning, they increased in excitatory layer IV neurons but markedly decreased in fast-spiking inhibitory interneurons (Urban-Ciecko et al., 2010). The results listed above speak for an increased inhibitory drive in the cortex that is reorganized by the conditioning.

An increase in the density of GAD immunoreactive neurons in the hollows of barrels representing the whiskers stimulated during the conditioning was observed 24 h after the end of conditioning, which indicated that it reflected a lasting change in cortical circuitry. The most plausible interpretation of this increase is that neurons with GAD levels below the threshold for immunodetection in control mice upregulated synthesis of the enzyme as a result of conditioning. We sought to identify the subclass of inhibitory interneurons that contributes to the enhancement of

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Abbreviations: ANOVA, analysis of variance; CB, calbindin; CR, calretinin; CS, conditioned stimulus; GAD, glutamic acid decarboxylase; GAD+, GAD immunopositive; RT, room temperature; Som, somatostatin; UCS, unconditioned stimulus.

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inhibitory interactions. The most likely were parvalbumin-containing neurons, as they are the most numerous class of GABAergic cells in cortical layer IV, which contains the barrels (Ren et al., 1992; Staiger et al., 1996). In several experimental situations described in the literature, changes in the density of parvalbumin-immunoreactive neurons (Prv+) paralleled changes in GAD-immuno-positive (GAD+) neurons (Lewis et al., 2005; Mix et al., 2010). We therefore expected that in mice after conditioning, we will find an increase in the population of neurons that contained both proteins. However, this was not the case, and we observed an increase in the density of GAD+ neurons but no changes in Prv+ neurons (Siucinska and Kossut, 2006). In the present paper, we attempted to identify the subclass of interneurons in the barrels, that increases GAD synthesis following conditioning. We performed a series of double-label immunocytochemical studies, in which a GAD-67 antibody and an antibody against one of the factors co-localizing with GAD in interneurons were used. We examined the density of somatostatin (Som)-, calbindin (CB)- and calretinin (CR)-containing cells in rows of layer IV barrels that received the conditioned input, in other rows of the same barrel field and in the corresponding rows in the unstimulated brain hemi-sphere, in conditioned, pseudoconditioned and naïve groups of mice. A significant increase in the density of GAD+/Som+ neurons was found following classical conditioning.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on 47 young adult (6 weeks old) C57BL/6J mice. The animals were kept in a temperature-controlled room with a natural light/dark (12-h:12-h) cycle and had free access to food and water. All work with mice was conducted in accordance with the European Community Council Directive (86/609/EEC) and was approved by the Animal Care and Use Committee of the Polish Academy of Sciences.

Classical conditioning-based sensory training

For 3 weeks before the start of the training procedure, the animals were habituated to immobility in a restraining apparatus (10 min per day). The conditioning procedure was conducted for 3 days with one session per day, as described by Siucinska and Kossut (1996). Briefly, the row B vibrissae on one side of the snout were stimulated for 9 s by three smooth strokes (each lasting 3 s) with a fine brush (CS), and during the last 0.5 s of the third stroke, a mild electric shock (unconditioned stimulus (UCS), 0.5 mA, 0.5 s) was applied to the tail. These paired stimuli were repeated four times per minute for 10 min. This CS + UCS group consisted of 15 mice. Additionally, there were two control groups: one consisted of naïve animals (NS group, $n = 16$), and one group was trained in a pseudoconditioning paradigm (Pseudo group, $n = 15$), where the same number of CS and UCS as used during conditioning was applied at random. In the Pseudo group no CS–UCS association could therefore develop. All sessions were videorecorded, and recorded films were used for the measurement of the behavioral index of learning. Trials in which the animal moved its head in response to stimulation of vibrissae were counted. Head movements occurring during the application of the tail shock and/or during the intertrial interval were not counted. Data are expressed as the percentage of trials in which head turns appeared with reference to all trials in every single minute of the training session.

Tissue preparation

Twenty-four hours after the last training session the animals were anesthetized with an overdose of Nembutal and perfused transcardially with 20 ml of 0.9% saline followed by 100 ml of 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS, pH 7.4). After perfusion, the brains were removed and postfixed in the same fixative for 3 h and then replaced with 20% sucrose for 2–3 days, followed by 30% sucrose for 2–3 days. Then, 25-μm-frozen cross sections were cut in the tangential plane.

Antibodies

Somatostatin (17 kDa) was visualized using a polyclonal antibody purchased from Santa Cruz Biotechnology, INC. (Dallas, Texas, USA) (Product No. sc-13099; dilution 1:100). This antibody was raised against amino acids 1–106 of Som of human origin. On the Western blot of the mouse brain tissue homogenate, this antibody stained a single specific band of 17 kDa (Fig. 2D). Glutamic acid decarboxylase (GAD)-67 was detected with a mouse monoclonal antibody from Millipore (Billerica, MA, USA) (Catalog No. MAB5406; dilution 1:1000). The immunogen was a recombinant GAD-67 protein. The specificity was controlled using a Western blot on mouse brain tissue, which revealed a single

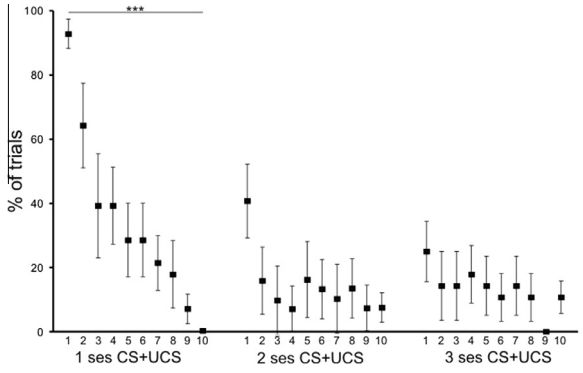

Fig. 1. Behavioral observations – head movement reduction in response to CS. Percentage of trials in subsequent minutes of training during which head turning in the direction of the stimulus was observed. 1ses CS + UCS—1st session of conditioning, 2ses CS + UCS—2nd session of conditioning, 3ses CS + UCS—3rd session of conditioning. Statistically significant differences between training trials are shown. Mean ± SEM; $F_{9,60} = 7.023$, $P < 0.0001$, ANOVA.