Reducing Contralateral SI Activity Reveals Hindlimb Receptive Fields in the SI Forelimb-Stump Representation of Neonatally Amputated Rats

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Pluto, Charles P., Nicolas L. Chiaia, Robert W. Rhoades, and Richard D. Lane. Reducing contralateral SI activity reveals hindlimb receptive fields in the SI forelimb-stump representation of neonatally amputated rats. J Neurophysiol 94: 1727–1732, 2005. First Published March 30, 2005; doi:10.1152/jn.00228.2005. In adult rats that sustained forelimb amputation on the day of birth, >30% of multiunit recording sites in the forelimb-stump representation of primary somatosensory cortex (SI) also respond to cutaneous hindlimb stimulation when cortical GABA_{A+B} receptors are blocked (GRB). This study examined whether hindlimb receptive fields could also be revealed in forelimb-stump sites by reducing one known source of excitatory input to SI GABAergic neurons, the contralateral SI cortex. Corpus callosum projection neurons connect homotopic SI regions, making excitatory contacts onto pyramidal cells and interneurons. Thus in addition to providing monosynaptic excitation in SI, callosal fibers can produce disynaptic inhibition through excitatory synapses with inhibitory interneurons. Based on the latter of these connections, we hypothesized that inactivating the contralateral (intact) SI forelimb region would “unmask” normally suppressed hindlimb responses by reducing the activity of SI GABAergic neurons. The SI forelimb-stump representation was first mapped under normal conditions and then during GRB to identify stump/hindlimb responsive sites. After GRB had dissipated, the contralateral (intact) SI forelimb region was mapped and reversibly inactivated with injections of 4% lidocaine, and selected forelimb-stump sites were retested. Contralateral SI inactivation revealed hindlimb responses in ~60% of sites that were stump/hindlimb responsive during GRB. These findings indicate that activity in the contralateral SI contributes to the suppression of reorganized hindlimb receptive fields in neonatally amputated rats.

INTRODUCTION

Neuron clusters in the SI forelimb-stump representation of adult rats that sustained neonatal forelimb amputation normally have receptive fields located exclusively on the stump. However, when cortical GABA_{A+B} receptors are blocked (GRB), many of these recording sites exhibit an additional receptive field located on the ipsilateral hindlimb (Lane et al. 1997). Results from previous studies suggest that these hindlimb inputs originate in the SI hindlimb representation in the same hemisphere and are transmitted to SI forelimb-stump neurons via a polysynaptic circuit through the intervening dysgranular cortex (Lane et al. 1999; Stojic et al. 2001). Hindlimb responses can be revealed when GRB is induced globally by topical application or locally by injection at the recording site, suggesting that the GABA receptors of interest are located on hindlimb responsive forelimb-stump neurons and/or on axons of other cells carrying hindlimb information into the deafferented region (Pluto et al. 2004). Because inhibitory interneurons typically have relatively short axons that synapse locally, it is likely that the GABAergic neurons that suppress hindlimb responses are themselves located within the SI forelimb-stump area. As GABA release by interneurons is usually triggered by excitatory inputs to these cells (McBain and Fisahn 2001), we attempted here to modulate the hindlimb suppression, independent of GRB, by reducing excitatory input to SI GABAergic neurons.

One source of excitatory input to SI GABAergic neurons is the contralateral SI via the corpus callosum. Callosal fibers make predominantly excitatory connections between homotopic regions of SI (Koralek and Killackey 1990), terminating on both pyramidal and inhibitory neurons (Carr and Sesack 1998). Callosal axons are thus capable of exerting monosynaptic excitation and/or disynaptic inhibition at postsynaptic targets in the opposite hemisphere (Kawaguchi 1992; Vogt and Gorman 1982). Callosal connections in SI appear to modulate inhibition and cutaneous receptive fields (Clarey et al. 1996; Rema and Ebner 2003; Shuler et al. 2001; Swadlow 2003). In this study, the contralateral SI forelimb region was reversibly inactivated with lidocaine in an attempt to weaken disynaptic inhibition and thereby reveal hindlimb receptive fields in the SI forelimb-stump representation independent of the use of GABA receptor antagonists.

METHODS

All protocols described here were developed in accordance with the National Institutes of Health Guide for the Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Medical College of Ohio.

Neonatal forelimb removal

Neonatal forelimb amputations were carried out using methods previously described (Lane et al. 1995). Within 12 h of birth, rat pups were anesthetized by hypothermia until immobile. The left forelimb was amputated at the proximal humerus with iridectomy scissors, and the brachial artery was sealed by electrocautery. The region was infiltrated with 0.7% bupivicaine, and the skin was closed with cyanoacrylate adhesive. The pups were re-warmed, returned to their mother, and allowed to reach 60 days of age before being used in terminal recording experiments. These rats grew and behaved normally and showed no signs of pain; body weight was normal in these animals.

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Receptive field mapping

Rats were anesthetized with 60 mg/kg ketamine hydrochloride and 15 mg/kg xylazine administered intraperitoneally and prepared for recordings as previously described (Lane et al. 1999). The trachea was cannulated, and the rat was placed on a thermoregulatory blanket and fixed in a stereotaxic device. Mechanical ventilation was set at 60–75 strokes/min (5–7 ml/stroke), and heart rate was monitored periodically. A state of light anesthesia was maintained by periodically testing the corneal reflex and administering 1g/kg urethan when needed; urethan was used because it maintains a long-lasting but relatively light level of anesthesia. The cisterna magna was opened to drain cerebral spinal fluid (to prevent herniation through the cranios- tomy), a mid-sagittal incision was made over the skull, and a craniotomy was performed over the cortex contralateral to the amputation. Warmed (37°C) neurobasal culture medium (Gibco-BRL) was applied periodically to prevent desiccation. A magnified (~20 times) digital photograph of the exposed cortex was used to mark electrode penetrations with reference to the surface vasculature. Multi-unit responses were recorded with extracellular tungsten electrodes (1.0 MΩ). Electrode penetrations were spaced 250–300 μm apart, and activity was recorded at layer IV depth (600–800 μm). Light, cutaneous stimuli were delivered separately to the stump, whisker pad, lower jaw, trunk, and hindlimb by lightly tapping the body surface with a modified artist’s brush. This type of stimulation could activate deeper proprioeceptive receptors (e.g., muscle spindle, joint, Golgi tendon organs) in addition to superficial touch receptors (e.g., Meissner, Merkel, Pacinian, Ruffini). The cortical surface vasculature was used to determine electrode placement in the retesting of unit clusters at specific recording sites. At the end of the recording session, each animal was given a lethal dose of carbon dioxide and perfused with heparinized saline followed by 4% formaldehyde dissolved in sodium phosphate buffer (pH 7.4). The brains were postfixed overnight, removed, and cut on a freezing microtome at 50 μm. Photographs of the exposed cortex were used to mark electrode penetrations. Multi-unit responses were averaged and compared by dependent t-test. Sites within the forelimb-stump region that were stump/hindlimb responsive during GRB were retested during lidocaine inactivation. The effect of contralateral lidocaine inactivation on the spontaneous activity levels of SI forelimb-stump neurons was examined in four amputated rats. Spontaneous activity was quantified by counting the number of spikes with amplitudes greater than twice the level of background activity using the Corel Draw 4 program for off-line analysis of oscilloscope traces (3 10-s sweeps at each of 4 recording sites in each animal under normal conditions and during lidocaine treatment). These values were averaged and compared by dependent t-test. In another two amputated rats, the forelimb-stump area was mapped prior to GRB mapping, to determine if the lidocaine-revealed hindlimb responses were influenced by prior GRB treatment.

Data analysis

All SI forelimb-stump recording sites were classified as responsive to cutaneous stimulation of the stump only, stump/hindlimb, stump/face (vibrissae and/or lower jaw), or stump/trunk, under normal conditions, and during GRB. All sites that were stump/hindlimb responsive during GRB were again tested during lidocaine inactivation of the opposite SI forelimb region. Frequency data (% of total sites) were compared by one-way ANOVA to test for hindlimb responses under each condition. Specific comparisons of hindlimb response frequencies between two different conditions were analyzed by the dependent t-test. Spontaneous activity values (spikes/s) were compared by the dependent t-test. The accepted level of significance for all statistical tests was P < 0.05.

Results

Receptive field mapping was performed in 15 adult (>60 days) Sprague-Dawley rats that had sustained forelimb amputation on the day of birth. A total of 806 multiunit SI forelimb-stump sites were assessed under normal conditions, during GRB, and during lidocaine inactivation of the opposite SI forelimb region. The same mapping protocol was also performed in three normal rats. In nine of the amputated rats, hindlimb receptive fields were found in 3.7 ± 3% of SI forelimb sites under normal conditions and in 12.7 ± 1% during GRB (P = 0.01). These values are similar to those reported in previous multiunit studies (Lane et al. 1997, 1999; Stojic et al. 2001).

Lidocaine inactivation of the opposite SI forelimb region

After completing the map of the SI forelimb-stump region under normal conditions and during GRB to identify hindlimb responsive sites, the opposite (intact) SI forelimb representation was mapped and lidocaine was injected centrally in this region (Fig. 1, A–C). Recordings were conducted at granular and infragrannular depths (600–1,000 μm) in the injected SI forelimb region to confirm that the activity of potential callosal efferent neurons was reduced. Within 3–4 min of lidocaine treatment, evoked and spontaneous activity was greatly diminished at several sites throughout the injected representation. In contrast, neuronal activity in the adjacent SI hindlimb and vibrissae representations was largely unaffected, indicating that inactivation was principally restricted to the SI forelimb region (Fig. 2).

GABA receptor blockade (GRB)

After mapping the SI forelimb-stump representation, 30 μl of a 50 μM biccuculline methiodide/salpetrol hydrochloride (Research Biochemicals International) solution was applied topically to SI to induce GRB. The forelimb-stump region was remapped 10–15 min later, when increased neuronal bursting activity indicated that GRB had taken effect. The bursting pattern induced by GRB was used to monitor the level of receptor antagonism, and tactile stimuli were delivered between periods of intense bursting. All sites were retested during GRB (~1 h); additional 30-μl drug applications were employed if needed. Evoked responses were considered significant if at least nine sets of action potentials were elicited in response to a train of 10 light taps delivered to the body surface at a frequency of ~3 Hz. After each GRB mapping session, the cortical surface was flushed with fresh neurobasal medium and 60 min allowed for the drug to washout.

Lidocaine inactivation of the opposite SI forelimb region

In these experiments, the SI forelimb-stump region was first mapped under normal and GRB conditions to define the region and to identify stump/hindlimb responsive sites. The opposite SI forelimb representation was then mapped and a total of 15–25 μl 4% lidocaine (Roxane Laboratories) was injected at two central sites within the representation, ~400 μm below the pial surface. Evoked responses at two to three sites across the lidocaine-treated SI forelimb area were monitored every 10 min to confirm that the area was inactivated. Evoked and spontaneous activity was also monitored at one to two sites in the adjacent SI hindlimb and vibrissae regions to confirm that only the SI forelimb region was inactivated. Sites within the forelimb-
Effects of contralateral lidocaine inactivation on hindlimb expression

Recordings in the SI forelimb-stump representation during lidocaine inactivation revealed hindlimb responses in 59.8% of those sites that were hindlimb responsive during GRB (Fig. 1D). Overall, hindlimb responses were found in 18.3 ± 5% of the total SI forelimb-stump sites during lidocaine inactivation. This value was significantly greater than the 3.3 ± 4% found during normal conditions (P = 0.0001) and significantly less than the 30.6 ± 7% found during GRB (P = 0.0001; Fig. 3).

In two amputated rats, lidocaine was injected and the SI forelimb-stump sites tested prior to GRB to determine whether residual effects of GABA antagonism contributed to the 18.3% of sites that exhibited hindlimb expression for the overall group data. In these animals, the percentage of hindlimb-expressing SI forelimb-stump sites (2 and 8% at baseline) increased to 12 and 17% during lidocaine inactivation, respectively. Subsequent GRB revealed 30% of sites as hindlimb responsive in both animals, and re-injection of lidocaine 1 h after GRB revealed hindlimb responses in 22 and 21% of sites, respectively (data not shown). These findings suggest that there was a minor enhancing effect of previous GRB on the ability of lidocaine treatment to reveal stump/hindlimb receptive fields. All lidocaine-revealed hindlimb responses in these rats were at sites that became hindlimb responsive during GRB, and the majority of these responses did not persist for >30 min even if supplemental injections (10 μl) were given.

In three normal rats, hindlimb responses were found in 3.7 ± 3% of sites initially, and in 12.7 ± 1% during GRB. These results are consistent with the previous finding in normal rats of hindlimb responses in 2.7% of sites under normal and in 11.7% during GRB conditions (Lane et al. 1997). In the normal rats studied here, contralateral lidocaine inactivation revealed hindlimb responses in 3.0 ± 3% of sites, indicating no significant effect of lidocaine treatment on hindlimb expression in the SI forelimb representation of normal rats.

Effects of contralateral lidocaine inactivation on spontaneous activity

The influence of inactivating the intact, contralateral SI forelimb representation on spontaneous activity in the SI forelimb-stump representation was examined in four amputated rats. On average, contralateral lidocaine inactivation significantly decreased the level of spontaneous activity at 16 multiunit SI forelimb-stump sites (4 per rat), from 6.9 at baseline to 2.9 spikes/s during lidocaine treatment (P = 0.0001; Fig. 4).

DISCUSSION

In the present study, hindlimb receptive fields were revealed in the SI forelimb-stump representation after deactivation of the contralateral (intact) SI forelimb representation with lido-
caine. However, this effect was transient and less robust (present at fewer recording sites) than that found when GRB was applied directly to the deafferented cortex. These results indicate that activity in the contralateral SI forelimb representation may contribute to the inhibition of hindlimb receptive fields in neonatally amputated rats. The finding that each of the hindlimb responsive sites revealed by lidocaine treatment was also hindlimb responsive during GRB strongly suggests that these two treatments result in disinhibition of the same cortical circuit.

Contralateral SI-mediated inhibition

GABAergic neurons, which are located in all layers of SI, receive excitatory asymmetric synaptic contacts from a number of sources (Keller and White 1986; 1987; Swadlow 1995). One source of excitatory input to inhibitory interneurons is callosal projection neurons that interconnect the two SIs (Carr and Sesack 1998; Somogyi et al. 1983). A small number of callosal projection neurons are glutamic acid decarboxylase or GABA positive (Fabri and Manzoni 2004; Gonchar et al. 1995). These findings establish callosal SI connections as a potential substrate for modulating inhibition in SI. Recent functional and neuroimaging studies further suggest that transcallosal modulation is important for the integration of bilateral tactile information (Shuler et al. 2001; Werhahn et al. 2002; Wiest et al. 2004).

Although SI predominantly processes input from the contralateral body surface, a number of studies have described ipsilaterally evoked responses and have further shown that the opposite SI plays a regulatory role in their expression. For example, ipsilateral responses in the SI vibrissae barrel cortex of rats and mice were noted after a 4- to 5-ms delay but were abolished when the opposite SI was ablated (Pidoux and Verley 1979). In the rat SI hindlimb region, 51% of single units responded to stimulation of both the contralateral and ipsilateral hindlimbs. Under deeper anesthesia, however, 77% of these cells lost the ipsilateral receptive field, indicating that these responses are more likely to be expressed under relatively light anesthesia (Armstrong-James and George 1988). More recent work has demonstrated ipsilateral responses in the vibrissae barrel cortex and shown that these responses have a suppressive influence on subsequent contralateral responses. These responses and their suppressive effects are, however, abolished by muscimol inactivation of the opposite SI (Shuler et al. 2001). In cortical area 3b of the flying fox, cutaneous receptive fields on the digits immediately expanded when a small region of the opposite area 3b was cooled to 10°C (Clarey et al. 1996). Together these findings indicate that SI neurons are capable of processing bilateral sensory input, and suggest that the opposite hemisphere may provide a “tonic” inhibition in SI (Calford 2002).

It is not yet known whether the density and/or distribution of callosal projections are altered in neonatally amputated rats, although results from previous developmental sensory system studies suggest that this may be the case. The relatively exuberant callosal projections found during development compete with thalamocortical axons for cortical occupancy (Ivy and Killackey 1982; Lent et al. 1990). In the cat, >70% of callosal axons are eliminated from birth to adulthood, and this transition is influenced by visual experience (Innocenti et al. 1985; Koppel and Innocenti 1983). Normally, callosal projections in rat SI eventually distribute in a “complementary” pattern mostly to dysgranular areas of layer IV, where they are less dense than in the supra- and infra-granular layers (Hayama and Ogawa 1997; Olavarria et al. 1984). This pattern is altered, however, after either neonatal transection of the infraorbital nerve or removal of the dorsal thalamus (Korsak and Killackey 1990). The density and distribution of callosal projections was also abnormal in rats that had undergone neonatal spinal overhemisection to remove dorsal column input bilaterally (Remple et al. 2004). Similarly, callosal terminals were altered in the deafferented visual cortex of hamsters that sustained neonatal enucleation (Fish et al. 1991; Rhoades and Dellacroce 1980), or transection of the optic radiations.
These findings raise the possibility that there is an abnormal distribution of callosal fibers in neonatally amputated rats due to the large-scale disruption of thalamocortical input to the SI forelimb-stump area. The observation that contralateral lidocaine inactivation does not enhance the expression of hindlimb receptive fields in the SI forelimb region of normal rats further suggests that this circuit is altered by neonatal amputation. Neuronal tracer experiments are currently underway to examine the areal and laminar distribution of callosal projections in these animals.

It is worth noting that although both GRB and contralateral lidocaine inactivation are capable of revealing hindlimb receptive fields, these manipulations have opposite effects on spontaneous activity within the SI forelimb-stump representation. While GRB treatment increases, contralateral lidocaine injections decrease, the spontaneous firing rate of these neurons. A previous study demonstrated that GRB significantly increases the spontaneous firing rate of individual neurons in the SI forelimb-stump of neonatally amputated rats (Stojic et al. 2001). Other studies have also shown increased spontaneous firing rates of neurons in the visual and SI cortex as well as the dorsal column nuclei of the cat (Dykes and Craig 1998; Dykes et al. 1984; Sillito et al. 1981), and in slices of rat SI (Chagnac-Amitai and Connors 1989) after application of bicuculline. By blocking inhibition in SI, GRB increases the overall activity of neurons and allows weak or previously suppressed (i.e., hindlimb) inputs to be expressed. On the other hand, contralateral lidocaine inactivation reduced spontaneous activity. This finding is consistent with those of Clarey et al. (1996), who reported a decrease in spontaneous activity when the opposite sensory cortex was cooled. The decreased spontaneous activity that results from contralateral SI deactivation indicates that callosal input generally has a net excitatory effect (Kawaguchi 1992; Vogt and Gorman 1982), but the unmasking of hindlimb responses suggests that callosal inputs also provide one source of drive to the GABAergic neurons that normally suppress hindlimb receptive fields.

Hindlimb receptive fields were only expressed for ~30 min after lidocaine injections, which is consistent with the transient (≤30 min) expansion of receptive fields noted by Clarey et al. (1996). The transitory nature of this effect suggests that inhibitory function is reestablished when input from the opposite hemisphere is reduced for a sustained period of time (25–30 min). The reestablishment of inhibition could reflect an increase in other sources of input and/or in the sensitivity of SI GABAergic neurons to these inputs (Clarey et al. 1996). Together with the fact that lidocaine inactivation revealed hindlimb responses in only 60% of the sites that were stump/hindlimb responsive during GRB, it seems probable that other factors contribute to the modulation of GABAergic inhibition in SI (Fig. 5). This could include other brain regions such as SII, the ventroposterior thalamus (Porter et al. 2001; Staiger et al. 1996; Swadlow 1995), as well as peripheral C-fiber activity (Calford 2002). Alternatively, inhibitory SI neurons have a high level of basal activity during normal behavior (McCasland et al. 1997), and hence a portion of the selective inhibition of hindlimb receptive fields (Stojic et al. 2001) may not require continuous input from outside sources. The variety of form and function among GABAergic neurons (Beierlein et al. 2003; Markram et al. 2004; McBain and Fisahn 2001; Reyes et al. 1998; Swadlow et al. 1998; Tamas et al. 2003), the differential locations and roles of GABA_A and GABA_B receptors (Chowdhury and Rasmusson 2003), and the interplay between stimulus-driven and tonic inhibition (Mody 2005) support the concept that multiple factors are involved in modulating the expression of receptive fields in SI. Our results suggest that the suppression of hindlimb receptive fields in the SI forelimb-stump representation of neonatally amputated rats is mediated by a dynamic circuit with multiple inputs as well as the capacity to compensate or adjust for a loss of activity in one of these inputs.

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