Role of NMDA Receptors in Adult Primate Cortical Somatosensory Plasticity

W.A. MYERS, J.D. CHURCHILL, N. MUJA, AND P.E. GARRAGHTY

1Program in Neural Science, Indiana University, Bloomington, Indiana 47405
2Department of Psychology, Indiana University, Bloomington, Indiana 47405

ABSTRACT

We have previously shown that most of the reorganization that typically follows median nerve transection in adult squirrel monkeys is dependent on normally functioning N-methyl-D-aspartate (NMDA) receptors. Here, we have evaluated two additional hypotheses: (1) is the immediate “unmasking” found after median nerve transection NMDA receptor-dependent? and (2) are NMDA receptors necessary for both the initiation and maintenance of the second phase of reorganizational changes, or only the former? To address these issues, we implanted osmotic minipumps subcutaneously to deliver an NMDA receptor antagonist (3-((6S)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid, CPP) systemically either before examining the immediate effects of median nerve transection, or after reorganization had presumably occurred. For the first set of experiments, NMDA receptor blockade was initiated either 1 or 4 weeks prior to multi-unit mapping in area 3b followed by transection of the median nerve and remapping of the cortex. In the second set of experiments, median nerve transection was followed 4 weeks later by either 1 or 4 weeks of NMDA receptor blockade prior to terminal mapping. We report that the immediate unmasking of new receptive fields after acute nerve injury is not prevented by NMDA receptor blockade; nor are completely reorganized cortical maps dependent upon NMDA receptors for their maintenance. We conclude that the immediate changes in cortical topography are not due to an NMDA receptor-dependent mechanism, but more likely due to release from tonic inhibition. Furthermore, the later phase of reorganization, as for some forms of hippocampal long-term potentiation (LTP), is dependent on normally functioning NMDA receptors for its initiation, but not for its maintenance. J. Comp. Neurol. 418:373–382, 2000.

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The topographic maps of adult primate somatosensory cortex retain remarkable flexibility in their ability to respond to altered patterns of nerve activity. Modified activity patterns can be derived from numerous sources such as nerve injury or exposure to novel experiences (e.g., Merzenich et al., 1983a; Sanes et al., 1988; Kaas et al., 1990; Jenkins et al., 1990; Garraghty and Kaas, 1991; Schwaber et al., 1993; for review see Garraghty et al., 1994). For example, Merzenich et al. (1983a) showed that partial deafferentation of the hand in adult monkeys led to topographical reorganization in somatosensory cortex. They reported that the deprived area of cortex became responsive primarily to areas of skin which surrounded the deafferented region. In a subsequent study, Merzenich et al. (1983b) reported that this reorganization occurs in two phases. During the initial phase, some new responses become evident immediately after the nerve cut. This immediate reorganization is followed by a more protracted second phase, lasting several weeks, during which the neurons throughout virtually all of the remaining deprived cortex become responsive to cutaneous stimulation of skin regions on the hand with intact innervation.

Two possible mechanisms for this cortical reorganization have been hypothesized: the sprouting of axon collaterals to create new connections, or changes in the efficacy of existing synapses allowing already present subthresh-
TABLE 1. Effect of Transection

<table>
<thead>
<tr>
<th>Animal</th>
<th>Acute effects of transaction</th>
<th>Chronic effects of transaction</th>
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<tbody>
<tr>
<td>92-46</td>
<td>Normal Map</td>
<td>X</td>
</tr>
<tr>
<td>92-13</td>
<td>Post-transection Remap</td>
<td>X</td>
</tr>
<tr>
<td>96-22</td>
<td>Normal Map (CPP for 25 days)</td>
<td>Map 57 days after transections</td>
</tr>
<tr>
<td></td>
<td>Post-transection Remap</td>
<td>(CPP1 days 30–55)</td>
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<tr>
<td>96-23</td>
<td>Normal Map (CPP for 25 days)</td>
<td>Map 57 days after transections</td>
</tr>
<tr>
<td></td>
<td>Post-transection Remap</td>
<td>(CPP days 32–57)</td>
</tr>
<tr>
<td>94-7</td>
<td>X</td>
<td>Map 36 days after transections</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(CPP days 31–36)</td>
</tr>
</tbody>
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1CPP, 3-(+)-2-carboxypiperazin-4-ylpropyl-1-phosphonic acid.

Old inputs to be expressed. Although both of these mechanisms could contribute to the ultimate “complete” reorganization, sprouting likely has no role in the immediate reorganization. Therefore, the first phase of reorganization is most likely due to changes in synaptic efficacy (e.g., “unmasking”; Merrill and Wall, 1978). The second phase of reorganization could be due to further changes in synaptic efficacy and/or to the sprouting of new connections. We have shown previously that a blockade of N-methyl-D-aspartate (NMDA) glutamate receptors prevents much of the reorganization that typically follows median nerve transection (Garraghty and Muja, 1996). This consequence of NMDA receptor blockade could stem from the interruption of a dynamic process of synaptic strengthening (e.g., Aniksztejn and Ben-Ari, 1995), or from a retardation in neurite outgrowth, as Sutula et al. (1996) have reported that blockade of these receptors impaired mossy fiber sprouting during kindling.

In the present experiments, we have extended our assessment of the role of NMDA receptors in injury-induced reorganization by addressing two questions: (1) are NMDA receptors involved in the immediate, “unmasking” phase of the injury-induced reorganization? If NMDA receptors are essential for the rapid expression of new receptive fields after nerve transection, then their blockade would prevent immediate reorganization; and (2) are NMDA receptors necessary for the maintenance of the completely reorganized cortical map once it is manifested? If the maintenance of new responses relies upon an NMDA receptor-dependent mechanism, then blockade of these receptors would result in the reversion of the newly formed map to its deprived state. A brief report of the findings has appeared in abstract form (Myers et al., 1996).

MATERIALS AND METHODS

In this study, median nerve transections were performed in five adult squirrel monkeys (Saimiri sciureus). In two of these monkeys, both median nerves were transected, with the second transection occurring during the terminal recording experiments. Table 1 summarizes the experimental manipulations that each animal received during this experiment. All surgical procedures were approved by the institutional animal care and use committee prior to implementation and followed those guidelines dictated in the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985).

Immediate effects of median nerve transection

One monkey was anesthetized with an intramuscular (i.m.) injection of a mixture of ketamine hydrochloride (25–30 mg/kg) and xylazine (0.5–1.0 mg/kg) and placed in a standard stereotaxic device for terminal electrophysiological mapping. Craniotomies and durotomies were performed to expose somatosensory cortex. A video image of the exposed brain was captured and displayed on a computer screen (N.I.H. Image software) and electrode penetration sites were recorded directly on the digitized image by using the mouse. Low impedance (0.9–1.2 MΩ) at 1 kHz) tungsten microelectrodes were used to record activity from small clusters of neurons at recording sites at depths that corresponded to the middle layers of cortex and were typically separated by 150–300 μm while delivering cutaneous stimulation with fine probes. Neural activity was conventionally amplified and displayed. Cutaneous receptive fields were defined as regions where gently touching the skin or moving hairs markedly increased neural activity and was subsequently outlined on drawings of hand, forearm, and face taken from photographs. Any recording site that could not be activated by cutaneous stimulation was categorized as noncutaneous (e.g., movement-related) or unresponsive. After mapping of the exposed somatosensory cortex was completed, a skin incision was made along the midline of the ventral side of the contralateral forearm. The median nerve was located by blunt dissection and separated from the surrounding tissue and vasculature under a dissecting microscope. The nerve was then cut about midway between the elbow and wrist. The cortex was then remapped to examine the acute effects of nerve transection. This part of the experiment served merely to replicate the original report of Merzenich et al. (1983b) on the immediate effects of median nerve transection on cortical topography.

Survival nerve transections

Three monkeys underwent median nerve transection, after which they survived for approximately 1 month before terminal electrophysiological mapping in the cortex. These surgeries were performed under aseptic conditions in a sterile surgical suite reserved solely for survival procedures (see Churchill et al., 1998b). The monkeys were anesthetized with a combination of ketamine (25–30 mg/kg) and xylazine (0.5–1.0 mg/kg). The ventral surface of the forearm was shaved and scrubbed alternately with Betadine and alcohol. The animal was then moved to the surgery where it was covered with a fenestrated drape that left only the ventral forearm exposed. The transection procedure itself was identical to that described above except that the epineural sheath of the transected proximal stump was slid up over the nerve 0.5–1.0 cm to permit evulsion of the exposed nerve. The empty epineural sheath was then reextended, folded back upon itself and ligated. The nerve stumps were repositioned and the skin incision closed with sutures. Postsurgically, the monkeys were treated with penicillin, Dopram, and dexamethasone. The evulsion/ligation was intended to prevent regeneration back into the deafferented median nerve territory of the hand.
Osmotic minipump implantation
In four monkeys, osmotic minipumps (Alzet model 2001 or 2004) were implanted subcutaneously for the delivery of 3-((+-)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), a potent and highly specific competitive NMDA receptor antagonist (Davies et al., 1986; Harris et al., 1986; Olverman et al., 1986; Ferkany et al., 1989). Minipumps were filled with either 10 or 40 mg CPP dissolved in 200μl saline. The lifetime of the minipump was either 1 or 4 weeks yielding an infusion rate of 59.5 μg of CPP/hour. The implantations were performed under sterile conditions. The animals were anesthetized with a mixture of ketamine (25–30 mg/kg) and xylazine (0.5–1.0 mg/kg). The back was shaved and scrubbed alternatively with Betadine and alcohol, and the animal moved into the surgery room where it was draped leaving only the rostral midline portion of the back exposed. An incision was made between the scapulae, and a subcutaneous pocket was formed by blunt dissection. The minipump was then placed in the pocket, and the wound was sutured.

Role of NMDA receptors in immediate unmasking
In three monkeys (including two of the above), the acute effects of median nerve transection on cortical map topography were evaluated after 7–27 days (7, 25, 27 days) of CPP administration. In these experiments, the hand area of cortex was mapped, the median nerve was transected, and the cortex was then remapped. This experimental condition also permitted the assessment of any effect that NMDA receptor blockade might have on the normal topographic map of the hand in area 3b.

Role of NMDA receptors in maintaining reorganization
Osmotic minipumps were implanted subcutaneously in three monkeys 30–32 days (30, 31, 32 days) after median nerve transection. Approximately 1 to 4 weeks after the initiation of CPP treatment (5, 25, 25 days), the subjects were reanesthetized and placed in a standard stereotaxic device for terminal electrophysiological mapping as described above.

Delineation of area 3b
For the purposes of this experiment, all maps were defined by using functional criteria. Recordings were concentrated in area 3b (i.e., S-I “proper”; Merzenich et al., 1978). The rostral border of area 3b with area 3a was functionally defined by an abrupt change in modality. Neurons at recording sites in area 3b (mean; 146: range; 75–182) responded to light cutaneous stimulation, whereas those in area 3a required much brisker taps to the skin, deep pressure, or joint manipulation to elicit a response. In normal monkeys, the transition from area 3b to the more caudal area 1 is characterized by a reversal in receptive field progression (e.g., Merzenich et al., 1978; Sur et al., 1982) and by the generally larger fields typically found in area 1 (e.g., Iwamura et al., 1983; Sur et al., 1985). In monkeys with systemic NMDA receptor blockade, however, this caudal border of area 3b with area 1 is even more distinctive, as neurons in area 1 are much less responsive to cutaneous stimulation (Garraghty and Muja, 1996), much like neurons in area 2 of normal monkeys (Pons et al., 1985). Following electrophysiological recordings, all monkeys were deeply anesthetized with urethane, perfused transcardially and prepared for either immunocytochemistry or autoradiographic labeling.

RESULTS
Mapping data are reported for the somatosensory cortices of monkeys in three conditions. In the first condition (n = 1), we simply observed the topographic changes present after acute median nerve transection in the absence of NMDA receptor blockade (cf., Merzenich et al., 1983b). In the second condition (n = 3), we examined the effects of acute median nerve transection on the cortical map of monkeys that had been treated for a specified period of time with the NMDA receptor antagonist, CPP. Because the cortex was mapped before the nerve transection, these subjects also permitted the opportunity to assess whether there were any effects of NMDA receptor blockade on normal topography. In the final condition (n = 3), we studied the consequences of NMDA receptor blockade after reorganization had presumably occurred (that is, approximately 1 month after median nerve transection). In all monkeys that were subject to NMDA receptor blockade with CPP, we observed a reduction in the responsiveness of neurons in area 1 to cutaneous stimulation. These findings are consistent with those that we have previously reported using the same NMDA receptor blocker and anesthetic (Garraghty and Muja, 1996).

Effects of combining acute median nerve transection with concurrent blockade of NMDA receptors
The cortical hand maps of monkeys that had undergone a combined acute median nerve transection with concurrent NMDA receptor blockade were not significantly different from the cortical maps of the control monkey that had only undergone an acute median nerve transection. The top half of Figure 1 depicts the cortical organization typically observed in normal monkeys, whereas the bottom half of this figure illustrates the effects of acute median nerve transection on cortical topography. Although much of the deprived cortex remained unresponsive to cutaneous stimulation, some new responses (primarily from the dorsal surface of the hand) were apparent. This result simply replicates in our hands the early report on the immediate effects of median nerve transection by Merzenich et al. (1983b).

The top portions of Figures 2–4 all show the representation of the hand in area 3b before nerve injury, whereas the bottom halves of these figures depict the effects of acute median nerve transection with concurrent NMDA receptor blockade. The animals in Figures 2–4 had been treated with CPP for 25, 27, or 7 days, respectively. In comparing the top portions of these figures with the top half of Figure 1, no obvious effects of NMDA receptor blockade on normal topography are evident. Likewise, the postinjury maps represented in the bottom parts of Figures 2–4 are not obviously different from the map presented in the bottom part of Figure 1. There are clear zones of cortex responsive to new skin sites, with most of the new receptive fields on the dorsum of the hand. As for the case illustrated in Figure 1, much of the deprived cortex remained unresponsive.
Effects of combining chronic median nerve transection with delayed blockade of NMDA receptors

The data represented in Figure 5 are from a monkey that survived median nerve transection for 57 days. NMDA receptors were blocked for the last 27 of those days. This map reflects the apparently complete reorganization that occurs within 3–4 weeks following median nerve transection (Merzenich et al., 1983b; Garraghty and Muja, 1996). As in those previous studies, most of the reorganization within the deprived cortex is accomplished by an expansion in the representation of the dorsal skin surface. The institution of NMDA receptor blockade had no detectable effect on the already reorganized maps. We suggest that the reorganization patterns shown in the Figures 5 and 6 are not

Fig. 1. **Top:** Summary map of the hand region in area 3b from a normal (non-N-methyl-D-aspartate [NMDA] receptor blocked) monkey. **Bottom:** Summary map from the same monkey immediately after acute median nerve transection. Much of the deprived cortex remained unresponsive to cutaneous stimulation. The partial reorganization appears to be due mainly to an immediate “unmasking” of the dorsal representation of digits 1 and 2. D1–D5, digits 1–5; P1–P4, pads 1–4; Pt, thenar pad; Ph, hypothenar pad; Pi, insular pad; L, lateral; C, caudal.

Fig. 2. **Top:** Summary map from monkey that had undergone N-methyl-D-aspartate (NMDA) receptor blockade for 25 days. **Bottom:** Summary map from the same monkey immediately after median nerve transection. The NMDA receptor blockade did not prevent the immediate “unmasking” of the dorsal representation. Noncutaneous regions of the cortex include area 3a, area 1, and the nonreorganized region of area 3b. DR Hand, dorsal radial hand; DU Hand, dorsal ulnar hand; dPh, distal hypothenar pad. FA, forearm. Other abbreviations as in Figure 1.
significantly different from the typical reorganization pattern found after median nerve transection (Merzenich et al., 1983a,b; Garraghty and Muja, 1996). Thus, NMDA receptors blockade did not cause a reorganized map to revert back to a relatively unresponsive state.

Finally, the top half of Figure 7 illustrates receptive fields and receptive field progressions in area 3b in an anesthetized monkey in the absence of NMDA receptor blockade. The bottom half of Figure 7 presents comparable data from a monkey in which NMDA receptors had been blocked for 25 days prior to recording. Receptive field sizes and progressions, and the overall topography (top halves of Figs. 1–4) of the area 3b hand map are indistinguishable with or without NMDA receptor blockade.

DISCUSSION

In the present experiments, we have evaluated the effects of NMDA receptor blockade on normal cortical somatotopy, the role of these receptors in the immediate unmasking of new receptive fields that follows peripheral nerve injury, and the contribution of these receptors to the maintenance of reorganized cortical maps after peripheral nerve injury. There are four basic findings: (1) the blockade of NMDA receptors has no apparent effect on the topography of the cortical map in cytoarchitectonic area 3b or on the responsiveness of area 3b neurons; (2) in contrast, in both intact and nerve-injured monkeys, the responsiveness of neurons in cytoarchitectonic area 1 is profoundly depressed in CPP-treated monkeys; (3) the immediate unmasking phase of injury-induced reorganization proceeds independently of NMDA receptor blockade; and (4) NMDA receptor activation is not needed to maintain the fully reorganized cortical map present 3–4 weeks after peripheral nerve damage.
Garraghty and Muja (1996) reported that initiating a systemic blockade of NMDA receptors at the time of median nerve transection prevented much of the reorganization that typically ensues within 3–4 weeks in monkeys without NMDA receptor blockade (Merzenich et al., 1983b). We interpreted that result as being due to a specific interruption of an NMDA receptor-dependent mechanism of plasticity, although the use of a systemic blockade prevents us from determining whether that mechanism of plasticity was cortical, subcortical, or both. Recently, Buonomano and Merzenich (1998) suggested an alternative interpretation of these data in that the blockade may have functionally inactivated the cortex, thereby masking the reorganization. Data from the present experiments demonstrate that the blockade of NMDA receptors does not functionally inactivate nondeprived area 3b; the maps of the hand in animals in which NMDA receptors were blocked prior to recording were indistinguishable from the maps recorded in blocked monkeys. Furthermore, the maps taken from animals in which NMDA receptor blockade was initiated after reorganization had occurred were not different from those reported previously for animals not chronically treated with an NMDA blocking agent (Merzenich et al., 1983a,b). Buonomano and Merzenich (1998) further suggested that the “blocked” reorganization might be mediated by NMDA receptors such that a blockade of the receptors at the time of mapping might mask the reorganization rather than prevent it. It is the case that the CPP-mediated receptor blockade was in effect when the mapping experiments were conducted in those experiments (Garraghty and Muja, 1996), but given that the original reports of Merzenich et al. (1983a,b), and other reports of complete reorganization (e.g., Garraghty and Kaas, 1991) were based on recordings in ketamine-anesthetized monkeys, it seems unlikely that the CPP was masking reorganization. Were that the case, it would seem that ketamine would have a similar effect, unless neurons in the reorganized cortical map were differentially sensitive to the effects of competitive (CPP) vs. noncompetitive (ketamine) NMDA receptor-blocking agents, or unless the function of NMDA receptors are different in the presence of combined competitive and noncompetitive blockade vs. either of those alone.

Area 1 and prolonged competitive blockade of NMDA receptors

From the first publication of separate representations of the contralateral body surface in cytoarchitectonic areas 3b and 1 of owl monkey somatosensory cortex (Merzenich et al., 1978), separate maps in areas 3b and 1 have been routinely reported in a number of primate species (macaque: Nelson et al., 1980; squirrel monkeys: Sur et al., 1982; cebus monkeys: Fellemman et al., 1983). Importantly for the present results, all of those data were collected in ketamine-anesthetized preparations, and area 1 neurons were found to be very responsive to cutaneous stimulation. Similarly, that data reported in Figure 1 were col-
Fig. 7. **Top:** An illustration of receptive fields and receptive field progressions in area 3b in an anesthetized monkey in the absence of N-methyl-D-aspartate (NMDA) receptor blockade. **Bottom:** An illustration of comparable data from a monkey in which NMDA receptors had been blocked for 25 days prior to recording. Receptive field sizes and progressions are indistinguishable. Dots represent recording sites; R, rostral; M, medial.
lected in a ketamine-anesthetized monkey with no prior exposure to CPP, and we found the 3b/1 border to be characterized by the classic reversal in receptive field progressions typically present. On the other hand, in monkeys chronically exposed to CPP prior to recording with ketamine anesthesia, we find a marked reduction in cutaneous responsiveness of area 1 neurons whether or not the median nerve has been transected (present results; Garraghty and Muja, 1996). Although this observation has been robust in our hands, we have no explanation for it. It has been suggested that NMDA receptors may be preferentially involved in corticocortical processing (Thompson, 1986; Shirokawa et al., 1989; Larson-Prior et al., 1991; Nicoll et al., 1992), whereas AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors are suggested to be preferentially involved in thalamocortical and callosal processing (Tsumoto et al., 1986; Tsumoto, 1990; Armstrong-James et al., 1993), and it is possible that area 1 responsiveness in squirrel monkeys is due in large part to inputs from area 3b (Garraghty et al., 1990). If the relative lack of cutaneous activation of area 1 in the CPP-treated animals is due to a functional interruption of corticocortical input from area 3b, it remains uncertain whether this interruption is due to the mere presence of a competitive NMDA blocker, chronic treatment with a competitive blocker, or a synergistic or synergic effect of combining competitive and noncompetitive NMDA receptor blockers.

NMDA receptors and immediate unmasking of new receptive fields after nerve injury

The topographic reorganization of somatosensory cortex in adult mammals is a complex process that involves multiple stages (e.g., Merzenich et al., 1983b; Churchill et al., 1998b). In the first phase, some new responses become apparent immediately following nerve transection (Merzenich et al., 1983b). In the second phase, which occurs over the next few days/weeks/months, the remainder of the deprived cortex becomes responsive (Merzenich et al., 1983b; Cusick et al., 1990). We have previously shown that activation of NMDA receptors is essential for at least the second of these phases to proceed, in that a blockade of these receptors initiated at the same time as nerve transection prevented much of the deprived cortex from gaining new responses (Garraghty and Muja, 1996). The present results demonstrate that the unmasking of latent inputs (see Garraghty and Sur, 1990; Schroeder et al., 1995, 1997), the first step of cortical reorganization, is not an NMDA receptor-dependent process. Immediately following nerve transection, the somatotopic maps of monkeys that had been subjected to NMDA receptor blockade 1–4 weeks prior to median nerve transection were indistinguishable from the one non-drug-treated animal in this study and those previously reported (Merzenich et al., 1983a,b). This finding is consistent with previous research that suggests unmasking may result from a reduction inafferent-driven inhibition. For example, Alloway and Burton (1991) have shown that the receptive fields of cortical neurons can increase in size by much as an order of magnitude after treatment with the GABA_A receptor antagonist bicuculline. Thus, relaxed cortical inhibition could permit the immediate expression of new receptive fields in deprived cortex. Consistent with this idea, Garraghty et al. (1991) report signifi-
memory, its transient nature renders it an unlikely candidate for a mechanism for long-term memory storage. It seems far more likely that LTP’s role in the hippocampus is related to the formation of memories, which are then stored in other areas of the brain. The synaptic changes seen in cortical reorganization, conversely, are of a much more permanent nature. Therefore, the manner by which cortical changes occur during reorganization may be more akin to those mechanisms used during the storage of memories than to those utilized during the encoding of memories.

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