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Glutamate receptor blockade at cortical synapses disrupts development of thalamocortical and columnar organization in somatosensory cortex

(activity-dependent mechanisms/axon remodeling/*N*-methyl-D-aspartate receptor/synaptic competition/topographic maps)

KEVIN FOX*†, BRADLEY L. SCHLAGGAR‡, STANISLAW GLAZEWSKI*, AND DENNIS D. M. O'LEARY‡

*Department of Physiology, University of Minnesota, Minneapolis, MN 55455; and ‡Molecular Neurobiology Laboratory, 10010 North Torrey Pines Road, The Salk Institute, La Jolla, CA 92037

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ABSTRACT **The segregation of thalamocortical inputs into eye-specific stripes in the developing cat or monkey visual cortex is prevented by manipulations that perturb or abolish neural activity in the visual pathway. Such findings show that proper development of the functional organization of visual cortex is dependent on normal patterns of neural activity. The generalization of this conclusion to other sensory cortices has been questioned by findings that the segregation of thalamocortical afferents into a somatotopic barrel pattern in developing rodent primary somatosensory cortex (S1) is not prevented by activity blockade. We show that a temporary block of *N*-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors in rat S1 during the critical period for barrel development disrupts the topographic refinement of thalamocortical connectivity and columnar organization. These effects are evident well after the blockade is ineffective and thus may be permanent. Our findings show that neural activity and specifically the activation of postsynaptic cortical neurons has a prominent role in establishing the primary sensory map in S1, as well as the topographic organization of higher order synaptic connections.**

The functional specialization of a primary cortical area is largely defined by its thalamocortical input. An important feature of thalamocortical afferents is their organization into a topographic map of the peripheral receptor sheet. In the primary somatosensory cortex (S1), this organization results in a complete map of the body surface relayed to the S1 by thalamic afferents from the ventroposterior nucleus (VP). A prominent example of this somatotopic organization is the barrel field of rodent S1, which contains in layer IV a representation of the animal's facial vibrissae in the form of an orderly array of discrete functional and anatomical specializations termed barrels (1). Each barrel corresponds topologically and functionally to a particular vibrissa and is composed of an aggregation of layer IV neurons coincident with a cluster of thalamic afferents from the medial division of VP (VPm).

In normal adult rodents, VPm afferents corresponding to an individual vibrissa are restricted to a single barrel and the zone immediately surrounding it (2, 3), as are short-latency responses for each vibrissa, which are believed to reflect monosynaptic input from VPm thalamus (4, 5). At birth, VPm thalamocortical afferents show no vibrissa-related patterning in S1, but gradually cluster into an adult-like pattern over the first few postnatal days (6, 7). Similarly, thalamocortical afferents from the lateral geniculate nucleus to cat visual cortex (V1) show no patterning at birth, but gradually segregate into eye-specific stripes in layer IV over the first few postnatal weeks (8). Activity in the primary visual pathway is necessary for the segregation of geniculocortical afferents into eye-specific stripes (9). By analogy, activity in the somatosensory pathway could be necessary for clustering of VPm afferents into a vibrissae-related, somatotopic pattern.

The idea that activity influences formation of the barrel field is plausible because neuronal activity can occur in barrel cortex during barrel formation [postnatal days 0 to 4 (P0–4)]; thalamocortical afferents are glutamatergic (10), excitatory glutamatergic synapses are functional at birth (11), and peripherally evoked activity occurs in SI on P0 (12). However, blocking vibrissae-related inputs to the developing barrel field or blocking activity in barrel cortex directly has no obvious effect on the formation of a normal barrel pattern when assayed using histochemical stains (13–15), a result that implies little or no role for activity in development of the barrel field. In the present study, we have reexamined this issue by blocking cortical activity during the critical period for development of the barrel pattern (16) and assaying the receptive field properties of individual cells in the barrel cortex several weeks later. When studied in this way, several activity dependent processes become apparent, implying some role for activity in the topographic organization of thalamocortical afferents in barrel cortex.

MATERIALS AND METHODS

2-Amino-5-Phosphonovaleric Acid (APV) Treatment. Rat pups obtained from time-pregnant females (Harlan Sprague–Dawley) received implants of APV-loaded Elvax (DuPont) within 6 hr of birth as described (13). Loaded Elvax was prepared by producing a 2% emulsion of aqueous D- or L-APV (1 M; Tocris Neuramin, Bristol, U.K.) in Elvax dissolved in methylene chloride and cured in a metal mold on dry ice (17). The solvent was evaporated for 5 days at 4°C and 100-μm thick sections were cut on a freezing microtome. Pups were anesthetized by hypothermia and 3 mm by 2 mm pieces of Elvax were implanted subdurally over the posteromedial barrel subfield (PMBSF), which is the representation of the large facial vibrissae in S1. PMBSF measures $\approx$1.2 mm square on P0, and $\approx$2 mm square on P3, by which time VPm afferents have clustered into a mature appearing pattern (6, 7). Thus, during the critical period for clustering of VPm afferents, the Elvax covered the entire PMBSF. By postnatal weeks 3 to 6, when the recording experiments were done, the Elvax still covered most of PMBSF.

Tests with [^{3}H]-APV (Tocris Neuramin) indicated that the Elvax released APV at a rate of $\approx$100 pM mm^{-2} per day for

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Abbreviations: S1, primary somatosensory cortex; VP, ventroposterior nucleus; VPm, medial division of VP; V1, visual cortex; APV, 2-amino-5-phosphonovaleric acid; PMBSF, posteromedial barrel subfield; NMDA, *N*-methyl-D-aspartate; POm, posterior medial.

†To whom reprint requests should be addressed at: Molecular and Medical Bioscience, University of Wales, Cardiff, Museum Avenue, P.O. Box 911, Cardiff, CF1 34S, United Kingdom. e-mail: foxkd@cardiff.ac.uk.

more than 3 months *in vitro* at room temperature; on average, the release rate was ≈50% higher at 37°C. Tritium was detected using standard liquid scintillation counting techniques using BCS scintillant (Amersham) and a Packard model 4530 counter. Control experiments showed that Elvax released APV for less time *in vivo*. The tritiated drug could be detected in S1 up to, but not beyond, 14 days postimplantation. Our previous tests demonstrated the effectiveness of the APV treatment by recording S1 responses to iontophoretically applied *N*-methyl-D-aspartate (NMDA) and AMPA (13). The amount of D-APV released from a fresh Elvax implant blocked NMDA and AMPA glutamate receptors to a depth of 700 μm within 4 hr of implantation in adult rats. Elvax that had been implanted over S1 for up to 8 days still released amounts of D-APV sufficient to block all glutamate receptors, whereas Elvax that had been implanted for 21 days no longer released effective amounts of D-APV (13). In the present study, receptive field properties were studied in rats at 21–44 days of age, well beyond the period of effective APV release. At these times, iontophoretic electrodes were lowered into S1 before or immediately after removing the Elvax implants and normal responses to iontophoretically applied NMDA were found for cells even in the most superficial locations (i.e., nearest to the Elvax implant), again indicating that the D-APV was no longer being released at concentrations sufficient to block NMDA or AMPA glutamate receptors.

We also tested whether the APV could be acting at the level of the VP. VP was dissected out of the thalamus by shaving 1 mm off the caudal and rostral aspects of the thalamus, 1 mm off the lateral aspect to remove the reticular nucleus, and 1 mm off the medial aspect to remove midline nuclei. The ventral half (hypothalamus) was discarded leaving an ≈2-mm cube of tissue. Several such cubes of VP tissue (usually four) were collected, frozen, and sonicated to emulsify it before treatment with a tissue solublizer (Soluene 350; Packard). Liquid scintillant was added and samples were counted as described above. Tritiated D-APV could not be detected in VP at any age, implying that D-APV did not effect thalamocortical afferents at the level of the thalamus.

Stimulation, Recording, and Histology. All methods for recording from neurons and stimulating vibrissae were the same as described (4). If the Elvax implant obscured the caudal C and D rows of barrels, it was removed before recording; if not, recordings were made close to the edge of the implant (within 100 μm).

The analysis presented here is for all vibrissae within the receptive fields evoking a response of at least 0.5 spikes per stimulus. Receptive fields were characterized for 165 layer II, III, and IV cells from 10 D-APV-treated rats, 160 from 9 L-APV-treated rats, 64 from 3 saline-treated rats, and 74 from 4 age-matched untreated rats. Mean receptive field size is calculated as the total number of vibrissae (with response magnitude of at least 0.5 spikes per stimulus) for all cells recorded, divided by the number of cells recorded. The fraction of short-latency responses making up the receptive field is calculated as the total number of vibrissae evoking short-latency responses divided by the total number of cells in the sample.

The location of each recording penetration was marked with a small focal lesion. Layer IV cells were classified as barrel cells if the marker lesion was located within 50 μm of the dense cytochrome oxidase (CO) staining (18); cells more than 50 μm from the dense CO staining were classified as septal cells. The principal input for a barrel cell was the vibrissa topographically related to the barrel in which the cell was located. For a septal cell, the nearest barrel defined the principal vibrissa. All other inputs were termed "inappropriate." A normal barrel field pattern was apparent from CO stained sections (Fig. 1), except in cases where the Elvax had been implanted poorly and damaged S1. Such cases were discarded.

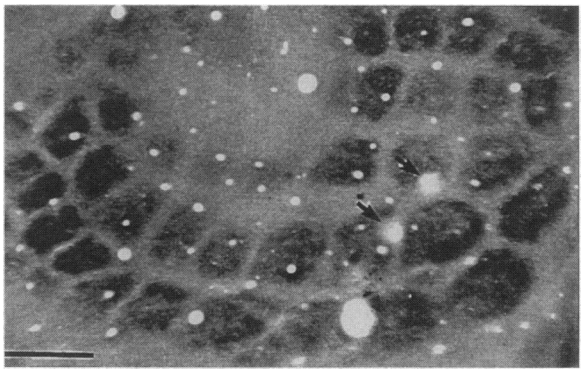

FIG. 1. The lack of effect of APV on formation of the barrel field. A section through layer IV of the barrel field is shown for an animal treated with D-APV-loaded Elvax from P0. The section is stained for cytochrome oxidase. Orientation: anterior left, lateral top. Two focal lesions mark the position of recording tracks in barrels D2 and C2 (arrows). (Bar = 500 μm.)

Bicuculline Iontophoresis. Vibrissae in and immediately outside the receptive field were tested before, during, and after iontophoresis of bicuculline [bicuculline methyliodide; Research Biochemicals (Natick, MA); 5–20 nA ejecting current]. The modal latency for each response was derived from latency histogram analysis. The modal latency was considered valid if the latency histogram showed a clear peak related to the poststimulus time histogram and the modal bin height was significantly different from the mean ($P < 0.05$, based on a Poisson distribution for bin height). The latter was particularly important as bicuculline significantly increased spontaneous activity. A change in modal latency was considered significant if it differed from the control by one latency category, and recovered to control values after bicuculline had been retained for at least 10 min. Latency categories were 5–10 msec, 11–15 msec, 16–20 msec, 21–30 msec, and >30 msec as described (4, 5).

RESULTS

Effect of APV on Clustering of VPm Afferents into a Vibrissae-Related Pattern. The effect of activity block on development of the barrel pattern was assessed using CO histochemistry at 3 to 6 weeks of postnatal age in the same animals for which the functional analyses described below were performed. Even in the D-APV-treated rats, the vibrissae-related pattern of CO staining appears normal (Fig. 1). This result corroborates our findings using acetylcholinesterase histochemistry, an early transient marker for VP afferents (13). Thus, even though D-APV treatment blocks the ability of VPm afferents to activate their postsynaptic target cells in S1, it does not prevent their clustering into a vibrissae-related pattern.

Effect of APV on Development of Topographically Ordered Synaptic Inputs to Layer IV. To determine the distribution of functional thalamic inputs in the barrel field of D-APV-treated animals, we analyzed in 3- to 6-week-old rats the spatial distribution of short-latency (≤10 msec) responses of individual layer IV neurons to stimulation of each vibrissa composing their receptive field. Short-latency responses are thought to represent direct monosynaptic excitation from the thalamus. In normal adult animals, short-latency responses for each whisker are restricted to a single barrel and its immediately surrounding septal zone (Fig. 2) (4, 5) as are the VPm fibers corresponding to an individual vibrissa (2, 3). However, in animals reared with D-APV treatment, short-latency responses to an individual whisker were not restricted to the single appropriate barrel (Fig. 2) but could be found in all surrounding barrels up to a distance of at least one barrel width away. This spatial blurring of topographic input was not observed in animals treated with the inactive isomer L-APV (Fig. 2). These results suggest that in D-APV-treated S1, VPm inputs do not

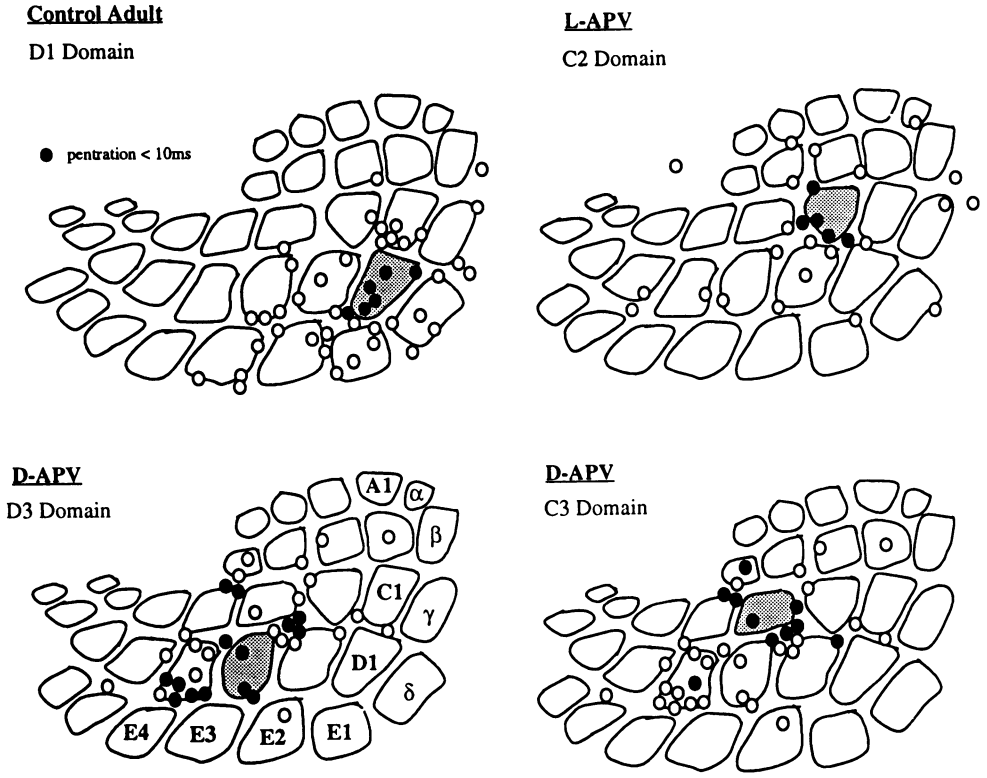

FIG. 2. The effect of APV on the distribution of short-latency inputs within the rat barrel field. (*Top Left*) The locations of penetrations containing layer IV cells responding within 10 msec (short-latency responses) to stimulation of the D1 vibrissa are represented as solid circles and penetrations without cells responding within this period by open circles. Only neurons within the D1 barrel (shaded) exhibit short-latency responses to D1 stimulation in control animals. (*Top Right*) The short-latency responses to C2 vibrissa stimulation are similarly confined to the area of the C2 barrel (shaded) in animals reared with L-APV implants above the barrel cortex. (*Lower Left*) In D-APV-treated cortex, short-latency responses are found outside the boundaries of the appropriate barrel (shaded area), shown here for D3 vibrissa stimulation and for C3 vibrissa stimulation (*Lower Right*). The nomenclature for barrel row and number is given in the lower left diagram (B1 designation omitted to prevent obscuring a recording site).

establish the one-to-one functional relationship between vibrissae and barrels seen in normal S1.

To quantify these results, we analyzed the number of short-latency responses exhibited by individual layer IV cells in D-APV-treated animals. Layer IV cells with short-latency responses located in barrels showed a significantly higher proportion of inappropriate short-latency responses (39%, $n = 54$), than in age-matched untreated animals (14%, $n = 44$) and normally reared adults (2%, $n = 36$) (4). This difference between barrel cells in D-APV-treated animals and age-matched untreated animals is highly significant ($p < 0.01$, χ^2 test, df = 2). Layer IV cells with short-latency responses in septal areas showed an even higher proportion of inappropriate short-latency responses with 67% ($n = 12$) receiving at least one extra short-latency response from an inappropriate vibrissa, compared with 10% ($n = 9$) for age-matched untreated animals and 13% ($n = 36$) for normally reared adults (4). These differences between septal cells in D-APV-treated animals and untreated controls are also highly significant ($P < 0.01$, χ^2 test, df = 4). Furthermore, 33% of septal cells in D-APV-treated animals exhibited a short-latency response to two or more inappropriate vibrissae, a condition not encountered at all in untreated controls (0%, $n = 19$). The receptive fields of some cells in D-APV-treated animals were composed of short-latency responses from as many as nine vibrissae (Fig. 3). These results demonstrate that individual layer IV neurons in the barrel field receive a greater functional convergence of short-latency vibrissae input in D-APV-treated cortex than in normal cortex.

A more detailed analysis revealed that barrel cells located in the sides of the barrels showed a higher frequency of inappropriate short-latency responses (70%) than did cells in the centers of the barrels (25%). Cells located in the barrel sides showed a similar rate of inappropriate short-latency responses as septal cells. However, cells in the barrel sides were not mistaken for septal cells as they were all clearly located within the border of the cytochrome oxidase staining of the barrel, which if anything underestimates the size of the barrel (2). These results imply that a spatial difference in frequency of somatotopic errors exists with minima at the centers of the barrels and maxima at the boundaries between neighboring barrels.

Effect of APV on Development of the Receptive Field Properties in Layer IV. The proportion of the receptive field occupied by short-latency responses was much greater for D-APV-treated animals than for control animals. The receptive fields of septal cells were of similar size across D-APV, L-APV, saline, and age-matched untreated animals (mean receptive field sizes of 3.2, 2.6, 3.0, and 2.5, respectively), though slightly smaller than found in adults for both barrel and septal areas (mean receptive field size, 3.9) (4). However, short-latency responses made up a higher proportion of the receptive field in D-APV-treated animals (47.5%) compared with age-matched untreated animals (14%). This implies that VPm afferents converge more highly on cells in D-APV-treated cortex.

Animals treated with L-APV in S1 also showed a higher than normal incidence of short-latency responses in the septal regions with ≈35% of septal cells ($n = 17$) showing inappropriate fast input. This is a higher incidence than found in normal animals of this age or in saline-treated controls, implying that L-APV does have some effect on development, possibly by acting on metabotropic glutamate receptors (19). However, the effect of L-APV is less than that of D-APV in several respects: no more than two inappropriate inputs were found for any cell, fewer inappropriate inputs were found overall, and the spatial distribution of inappropriate inputs was more restricted (Fig. 2).

Effect of Bicuculline on Receptive Field Properties in Layer IV of S1 Barrelfield. One possible explanation for the effect of D-APV treatment is that inappropriate VPm connections are normally present in layer IV of untreated animals but are masked by inhibitory connections. We tested this idea in normally reared 4-to 6-week-old rats by iontophoresis of bicuculline, a $GABA_A$ antagonist, in S1 to unmask subthreshold vibrissae responses of layer IV cells. Bicuculline treatment increased the response duration, response magnitude, receptive field size, and usually the spontaneous firing rate of layer IV cells (277 vibrissae tested for 42 cells). In 46 cases, bicuculline unmasked new vibrissae in the receptive field (Fig.

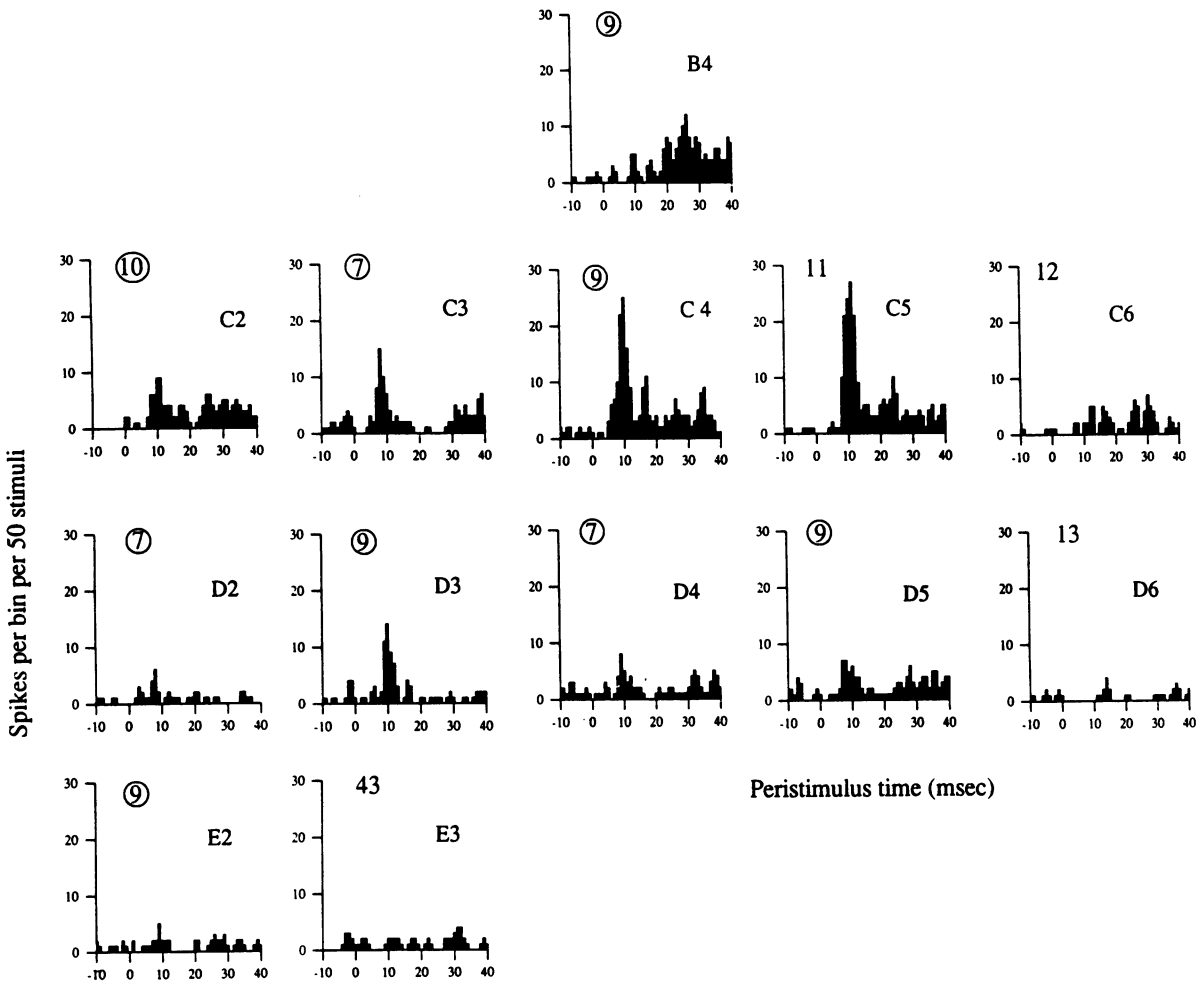

FIG. 3. Convergence of short-latency input onto cortical cells chronically treated with D-APV from P0 was far greater than for normal animals. Poststimulus time histograms are shown for one D-APV-treated layer IV cell's response to stimulation of the 13 vibrissae that evoked a response. The modal latencies for each vibrissa are shown in the top left of each histogram, and were derived from latency histograms (not shown). This cell, located in the septum closest to C4 barrel, responded with short latency to the C4 vibrissa as would be expected, but also to eight other vibrissae inappropriate for this barrel [short latencies (≤10 ms) have been circled].

4), but all of this type of unmasked responses were of intermediate to long latency (mean 22.5, range of 12–62 msec) with none of short latency. In 20 of the remaining 231 cases, bicuculline altered the latency classification of a vibrissa that evoked a response before bicuculline application. In two of these cases, the modal response latency was decreased to 10 msec but the vibrissa was appropriate for the barrel. In two other cases, the modal response latency was decreased to 10 msec and the vibrissa was inappropriate for the barrel but appropriate for a near neighbor. Unmasked short-latency responses were therefore encountered far less frequently (<1% of vibrissae-responses tested and <3% of cells tested) than would be necessary to explain the occurrence of multiple vibrissa short-latency responses in D-APV-treated animals. Further, because even the very few short-latency responses that were unmasked occurred for vibrissae related to adjacent barrels, they could not explain the more widespread spatial distribution of inappropriate short-latency responses found in D-APV-treated animals. These findings make it highly improbable that the effect of the early, transient D-APV treatment is due in some way to an unmasking of inappropriate connections that normally exist in the adult S1.

Effect of APV on Columnar Properties of S1 Barrelfield. To determine whether the columnar organization (20) is also affected by D-APV treatment, we recorded cells in the supragranular layers. In 13 of 36 penetrations (36%) in D-APV-treated animals, there was an erratic change in the principal vibrissa within the column, including four cases in which an inappropriate vibrissa was the only vibrissa to drive the cells in superficial layers (Fig. 5). Such fractured columns were not seen in L-APV-treated animals (0/28 penetrations), age-matched saline-treated controls (0/10), or in untreated animals (0/13). This finding implies that D-APV increases the incidence of columnar errors in topographic representation, as well as inappropriate thalamocortical inputs to layer IV as described above.

Further evidence of a disruption in columnar connectivity in D-APV-treated rats was apparent from the failure of many cells in the supragranular layers to respond to their columnar input, despite the fact that neurons immediately below them in layer IV responded at normal levels. This was determined by measuring the response magnitude of each cell to stimulation of its principal vibrissa. Some 45% of the cells in D-APV-treated animals showed no detectable response to principal vibrissa stimulation and a further 28% showed poor responses. Consequently, 72% of the cells located in layers II/III of D-APV-treated cortex had responses that fell below the 0.5 spikes per stimulus threshold for inclusion in the other forms of analysis reported in this paper. In contrast, layer IV neurons in the same animals showed normal levels of response magnitude compared with L-APV-treated animals and age-matched untreated animals; mean responses were respectively 1.4 ± 0.14 (mean ± SEM, 10 rats), 1.24 ± 0.10 (9 rats), and 1.2 ± 0.15 (7 rats). This finding implies that the

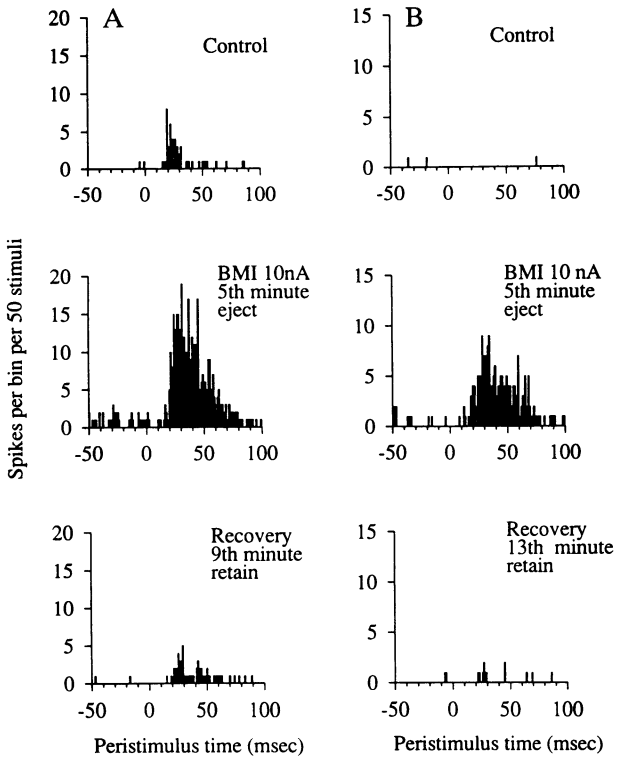

FIG. 4. The effect of disinhibition on vibrissae response latencies. (*A*) An example of a neuron that exhibited some response to the stimulation of the delta vibrissa before bicuculline application. The effect of bicuculline application using 10 nA ejecting current is to increase the magnitude and duration of the response without altering the response latency (of 20 msec). (*B*) An example of a neuron that did not respond to stimulation of the delta vibrissa before application of bicuculline using 4 nA ejecting current. The unmasked response exhibited a latency of 12 msec.

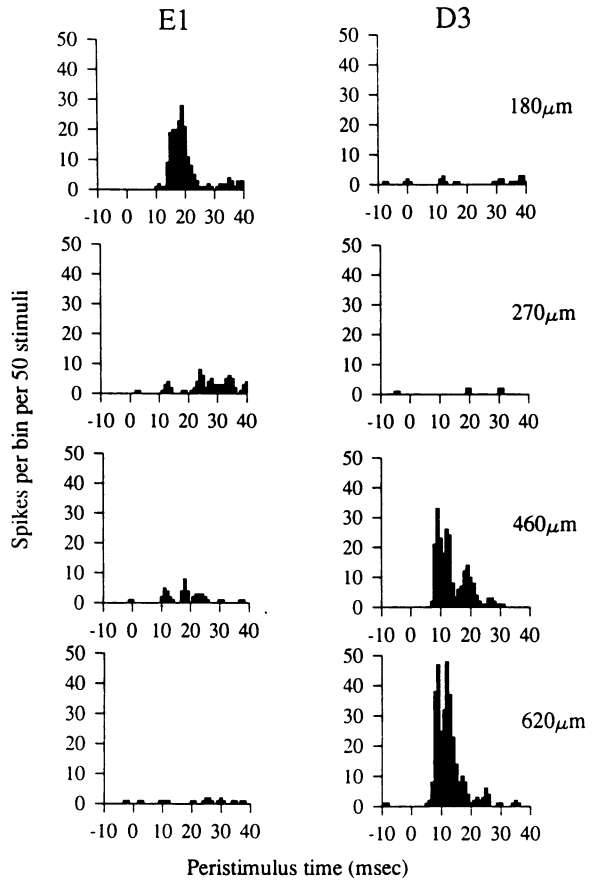

FIG. 5. Columnar organization in D-APV-treated cortex. An example of a fractured column in a D-APV-treated animal. The supragranular layer neurons respond to stimulation of the E1 vibrissa but not D3, which is the anatomically appropriate vibrissa for this penetration. In layer IV, the neurons in the barrel do respond to the anatomically appropriate vibrissa but not to E1. No other vibrissae evoked responses in this particular penetration. This represents a connection error of ≈1 mm tangentially across the cortex. The location of the penetration was known from a small lesion placed in layer IV at the end of the penetration. The brain appeared normal in sections through the barrel field. The electrode was angled normal to the surface of the cortex on a trajectory though the D3 column only. For the electrode to have passed through the E1 column before the D3 barrel, we would have had to have made an error in orienting the electrode of at least 80°.

effects of D-APV treatment on cortical connections are unlikely to be due to nonspecific damage or general deterioration of responsiveness.

DISCUSSION

In normal adult rodents, short-latency responses for each vibrissa are restricted to a single barrel and the septal zone immediately surrounding it (4, 5), but in the D-APV-treated rats, short-latency responses to an individual vibrissa were also found in surrounding barrels. Acute application of bicuculline to S1 in normal mature rats did not mimic these effects of early D-APV treatment, which implies that connections that can generate inappropriate short-latency responses are not normally present in adult S1. The first responses recorded in the D-APV-treated S1 after vibrissae stimulation are in the range of 5–10 msec. As they are the first responses in S1, it is likely that they are evoked by the direct trisynaptic circuit from the periphery to S1 ending with the link from VPm to layer IV. This idea is corroborated by the finding that in normal adults responses of the same short-latency are confined to the appropriate barrel for a particular vibrissa (4, 5, 21), as are VPm afferents (2, 3). The other thalamic source of sensory input to the S1 barrel field is the posterior medial (POm) nucleus. Whereas VPm afferents project to the barrels, POm afferents project to the septae between barrels (22, 23). However, unlike VPm cells, POm cells exhibit long-latency responses to vibrissae stimulation that exceed the latency exhibited by layer IV cells (24, 25). Therefore, POm afferents cannot account for the short-latency responses recorded in layer IV.

There are two means by which VPm axons driven by one vibrissa could evoke responses in cortical cells over a wider distance than normal; either VPm axons distribute over a wider area than normal or the dendrites of cortical cells extend over a wider area than normal. It seems unlikely that increased dendrite extension could explain inappropriate short-latency responses of barrel cells found at more than half of one barrel away (beyond 200 μm). Markers of barrel architecture, including acetylcholine-esterase (13), CO (present study), and cytotactin and Nissl stains (26), show a normal pattern in the D-APV-treated S1, suggesting that the dendritic arbors of layer IV barrel cells also develop in at least a roughly normal fashion because they are a prominent component in configuring barrel architecture. It also seems unlikely that aberrant dendritic coverage could account for the increase in septal cells with short-latency responses to inappropriate vibrissae. Although some septal cells normally extend dendrites to two adjacent barrels (27), the finding that many septal cells have several inappropriate short-latency responses (up to nine) in D-APV-treated rats would require that they extend dendrites into several disparately located barrels. Thus, the most straightforward interpretation is that the topographically aberrant short-latency responses in the D-APV-treated animals are mainly due to inappropriate distributions of VPm afferents.

The inappropriate distributions of VPm afferents in D-APV-treated S1 may be due to the retention of topographically inappropriate VPm projections that normally form only transiently in S1. Although the initial distribution of VPm afferents is continuous within layer IV (2, 3), individual VPm axons do not arborize widely within layer IV and most are more or less appropriately targeted (28, 29). However, at birth ≈30% of VPm axons do project to a location in layer IV more than 1.3 presumptive barrels away from their topographically appropriate site (28, 29). This distribution of VPm afferents in normal developing S1 is similar to that in D-APV-treated S1 in which ≈40% of barrel cells receive a short-latency input from an inappropriate vibrissa, and that these inputs are usually from a neighboring vibrissa. Because the topographically inappropriate VPm projections persist well beyond the effectiveness of the D-APV treatment, it is likely that they are permanent and that they can be stabilized by a temporary activity block during the critical period.

Our results draw a distinction between mechanisms responsible for the development of the large-scale somatotopic barrel pattern in layer IV and the refinement of topographic order within that pattern. The clustering of VPm afferents into the large-scale somatotopic pattern does not require neural activity, whereas the topographic refinement of this projection resulting in the one-to-one functional relationship between a vibrissa and a barrel depends on functional glutamate receptors and the ability of VPm afferents to activate their postsynaptic target cells in layer IV. Topographic errors are also induced by univibrissae rearing if begun within the critical period by clipping all but a single vibrissa without damaging the follicle (30). In the univibrissae animals, a normal-appearing barrel pattern is maintained, but inappropriate short-latency responses to the remaining vibrissa can be recorded in barrels corresponding to the deprived vibrissae (21). These results support those from our D-APV study in implying that elimination of topographic errors is activity dependent in early postnatal S1.

Our findings are consistent with a model in which the normal removal of inappropriate VPm afferents is based on a Hebbian-like mechanism that requires postsynaptic detection of correlated (i.e., appropriate) versus noncorrelated (inappropriate) inputs (31–33). In V1, correlated activity causes aggregation of correlated geniculocortical axons (9, 34), whereas extreme levels of uncorrelated activity lead to extreme segregation of geniculocortical axons (35). The NMDA receptor is important for segregation of eye-specific stripes in the three-eyed frog tectum (36) and has been hypothesized to be a postsynaptic detector of correlated activity among afferents because of its voltage-gated properties and involvement in forms of synaptic strengthening such as long-term potentiation (37). In our experiments, D-APV treatment blocked both NMDA and non-NMDA glutamate receptors (13). However, NMDA channels carry the greater part of the postsynaptic current in cortical neurons during the first postnatal week (11, 38). These currents are of long duration, which allows temporal summation over a longer time period than in adult animals. This may be important for detecting correlated activity in neonates when synaptic responses are weak and variable. Further, VPm afferents to S1 can undergo long-term potentiation during the first postnatal week (39). This mechanism is dependent on NMDA receptors and, if involved in normal development, would have been blocked by the D-APV treatment used in our experiments.

The columnar projection to layers II/III was also disrupted by D-APV treatment. In normal adult and adolescent rats, superficial layer neurons respond to the same principal vibrissa as the layer IV neurons that lie beneath them and with approximately the same intensity of response (4, 40). In the D-APV-treated rats, many penetrations exposed the presence of somatotopic errors such that the neurons failed to respond to their principal vibrissa but instead responded to a vibrissa corresponding to a neighboring barrel. Again, this finding implies that error elimination is an activity-dependent process. In this instance, though, the inappropriate responses may be due to a maintenance or aberrant development of erroneous intracortical connections, rather than thalamocortical. Furthermore, in the D-APV-treated animals, neurons in layers II and III responded poorly to principal vibrissa stimulation even though cells in layer IV beneath them responded at normal levels. A plausible explanation for this finding is that D-APV diminishes the formation of intracortical connections by blocking thalamocortical and intracortical excitatory transmission. This is consistent with the finding that infraorbital nerve section at P7, which abolishes sensory input to barrel cortex after the layer IV barrels have formed, leads to shorter intracortical connections in extragranular layers (41).

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1. Woolsey, T. A. & Van Der Loos, H. (1970) *Brain Res.* **17,** 205–242.
2. Jensen, K. F. & Killackey, H. P. (1987) *J. Neurosci.* **7,** 3529–3543.
3. Senft, S. L. & Woolsey, T. A. (1991) *Cerebr. Cortex* **1,** 308–335.
4. Armstrong-James, M. & Fox, K. (1987) *J. Comp. Neurol.* **263,** 264–281.
5. Armstrong-James, M., Fox, K. & Das Gupta, A. (1992) *J. Neurophysiol.* **68,** 1345–1358.
6. Erzurumlu, R. S. & Jhaveri, S. (1990) *Dev. Brain Res.* **56,** 229–234.
7. Schlaggar, B. L. & O'Leary, D. D. M. (1994) *J. Comp. Neurol.* **346,** 80–96.
8. Le Vay, S., Stryker, M. P. & Shatz, C. J. (1978) *J. Comp. Neurol.* **179,** 223–244.
9. Stryker, M. P. & Harris, W. A. (1986) *J. Neuosci.* **6,** 2117–2133.
10. Agmon, A. & O'Dowd, D. K. (1992) *J. Neurophysiol.* **68,** 345–348.
11. Kim, H. G., Fox, K. & Connors, B. W. (1995) *Cerebr. Cortex* **5,** 148–157.
12. McCandlish, C. A., Li, C. X. & Waters, R. S. (1993) *Exp. Brain Res.* **92,** 369–374.
13. Schlaggar, B. L., Fox, K. & O'Leary, D. D. M. (1993) *Nature (London)* **364,** 623–626.
14. Henderson, T. A., Woolsey, T. A. & Jacquin, M. F. (1992) *Dev. Brain Res.* **66,** 146–152.
15. Chiaia, N. L., Fish, SEM, Bauer, W., Bennett-Clarke, C. A. & Rhoades, R. W. (1992) *Dev. Brain Res.* **66,** 244–250.
16. Belford, G. R. & Killackey, H. P. (1980) *J. Comp. Neurol.* **193,** 335–350.
17. Langer, R., Brown, L. & Edelman, E. (1985) *Methods Enzymol.* **112,** 399–422.
18. Wong-Riley, M. (1979) *Brain Res.* **171,** 11–28.
19. Coleman, P. A. & Miller, R. F. (1988) *J. Neurosci.* **8,** 4728–4733.
20. Mountcastle, V. B. (1957) *J. Neurophysiol.* **20,** 408–434.
21. Fox, K. (1992) *J. Neurosci.* **12,** 1826–1838.
22. Koralek, K-A., Jensen, K. F. & Killackey, H. P. (1988) *Brain Res.* **89,** 346–351.
23. Lu, S.-M. & Lin, C.-S. (1993) *Somatosen. Mot. Res.* **10,** 1–16.
24. Diamond, M. E., Armstrong-James, M., Budway, M. J. & Ebner, F. F. (1992) *J. Comp. Neurol.* **319,** 66–84.
25. Hooglan, P. V., Welker, E. & Van der Loos, H. (1987) *Exp. Brain Res.* **68,** 73–87.
26. Ruff, N. L., Tuttle, R. & O'Leary, D. D. M. (1995) *Soc. Neurosci. Abstr.* **21,** 568.
27. Harris, R. M. & Woolsey, T. A. (1981) *J. Comp. Neurol.* **196,** 357–376.
28. Agmon, A., Yang, L. T., O'Dowd, D. K. & Jones, E. G (1993) *J. Neurosci.* **13,** 5365–5382.
29. Agmon, A., Yang, L. T., Jones, E. G. & O'Dowd, D. K. (1995) *J. Neurosci.* **15,** 549–561.
30. Li, X., Glazewski, S., Lin, X., Elde, R. & Fox, K. (1995) *J. Comp. Neurol.* **358,** 1–17.
31. Hebb, D. O. (1949) *Organization of Behavior* (Wiley, New York).
32. Stent, G. S. (1970) *Proc. Natl. Acad. Sci. USA* **70,** 997–1001.
33. Goodman, C. S. & Shatz, C. J. (1993) *Cell Suppl.* **72,** 77–98.
34. Blasdel, G. G. & Pettigrew, J. D. (1979) *J. Neurophysiol.* **42,** 1692–1710.
35. Shatz, C. J., Lindstrom, S. & Wiesel, T. N. (1977) *Brain Res.* **131,** 103–116.
36. Cline, H. T., Debski, E. & Constantine-Paton, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4342–4345.
37. Constantine-Paton, M., Cline, H. T. & Debski, E. (1990) *Annu. Rev. Neurosci.* **13,** 129–154.
38. Burgard, E. C. & Hablitz, J. J. (1993) *J. Neurophysiol.* **69,** 230–240.
39. Crair, M. C. & Malenka, R. C. (1995) *Nature (London)* **375,** 325–328.
40. Glazewski, S. & Fox, K. (1995) *J. Neurophysiol.* **75,** 1714–1729.
41. McCasland, J. S., Bernardo, K. L., Probst, K. L. & Woolsey, T. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1832–1836.