Blockade of GABAergic Inhibition Reveals Reordered Cortical Somatotopic Maps in Rats That Sustained Neonatal Forelimb Removal

RICHARD D. LANE,1 HERBERT P. KILLACKEY,2 AND ROBERT W. RHOADES1

1Department of Anatomy and Neurobiology, Medical College of Ohio, Toledo, Ohio 43699; and 2Department of Psychobiology, University of California, Irvine, California 92717

INTRODUCTION

The manner in which cortical functional organization is altered after damage to sensory organs or peripheral nerves and understanding the mechanisms underlying such reorganization have become foci of intense interest (see Kaas 1994 for a review). Results from a large number of studies in the somatosensory system have indicated that either damage to the periphery or transection of peripheral nerves results in rapid reorganization of cortical somatosensory maps such that regions of cortex in which the damaged or denervated body part was represented develop responses to other portions of the body surface (e.g., Calford and Tweedale 1991; Franck 1980; Merzenich et al. 1983a; Mogilner et al. 1993; Rasmusson 1982; Wall and Cusick 1984; Wall et al. 1992; and many others).

An issue of particular interest in the study of cortical reorganization that follows peripheral nerve injury or amputation is the extent to which alterations are a result of changes within the cortex and/or of subcortical functional changes relayed to it (e.g., Garraghty and Kaas 1991a; Millar et al. 1976; Pettit and Schware 1993). Results from several studies are consistent with the conclusion that, for at least some types of deafferentation, reorganization demonstrated in the cortex may largely reflect subcortical changes (Garraghty and Kaas 1991a,b; Rhoades et al. 1987).

We (Lane et al. 1995) recently reported that amputation of the forelimb at birth resulted in invasion of the cuneate (CN) by sciatic nerve axons and the development of CN cells including thalamic projection neurons with receptive fields that include both the forelimb stump and the hindlimb. However, recordings from unit clusters in lamina IV of the primary somatosensory cortex (SI) of these animals revealed the presence of only a few sites in the forelimb stump representation where responses to hindlimb stimulation could also be recorded. In the present study we tested the possibility that input from the hindlimb was suppressed in lamina IV of the cortical stump representation via GABAergic inhibitory mechanisms by mapping this cortical region, applying the γ-aminobutyric acid-A (GABA_A) and GABA_B receptor antagonists bicuculline and phaclofen (50 μM each), and then remapping the same sites. In six neonatally manipulated rats, 15 of 242 sites (6.2%) in the stump representation responded to hindlimb stimulation before GABA receptor blockade and 107 (44.2%) of the same sites responded to stimulation of the hindlimb during blockade (P < 0.05). In six normal adult rats, 7 of 264 sites (2.7%) in the forelimb cortex may largely reflect subcortical changes (Garraghty and Kaas 1991a,b; Rhoades et al. 1987).

METHODS

Neonatal forelimb removals

Pups >12 h old were anesthetized by hypothermia until immobile. The left forelimb was amputated with iridectomy scissors...
below the shoulder and the brachial artery was sealed by electrosurgery. The stump was infiltrated with local anesthetic (0.7% bupivacaine) and the skin was closed with cyanoacrylate adhesive. The pups were rewarmed, returned to their mothers, and used in recording experiments when they reached >60 days of age.

**Recordings from SI**

Standard multiple-unit recording and receptive field mapping techniques were used to assess the representation of the body surface in SI (Lane et al. 1995). Recordings in animals that sustained neonatal forelimb removals were made from the cortex contralateral to the amputation. Recordings from normal animals were made from the right side. Rats were initially anesthetized with a combination of ketamine (100 mg/kg) and xylazine (20 mg/kg) and the trachea was cannulated. The rats were placed in a stereotaxic head holder and mechanically ventilated. A midline incision was made in the scalp, the skull overlying the dorsal cortex was removed, and the dura was incised and reflected. The surface of the cortex was photographed at ×44 to record the placement of microelectrode penetrations. The cortical surface was kept wet by application of culture medium (Delbecco’s modified essential medium, Gibco) warmed to 37°C. The rats were maintained in a state of light anesthesia for the duration of the recording session with periodic injections of urethan (200 mg ip).

Unit clusters and occasional single units were recorded with varnish-coated tungsten microelectrodes (Z = 0.9–1.3 MΩ) and cutaneous receptive fields were defined in the manner previously described by Rhoades et al. (1993) and Lane et al. (1995). Cutaneous receptive fields were mapped with tactile stimuli delivered with brushes and blunt probes. Regions of the skin from which responses were evoked were plotted on body surface drawings. Electrode penetrations spaced ∼300 µm apart were made in a roughly rectangular array and multiunit activity was recorded at depths between 500 and 750 µm (the approximate depth of lamina IV). The location of each electrode penetration was recorded on the photograph. Several peripheral electrode tracks in each experiment were marked with electrolytic lesions. At the end of the mapping experiment, the animal was given a lethal dose of carbon dioxide.

**Application of bicuculline and phaclofen to SI**

In an initial series of experiments that included six adult rats that sustained neonatal forelimb removal and six normal adult animals, the stump or forelimb representation in cortex was mapped in the manner described above. Then a 30-µl solution that contained equal parts of 50 µM bicuculline methiodide and 50 µM phaclofen (both supplied by Research Biochemicals International) was applied and the stump region was remapped. In a second series of experiments, the stump representations of nine amputated rats were mapped, and then 15 µl of either bicuculline (4 rats) or phaclofen (5 rats) alone was applied and the stump representations were remapped. Statistical analysis was performed with the use of analysis of variance to test for significant differences between the bicuculline-treated, phaclofen-treated, and both bicuculline- and phaclofen-treated groups. In cases in which the F test showed significant (P < 0.05) between-group differences, a Newman-Keuls post hoc test was performed to identify those experimental groups with significantly different means.

**RESULTS**

**Normal forelimb representation and effects of GABA blockade**

The normal organization of the forelimb and hindlimb representations within SI are shown in Fig. 1A. The borders drawn denote the limits of the regions that responded to stimulation of the forelimb or hindlimb. The most important feature of these representations with respect to the present problem is that only a very small number of cortical sites within the representation of the forelimb responded to hindlimb stimulation. Of 264 sites that responded to stimulation of the forelimb in six normal adult rats, only 2.7% (n = 7) could also be activated by stimulation of the hindlimb. All of these were within 250 µm of the border between the forelimb and hindlimb representations (Fig. 2A).

Figure 1B shows the forelimb representation from the same animal during application of bicuculline and phaclofen. GABA blockade in the normal animal resulted in relatively modest changes in cortical functional maps that could be revealed by multiunit recording. Most importantly, there was a small, but significant, increase in the number of sites within the forelimb representation at which responses could be evoked by stimulation of the hindlimb. Of 264 sites that responded to stimulation of the forelimb during GABA blockade, 11.7% (n = 31) could also be activated by stimulation of the hindlimb (Fig. 2B; P < 0.02 compared with the untreated cortices from the same animals). As can be seen in Fig. 2B, there was some variability with respect to the number of hindlimb-responsive sites within the forelimb representation revealed by GABA blockade in different animals. Percentages ranged from 6.4 to 19.3% in the six animals tested. However, a constant feature of the altered maps observed after administration of bicuculline and phaclofen was that the vast majority (75.9%) of the sites within the forelimb representation that also responded to hindlimb stimulation were located within 250 µm of the border between the forelimb and hindlimb representations (Fig. 2B).

Although not quantified, the multiunit recordings made during GABA blockade also revealed sites near the rostral-lateral border of the forelimb representation that could also be excited by stimulation of the face including the whisker pad (Fig. 1B).

It is important to note that although GABA blockade increased the spontaneous activity of SI cortical neurons (Fig.
FIG. 1. Map of the forelimb and hindlimb representations in the somatosensory cortex of a normal rat (normal #2) before (A) and during (B) application of 50 μM bicuculline methiodide and 50 μM phaclofen to the cortical surface. The vast majority of sites mapped in A has only forelimb receptive fields (∙). One site (●) located along the border between forelimb (FL) and hindlimb (HL) had a receptive field that included both the forelimb and hindlimb. During γ-aminobutyric acid (GABA) blockade (B), there were minor changes in the forelimb representation including several new sites with forelimb-hindlimb and forelimb-vibrissae (●) dual receptive fields. Filled triangles: sites expressing only hindlimb receptive fields. Filled squares: sites with receptive fields located on either the lower jaw (LJ), neck (NK), vibrissae (WK), or trunk (TK) regions. Open circles: unresponsive sites. Orientation arrows in A: anterior (a) and lateral directions for the cortical maps. Bar = 1 mm.

3, compare A3 and B3), responses to peripheral stimulation were still very distinct (Fig. 3, compare A1 and B1), and mapping was no more difficult during GABA blockade than in the normal cortex.

Stump representation after neonatal forelimb removal and effects of GABA blockade

The organization of the stump and hindlimb representations within SI in an adult rat that sustained neonatal forelimb amputation is shown in Fig. 4A. As in Fig. 1, borders denote the limits of the regions that responded to stimulation of the forelimb stump or hindlimb. The most important feature of the forelimb stump representation with respect to this study is the fact that few sites within it also responded to stimulation of the hindlimb. Of 242 sites that responded to stimulation of the stump in six neonatally manipulated adult rats, only 6.2% (n = 15) could also be activated by stimulation of the hindlimb. Of 242 sites that responded to stimulation of the stump during GABA blockade, 44.2% (n = 107) could also be activated by stimulation of the hindlimb (Fig. 5B; P < 0.002 vs. the untreated cortex of normal adult rats and P < 0.0002 vs. the cortex of normal rats after GABA blockade).

Stump representation after neonatal forelimb removal and effects of GABA blockade

Thus neonatal forelimb amputation does result in statistically significant cortical functional changes, but, as in our previous experiments, this reorganization was modest compared with the changes observed in the CN of identically treated animals (Lane et al. 1995).

Figure 4B shows the stump representation from the same animal during application of bicuculline and phaclofen. GABA blockade revealed several changes in the functional reorganization of the stump representation. Most importantly with respect to the issue addressed by the present study, there was a large increase in the number of sites within the stump representation at which responses could be evoked by stimulation of the hindlimb. Of 242 sites that responded to stimulation of the stump during GABA blockade, 44.2% (n = 107) could also be activated by stimulation of the hindlimb (Fig. 5B; P < 0.002 vs. the untreated cortex of neonatally manipulated rats and P < 0.0002 vs. the cortex of normal rats after GABA blockade).

In contrast to the limited and variable effect of GABA blockade in the normal animals (see Fig. 2B), application of bicuculline and phaclofen in the animals that sustained neonatal forelimb removal consistently revealed sites within
the stump representation that responded to stimulation of the hindlimb. Moreover, the vast majority (79.4%) of these sites was >250 μm from the border of the hindlimb representation.

The effect of GABA blockade is best demonstrated by recordings from a site near the center of the stump representation before and during bicuculline and phaclofen administration (Fig. 6). Before GABA blockade, multiple unit activity at this site could be evoked only by stimulation of the stump (Fig. 6A1). During GABA blockade, stimulation of either the stump or the hindlimb elicited responses from cells recorded at this site (Fig. 6, B1 and B2).

As in the normal animals, the multunit recordings made during GABA blockade also revealed sites near the rostralateral border of the forelimb representation that could also be excited by stimulation of the face including the whisker pad (Fig. 4B). The magnitude of this reorganization was not evaluated quantitatively.

Relationships between the forelimb and stump maps and the dense CO aggregates corresponding to those representations are shown in Fig. 7. The borders of the CO densities corresponding to the forelimb, hindlimb, stump, lower jaw, and vibrissae (Fig. 7, B and D) have been overlaid on maps made during bicuculline plus phaclofen treatment of the recording sites of a normal rat (Fig. 7A) and a rat that sustained neonatal amputation of the contralateral forelimb (Fig. 7C). Comparison of the functional maps and the histochemistry supports two conclusions. First, the functional representations of the forelimb and stump extend slightly beyond the boundaries of the dense CO clusters. Second, the sites that responded to the hindlimb stimulation in the amputated animal extend throughout the region of dense CO stain-
GABA AND CORTICAL REORGANIZATION

FIG. 3. Traces recorded from a microelectrode positioned near the center of the forelimb representation of a normal rat cortex and left undisturbed during the period before (A) and during (B) application of GABA inhibitors to the cortical surface. Traces: responses to cutaneous stimulation (↓ below the traces 1 and 2) as well as the spontaneous activity at the recording site (trace 3). Trace 1: response to touch stimulation of the forelimb. Trace 2: lack of response to a hindlimb touch. Note the increase in the amplitude of the forelimb response (compare A1 and B1) as well as the increased spontaneous activity (compare A3 and B3) during the application of the GABA inhibitors. The GABA inhibitors did not alter the lack of response to a hindlimb touch at this recording site (B2). Traces 1 and 2 were recorded at a sweep rate of 50 ms per horizontal scale, whereas trace 3 has a slower sweep rate of 2 s per horizontal scale. Vertical scale (0.2 mV) in A, bottom applies to all traces.

The present study is limited by the fact that we recorded only multiple-unit activity and employed bath application of GABA blockers. This approach was taken to permit compar-

Effects of bicuculline and phaclofen alone on occurrence of hindlimb-responsive sites within the stump representation of neonatally manipulated animals

Application of either bicuculline or phaclofen alone revealed large numbers of hindlimb-responsive sites within the stump representation of neonatally manipulated rats (Fig. 8). In the four rats treated with bicuculline alone, 12 of 184 sites (6.5%) in the stump representation could be activated by stimulation of the hindlimb before treatment and 61 (33.2%) of these same sites could be activated by stimulation of the hindlimb during GABA<sub>A</sub> receptor blockade with bicuculline (Fig. 9A). In the five rats treated with phaclofen alone, 18 of 251 sites (7.2%) in the stump representation could be activated by stimulation of the hindlimb before treatment and 64 (25.5%) of these same sites could be activated by stimulation of the hindlimb during GABA<sub>B</sub> receptor blockade with phaclofen (Fig. 9B). Comparison of results from neonatally manipulated rats that received either bicuculline or phaclofen alone with those from similar animals treated with both GABA blockers revealed no significant difference between the effectiveness of delivering both agents or each alone in revealing hindlimb-responsive sites within the cortical stump representation (P > 0.05).

DISCUSSION

The results described in the preceding section indicate that hindlimb input to the portion of SI representing the forelimb stump is suppressed in adult rats that have sustained neonatal forelimb removal and that GABAergic inhibition, mediated by both GABA<sub>A</sub> and GABA<sub>B</sub> receptors, is involved in this process.

The present findings provide an explanation for the surprising results of our previous study (Lane et al. 1995) of subcortical and cortical reorganization in rats that sustained neonatal forelimb removal. That study showed that lesion-induced anatomic and functional subcortical changes were only minimally expressed in the cortex. This finding was difficult to understand because of the large number of studies that have demonstrated rapid cortical functional reorganization after peripheral lesions or sensory deprivation (e.g., Cusick et al. 1990; Garraghty and Kaas 1991b; Kalaska and Pomeranz 1979; Kelahan and Doetsch 1984; Merzenich et al. 1983a, b; Mogilner et al. 1993; Rasmusson 1982). Although the results of the present experiment were instructive, several important technical limitations of our approach must be acknowledged.

Technical limitations

The present study is limited by the fact that we recorded only multiple-unit activity and employed bath application of GABA blockers. This approach was taken to permit compar-
FIG. 4. Example of the stump area within the somatosensory cortex of a neonatally amputated rat (DOB-FLX #28) before (A) and during (B) application of bicuculline and phaclofen to the cortical surface. The majority of sites (●) mapped in A has only forelimb receptive fields. Several sites (△) located both along the hindlimb border and within the stump representation (ST) have both stump and hindlimb receptive fields. Other sites (□) bordering the neck and vibrissae regions have receptive fields including both the stump and head. B: GABA blockade produced a large increase in the number and a wider distribution of sites in the stump representations with receptive fields that included the hindlimb. In addition, several sites near the vibrissae border now have receptive fields that include both the stump and vibrissae. Filled triangles: expressing only hindlimb receptive fields. Filled squares: sites with receptive fields located on either the lower jaw, neck, vibrissae, or trunk regions. Open circles: unresponsive sites. Orientation arrows in A: anterior (a) and lateral directions for the cortical maps. Bar = 1 mm.

ison of the results of this study with those obtained previously (Lane et al. 1995). Clearly, much more information regarding the hindlimb input to individual neurons can be obtained with single-cell recording and microiontophoretic application of GABA antagonists. However, such an approach would not permit rapid mapping of the entire forelimb/stump representation as was carried out here.

The manner in which we applied bicuculline and phaclofen also did not permit determination of concentrations of these agents in lamina IV where our recordings were made. The fact that robust functional changes were observed during application of either or both GABA blockers indicates that effective concentrations were achieved in lamina IV. Nevertheless, the lack of information regarding concentrations in this layer raises a potential question about the equivalent effects of bicuculline and phaclofen and the lack of additivity of effects of these two agents. Results from other studies suggest that phaclofen and bicuculline, at the concentrations used (50 μM), were likely to act only on their appropriate receptors. Bowery et al. (1983) reported that the median inhibiting concentration for bicuculline-induced displacement of GABA or baclofen from GABA_B receptors was >50 μM (also see Hill and Bowery 1981). They also demonstrated that the median inhibiting concentration for baclofen-induced displacement of GABA from GABA_B receptors was >50 μM. Moreover, the concentrations used in the present study are similar to those employed in other in vitro experiments in which these agents have been applied to cortical slices (e.g., Dutar and Nicoll 1988; Maurin 1988).

Importance of GABAergic mechanisms in the cortices of experimentally manipulated and normal animals

The observation that altered functional organization apparent subcortically required a reduction in cortical inhibition for functional expression at this level has several precedents in the literature. For example, Duffy et al. (1976) demonstrated that intravenous administration of bicuculline resulted in a marked increase in the cortical expression of deprived-eye inputs in monocularly deprived cats. Sillito et
al. (1981) refined this paradigm and showed that iontophoretic application of bicuculline resulted in the reversible expression of deprived eye inputs to visual cortical neurons in similarly treated cats. Although results for some neurons recorded by Sillito et al. (1981) might be explained by a bicuculline-induced nonspecific increase in responsiveness, such an effect cannot explain the data from cells that became equally responsive to stimuli delivered to the deprived and nondeprived eyes.

There are also a number of studies that have demonstrated that GABAergic mechanisms play an important role in modulating expression of excitatory inputs to neurons in the normal somatosensory and motor cortices. However, the majority of these experiments has addressed only the effects of blocking the GABA<sub>A</sub> receptor. Dykes et al. (1984) showed that iontophoretic application of bicuculline onto neurons in the cat somatosensory cortex resulted in the development of cutaneous receptive fields by previously unresponsive somatosensory cortical neurons and also increased receptive field size for some already responsive cells. Similarly, Alloway and Burton (1991) found that bicuculline treatment of neurons in cortical area 3b of monkeys increased the receptive field size of these cells by a factor of 3–4. The importance of GABAergic mechanisms in shaping receptive fields of cortical sensory neurons is not restricted to the somatosensory system. Ramoa et al. (1988) reported that continuous infusion of bicuculline into the visual cortex resulted in the development of neurons with abnormally large receptive fields. Jacobs and Donoghue (1991) demonstrated a similar effect of GABA<sub>A</sub> receptor blockade in the motor cortex. Normally, delivery of small amounts of current to the vibrissae representation in the motor cortex results in

**FIG. 5.** A: percentages of sites in the stump representation expressing a hindlimb receptive field in adult rats that sustained neonatal amputation of the contralateral forelimb (DOB-FLX rats). Pair of bars at far left: average values from 6 amputated rats. Error bars: means ± SD. Remaining bars: individual values for 4 rats from this group. There are significantly more sites expressing a hindlimb receptive field in the stump region both near and distant from the hindlimb border than in the normal rats (Fig. 2). However, these sites still represent a minor component of all the cortical stump sites. B: GABA blockade produced by the application of bicuculline and phaclofen greatly increases the expression of hindlimb receptive fields in the forelimb representation, both near and >250 μm from the hindlimb border.
movement of the whiskers, but not the limbs. However, when bicuculline was applied to the motor cortical forelimb representation, delivery of the same current to the vibrissae field did result in forelimb as well as vibrissae movement.

Kaneko and Hicks (1990) have provided results indicating that activation of GABA$_B$ receptors with baclofen reduces the responsivity and decreases the receptive field sizes of somatosensory cortical neurons in cat. Interestingly, the effects of baclofen were only partially and variably blocked by phaclofen. Kaneko and Hicks concluded that the effects of GABA in cat SI were principally due to the activation of GABA$_A$ receptors and that GABA$_A$ mechanisms revealed by application of baclofen were probably either presynaptic or located at sites distant from the soma. These conclusions are at least partially consistent with previous and subsequent observations indicating that GABA$_B$ receptors are located both pre- and postsynaptically in the neocortex (Deisz and Prince 1989; Fukuda et al. 1993; Howe et al. 1987). The importance of GABAergic mechanisms in modulating the functional efficacy of inputs to deprived cortical regions suggests that increases in expression of elements involved in GABAergic neurotransmission might occur in these areas.

Immunocytochemical and radioligand binding studies generally indicate just the opposite. The results of a large number of studies in both the visual and somatosensory systems have indicated that either deprivation of afferent activity or destruction of primary sensory neurons results in downregulation of GABAergic markers including GABA, glutamic acid decarboxylase (GAD), and GABA$_A$ receptors (Akhtar and Land 1991; Chalmers and McCulloch 1991; Hendry and Jones 1986; Hendry et al. 1990, 1994; Huntsman et al. 1994; Kossut et al. 1991; Land et al. 1995; Ribak and Robertson 1986; Warren et al. 1989; Welker et al. 1989). There are only limited opposing results suggesting that deprivation may increase GABAergic markers. Shaw and Cynader

---

**FIG. 6.** Traces recorded from a microelectrode positioned near the center of the cortical stump representation of an adult rat that sustained neonatal amputation of the contralateral forelimb. The recording electrode was left undisturbed during the period before (A) and during (B) the application of the GABA inhibitors to the cortical surface. Traces: responses to cutaneous stimulation († below traces 1 and 2) as well as the spontaneous activity at the recording site (trace 3). Trace 1: response to stimulation of the forelimb. Trace 2: response (or lack thereof) to a hindlimb touch. As was similarly noted for the normal rats (Fig. 3), GABA blockade causes an increase in the amplitude of the stump response (compare A1 and B1) as well as an augmentation of the spontaneous activity (compare A3 and B3). In addition, GABA blockade produced a robust de novo hindlimb response at this recording site (B2) where there had previously been no detectable response to hindlimb stimulation (A2). Calibration: 0.2 mV, 50 ms (traces 1 and 2); 0.2 mV, 2 s (trace 3).
FIG. 7. Comparisons of the electrophysiologically mapped and cytochrome oxidase (CO)–histochemically defined borders of the forelimb/stump areas of normal and neonatally manipulated DOB-FLX rats. Paired photomicrographs of the cortical surfaces show the map of the forelimb (A) or stump (C) representation after 50 μM bicuculline methiodide plus 50 μM phaclophen treatment and the corresponding tangential sections stained for CO (B and D). The forelimb or stump borders defined by the CO densities shown in B and D have been superimposed on the mapped cortices in A and C, respectively. At several sites, the mapped borders of both the forelimb and the stump extend out a distance ≥300 μm beyond the CO borders for both the normal and DOB-FLX animals. Unlike the distribution in the normal animal (A), the majority of the dual stump and hindlimb receptive field sites (△) in the DOB-FLX animal (C) are located within the CO-defined border of the stump area. Filled circles: forelimb or stump-only receptive fields. Open squares: sites with a forelimb/stump as well as a vibrissae receptive field. Asterisks: sites of burn marks used to orient the CO pattern on the cortical maps. Orientation arrow in C: anterior (a) and lateral directions for the cortical maps. Bar = 1 mm.

(1988) reported that unilateral eyelid suture increased the density of GABA_A receptors in the cat’s visual cortex, and Pinard et al. (1991) noted that enucleation in rats resulted in a transient increase in binding of a benzodiazepine to GABA_A receptors in the deprived visual cortex.

Although GABAergic mechanisms appear to be involved in expression of inputs to the cortex, it is difficult to understand why they would inhibit expression of inputs in the present study as well as that of Lane et al. (1995), but apparently not in a number of other experiments in which peripheral denervations or deprivation have been demonstrated to result in cortical functional reorganization. In considering this, it is important to remember that the forelimb-amputated animals did exhibit slight, but statistically significant, cortical reorganization. This reorganization was substantially amplified by GABA blockade. The very mod-
FIG. 8. Examples of the stump representation within the somatosensory cortices of 2 adult rats that sustained neonatal amputation of the contralateral forelimb (DOB-FLX #29 and #23). Receptive fields were mapped before (A and C) and during cortical application of either bicuculline (B) or phaclofen (D). Before GABA blockade, both cortices had small numbers (3-6) of sites (△) where multiple unit responses were evoked by stimulation of either the hindlimb or stump. The majority of the recording sites exhibited only stump receptive fields (●). The number and distribution of sites with dual stump and hindlimb receptive fields within the stump area increased after the blockade of either the GABA<sub>A</sub> receptors (B) or GABA<sub>B</sub> receptors (D). Orientation arrows in C: anterior (a) and lateral directions; arrows apply to all 4 cortical maps. Bar = 1 mm.
est reorganization observed after neonatal damage in the present study actually appears similar to that reported by Wall and Cusick (1984) after sciatic nerve damage in adult rats, and it may be that changes observed in other situations are also gated to some degree by GABAergic mechanisms. The extent to which GABAergic inhibition may suppress cortical functional reorganization is likely to vary significantly as a function of the effects of deprivation on expression of this transmitter and its receptors. The papers reviewed above suggest that these effects may be somewhat variable, and evaluation of results from the rodent somatosensory system suggests strong dependence on both the age of the animal and the type of manipulation. Akhtar and Land (1991) showed that peripheral deprivation in adulthood, but not shortly after birth, resulted in reduced GAD immunoreactivity in the contralateral cortex. Land et al. (1995) showed further that perinatal peripheral deprivation had no effect on cortical GABA_{A} receptors. In apparent contrast to these results, Kossut et al. (1991) observed that vibrissa follicle ablation in neonatal mice resulted in downregulation of GABA immunoreactivity in the corresponding cortical barrels. Welker et al. (1989) reported similar results for GAD immunoreactivity in the barrel cortex after follicle ablations in adult mice, and Skangiel-Kramska et al. (1994) demonstrated reductions in GABA receptors under similar conditions. Ongoing experiments in our laboratory suggest that the number of GABA-positive neurons in lamina IV of the stump representation is not reduced by neonatal forelimb removal. All of these results suggest that the response of cortical GABAergic neurons and receptors to peripheral deprivations and thus their ability to modulate functional reorganization varies significantly with the type of deprivation and the age at which it is carried out.
Anatomic substrate for functional reorganization observed in stump cortex after GABA blockade

Comparison of the results from the normal rats and those that sustained neonatal forelimb amputation indicate that some form of reorganization associated with neonatal forelimb removal is necessary for GABA-blockade-induced expression of hindlimb-related input to the portion of the cortex in which the forelimb/stump is represented. GABA blockade resulted in a small, but significant, increase in the percentage of sites within the forelimb representation from which responses to hindlimb stimulation could be recorded in normal adult rats, but virtually all of these were near the border with the hindlimb representation. GABA blockade in the rats that sustained neonatal forelimb amputation resulted in a significantly greater rise in the percentage of sites within the stump representation from which hindlimb-evoked activity could be recorded, and the vast majority of these sites was >250 μm from the forelimb-hindlimb border.

There are at least three potential substrates for the hindlimb responses observed in the cortical stump representation during cortical GABA blockade in rats that sustained neonatal forelimb amputations. The first is that GABA blockade permits the expression of input from thalamocortical cells that respond to stimulation of the both the stump and the hindlimb. Our previous results (Lane et al. 1995) showed that CN neurons with receptive fields containing both the stump and hindlimb project to the thalamus, and it is reasonable to suggest that thalamocortical neurons within the ventral posterolateral nucleus may have similar receptive fields. Limited thalamic recordings from rats that have sustained neonatal forelimb amputations have provided results consistent with this hypothesis (A. S. Stojic, R. D. Lane, and R. W. Rhoades, unpublished observations). A second possibility is that early forelimb removal results in reorganization of thalamocortical projections such that thalamic neurons from the normal hindlimb representation in ventral posterolateral nucleus send axons into the cortical stump representation and that these projections are only expressed during GABA blockade. There is precedent for substantial reorganization of thalamocortical projections after neonatal peripheral damage in the rodent somatosensory system (Verley and Onnen 1981). Finally, it is possible that GABA blockade permits expression of lesion-induced intracortical projections from the hindlimb to the stump representation. The importance of functionally latent intracortical projections in lesion-induced functional reorganization has been suggested and/or demonstrated by a large number of investigators (e.g., Das and Gilbert 1995; Jacobs and Donoghue 1991; Smits et al. 1991). Which of these pathways is/are involved in the functional cortical reorganization revealed by GABA blockade in the neonatally manipulated animals will be determined by currently ongoing experiments.

This work was supported by National Institutes of Health Grants NS-28888 and DE-07734. Address for reprint requests: R. D. Lane, Dept. of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699.

Received 1 May 1996; accepted in final form 17 January 1997.

REFERENCES


GABA AND CORTICAL REORGANIZATION


KANEKO, T. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.


KAN, A. M. AND HICKS, T. P. GABA\(_B\)-related activity involved in synaptic kainic acid lesions of the trigeminal brain stem complex.