Role of Inhibition in Cortical Reorganization of the Adult Raccoon Revealed by Microiontophoretic Blockade of GABA_A Receptors

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Tremere, Liisa, T. Philip Hicks, and Douglas D. Rasmusson. Role of inhibition in cortical reorganization of the adult raccoon revealed by microiontophoretic blockade of GABA_A receptors. J Neurophysiol 86: 94–103, 2001. Cortical reorganization was induced by amputation of the 4th digit in 11 adult raccoons. Animals were studied at various intervals, ranging from 2 to 37 wk, after amputation. Recordings were made from a total of 129 neurons in the deafferented cortical region using multibarrel micropipettes. Several types of receptive fields were described in reorganized cortex: restricted fields were similar in size to the normal receptive fields in nonamputated animals; multi-regional fields included sensitive regions on both adjacent digits and/or the underlying palm and were either continuous over the entire field or consisted of split fields. The proportion of neurons with restricted fields increased with time after amputation and was greater than previously found in subcortical regions. A GABA_A receptor antagonist (bicuculline methiodide), glutamate, and GABA were administered iontophoretically to these neurons while determining their receptive fields and thresholds. Bicuculline administration resulted in expansion of the receptive field in 60% of the 93 neurons with cutaneous fields. In most cases (33 neurons) this consisted of a simple expansion around the borders of the predrug receptive field, and the average expansion (426%) was not different from that seen in nonamputated animals. In some neurons (n = 4), bicuculline produced an expansion from one digit onto the adjacent palm or another digit, an effect never seen in control animals. Bicuculline also changed the split fields of seven neurons into continuous fields by exposing a responsive region between the split fields. Finally, bicuculline changed the internal receptive field organization of 10 neurons by revealing subfields with reduced thresholds. In contrast to the situation in nonamputated animals, iontophoretic administration of glutamate also produced receptive field expansion in some neurons (n = 6), but the size and/or shape of the change was different from that produced by bicuculline, indicating that the effects of bicuculline were not due to an overall facilitation of neuronal activity. These results are consistent with the hypotheses that an important component of long-term cortical reorganization is the gradual reduction in effective receptive field size and that intracortical inhibitory networks are partially responsible for these changes.

INTRODUCTION

The term “cortical reorganization” refers to the changes in the sensory responsiveness of cortical neurons that occur following removal of their dominant sensory input. Such changes occur in adult, as well as developing, mammals and consequently have important implications for humans with peripheral nerve damage. The mechanisms responsible for cortical reorganization are still unknown, but clearly involve changes in synaptic strength as well as the formation of new connections. Many of the studies on cortical reorganization have been carried out in the raccoon (Kelahan and Doetsch 1984; Kelahan et al. 1981; Rasmusson 1982), a species that offers the advantage of extremely large (~25 mm²) representational areas of each digit that can be reliably identified on the basis of the unique “triradiate sulcus” (Welker and Seidenstein 1959). In addition, the segregation of digit inputs to each representational area enables one to produce extensive deafferentation of a digit region by means of digit amputation.

Deafferentation in both the visual and somatosensory systems has been shown to produce changes in markers for neurotransmitters such as γ-aminobutyric acid (GABA) (Jones 1993) and glutamate (Carder and Hendry 1994). The decrease in the efficacy of GABAergic synapses is particularly interesting because it could alter synaptic efficacy by increasing the excitability of cortical neurons and by lowering a “modification threshold” that would permit strengthening of other synapses (Bear et al. 1987; Bienenstock et al. 1982).

One important role of GABAergic transmission in normal somatosensory cortex is to restrict the size of receptive fields (RFs) of many neurons (Alloway and Burton 1991; Alloway et al. 1989; Batuev et al. 1982; Dykes et al. 1984; Hicks and Dykes 1983). This action was first demonstrated using the microiontophoretic administration of a GABA_A receptor antagonist such as bicuculline. Two implications of this finding are that cortical neurons normally receive excitatory inputs from a greater spatial range than is indicated by their RFs and that some of these inputs are being selectively blocked by inhibitory synapses. The RF seen during the microiontophoretic administration of bicuculline therefore provides an indication of the total spatial convergence onto these cortical neurons.

To determine the role of GABA_A inhibition in somatosensory processing during reorganization, we first studied bicuculline effects in control raccoons (Tremere et al. 2001). The results indicated that when expansion of RFs occurs it is limited to the same part of the forepaw that the original RF was on; i.e., when the RF is originally on a digit, RF expansion is restricted to that digit. Following amputation of a digit in this species, neurons in the deafferented cortical region gradually

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acquire new RFs on one or both adjacent digits (Kelahan and Doetsch 1984; Rasmusson 1982). Reorganization therefore cannot be explained by unmasking or removing inhibition of inputs from these adjacent digits.

On the other hand, the down-regulation in GABA activity that occurs after peripheral denervation raises the possibility that alteration of cortical inhibition plays a significant role during the reorganization process that occurs over several months. Consequently, the present study examined antagonism of GABA_A receptors within the clearly defined deafferented digit region of the raccoon cortex during the course of reorganization. The use of microiontophoretic application of antagonists is also useful in distinguishing cortical from subcortical mechanisms of reorganization since any drug effects will be restricted to the cortex. In this paper we show that the organization of both excitatory and inhibitory RFs is different in reorganized cortex than it is in the normal cortex and that the progressive changes in RF structure that follow deafferentation are partially regulated by GABA_A receptors.

METHODS

Recordings were made from 11 adult raccoons, ranging in weight from 6.1 to 11.3 kg. The animals were trapped in the wild by a licensed trapper and were maintained in communal housing with ad lib food and water. All procedures for handling and experimentation were approved by the University Ethics Committee and were in accordance of the guidelines set down by the Canadian Council of Animal Care.

The fourth digit of the right forepaw was amputated in all but one animal. The left forepaw was used in the latter case because this animal had previous damage to the right forepaw. Our previous experiments have not found any right-left differences in raccoon cortex or in response to denervation. The surgical procedure was the same as described previously (Rasmusson 1996a). Briefly, the digit was removed at the metacarpo-phalangeal joint under sterile surgical conditions with the animal anesthetized with halothane. The nerves were ligated as far proximal as possible before being cut, and the dorsal hairy skin was sutured to the glabrous skin of the palm. Immediately after surgery, cesazolin sodium (Keskoal, Lilly; 15 mg/kg im) was injected to prevent infection, and buprenorphin (Buprenex, Reckitt-Coleman; 0.3 mg/kg im) was injected to reduce postoperative pain.

Recordings were made from primary somatosensory cortex contralateral to the amputation at 2-, 8-, 11-, 12-, 15-, 18-, 19-, 24- (n = 2), 34-, or 37-wk intervals after the amputation. The construction of the micropipette assemblies and general recording procedures are described in detail in Tremere et al. (2001). The animal was initially anesthetized with halothane and maintained with α-chloralose throughout the recording session and was given 2 ml of a corticosteroid (Solu-Delta-Cortef, Upjohn) to reduce cerebral edema. Body temperature was maintained near 37°C by a feedback-controlled heating pad (Harvard). The animal’s head was stabilized in a Kopf stereotaxic frame before opening the skull and dura. The brain was covered with warmed Elliott’s solution (Abbott, Montreal) throughout the experiment.

Recordings were made using one barrel of a 5- or 7-barrel pipette (A-M Systems, Carlsborg, WA) containing a 7-μm-diam carbon fiber. The pipettes were pulled in a vertical Narishige microelectrode puller, and the carbon fiber was electrochemically etched to yield a final impedance of 0.5–4 MΩ at 1 kHz. The other barrels contained bicuculline methiodide (BMI, Sigma, 5 mM in 0.9% saline, pH 3.3), l-glutamate (Sigma, 0.5 M, pH 8.0), GABA (Sigma, 0.5 M, pH 3.5), and 0.9% saline, for current balancing. The impedance of these barrels was 10–15 MΩ. Administration of drugs was controlled using a

Neurophore apparatus (Medical Systems, Greenvale, NY), and a retaining current of 9–20 nA was applied to each barrel.

When the activity of a single neuron was isolated, the boundaries of its RF were determined using von Frey hair monofilaments (Stoelting, Wood Dale, IL). When measuring RF area, the RF was defined using the lowest suprathreshold monofilament and was marked directly on the skin with a felt tip pen. The length and width of this field were measured with calipers and recorded. The RF was also sketched on drawings of the raccoon forepaw. The activity of each cell was recorded for at least 5 min prior to any drug administration. Since the amount of drug ejected depends on many variables such as tip diameter, capacitance, transport number, as well as ejection current (Hicks 1983), the ejection current was determined empirically for each cell with the major concern being the avoidance of excessive stimulation with either glutamate or BMI. The RF was mapped repeatedly before, during, and after drug administration using the same von Frey monofilament, and the maximal length and width of the RF were measured in the predrug and drug states.

The deafferented region of cortex that normally represents the fourth digit was identified from the gyral patterns surrounding the triradiate sulcus and confirmed from recordings in the intact third digit, fifth digit, and palm representations. Data obtained from neurons in the third and fifth digit cortex were consistent with our observations in intact, control animals (Tremere et al. 2001) and will not be dealt with here.

The data obtained in amputated animals were compared with those from our previous publication (Tremere et al. 2001) that used the same techniques in nonamputated animals. Comparisons of frequency data were made using the χ² test or the nonparametric Kolmogorov-Smirnov test (Siegel and Castellan 1988). Other comparisons were made using an unpaired Student’s t-test. All statistical analyses were carried out using the Statview 5.0 program (SAS Institute, Cary, NC).

RESULTS

Electrode penetrations were made at 150 sites in the deafferented 4th digit representation, and a total of 129 cells were studied in this region. It appeared to be slightly more difficult to find active cells in deafferented than in control animals. The average number of cells isolated per penetration was 0.86 in the deafferented cortex compared with 1.71 in the 4th digit representation of nondeafferented animals, but this difference was not statistically significant (t = 1.82, P = 0.08). The number of cells obtained per penetration was particularly low (<0.5) in the two animals that were studied at 12 and 15 wk postamputation, whereas in all of the remaining animals the values were between 0.75 and 2.0. The majority of the neurons (n = 93, 73%) were rapidly adapting (RA) with RFs on the glabrous skin. A small number responded to stimulation of hairy skin (n = 6) or squeezing a joint or claw (n = 7). The remaining 23 neurons (18%) did not respond to stimulation of any part of the forepaw.

The depth below the cortical surface at which the neuron was encountered ranged from 40 to 1,400 μm. The greatest number of sampled neurons were around 500 μm (Fig. 1). While the range of depths was slightly larger than in our sample of neurons in nonamputated animals (Tremere et al. 2001), there was no statistically significant difference between the two groups in terms of depth profiles (Kolmogorov-Smirnov test, P = 0.68).

Novel RF types in reorganized cortex

In nonamputated raccoons, virtually all of the neurons in the digit representational areas of S1 cortex respond exclusively to
stimulation of the glabrous surface of the digit, and the RFs are restricted to a small portion of the digit. Similarly restricted RFs were found in the amputated animals, although the restricted fields often lacked the sharp boundary that is characteristic of the control animals, making it more difficult to quantify RF area. These restricted RFs in reorganized cortex were most often on either the third or fifth digit, but were sometimes on the palm near the scar at the site of the amputation.

In addition to these restricted RFs, several other types of RF were often encountered in reorganized cortex (Fig. 2). Previous descriptions have called these RFs “heterotopic” or simply “large” (Kelahan and Doetsch 1984; Turnbull and Rasmusson 1991), but, to emphasize the unusual spatial organization of the RFs characteristic of reorganized cortex, we will use the term multi-regional (MR), as the RFs included parts of two or three regions of the forepaw, namely the two adjacent digits and/or the palm. Multi-regional RFs could be further grouped according to whether the RF consisted of spatially distinct RFs on two or more regions (multi-regional-split, MR-S) or was continuous over these regions (multi-regional-continuous, MR-C).

The largest proportion of RFs on glabrous skin fell into the restricted category (49 of 93, 53%). The RF sizes of these restricted fields were not significantly different from the size of RFs on the distal digit in control animals (12.9 vs. 15.4 mm², respectively; \( t = 1.0, P = 0.32 \)). Forty-four neurons had multi-regional RFs, 22 neurons (24%) in each of the MR-S and MR-C categories. In the MR-C group, the most common combination of regions was basically a U-shaped RF with responsive regions on both digits 3 and 5 and the distal part of palmar pads C and D proximal to these digits (Fig. 2C). The threshold was usually not uniform throughout these MR-C fields, but there were lower threshold subfields that were spatially joined by higher threshold regions. The low-threshold subfield was usually on the distal pad of a digit or near the wound. In the case of the MR-S group, the majority of cells had subfields on the distal pads of digits 3 and 5.

The frequency of occurrence of these different types of RF changed with time after the amputation. This is illustrated in Fig. 3, in which animals tested at similar intervals after the amputation are grouped to give larger sample sizes at each interval. The change in the proportion of neurons in each of these four categories over time is statistically significant (\( \chi^2 = 35.9, P < 0.001 \)). The most dramatic changes are the increase in restricted RFs, from 5% at the earliest intervals to over 50% at later intervals, and the decrease in nonresponsive cells, from 40% to almost zero. At the earliest intervals almost all of the responsive neurons have multi-regional RFs, but this proportion drops to 35–40% at longer intervals. These data support two hypotheses suggested in earlier reports. First, many RFs in deafferented cortex differ from normal RFs in being unusually large, often extending beyond the confines of a single digit. Second, there is a progressive decrease in RF size with increased time after amputation.

Comparison with reorganization in thalamus and cuneate

The proportion of neurons falling into these three categories of RF was compared with previous results in the reorganized thalamus (Rasmusson 1996a,b) and cuneate nucleus (Rasmusson and Northgrave 1997). These data are presented in Fig. 4, which illustrates that restricted RFs predominate in the cortex, whereas multi-regional fields are most common in subcortical regions. The differences between the three levels are statistically significant (\( \chi^2 = 30.3, P < 0.001 \)).
Effects of microiontophoretic administration of drugs

Neuronal excitability. BMI was administered to 113 cells in deafferented cortex. The range of ejection currents for BMI was 10–85 nA, and the maximal time of application was 6 min. In our experience (Tremere et al. 2001), equilibrium is reached within 5 min, and responses do not show any additional change with longer administration. At these levels, BMI produced a clear increase in spontaneous activity in 18 cells and decreased the threshold for mechanical activation in 37, with both effects occurring in 9 neurons. Glutamate was tested on 77 cells in the deafferented cortex. Glutamate resulted in a decreased threshold in 38 cells and an increase in spontaneous activity in 33 cells, with 12 neurons showing both effects. GABA was administered to 31 cortical neurons in reorganizing cortex. Fourteen cells showed an increased threshold to mechanical stimulation, and 14 showed a decrease in spontaneous activity, with both effects seen at 4 cells. While these observations give only a general impression about the effectiveness of each drug in altering neuronal responsiveness, the absence of a change does not imply that the neuron does not possess the relevant receptors. Nevertheless it is important to note that these proportions were not statistically different from those observed in nonamputated animals (glutamate, $\chi^2 = 0$; BMI, $\chi^2 = 0.12$; GABA, $\chi^2 = 0.09$), indicating that deafferentation did not result in a large change in responsiveness to these drugs.

BMI effects on RF organization. BMI was tested on a total of 113 neurons in deafferented cortex. Fifteen of these neurons did not respond to peripheral stimulation before BMI administration; in none of these was a RF unmasked by BMI. BMI also did not produce any appreciable change in the RF of any of the neurons that responded only to stimulation of deep tissue or of hairy skin. Of the 93 neurons with purely cutaneous RFS, BMI produced changes in RF size or structure in 56 (60%). Three types of changes were produced by BMI. The first type of change was a simple expansion of the predrug RF with preservation of the original RF shape; the second was an expansion that drastically altered the RF shape and necessitated a redefinition of the RF category for that cell; the third was the appearance of lower threshold subfields within the original RF without an increase in total RF area.

Simple expansion of the RF was seen in 33 of 93 neurons with cutaneous RFS (35%). Expansion was more common among the neurons with restricted RFSs (21 of 49, 45%) than in the MR-S or MR-C groups. This expansion was similar to that seen in control animals (e.g., Fig. 5A). The amount of expansion was determined by measuring the RF area before and after BMI in a subset of 15 neurons that had RFSs with discrete boundaries. The average expansion in these neurons was not significantly different from that seen in nonamputated animals, regardless of whether this was measured as absolute change (28.3 mm$^2$ vs. 40.8 mm$^2$, $t = 143$, $P = 0.15$) or relative change (426% of the original RF size vs. 286% in nonamputated; $t = 1.16$, $P = 0.25$).

Fewer neurons in the multi-regional groups underwent simple RF expansion, but the number was still substantial. The RFSs of 7 of 22 neurons in the MR-S group (32%) showed simple expansion under the influence of BMI. The expansion could occur around all of the subfields (Fig. 5B, left), but in some cells only one of the subfields became larger in the presence of BMI (Fig. 5B, right). Most ($n = 5$) of the MR-S neurons that showed simple expansion were observed in the animals studied at shorter intervals (<12 wk) after amputation. In the MR-C group, 5 of 22 neurons (23%) showed RF expansion under the influence of BMI (e.g., Fig. 5C). In contrast to the MR-S group, simple expansion of MR-C RFSs was seen only in animals in which recordings were made at longer intervals (>24 wk).

In 13 neurons (14%) BMI produced dramatic changes in RF properties that required them to be re-classified. In two of these neurons, BMI revealed a responsiveness to stimulation of several claws. This type of multimodal convergence is rarely seen in this part of normal raccoon cortex, but is common in more anterior areas (Johnson et al. 1982). In the remaining 11 neurons, the RFSs remained entirely on the glabrous skin after BMI administration. Seven of these neurons originally possessed MR-S fields, and BMI administration produced a RF expansion that included the entire skin region between the subfields. These RFSs in the presence of BMI would therefore be re-classified as MR-C fields (e.g., Fig. 6A). In four neurons in the restricted group, BMI revealed an expansion onto another region of the forepaw; they were therefore reclassified as multi-regional fields. Two of these neurons acquired a split RF (MR-S, e.g., Fig. 6B), while in the other two the new RF was continuous with the original RF (MR-C, e.g., Fig. 6C).

The third type of change in RF organization that was pro-
Reduced by BMI administration was the appearance of one or more lower threshold subfields within the RF. This occurred almost exclusively in neurons that were originally in the MR-C classification (9 of 10 cases). Three examples are shown in Fig. 7. These new subfields were easily distinguished because they had a considerably lower threshold than the pre-BMI RF and produced a greater response when stimulated with the original von Frey monofilament. In all cases the appearance of the subfields occurred within the boundaries of the predrug RF.

The RFs of 37 neurons (33%) were not altered by ejection of BMI. This included 22 cells with restricted RFs, 8 with MR-S RFs, and 7 with MR-C RFs. The three categories (restricted, MR-S, and MR-C) were not statistically different in terms of the proportions of cells that were affected by BMI ($\chi^2 = 1.52$, $P = 0.47$). Failure to see a change does not necessarily indicate that these neurons did not possess GABA$_A$ receptors, as other factors, such as their location with respect to the pipette tip, are likely responsible.

The incidence of these different RF changes at different intervals after amputation are presented in Table 1. Although the percentage of neurons whose RFs are altered by BMI appears to be increasing with time after amputation (from 50 to 71%), the small number of neurons at some intervals did not allow this to be verified statistically ($\chi^2 = 2.46$, $P = 0.4$).

EFFECTS OF GLUTAMATE ON RFs. In contrast to the situation in nonamputated animals, glutamate did change the RF properties...
of a small number of neurons ($n = 6$); four of these had a restricted RF (Fig. 8, A–D), one a MR-C field (Fig. 8E) and one a MR-S field (Fig. 8F). In each of these neurons, BMI also produced expansion. In four neurons, glutamate and BMI had similar effects with BMI producing greater expansion than glutamate. In the other two neurons the effects of the two drugs were quite different. One of these (Fig. 8B) had a restricted field on the distal 5th digit that expanded to cover the entire digit when BMI was administered. Glutamate, on the other hand, did not expand the field on the 5th digit, but revealed a second field on the distal 3rd digit. The other exception had a MR-S field (Fig. 8F). Glutamate produced proximal expansion of both subfields, whereas BMI affected only one of the subfields, producing an expansion in the distal direction on the 3rd digit.

In addition to these 6 neurons in which RF size was altered by glutamate, 12 neurons showed a clear decrease in threshold in only part of the RF. Finally, the RFs of 5 cells were made smaller by the administration of glutamate. This may be due to a greater activation on neighboring inhibitory neurons.

**DISCUSSION**

Peripheral denervation, as in the case of digit amputation, results in a reorganization within the CNS so that regions that have lost their dominant input gradually come to respond to new sensory inputs. Here we have demonstrated that local inhibitory connections, acting via GABA$_\text{A}$ receptors, play a role in the changes that occur during reorganization. While this complex process is often discussed in terms of simple alternative mechanisms, such as unmasking versus sprouting, or cortical versus subcortical loci, it likely involves multiple mechanisms occurring at several sites in the somatosensory pathways. The present study provides information about three important issues related to reorganization. One is the time course of reorganization and the progression of changes in RF structure; the second is the role of cortical inhibitory mechanisms in reorganization; and the third is the relative contribution of cortical and subcortical structures to the changes that are seen in the cortex.

**Time course**

It is useful to divide the process of reorganization into several, probably overlapping stages (Cusick et al. 1990; Turnbull and Rasmusson 1991). During the first phase, immediately after nerve injury or amputation, there is an unmasking of secondary inputs to the region that may be either actively suppressed by the dominant input or simply not obvious when the dominant input is present. Although the intent of studying the immediate effects of denervation is to observe the starting point of long-term plasticity, it is important to recognize that rapid changes in synaptic strength may be occurring during the course of these recording experiments that last for many hours (Mioche and Singer 1989). In the raccoon digit amputation model, this phase is characterized by the cells being largely nonresponsive or showing inhibition to stimulation of an off-focus digit with or without rebound excitation at the end of stimulation (Rasmusson and Turnbull 1983; Turnbull and Rasmusson 1991). During the first phase, immediately after nerve injury or amputation, there is an unmasking of secondary inputs to the region that may be either actively suppressed by the dominant input or simply not obvious when the dominant input is present. Although the intent of studying the immediate effects of denervation is to observe the starting point of long-term plasticity, it is important to recognize that rapid changes in synaptic strength may be occurring during the course of these recording experiments that last for many hours (Mioche and Singer 1989). In the raccoon digit amputation model, this phase is characterized by the cells being largely nonresponsive or showing inhibition to stimulation of an off-focus digit with or without rebound excitation at the end of stimulation (Rasmusson and Turnbull 1983; Turnbull and Rasmusson 1991).
musson 1990). In other models, nerve section may reveal minor connections that reflect either peripheral overlap of the sectioned and surviving nerves or overlap in the projection pathways. For example, median nerve section in the monkey reveals RFs on the dorsal surface of the hand in some parts of the affected cortex (Schroeder et al. 1997).

During the second phase of reorganization, the neurons in denervated cortex begin responding to stimulation of new regions of skin. In the raccoon this occurs over the course of several weeks and involves the disappearance of the strong inhibitory responses, an increase in spontaneous activity (Rasmusson et al. 1992) and the appearance of large RFs with poorly defined borders (Kelahan and Doetsch 1984; Rasmusson 1982). In both the visual and somatosensory system, it has been shown that denervation results in down-regulation of GABAergic markers (cf. next section). These changes are consistent with the model of plasticity that involves the ability to vary a “modification threshold” on the basis of overall cell activity (Bear et al. 1987; Bienenstock et al. 1982). For new synapses to be strengthened, the modification threshold would have to be lowered so that weak inputs would be sufficient to activate the postsynaptic neuron and consequently initiate plasticity.

Finally, once the periphery is capable of firing the deafferented neurons, the pattern and intensity of tactile input may selectively enhance some synapses and weaken others (Jenkins et al. 1990). The strengthening of some of the synaptic inputs and the reestablishment of inhibitory shaping could cause the large, diffuse RFs to become restricted. Several months after amputation in the raccoon, the new RFs are much smaller and are similar in size to those in nonamputated animals, although now on an adjacent digit (Kelahan and Doetsch 1984; Rasmusson 1982; Turnbull and Rasmusson 1991). This final stage in the progression has also been described as a “consolidation” of reorganization (Churchill et al. 1998), which emphasizes the similarities of these later changes to those underlying learning and memory.

The incidence of restricted and multi-regional RFs seen in the present study (Fig. 3) is consistent with this interpretation of the time course of reorganization. The proportion of neurons with restricted RFs increases dramatically between 8 and 11 wk. This is not, however, a direct test of the hypothesis that a given neuron first develops a multi-regional field that gradually becomes restricted to a small part of this large field. Conclusive evidence would require long-term recordings from the same cell throughout the reorganization process. While this technique has been applied in the study of both somatosensory and visual plasticity for periods of several hours (Katz et al. 1999; Mioche and Singer 1989), it is not practical to follow the same cell for many days or weeks. More convincing support for a continual progression from large, diffuse fields to small RFs comes from the finding of the present study that blocking GABA_A receptors on neurons with restricted RFs can reveal multi-regional fields and on neurons with split fields can unmask a responsive region between the subfields. These observations are also consistent with the idea that the spatial properties of RFs are determined by dynamic, self-organizing processes (Merzenich 1987).

Role of GABAergic inhibition in reorganization

The second major finding of this paper concerns the relationship between inhibition and the development of new RFs during reorganization (cf. reviews, Jones 1993, 2000). Following the discovery that visual deprivation (by enucleation) produces biochemical GABAergic changes in visual cortex (Hendry and Jones 1986), similar effects were shown in the somatosensory cortex. The density of immunoreactive labeling of the synthesizing enzyme, glutamate decarboxylase (GAD), is decreased by whisker trimming in rats and mice (Akhtar and Land 1991; Welker et al. 1989b) and by digit amputation in the

Fig. 8. Comparison of BMI and glutamate effects in 6 different neurons (A–F). Original RF is shown in black and expanded field in the presence of drug in gray.
removal (Lane et al. 1997, 1999; Stojic et al. 2000). Since this recovery of GABAergic mechanisms.

recovery of sharp borders may be another reflection of the lateral or surround inhibition is to enhance response differences as in normal cortex. A well-recognized function of reorganization proceeds. It is also worth noting that, early in

intervals after amputation, consistent with the idea that the functionality of the GABAergic synapses is recovering as expected that there would be an increase in spontaneous activity; this has been demonstrated electrophysiologically in rat barrel cortex after whisker trimming (Simonds and Land 1987) and in raccoon cortex 1 wk after digit amputation (Rasmusson et al. 1992). Both GAD immunoreactivity and GABA_A receptor binding have been shown to return to normal levels within several months after whisker destruction in mice (Skangiel-Kramska et al. 1994; Welker et al. 1989b) and GABAergic markers in the visual system recover after restoration of visual input (Hendry and Jones 1988). Similarly, training or excessive sensory stimulation produces an increase in GABAergic measures (Stiucinska et al. 1999; Tremere et al. 2001; Welker et al. 1989a). These results indicate that the level of afferent activity is regulating GABA expression.

Normally, GABAergic synapses in somatosensory cortex function to restrict RF size, as demonstrated by the fact that GABA_A receptor blockade produces RF expansion in control animals (Dykes et al. 1984; Hicks and Dykes 1983; Kyriazi et al. 1996). The present study shows that, in cortex that is undergoing reorganization, GABA_A receptor blockade can produce different types of RF expansion. One is similar to that seen in control animals, with a two- to threefold increase in RF area occurring around the original RF. In contrast to the control situation, however, some neurons in deafferented cortex showed an expansion onto another digit or the palm. In other words, in reorganized cortex, RF expansion did not always respect the boundaries between digits or between a digit and the palm. This is evidence of significant convergence, within the cortex, of information from a much larger region than seen before amputation. In addition, a qualitatively different type of expansion was observed in which a new field could be revealed due to plasticity at these lower relay nuclei. Furthermore, the issue of selectivity of inhibition can now be addressed by observing the effects of BMI with the effects of glutamate on the same neurons. If GABA receptor blockade has simply a nonselective effect, similar changes would be expected with BMI (via removal of inhibition) and glutamate (via excitation). In contrast to control animals (Tremere et al. 2001), glutamate did produce RF expansion in a small number of neurons in reorganized cortex (Fig. 8). Thus these neurons must receive subthreshold excitatory inputs that were only able to reach threshold when the neuron was depolarized by glutamate. However, most cells that were influenced by BMI were not affected by glutamate, and, in those neurons that were altered by both, the BMI-induced expansion was larger and/or spatially different from the glutamate-induced expansion. This again suggests that inputs from different parts of a neuron’s RF must be sufficiently separated on the dendritic tree that inhibitory synapses can suppress some of the inputs but not others.

An important question is whether the functional pruning of inputs is random for a particular neuron or whether some large-scale control mechanism regulates the overall organization of the deafferented cortex. A consistent finding in raccoon experiments (Kelahan and Doetsch 1984; Rasmusson 1982; Turnbull and Rasmusson 1991) has been that while the deafferented cortex is not randomly organized, it is far less organized than the original state, and there is less somatotopy than in primate models of cortical reorganization (Merzenich et al. 1983, 1984).

Subcortical versus cortical contributions to reorganization

The results of the present study also relate to the relative importance of subcortical and cortical plasticity. While the majority of studies on reorganization have looked only at the cortex, many studies have also found changes in the thalamus, dorsal column nuclei, and spinal cord (cf. recent review, Jones 2000). Obviously any changes that occur in these subcortical regions must contribute to the responses of cortical neurons. Digit amputation in the raccoon results in long-term reorganization in both the ventrobasal thalamus (Rasmusson 1996a,b) and the cuneate nucleus (Rasmusson and Northgrave 1997) that is similar to that seen in the cortex: the deafferented regions contain neurons that now respond to adjacent digits and the palm. Since the same denervation and RF mapping techniques were used in these studies on subcortical and cortical reorganization, it is likely that some of the cortical changes are due to plasticity at these lower relay nuclei. Furthermore, the short latencies of novel responses in the cuneate and thalamic
nuclei make it physically impossible for them to be occurring as a result of a relay through the reorganized neocortex.

The impression gained from this series of studies is that small, restricted RFs are less common in deafferented subcortical regions than in the cortex. A comparison of data from the cortex (present study) with these subcortical regions in terms of restricted, MR-S and MR-C RFs (Fig. 4) confirms this hypothesis. Most notable is the decrease in MR-S fields as one ascends from cuneate to thalamus to cortex with a corresponding increase in restricted RFs. While these data are taken from different animals and the times after amputation are different in each study, the cortical sample includes both short and long intervals, whereas the subcortical data are all from animals studied at least 2 mo after amputation. If only the long intervals were included in the cortical sample, the differences from the subcortical samples would be even greater. A similar difference between subcortical and cortical levels was seen in the neonatal forelimb amputation model (Lane et al. 1995; Stojic et al. 1998); these results were interpreted as a suppression within the cortex of some of the subcortical changes.

The microiontophoretic technique used here to block selectively a subpopulation of GABA receptors can contribute to this issue because the drug administered in this way can only affect local, in this case cortical, synapses. The observation that BMI administration within the cortex results in RF expansion is strong evidence that some of the thalamocortical inputs are indeed being suppressed within the cortex but does not, of course, tell us whether local or extrinsic GABAergic neurons are responsible. Similar studies at the subcortical relays would be useful in determining the relative importance of each level in shaping RF properties.

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