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Research Report

Effects of $GABA_A$ receptor inhibition on response properties of barrel cortical neurons in C-Fiber-depleted rats

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Abstract

C-fiber depletion results in expansion of low threshold somatosensory mechanoreceptive fields. In this study, we investigated the role of intact C-fibers in GABAA-mediated inhibition in barrel cortical neurons. We used electronically controlled mechanical stimulation of whiskers to quantitatively examine the responses of barrel cells to whisker displacements. After systemic injection of picrotoxin neuronal responses were recorded at 5 min intervals for 20 min and then at 10 min intervals for 100 min. Picrotoxin injection caused a 3-fold increase in response magnitude of adjacent whisker stimulation and 1.4-fold increase in response magnitude of principal whisker stimulation with a maximum enhancement 50 min after the injection. There was no significant change in spontaneous activity following picrotoxin injection. The response enhancement and receptive field expansion observed in normal rats were completely absent in the C-fiber-depleted rats. These results suggest that the GABAA-mediated inhibition that modulates the receptive field functional organization of the barrel cortex depends on intact C-fibers.

Theme: Sensory system
Topic: Somatosensory cortex and thalamocortical relationships

Keywords: C-fibers; Barrel cortex; Capsaicin; GABA

1. Introduction

Layer IV cortical neurons in rodent primary somatosensory cortex form morphologically distinct structures called "barrels". Cells within each barrel respond selectively to displacement of an anatomically corresponding vibrissa, called the principal whisker (PW) [27,28]. Displacement of neighboring vibrissa, called adjacent whisker (AW), evokes lower magnitude and longer latency responses [2,24,26].

Gamma amino butyric acid (GABA) plays an important role in the functional organization of the whisker-barrel system [14]. Nearly 15% of barrel cells are GABAergic [6,16,24] and the thickest density of GABA receptors is found in layer IV [15,22].

Several studies have shown that, following sensory deprivation, $GABA_A$ receptor density and distribution change in multiple brain areas [4,15,21,22]. For example, it has been shown that electrocautery ablation of whisker follicles leads to a marked decline in $GABA_A$ receptor immunoreactivity in lamina IV of cortical barrels associated with the ablated follicles [15]. But the neuronal basis of GABA system contribution in cortical cell receptive field plasticity is mainly unknown.

In most studies of cortical receptive filed plasticity different types of afferents are damaged by removing the whisker follicle or by electrical lesion. In these studies the contribution of each type of afferents in the observed plasticity cannot be examined directly and specifically.

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Neonatal administration of capsaicin destroys most of C-fiber afferents with little effect on myelinated afferents [9,13,23]. It has been shown that the receptive field properties of low-threshold mechanical somatosensory cells in the central nervous system are influenced by C-fibers. C-fiber depletion results in expansion of low threshold somatosensory mechanoreceptive fields [7,10, 11,13,18,25]. For example, C-fiber depletion increases the receptive field size of the barrel cortical cells [25] and cells in trigeminal nucleus principalis [13]. Similar results are obtained in various brain areas when acute inactivation of C-fibers by local subcutaneous injection of capsaicin is used [4,10]. C-fiber depletion also results in a reduction in $GABA_A$ receptors in the dorsal horn of the spinal cord [4,21]. Reduction in $GABA_A$ receptor density has been reported in the spinal cord [4] and cortical layer IV [15,22] following complete loss of afferent inputs which include C-fibers as well as myelinated fibers.

The drastic change in $GABA_A$ receptor density and distribution in the barrel cortex suggest that at least part of the physiological changes following sensory deprivation could be due to the change in the cortical GABA system. Some studies point to this possibility. For example, it has been shown that pharmacological blockade of cortical $GABA_A$ receptors increases the excitatory receptive field size of barrel cells while diminishing the extent of the surround inhibition [14].

In the current study we examined the contribution of C-fiber depletion on $GABA_A$ related receptive field plasticity. We recorded the barrel cortical single unit responses to electronically controlled PW and AW displacements following blockade of $GABA_A$ receptors in C-fiber-depleted and normal rats.

2. Material and methods

2.1. Animal preparation

The experiment was done on 22 adult male rats (Sprague–Dawley) weighing 250–400 g. Capsaicin treatment and electrophysiological recording have been described previously [7,11]. Briefly, neonatal rats received capsaicin (50 mg/kg, Sigma) or its vehicle (ethanol, Tween-80, 0.9% saline in a ratio of 1:1:8) intraperitoneally on the first postnatal day. Then 10 capsaicin-treated (Cap) and 12 vehicle-treated (Con) rats were allowed to grow to adulthood. It has been shown that neonatal capsaicin treatment within 48 h of birth effectively destroys C-fiber afferents [9,13,23]. In order to verify C-fiber depletion, corneal chemosensitivity test was used [12]. One drop of 1% ammonium hydroxide was administered into the right eye of the animal and its wiping was counted for 10 s. In this experiment the number of eye wiping in Cap rats (3.6 ± 1.05) was significantly ($P < 0.001$) less than that of Con rats (14.6 ± 0.87). It shows that capsaicin markedly destroyed C-fiber in the Cap group.

For the electrophysiological recording session, each rat was anesthetized with urethane (1.5 mg/kg) and placed in a stereotactic frame. After an incision through the skin, the skull overlying the right primary somatosensory cortex was drilled 1–4 mm posterior and 4–7 mm lateral to the bregma. Throughout the experiment the physiological condition of the rat was monitored by assessing the heart and respiratory rates and the temperature was maintained at 37.5 °C by a servo-controlled heating blanket (Harvard apparatus, England).

2.2. Physiological recording and whisker stimulation

Extracellular single unit recording from layer IV of barrel cortical neurons were obtained using glass microelectrodes. Microelectrodes with a tip diameter of 2–5 μm were filled with 3 M NaCl solution and advanced through the cortex perpendicular to its surface. The neuronal signals were amplified and band-pass filtered at 300–10000 Hz. Single cortical units were isolated in layer IV of barrel cortex using an amplitude window discriminator (WPI, USA). A delay line helped us to visualize whole spike waveforms. The receptive field of isolated neurons was assessed first by a hand-held probe and then by electromechanical stimulators. The whisker whose displacement elicited the most vigorous response with the shortest latency was identified as the principal whisker and the whisker caudal to it as an adjacent whisker. Then these two whiskers were trimmed to a length of 10 mm from the base and inserted into two independent stimulator tubes so that the tip of the tube was 5–6 mm away from the skin. Each stimulator was made of a loud speaker, which was attached in the center to the end of a glass tube. By controlling the electrical voltage delivered to the speakers, the other end of tubes independently produced ramp and hold movements in upward or downward directions (amplitude, 700 μm; rise time, 7 ms; and duration, 200 ms).

2.3. Experimental procedure and drug administration

Principal and adjacent whiskers were deflected individually 40 times in a pseudorandom order with 1 s gap between consecutive stimulations. A homemade computer software and hardware controlled stimulus presentation and data storage. After a baseline recording before injection, Picrotoxin, a $GABA_A$ receptor antagonist was injected. Picrotoxin was administrated in a subconvulsive doze of 2 mg/kg via a cannula, which had been inserted into the peritoneum before electrode insertion. Stimulations and recording were performed following picrotoxin injection every 5 min for 20 min and then every 10 min for 100 min. At the end of each recording session, an electrolytic lesion was produced at the recording site. Then the rat was perfused and its brain was removed. After fixation, the brain

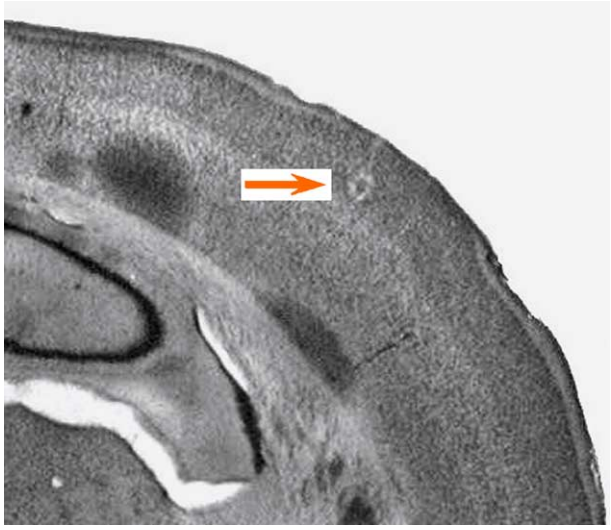

Fig. 1. A photomicrograph of a coronal section of barrel cortex stained with Nissl. Asterisk shows the recording site in layer IV of barrel cortex.

was cut into 60–80 μm slices and the recording position was confirmed after Nissl staining. A representative photomicrograph illustrating the location of recording site in layer IV of barrel cortex is shown in Fig. 1.

2.4. Data analysis

For each neuron, peristimulus time histograms (PSTH) were constructed with 1 ms bins for principal and adjacent whisker deflections at different times relative to picrotoxin injection. Spontaneous activity was measured in the 100-ms period immediately before the whisker displacement. Response latency was defined as the first bin of two consecutive 1 ms bins following whisker displacement where spike probability exceeded mean + 3.29 × SD (i.e., $P < 0.001$) of spontaneous activity. Mean response magnitude was measured for 25 ms after the response latency. The size of the response window was not critical to the results and the 25-ms window was chosen to cover response duration of different neurons. Response duration was defined as the duration after response latency when neural activity remained above latency threshold level.

Relative change of response magnitudes following picrotoxin injection was calculated as the ratio of the response magnitude at a particular time after injection to the response magnitude before injection. Absolute change of response magnitude was defined as the response magnitude after injection minus response magnitude before injection.

3. Results

We analyzed activity of 22 neurons recorded from 22 adult male Sprague–Dawley rats in response to individual deflection of principal and adjacent whiskers before and after administration of picrotoxin (a $GABA_A$ antagonist). Ten of the neurons were recorded from rats neonatally treated with capsaicin and 12 from vehicle-treated animals. After picrotoxin injection neuronal responses were recorded at 5 min intervals for 20 min and then at 10 min intervals for 100 min. Figs. 2 and 3 show barrel neuronal responses to principal (A–C) and adjacent (D–F) whisker deflection in Con and Cap groups, before, 50 min and 120 min after picrotoxin injections, respectively.

We have previously reported that neonatal treatment with capsaicin increases magnitude of responses to individual displacement of principal and adjacent whiskers and also increases duration of responses to adjacent whisker deflections without significant changes in response latencies [12]. Similar changes were found in the current study.

3.1. Effect of picrotoxin on individual whisker deflection in the Con group

In the control group, following picrotoxin injection, response magnitude to deflection of principal or adjacent

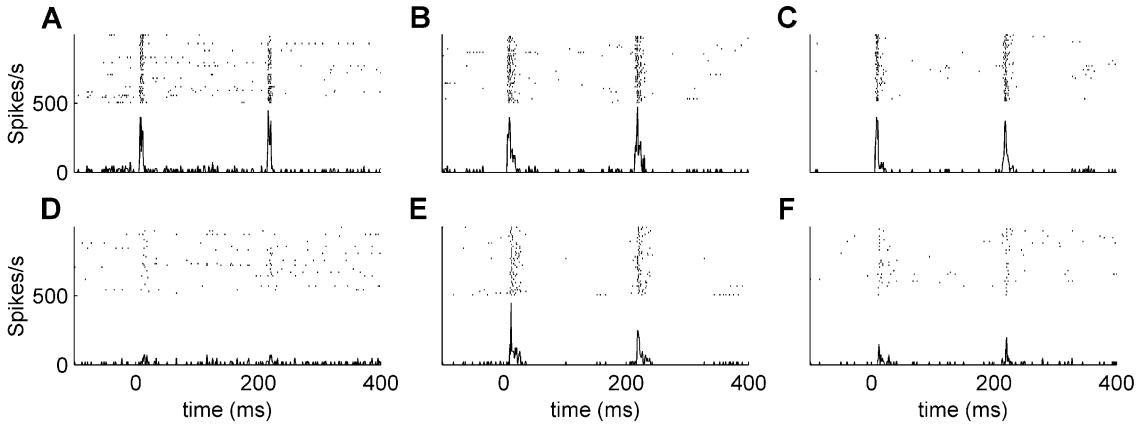

Fig. 2. Effect of picrotoxin on responses of a single neuron in the control group to principal and adjacent whiskers. Panels A–C show PSTH and rasters of neural response to deflection of principal whisker before, 50 min, and 120 min after picrotoxin injection, respectively. Panels D–F show responses to adjacent whisker deflection at the same times as in panels A–C.

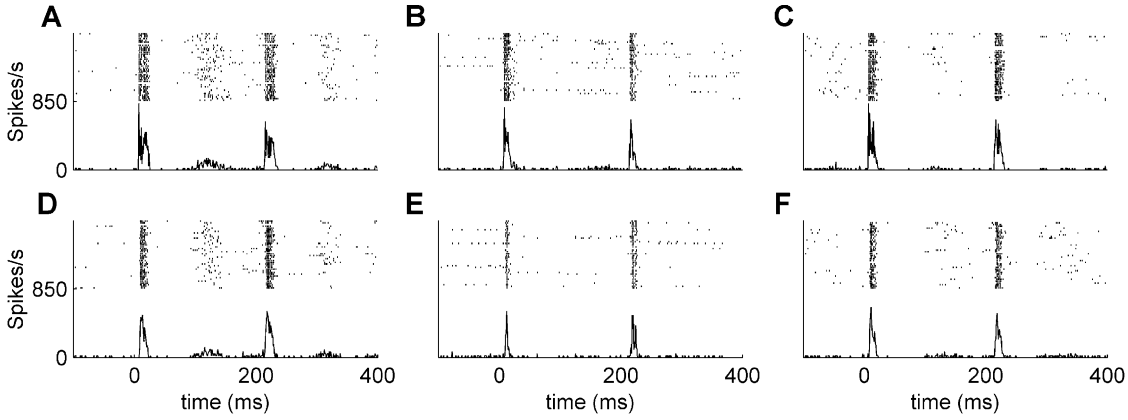

Fig. 3. Effect of picrotoxin on responses of a single neuron in the capsaicin-treated group to principal and adjacent whiskers. Panels A–C are responses to principal whisker before, 50 min, and 120 min after picrotoxin injection, respectively. Panels D–F show responses of the neuron to adjacent whisker deflections at the same times as in panels A–C.

whiskers increased with a maximum enhancement 50 min after injection. As shown in Fig. 4A, at this time, response magnitude to adjacent whisker deflection increased nearly three times compared to that of before injection (ratio of response magnitudes after and before injection, mean ± SD, 3.2 ± 2.0; median, 2.9). On the other hand, firing rates of principal whisker deflection increased by 1.4 times (mean ± SD, 1.4 ± 0.4; median, 1.4) 50 min after injection. A two-way ANOVA performed to investigate effects of principal/adjacent whisker deflection and recording time on relative increase of response magnitudes showed a significant effect for both factors (principal/adjacent whisker deflection, $P < 10^{-8}$, recording time, $P < 0.05$).

Although, the relative increase of response magnitude was much larger for adjacent whisker deflection compared to principal whisker, the absolute increases in response magnitudes following picrotoxin injection were close to each other (Fig. 4B). Fifty min after injection, deflection of principal whisker elicited 0.7 ± 0.9 (mean ± SD) more spikes compared to the values before injection. At the same time, 0.9 ± 0.5 more spikes were generated by adjacent whisker displacement. However, the difference between adjacent and

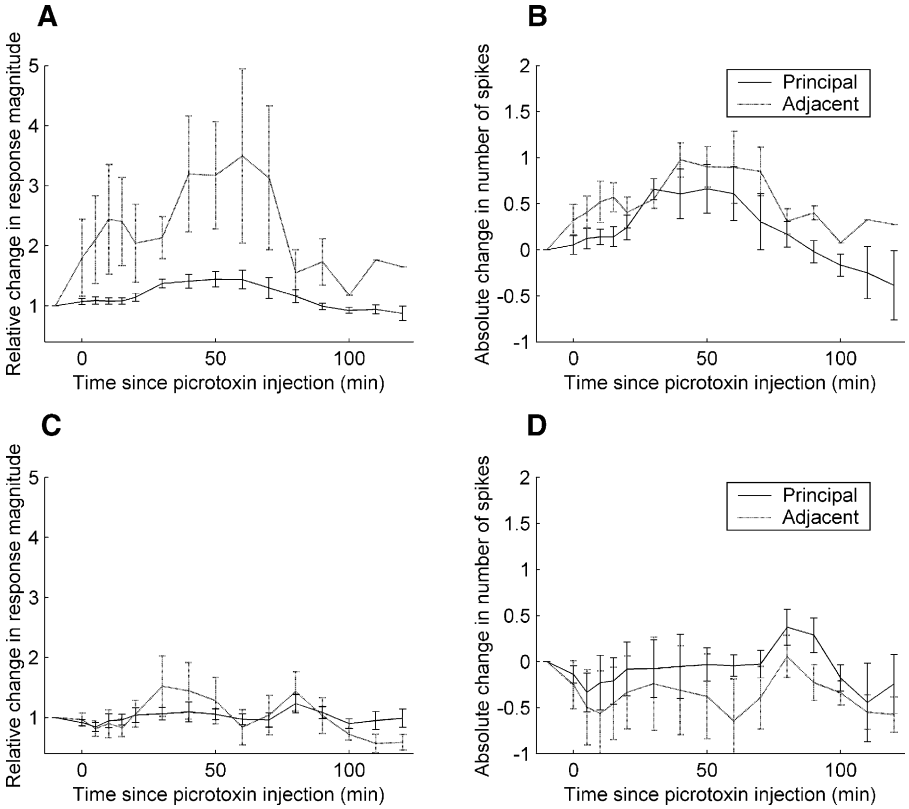

Fig. 4. Effect of picrotoxin on relative and absolute changes in response magnitudes to principal and adjacent whiskers in control (A, B) and capsaicin-treated (C, D) rats. Error bars show standard error of mean.

principal whiskers in the number of extra spikes was significant ($P < 0.005$) as revealed by a two-way ANOVA with recording time and principal/adjacent whisker deflection as main factors.

Unlike response magnitudes to whisker displacement, spontaneous neural activity was not significantly modulated by picrotoxin injection and remained fairly constant at different times following injection ($P = 0.5$, one-way ANOVA with recording time as the main factor).

3.2. Effect of picrotoxin on individual whisker deflection in the Cap group

Unlike the control group, neurons recorded from the capsaicin-treated group did not elicit any response magnitude modulation following picrotoxin injection (Figs. 4C–D). The ratio of response magnitude after injection to that of before injection remained close to 1 for both principal and adjacent whisker deflections at all times after injection. A two-way ANOVA showed no significant effect for recording time or principal/adjacent whisker deflection on relative change of response magnitude (recording time, $P = 0.1$; principal/adjacent, $P = 0.9$; interaction of factors, $P = 0.8$). Similarly, the absolute change in response magnitude remained near 0 at all times.

Statistical comparison between the Cap and Con groups, using a three-way ANOVA with capsaicin treatment, principal/adjacent whisker deflection and recording time as the main factors, revealed a significant effect of capsaicin treatment on relative change of response magnitude following picrotoxin administration ($P < 10^{-8}$). These results indicate that following neonatal depletion of C-fibers by capsaicin, modulation of neural response magnitudes by picrotoxin disappears.

One of the differences between the Cap and Con groups is that the difference between relative changes of response magnitudes of principal and adjacent whiskers in the Con group disappears in the Cap group. For instance, at 50 min after picrotoxin injection this difference is 1.6 ± 1.8 in the control group while it is only 0.3 ± 1.1 in the capsaicin-treated group (Wilcoxon test, $P < 0.05$).

3.3. Effect of picrotoxin on response latency or duration

Following picrotoxin injection, we did not observe any significant changes in neural response latencies and durations to deflection of principal or adjacent whisker combined deflection. An ANOVA revealed that picrotoxin did not cause systematic modulation of these response parameters in either Cap or Con group ($P > 0.5$).

4. Discussion

Our results show that, in the normally reared rats, blocking the $GABA_A$ receptors using systemic injection of picrotoxin increased the responsiveness of barrel cells to displacement of principal (PW) and adjacent (AW) whiskers. But this facilitation effect was more prominent for the AW stimulation resulting in an expansion of the receptive size. These findings are in agreement with the report of Kyriazi et al. [14] who showed a larger effect on AW responses compared to those of PW following blocking the $GABA_A$ receptors using cortical iontophoresis of the $GABA_A$ blocker, bicuculline, into layer IV barrel cortex of normal rats. In addition, we found that the picrotoxin-induced increased excitability to displacement of individual whiskers were not present or were highly diminished in C-fiber-depleted rats. These results suggest that the $GABA_A$-mediated inhibition that modulates the receptive field functional organization of the barrel cortex depends on intact C-fibers.

There are two main explanations for the effect of $GABA_A$ antagonists on neuronal response properties. First, they may cause a nonspecific excitation in cortical cells that leads to functional expression of subthreshold inputs and an increase in neuronal spontaneous and evoked firing. Second, they may unmask the inputs that, because of inhibitory interaction, cannot normally drive the target cells [1,14]. Increase in evoked neuronal responses in our study shows that picrotoxin reduced the inhibitory action of GABA and through it expanded the neuronal receptive field size. Since the spontaneous activities and response latencies of recorded neurons of our study did not change following application of picrotoxin it is unlikely that this $GABA_A$ antagonist influenced the general level of excitation of barrel cells by means of removing the tonic inhabitation exerted by the GABAergic cells [1].

Our result, in agreement with other studies, shows that the receptive field properties of low-threshold mechanical somatosensory cells are influenced by C-fibers [3,7,10,11,13,18,25]. Capsaicin-induced C-fiber depletion causes expansion of excitatory receptive fields and changes neuronal properties in spinal [17,25], trigeminal [5,13], and barrel cortical cells [18,25]. Displacement of AW before PW stimulation suppresses the responses to PW stimulation in normal rats [7,19,20]. We have previously shown that depletion of C-fibers reduces the suppressive effect of paired whisker stimulation at all of the tested inter-stimulus intervals suggesting that neonatal C-fiber depletion reduces the AW-evoked inhibition within barrel cortex [7]. It has been suggested that intact C-fibers exert a tonic inhibition that normally affects the receptive field properties of somatosensory cells mainly by limiting the extent of the receptive fields of such cells [3,10,11]. Such increase in receptive field sizes of cortical cells may not be a simple reflection of the changes that occur in the lower levels of sensory system and may have, at least, some cortical origin [7,10,11].

$GABA_A$ receptors play an important role in shaping the normal neuronal receptive field size [1,14]. It is believed that it has a critical function in sensory deprivation. For

instance, it has been shown that following the complete peripheral deafferentiation $GABA_A$ receptor density decreases in dorsal horn of spinal cord [4] and layer IV of cortex [15,22]. Similar effect of reduction in $GABA_A$ receptor density has been reported in spinal cord of capsaicin-treated rats [4,21]. Since we used systemic injection of picrotoxin, the physiological changes observed in C-fiber-depleted rats of our study might be due to the impact of the drug on $GABA_A$ receptors at different levels of the whisker-to-barrel system.

Our study cannot role out the possibility that C-fibers also exert their effect on somatosensory inhibitory mechanisms through $GABA_B$-mediated inhibition. Specific blockers of $GABA_B$ receptors should be used in capsaicin-treated animals to further explore such possibility.

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