Sensory Learning Differentially Affects GABAergic Tonic Currents in Excitatory Neurons and Fast Spiking Interneurons in Layer 4 of Mouse Barrel Cortex

Joanna Urban-Ciecko,1 Małgorzata Kossut,2 and Jerzy W. Mozrzymas1

1Laboratory of Neuroscience, Department of Biophysics, Wroclaw Medical University, Wrocław; and 2Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland

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Urban-Ciecko J, Kossut M, Mozrzymas JW. Sensory learning differentially affects GABAergic tonic currents in excitatory neurons and fast spiking interneurons in layer 4 of mouse barrel cortex. J Neurophysiol 104: 746–754, 2010. First published June 23, 2010; doi:10.1152/jn.00988.2009. Pairing tactile stimulation of whiskers with a tail shock is known to result in expansion of cortical representation of stimulated vibrissae and in the increase in synaptic GABAergic transmission. However, the impact of such sensory learning in classical conditioning paradigm on GABAergic tonic currents has not been addressed. To this end, we performed whole cell patch-clamp slice recordings of tonic currents from neurons (excitatory regular spiking, regular spiking nonpyramidal, and fast spiking interneurons) of layer 4 of the barrel cortex from naïve and trained mice. Interestingly, endogenous tonic GABAergic currents measured from the excitatory neurons in the cortical representation of “trained” vibrissae were larger than in the “naïve” or pseudoconditioned ones. On the contrary, sensory learning markedly reduced tonic currents in the fast spiking interneurons but not in regular spiking nonpyramidal neurons. Changes of tonic currents were accompanied by changes in the input resistances—decrease in regular spiking and increase in fast spiking neurons, respectively. Applications of nipectotic acid, a GABA uptake blocker, enhanced the tonic currents, but the impact of the sensory learning remained qualitatively the same as in the case of the tonic currents. Similar to endogenous tonic currents, sensory learning enhanced currents induced by THIP (superagonist for δ subunit-containing GABA\(_A\) receptors) in regular spiking neurons, whereas the opposite was observed for the fast spiking interneurons. In conclusion, our data show that the sensory learning strongly affects the GABAergic tonic currents in a cell-specific manner and suggest that the underlying mechanism involves regulation of expression of δ subunit-containing GABA\(_A\) receptors.

INTRODUCTION

GABA is a major inhibitory neurotransmitter in the mammalian CNS and plays a crucial role in the regulation of neuronal excitability. GABA acting at ionotopic GABA\(_A\) receptors (GABA\(_A\)Rs) has been recognized to mediate two forms of inhibition: phasic, which is mediated by synaptic receptors, and the tonic one mediated mainly by extrasynaptic GABA\(_A\)Rs (Farrant and Nusser 2005; Kullmann et al. 2005; Mody and Pearce 2004; Nusser and Mody 2002; Semyanov et al. 2004). The major source of ambient GABA that activates the tonic conductance is the neurotransmitter spill over from the synaptic cleft (Overstreet and Westbrook 2001; Rossi and Hamann 1998), although nonvesicular agonist release has been also implicated (Rossi et al. 2003). Moreover, GABA transporters (GATs) have been shown to affect tonic inhibition by limiting spillover from the synapse (Isaacson et al. 1993) in the cerebellum (Rossi et al. 2003), hippocampus (Nusser and Mody 2002), and layers 1 and 2/3 of sensorimotor cortex (Bragina et al. 2008; Keros and Hablitz 2005).

Inhibition is known to play a crucial role in shaping the neuronal response in the barrel cortex. An individual barrel is a cytoarchitectonic structure in the layer 4 corresponding to one of the vibrissae located on the contralateral side of the snout (Woolsey and Van der Loos 1970). Thalamic afferents, activated by a single whisker deflection, excite both excitatory and inhibitory neurons in layer 4, resulting in excitation immediately followed by inhibition of layer 4 excitatory neurons (Swadlow 1995). Such feedforward inhibition is responsible for suppression of neuronal responses to either nonpreferred direction deflections of whiskers (Wilent and Contreras 2005) or deflection of nonpreferred whiskers (Miller et al. 2001), indicating thus that the GABAergic system in the barrel cortex controls receptive field properties.

Most interestingly, the GABAergic system in the barrel cortex has been shown to be subject to profound plastic changes induced by alteration in sensory input intensity or sensory learning. In particular, prominent plastic changes in the GABAergic system were reported after either elimination (Jiao et al. 2006; Micheva and Beaulieu 1995; Welker et al. 1989a) or deflection of nonpreferred whiskers (Woolsey and Van der Loos 1970). Thalamic afferents, activated by a single whisker deflection, excite both excitatory and inhibitory neurons in layer 4, resulting in excitation immediately followed by inhibition of layer 4 excitatory neurons (Swadlow 1995). Such feedforward inhibition is responsible for suppression of neuronal responses to either nonpreferred direction deflections of whiskers (Wilent and Contreras 2005) or deflection of nonpreferred whiskers (Miller et al. 2001), indicating thus that the GABAergic system in the barrel cortex controls receptive field properties.

Most interestingly, the GABAergic system in the barrel cortex has been shown to be subject to profound plastic changes induced by alteration in sensory input intensity or sensory learning. In particular, prominent plastic changes in the GABAergic system were reported after either elimination (Jiao et al. 2006; Micheva and Beaulieu 1995; Welker et al. 1989a) or deflection of nonpreferred whiskers (Micheva and Beaulieu 1995; Welker et al. 1989a). Using 2-deoxyglucose mapping, it has been found that classical-conditioning in which stimulation of vibrissae is paired with tail shock resulted in an expansion of the cortical representation of stimulated whiskers (Siucinska and Kossut 1996). Subsequent experiments showed the increase in mRNA and protein level of GAD67 (Gierdalski et al. 2001), and GABA immunoreactivity (Siucinska 2006; Siucinska and Kossut 2006; Siucinska et al. 1999) in the barrels receiving inputs from stimulated whiskers. These effects were accompanied by selective reduction of layer 2/3 field potentials evoked by stimulation of layer 4 in transcolumnar but not intracolumnar connections; layer 4 field potentials evoked by stimulation applied to layer 6 of the barrel column corresponded to the “trained” vibrissae (Urban-Ciecko et al. 2005). Moreover, this conditioning paradigm has been recently reported to selectively increase the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in layer 4 excitatory neurons (Tokarski et al. 2007) in ex vivo brain slices.
Despite broad interest in characterizing the barrel cortex neural network properties, relatively little is known about involvement of tonic inhibition in its activity and plasticity. Recently, Krook-Magnuson et al. (2008) showed that inhibitory neurons, especially fast spiking, have greater tonic inhibition than excitatory cells in layer 4 and suggested that the tonic inhibition selectively controls feedforward circuits in mouse barrel cortex. However, whether the associative learning, such as classical conditioning, affects the GABAAergic tonic currents plasticity remains an open question. To address this issue, we studied the impact of classical conditioning on the tonic currents in the barrel cortex. We provide, to the best of our knowledge, the first evidence that associative learning strongly affects the tonic current in the barrel cortex in a neuron type–specific manner.

**METHODS**

Experiments were performed on adult female Swiss mice (5–7 wk old during conditioning) in accordance with accepted standards of humane care and approved by the Local Bioethics Committee for Experiments on Laboratory Animals and in accordance with the Institute for Laboratory Animal Research (ILAR) Guide for Care and Use of Laboratory Animals. Female mice were less aggressive, and habituation to the neck restraint apparatus took a shorter time than in males. According to the data of Krook-Magnuson et al. (2008), the tonic current recorded from neurons in the same model did not show any significant difference between males and females.

The conditioning procedure was performed as described previously by Siucinska and Kossut (1996). Mice were habituated to a neck restraint apparatus for 10 min/day for 1–2 wk before training. The first group of mice (termed CS + UCS, n = 48) obtained conditioned stimulus (CS), which consisted of stroking the whiskers of the row B on the left side of the muzzle with a hand-held fine brush. The CS lasted 9 s and was comprised of three strokes that were 3 s each. During the last second of the last stroke, an electrical tail shock was applied [unconditioned stimulus (UCS); 0.5 mA @ 100 V, 0.5 s]. After a 6-s interval, the trial was repeated. CS + UCS pairing was repeated 4 times/min, 10 min/d, for 3 days.

In the second group of mice (termed PSEUDO CS + UCS, n = 10), animals received stimulation of row B vibrissae, which was applied for the same duration as in the conditioning group, but a single tail-shock representing UCS was given at random relative to whisker stimulation. The PSEUDO CS + UCS group of mice received the same number of stimuli per session as animals in the CS + UCS pairing groups, and pseudoconditioning was repeated 4 times/min, 10 min/d, for 3 days.

The third group of mice (NAIVE, n = 64) was intact or habituated to a neck restraint apparatus for 1–2 wk.

To have behavioral evidence that learning occurs during the CS and UCS pairings, 10 mice were filmed during conditioning (n = 5) and pseudoconditioning (n = 5) sessions. The trials during which a mouse moved its head and reacted vigorously in response to stimulation of vibrissae were counted. The observed reduction in head movements is akin to freezing observed during fear conditioning where footshock is applied [unconditioned stimulus (UCS); 0.5 mA @ 100 V, 0.5 s]. After a 6-s interval, the trial was repeated. CS + UCS pairing was repeated 4 times/min, 10 min/d, for 3 days.

One day after the end of conditioning or pseudoconditioning, mice were decapitated. Their brains were rapidly removed and immersed in cold artificial cerebrospinal fluid (ACSF) consisting of (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 1.3 MgCl₂, 2.5 CaCl₂, and 10 d-glucose, bubbled with 95% O₂:5% CO₂. After a ≥3-h recovery time, a single slice was transferred to the recording chamber that was mounted on an upright microscope (Nikon Eclipse) and superfused with warm (32 ± 0.5°C) ACSF of the composition used for recovery. Barrels were identified within the slice under low magnification, and individual neurons were visualized with a long-range water immersion objective (40×) using infrared differential interference contrast. Patch electrodes had 4.5–5.5 MΩ when filled with the internal solution containing (in mM) 140 KCl, 1 MgCl₂, 0.5 EGTA, 10 HEPES, and 4 MgATP, pH 7.2, osmolality 290–300 MΩsm. Signals were recorded using the Multiclamp 700B patch-clamp amplifier and digitized at 20 kHz using a Digidata 1322 interface (Molecular Devices). For acquisition and analysis, pCLAMP 9.2 software (Molecular Devices) was used. Series resistance was monitored using standard routines provided by pClamp 9.2 software and a Digidata 1322 interface. In the case of adult animals (5–7 wk), relatively high values of series resistance (30–40 MΩ) were observed. Occurrence of large series resistances was strictly related to the animal age. When using, for the sake of comparison, the same protocols (same solutions and pipette resistances between 4.5 and 5.5 MΩ) on slices from younger animals (<21 days), stable series resistances considerably <20 MΩ were commonly obtained. An attempt to reduce the pipette resistance by pulling larger electrodes was not successful because of difficulties in penetrating the tissue. Substantial series resistance compensation affected the cell viability and reduced time available for recording that often precluded completion of protocols [firing pattern, IPSC recording, picrotoxin (PTX) treatment]. In this situation, recordings were done without compensation, but special attention was paid to consider only stable recordings (alteration in series resistance <20% of the initial value during the entire recording period).

Whole cell patch-clamp recordings were made from layer 4 neurons located in the barrel B (termed the trained barrel). Usually, one to two slices were examined from each mouse, and one cell was examined per one slice. Membrane capacity was measured for each neuron and the intensities of tonic currents were normalized to respective capacity.

Categorization of cell type was based on the visual inspection of morphological features of neurons and the criteria for firing pattern described by Beierlein et al. 2003, and three groups of neurons were considered: excitatory–regular spiking (RS), inhibitory–regular spiking nonpyramidal (RSNP; termed low-threshold spiking by Beierlein et al. 2003), and fast spiking (FS). In agreement with observations by Gibson et al. (1999), excitatory neurons could be distinguished from GABAergic interneurons based on morphology of the cell body. GABAergic interneurons have large, usually vertically oriented somata as shown in Supplemental Fig. S1, B and C. The main criterion for categorization of the cell types was based on the response to 300-ms current injection applied every 6 s, starting from −200 pA, with 25-pA increments to obtain a full train of spikes (Supplemental Fig. S1, D–F). Both RS excitatory neurons and RS inhibitory neurons (RSNP) always generated adapting trains of spikes. An important clue to distinguish excitatory neurons from inhibitory ones (RSNP) came from the analysis of the afterhyperpolarization (AHP) time course in the first and last action potential in the spike train elicited by a threshold stimulus. In agreement with previous observations by Beierlein et al. (2003), in RS excitatory neurons, AHP in the last action potential reached a more negative value than that in the first one, and the opposite was observed for the RSNP neurons (Supplemental Fig. S1, D and E). Moreover, as described by Beierlein et al. (2003), AHP in RS showed a U-shaped time course (Supplemental Fig. S1G), whereas in RSNP interneurons, a biphasic AHP time course was typically observed (Supplemental Fig. S1H).

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Spontaneous postsynaptic currents (sPSCs) were recorded at the holding potential of −75 mV as inward currents. To assess the tonic currents, the following drugs were used: the GABA_A receptors blocker PTX (100 µM), a δ-subunit containing GABA_A receptors superagonist {4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; 20 µM (THIP)}, or nipecotic acid (1 mM, a nonselective GABA transporter inhibitor). All the drugs were purchased from Sigma, except TTX (Latoxan).

The values of tonic currents were calculated as baseline shifts after application of the drugs, normalized to the whole cell membrane capacitance, and expressed as absolute current density. The baseline current was measured as the average current of five 50- to 100-ms epochs free of sPSCs. Baseline shifts were calculated as changes in holding current after 3 min of treatment with the appropriate drug (Keros and Hablitz 2005).

Input resistance was measured either from the response to small hyperpolarizing current steps or depolarizing ones (Supplementary Fig. S1, D–I, exemplary traces showing the time course of membrane voltage in response to current stimuli). Data are presented as mean ± SE unless noted.

For each considered neuronal type (RS, FS, RSNP), we analyzed the values of tonic current in the naïve group (this group consisted actually of 2 groups: 1, tonic current determined using PTX only; 2, using PTX together with CNQX, APV, CGP, TTX; Fig. 1), the group subjected to classical conditioning (CS + UCS), and the PSEUDO CS + UCS group. Thus for a given neuronal type, we have tested whether there were differences in one parameter, namely tonic current intensity between naïve, CS + UCS and PSEUDO CS + UCS groups.

For this task we applied one-way ANOVA followed by either Tukey-Kramer multiple comparison test or Student-Newman-Keuls test. The second test was applied in the case when number of samples in one of the examined groups was much smaller than in the other groups. Sigma-Plot (Systat Software) software was used to perform the automated test verifying the normality of data distributions, and in the case of non-normality, the Kruskal-Wallis ANOVA test was applied.

In the case in which only two groups were considered (naïve and CS + UCS; Figs. 2 and 3) for a given neuronal type, a standard unpaired t-test was applied. A separate test was done to see whether there were significant differences in tonic currents between different types of neurons, but this test was restricted only to naïve animals. This test was performed using one-way ANOVA applied to three respective data columns (naïve groups for RS, FS, and RSNP); the t-test was applied in the case when n ≤ 4. Statistical significance was set at 0.05.

RESULTS

Behavioral observations

Before performing the electrophysiological experiments aiming at describing the impact of sensory learning at the neuronal level, it is important to verify whether the applied
Learning paradigm is effective at the behavioral level. Analysis of head movements (see METHODS) during application of CS showed that the reduction of head movement frequency from the first to the third training session amounted to 49.9%, from 11.9 to 5.6 per session \((n = 5, P < 0.01, \text{paired } t\text{-test})\), indicating the formation of association between CS and UCS. No change in head turning frequency was seen in the PSEUDO CS + UCS group.

Sensory learning affects tonic currents in layer 4 in a neuron-specific manner

Tonic currents were shown by application of PTX (100 \(\mu\)M) after \(\approx 3\) min of baseline recording at \(-75\) mV in whole cell mode (Fig. 1, A and B) in ACSF containing the following drugs: glutamate receptors blockers DNQX (6,7-dinitroquinoxaline-2,3-dione, 20 \(\mu\)M) and APV [(\(\pm\)-2-amino-5-phosphono pentanoic acid, 100 \(\mu\)M], a blocker of GABA\(_A\) receptors CGP 55845 \{(2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl\} (phenylmethyl)-phosphonic acid), 1 \(\mu\)M], and TTX (1 \(\mu\)M, to block action potentials). The values of tonic currents were normalized to the respective membrane capacities. In naïve mice, the values of capacities were statistically larger in excitatory neurons (RS, 66.25 ± 4.08 pF, \(n = 35\)) than in both inhibitory neurons (FS, 41.15 ± 2.88 pF, \(n = 33\)).

FIG. 2. The extent of superagonist for \(\delta\) subunit–containing GABA\(_A\) receptors THIP (superagonist for \(\delta\) subunit–containing GABA\(_A\) receptors)-induced enhancement of tonic currents in layer 4 neurons depends on the neuron type and is modulated by training in a cell-specific manner. A: examples of the whole cell current recordings (at \(-75\) mV) from barrel B neurons: RS, FS, and RSNP representing the 2 groups of experimental animals: naïve and CS + UCS. Negative deflections represent inward synaptic currents. B: mean (±SE) absolute current density recorded from neurons of barrel B from the 2 groups of animals. Bars above the current traces indicate timing of drug applications. *\(P < 0.05\) t-test. Numbers under bars indicate number of recorded cells in each group.

FIG. 3. Effect of an aspecific GABA uptake blocker (nipecotic acid) on tonic currents in layer 4 neurons. A: examples of the whole cell current recordings (at \(-75\) mV) from barrel B neurons: RS, FS, and RSNP representing the 2 groups of experimental animals—naïve and CS + UCS. Negative deflections represent inward synaptic currents. B: mean (±SE) absolute current density recorded from neurons of barrel B from the 2 groups of animals. Bars above the current traces indicate timing of drug applications. *\(P < 0.05\) t-test. Numbers under bars indicate number of recorded cells in each group.
RSNP, 48.16 ± 2.35 pF, n = 21; P < 0.05, ANOVA followed by Tukey-Kramer multiple comparison test). There were no significant differences in the capacitances between the two groups of inhibitory neurons (P > 0.05, ANOVA) and between CS + UCS, PSEUDO CS + UCS, and naive groups of mice among the same type of neurons (P > 0.05, ANOVA).

As shown in Fig. 1, A and B, PTX application caused a reduction of the inward current density in all cell types (baseline shifts). Interestingly, in naive mice, excitatory neurons showed smaller tonic currents densities (RS, 0.20 ± 0.02 pA/pF, n = 10) than both groups of inhibitory cells (FS, 1.3 ± 0.13 pA/pF, n = 9; RSNP, 0.71 ± 0.09 pA/pF, n = 9; P < 0.05, Tukey-Kramer multiple comparison test; Fig. 1C). Moreover, among inhibitory cells, the absolute current density measured in FS cells was significantly larger than in RSNP neurons. We next tested whether the GABAergic tonic currents in naive mice were sensitive to network-driven activity including glutamatergic synaptic transmission. To this end, the current baseline shifts were measured after PTX application in the absence of DNQX, APV, CGP 55845, and TTX. As shown in Fig. 1C, for each considered cell type, the application of PTX in the absence of these blockers produced very similar baseline shifts (gray bars) as in experiments in which they were present (Fig. 1C, white bars).

In the next set of experiments, the effects of behavioral training on tonic GABAergic currents were studied. The mean absolute current density recorded from RS excitatory neurons of the trained barrels of slices prepared from the CS + UCS group of mice (0.31 ± 0.02 pA/pF, n = 7) was ~50% larger in comparison to that in the PSEUDO CS + UCS (0.18 ± 0.03 pA/pF, n = 5) and naive groups of mice (0.20 ± 0.02 pA/pF, n = 10; P < 0.05, ANOVA) followed by Tukey-Kramer multiple comparison test; Fig. 1C). There were no significant differences in the absolute current density between excitatory cells in the PSEUDO CS + UCS and naive groups of mice (P > 0.05, ANOVA). In contrast, the mean absolute current density recorded from FS interneurons in the trained barrels from the CS + UCS group of mice (0.41 ± 0.05 pA/pF, n = 9) was approximately three times smaller in comparison to that in the PSEUDO CS + UCS (1.57 ± 0.36 pA/pF, n = 5) and naive groups of mice (FS, 1.3 ± 0.13 pA/pF, n = 7; P < 0.05, ANOVA) followed by Tukey-Kramer multiple comparison test; Fig. 1C). There were no significant differences in the absolute current density between these inhibitory cells in the PSEUDO CS + UCS and naive groups of mice (P > 0.05, ANOVA).

Recordings of tonic currents from RSNP neurons showed that there were no differences in the absolute tonic current density between neurons of the barrels B in any considered experimental group (CS + UCS, 0.89 ± 0.12, n = 5; PSEUDO CS + UCS, 0.59 ± 0.05, n = 5; naive, 0.71 ± 0.09 pA/pF, n = 9; P > 0.05, ANOVA) followed by Tukey-Kramer multiple comparison test; Fig. 1C).

**Effect of nipeictic acid on tonic currents in layer 4 neurons**

To examine the possible role of GABA transporters subtypes 1 and 2 (GATs 1/2) in modulating tonic currents in layer 4 neurons of the barrel cortex, we applied nipeictic acid, a nonselective GAT antagonist. As shown in Fig. 3A, the drug induced a significant increase in the holding current in all types of neurons. An increase in the tonic currents after application of nipeictic acid is consistent with previous observations of tonic currents shown by PTX application (Fig. 1) and THIP-induced currents (Fig. 2). In particular, in control mice, both groups of inhibitory neurons showed a larger increase in tonic current density after treatment with nipeictic acid (FS, −4.04 ± 1.06 pA/pF, n = 9; RSNP, −2.75 ± 0.18 pA/pF, n = 4) than the excitatory neurons (−1.74 ± 0.23 RS, pA/pF, n = 10; P < 0.05, t-test; Fig. 3B). Moreover, nipeictic acid–induced enhancement of current density in RS neurons was ~50% larger in the trained barrels of slices prepared from the CS + UCS group (n = 10) in comparison to that in the naive group of mice (n = 10, P < 0.05, t-test; Fig. 3B). The opposite effect of the sensory learning on nipeictic acid–induced enhancement of tonic currents was observed for the FS interneurons. Indeed, as shown in Fig. 3B, current density increase after nipeictic acid administration in FS cells in the trained barrels of slices prepared from the CS + UCS group of mice (n = 8) was ~60% smaller in comparison to that in the naive group of mice (n = 9, P < 0.05, t-test). Similar to the tonic and THIP-evoked currents, in the case of RSNP cells, we did not observe statistically significant differences in nipeictic acid–induced enhancement of tonic currents between neurons of barrels B in slices prepared from mice of CS + UCS (n = 7) and naive groups (n = 4).
Impact of learning-induced tonic conductance alterations on membrane input resistance

The data presented above clearly indicate that the sensory learning markedly affects the tonic currents of specific neurons in the barrel cortex, and therefore, it is interesting to check whether these changes are accompanied by alterations of resting membrane voltage and input resistance. Because responses evoked by low GABA concentrations are characterized by strong outward rectification (Pytel and Mozrzymas 2006; Pytel et al. 2005), we decided to assess the input resistance by using either hyper- or depolarizing steps. Consistent with the observed impact of sensory learning on the tonic currents, we observed that input resistance measured at depolarizing subthreshold steps in RS neurons was significantly lower in the “trained” barrels of slices prepared from the CS + UCS group of mice (181.69 ± 9.04 MΩ, n = 29) in comparison to that in the PSEUDO CS + UCS group (241.91 ± 11.50, n = 10) and the naive group (216.09 ± 13.23 MΩ, n = 32; P < 0.05, ANOVA followed by Student-Newman-Keuls test; Fig. 4), whereas the input resistance in FS cells was higher in CS + UCS mice (93.45 ± 4.45 MΩ, n = 24) than in PSEUDO CS + UCS and in naive animals (71.44 ± 3.04, n = 27 and 64.91 ± 9.23, n = 5, respectively). However, there were no significant changes in input resistance of RSNP cells between the groups of mice. No changes in input resistance between trained and naïve groups were observed when applying hyperpolarizing steps (data not shown). It is likely that a decrease in GABA-evoked current intensity on membrane hyperpolarization could obscure the difference between tonic currents in naïve and trained animals. Moreover, the applied sensory learning paradigm had no significant effect on the resting membrane potential of any of considered cell types (data not shown).

Effect of sensory learning on IPSCs

The impact of sensory learning on IPSCs has been addressed in our previous study (Tokarski et al. 2007). However, in the study by Tokarski et al. (2007), different experimental conditions (low [Cl] internal solution) were used, and the group of FS interneurons has not been considered. For this reason, we addressed the impact of sensory learning on mIPSCs for all considered neuronal types. In agreement with Tokarski et al. 2007, the mean frequency of mIPSCs recorded from RS excitatory neurons of the trained barrels of slices prepared from the CS + UCS group of mice (8.85 ± 0.71 Hz, n = 7) was significantly larger in comparison to that in the PSEUDO CS + UCS (5.28 ± 0.95 Hz, n = 5; P < 0.05, ANOVA followed by Tukey-Kramer multiple comparison test) and naïve groups of mice (6.25 ± 0.20 Hz, n = 10; P < 0.05, ANOVA). There were no significant differences in the mean frequency of IPSCs between cells of the barrels B in the PSEUDO CS + UCS and naïve groups of mice (P > 0.05, ANOVA). We did not find statistically significant differences in the mean amplitude of mIPSCs between RS excitatory neurons of barrels B in slices prepared from mice in the CS + UCS group (6.25 ± 0.96 pA; P > 0.05, ANOVA) and naïve group (83.08 ± 9.31 pA; P > 0.05, ANOVA). There were no statistically significant differences either in the mean frequency or in the mean amplitude of mIPSCs between FS neurons of barrels B in slices prepared from mice of CS + UCS group (5.52 ± 0.81 Hz and 80.44 ± 9.41 pA, respectively, n = 9; P > 0.05, ANOVA followed by Tukey-Kramer multiple comparison test), PSEUDO CS + UCS group (59.30 ± 25.28 pA; P > 0.05, ANOVA), and naïve group (83.08 ± 9.31 pA; P > 0.05, ANOVA). Moreover, we did not find statistically significant differences in the mean frequency or in the mean amplitude of mIPSCs in RSNP neurons between the CS + UCS group (4.15 ± 0.54 Hz and 76.69 ± 16.82 pA, respectively, n = 5; P > 0.05, ANOVA followed by Tukey-Kramer multiple comparison test), PSEUDO CS + UCS group (4.76 ± 1.08 Hz and 94.53 ± 15.00 pA, respectively, n = 5; P > 0.05, ANOVA), and naïve group (5.57 ± 0.75 Hz and 81.09 ± 6.82 pA, respectively, n = 9; P > 0.05, ANOVA). Moreover, we did not find statistically significant differences in the mean frequency or in the mean amplitude of mIPSCs in RSNP neurons between the CS + UCS group (4.15 ± 0.54 Hz and 76.69 ± 16.82 pA, respectively, n = 5; P > 0.05, ANOVA followed by Tukey-Kramer multiple comparison test), PSEUDO CS + UCS group (4.76 ± 1.08 Hz and 94.53 ± 15.00 pA, respectively, n = 5; P > 0.05, ANOVA), and naïve group (4.14 ± 0.61 Hz and 109.32 ± 9.13 pA, respectively, n = 9; P > 0.05, ANOVA).

Effects of THIP on sIPSCs

Recording sIPSCs kinetics (in the presence of DNQX, APV, and CGP) showed that THIP at a concentration of 20 μM had no significant effect on sIPSCs kinetics in RS neurons of naive mice (n = 3, FS and RSNP were not examined). There were no statistically significant differences in mean amplitude (control: 142.40 ± 50.80 pA; THIP: 112.58 ± 37.83 pA; P > 0.05, paired t-test), 10–90% rise time (control: 0.53 ± 0.03 ms; THIP: 0.66 ± 0.06 ms; P > 0.05, paired t-test), 90–10% decay time (control: 6.31 ± 0.20 ms; THIP: 5.97 ± 0.11 ms; P > 0.05, paired t-test), or half-width (control: 3.18 ± 0.07 ms; THIP: 3.10 ± 0.41 ms; P > 0.05, paired t-test). However, there was a statistically significant decrease in the mean frequency of sIPSCs after the application of THIP (control: 6.58 ± 0.66 Hz; THIP: 3.64 ± 0.20 Hz; P < 0.05, paired t-test).

Discussion

The major finding of this study is that classical conditioning, consisting of pairing of whiskers stimulation with a tail shock, induces cell type–specific changes in the tonic current intensity in layer 4 of the barrel cortex (trained animals compared with naïve and PSEUDO CS + UCS animals). Interestingly, tonic inhibition in the excitatory neurons increases after associative learning, whereas the opposite change in tonic conductance takes place in FS interneurons. It is worth noting that, in
control conditions (naive and PSEUDO CS + UCS mice), tonic current density is considerably smaller in RI than in FS interneurons; therefore such inverse modulation of tonic conductance suggests that the considered sensory learning tends to reverse the relations between tonic inhibition in these neurons. Importantly, the effects of classical conditioning on the tonic currents were associated with changes in the input resistance. It needs to be emphasized that these modulatory phenomena occurred only in the group of mice subjected to conditioning (and not to pseudoconditioning), showing the involvement of associative mechanisms in their induction. Moreover, behavioral evidence, showing a rapid decrease in head movements toward the CS already during the first conditioning session in the CS + UCS group, but not in the PSEUDO group, suggests the conditioned mice learned that the CS predicts the painful UCS. We previously showed that, in a test session 24 h after the end of training, the mice remember the meaning of CS and show low frequency of head movements, which can be used as an index of learning (Cybulskas-Klosowicz et al. 2009).

In a previous study addressing the GABAergic tonic currents in layer 4 of the barrel cortex (Krook-Magnuson et al. 2008), cell type–specific differences in tonic inhibitory currents were shown by application of bicuculline methobromide (the GABA_A receptor antagonist) or THIP, applied in the presence of low GABA concentrations. Although in this study, to show the tonic currents, we applied PTX (the GABA_A open channel blocker) in the absence of exogenous GABA, our results regarding tonic currents in naive mice basically confirm those of Krook-Magnuson et al. (2008). Larger GABAergic tonic currents in the inhibitory interneurons in comparison to excitatory cells were also found in the hippocampus (Semyanov et al. 2003). However, it needs to be stressed that GABAergic interneurons are very heterogenous, and larger tonic currents in these cells with respect to the excitatory neurons is not a rule. Large tonic current in response to THIP was observed in layer 2/3 pyramidal neurons, whereas it was absent in somatostatin–positive interneurons of the frontoparietal cortex of mice (Vardy et al. 2008). Moreover, THIP induced larger tonic currents in layer 2/3 than in layer 5 pyramidal neurons in slices of the frontoparietal cortex of mice (Drasbek and Jensen 2006). Recordings from layer 2/3 pyramidal neurons and layer 1 interneurons in rat sensorimotor cortex did not show cell type–specific differences in tonic currents in response to bicuculline (Keros and Hablitz 2005). It seems that proportions between tonic currents in interneurons and excitatory neurons represent the specificity of the considered model.

It is interesting that, in our experiments, the values of tonic currents were not sensitive to agents blocking the network activity (TTX) or glutamatergic synaptic currents (APV, DNQX). The lack of significant changes in the value of tonic inhibition, when these drugs were omitted, suggests that, in our model, most of recorded tonic currents result from neurotransmitter released on miniature synaptic current activity. Our results seem to be consistent with data from other groups that reported that neocortical neurons rarely fire action potentials in vivo (Moore and Nelson 1998; Zhu and Connors 1999) and in vitro (Simkus and Stricker 2002; Tokarski at al. 2007). Similar to our results, action potential–independent release of GABA was reported for mature (but not immature) cerebellar granule cells (Wall and Usowicz 1997). We are aware, however, that the lack of dependence of the tonic current on TTX is not a general rule. For instance, tonic currents are strongly dependent on action potential firing in relay neurons of dorsal lateral geniculate nucleus (dLGN) of adult mice, and in these cells, tonic currents are also sensitive to TTX (Bright et al. 2007). We believe that the lack of dependence of tonic currents on TTX reflects the specific properties of neurons in the considered model. Intriguingly, we found that PTX application showed larger tonic currents in FS than in RI inhibitory cells, whereas exogenous THIP application induced similar enhancement of tonic currents in both cell types. The latter observation on THIP-induced currents is in agreement with data reported by Krook-Magnuson et al. (2008). This observation suggests that tonic currents are not mediated exclusively by δ-subunit–containing receptors but rather that different sets of GABA_A receptors may contribute to the tonic currents in these two inhibitory cell types. A variety of subunits of the GABA_A receptors have been shown to be involved in tonic inhibition in different regions of the brain (Farrant and Nusser 2005). High-affinity, δ-subunit–containing receptors play a crucial role in mediating tonic currents in the cerebellum (Brickley et al. 1996) and hippocampus (Stell and Mody 2002), as well as in the barrel cortex (Krook-Magnuson et al. 2008). However, it has been found that GABA_A receptors containing the α1-subunit underlie tonic currents in layer 2/3 pyramidal neurons, and GABA_A Rs with α1 or α5 subunits mediate tonic currents in layer 5 pyramidal neurons of the somatosensory cortex (Yamada et al. 2007). It is thus possible that different proportions between PTX–shown tonic currents (naive vs. trained) and THIP-evoked currents are reflecting different subunit compositions of GABA_A-R–mediating tonic currents in FS and RSNP inhibitory neurons.

An obvious candidate to explain cell type dependence of tonic currents in excitatory and inhibitory neurons and between two classes of considered inhibitory interneurons could be the spatiotemporal difference in the ambient GABA concentration. To check for the impact of GABA uptake system in our model, we used a nonspecific blocker, nipecotic acid. Importantly, blockade of GABA uptake by this agent induced robust currents that exceeded severalfold the tonic currents shown by PTX application (Figs. 1 and 3), indicating a prominent role of the uptake system. However, the proportions between tonic current values in naive and trained animals in RI and FS neurons roughly mirrored those observed in the presence of nipecotic acid. The most parsimonious explanation of this finding could be that blockade of the uptake system with nipecotic acid produced a proportional rescaling of ambient GABA, suggesting the lack of major spatial nonhomogeneities in the extracellular GABA concentration. However, such a proposal would require further evidence using more specific blockers against different types of GABA transporters. As many as four GATs have been described in the rat brain, GAT-1, GAT-2, GAT-3, and BGT-1 (Borden 1996), that are differentially targeted to neuron and glia (Krogsgaard-Larsen et al. 1987). Moreover, nipecotic acid has been found to act as a weak GABA_A receptor agonist (Barrett-Jolley 2001), indicating that part of currents measured after administration of this drug might result from this nonspecific action. To our knowledge, no systematic study exploring the role of GATs in modulating tonic currents in layer 4 barrel cortex has been published thus far, and this issue, including the role of GABA...
uptake in plasticity related to sensory learning, remains to be explored.

Although ambient GABA around distinct types of neurons might differ because of activity of the uptake system, it seems that exogenously applied THIP at relatively high concentration (20 μM) is likely to arrive to distinct neuronal targets at a similar concentration. Thus cell type–specific changes in the THIP-evoked enhancement of tonic currents induced by sensory learning indicate that classical conditioning altered expression of δ-subunit–containing GABA_A receptors. Thus cell type–specific changes in the tonic currents after nipeptoc acid administration induced by conditioning may also largely reflect altered expression of these GABA_A receptors. Alterations in δ-subunit expression seems to be commonly involved in various mechanisms of neuronal plasticity. For instance, selective changes in expression of this subunit were observed in a mouse model of temporal lobe epilepsy, where expression of the δ-subunit–containing GABA_A receptors was downregulated in dentate gyrus granule cells, whereas δ-subunit expression in interneurons was increased (Peng et al. 2004). A pronounced change in the expression of the δ-subunit was also found in models of chronic alcohol treatment (Cagetii et al. 2003). An important issue is whether THIP at a concentration of 20 μM can be still considered a specific δ subunit–containing GABA_A superagonist. Most importantly, in our experiments, THIP at this concentration had no effect on mIPSC kinetics, similar to previous finding of Krook-Magnuson et al. (2008), who used exactly the same THIP concentration in the same experimental model. Moreover, data collected in other models provide evidence that THIP at concentrations of several micromoles activates mainly tonic current and has no significant effect on IPSCs. Gao and Smith (2010) have found that application of 10 μM THIP had no effect on mIPSCs in the dorsal motor nucleus. Maguire et al. (2005) reported that, in dentate gyrus granule cells, 5 μM THIP did not affect mIPSCs. Similarly, Belelli at al. (2005) found that 3 μM THIP had no significant effect on mIPSCs kinetics. Thus it seems that, in our experiments, the major target for THIP at the 20 μM concentration is the nonsynaptic GABA_A receptor, presumably with a predominant proportion of those containing the δ subunit.

The physiological role of the conditioning-induced plasticity of tonic currents in the barrel cortex is not clear. However, as already mentioned, GABAergic feedforward inhibition has been found to be involved in suppression of neuronal responses to nonpreferred whiskers or whisker deflections, indicating a role in shaping the receptive field properties. We may thus speculate that opposite modulation of tonic currents in RS excitatory and FS inhibitory neurons, described in this study, may be involved in a modulation of information transfer from a trained whisker and refinement of the respective receptive fields. In particular, it is likely that conditioning-induced downregulation of tonic inhibition (and of input resistance) in FS interneurons results in increased excitability of these neurons, giving rise to an enhancement of GABAergic drive to excitatory cells, observed as an increase in the sIPSC frequency in layer 4 excitatory neurons within the trained barrel (Tokarski et al. 2007). Importantly, the thalamocortical afferents in the rodent barrel cortex terminate mainly on excitatory and fast-spiking inhibitory neurons, whereas rarely and only weakly do they contact RSNP neurons (Beierlein at al. 2003). It may thus be speculated that the plasticity phenomena induced by sensory learning concern mainly those cells in the barrel cortex that receive major thalamocortical inputs and might primarily affect the feedforward circuits. Thus the role of RSNP cells in cortical circuits is likely to be different from that of FS cells. In addition, electrophysiological sampling and immune-cytchemical markers showed fewer RSNP cells than FS neurons (Amiati et al. 2002; Gibson et al. 1999; Kawaguchi and Kubota 1997; Sun et al. 2006).

The results of this study extend previous studies that provided solid evidence that the GABAergic system in the barrel cortex is a subject of a profound plasticity induced by classical conditioning. In particular, a change in the following GABAergic markers in trained barrels has been shown: an increase in the density of GABA-IR neurons and elevation in mRNA and protein level of GAD67 but not GAD65 (Gierdalski et al. 2001; Lech et al. 2001; Siucinska 2006; Siucinska and Kossut 2006; Siucinska et al. 1999). Lech et al. 2005 also reported training-related increase in the level of GABA_A α1 subunit mRNA; however, no changes in the binding of muscimol to GABA_A receptors (Jablonska and Skangiel-Kramska 1995) and no change in the amplitude of sIPSCs (Tokarski et al. 2007) have been shown. These results add important functional aspects related to tonic inhibition in the barrel cortex and further show that sensory learning might produce complex cell-specific plastic effects mediated by different (in this case apparently opposite) mechanisms.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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