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Journal
The Journal of comparative neurology, 397(1)

ISSN
0021-9967

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Publication Date
1998-07-20

Peer reviewed

THE JOURNAL OF COMPARATIVE NEUROLOGY 397:1–9 (1998)

Regulation of α7 Nicotinic Acetylcholine Receptors in Mouse Somatosensory Cortex Following Whisker Removal at Birth

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ABSTRACT

Previous studies in postnatal mouse demonstrating high levels of α7 nicotinic acetylcholine receptors on layer IV somatosensory cortical neurons coincident with the onset of functional synaptic transmission led us to investigate whether the number and/or the localization of these receptors could be regulated by activity. Accordingly, we examined α-bungarotoxin binding in mouse somatosensory cortex following removal of all of the vibrissae on one side of the face, either by vibrissal follicle cauterization or daily plucking beginning on the day of birth. Following vibrissa plucking, the levels of [^{125}I]α-bungarotoxin binding on postnatal day 6 were significantly higher (23 ± 7%) in the denervated cortex (contralateral to the peripheral manipulation) than the intact cortex. Cauterization also resulted in significantly higher (14 ± 3%) [^{125}I]α-bungarotoxin binding in the contralateral vs. the ipsilateral cortex. In contrast, there was no difference in [^{125}I]α-bungarotoxin binding in the left and right cortices of unoperated control animals. At postnatal day 14, levels of [^{125}I]α-bungarotoxin binding in layer IV were very low in control animals as well as in animals subjected to whisker plucking or cautery. These findings suggest that reducing activity in the somatosensory pathway regulates the density of α7 nicotinic acetylcholine receptors during the first postnatal week. However, the normal decrease in receptor density that is seen during the second postnatal week of development proceeds despite altered sensory activity. J. Comp. Neurol. 397:1–9, 1998.

Indexing terms: barrel cortex; α7 nicotinic acetylcholine receptor; development; α-bungarotoxin; cautery

Activity-dependent regulation of neurotransmitters and their receptors has been studied extensively in the mammalian visual cortex. The unique anatomical and functional organization of this sensory system, specifically, the organization of inputs from the left and right eyes into adjacent ocular dominance columns in the primary visual cortex of primates (Hubel and Wiesel, 1968, 1969), has facilitated direct comparison of the effects of different activity levels in the two eyes on components of cortical neurotransmitter systems (Hendry and Jones, 1986, 1988; Benson et al., 1991; Huntsman et al., 1994). In adult rodents, organizational features in the somatosensory cortex, including topographically organized clusters of cells in layer IV, called barrels (Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974), make this sensory system similarly suited to the study of activity-dependent regulation of neurotransmitters. A number of studies have demonstrated that components of the γ-aminobutyric acidergic (GABAergic) transmission system in the somatosensory cortex can be regulated by activity in the adult rodent. Trimming whiskers of adult rats leads to a reversible decrease in the levels of glutamic acid decarboxylase (GAD) immunoreactivity in barrels corresponding to trimmed hairs (Akhtar and Land, 1991). Conversely, in-

Grant sponsor: NIH; Grant numbers: NS30109 and S27501; Grant sponsor: RCDA; Grant number: NS01854.

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Received 4 November 1996; Revised 4 February 1998; Accepted 2 March 1998

creasing levels of sensory stimulation results in an increase in GAD immunoreactivity (Welker et al., 1989).

The early postnatal somatosensory cortex is an attractive system in which to examine the role of activity in the development of cortical neurotransmitter systems. The formation of cortical barrels (Rice et al., 1985) and innervation of the cortex by thalamic afferent fibers (Senft and Woolsey, 1991; Agmon et al., 1993) occurs during the first postnatal week, making manipulations of this system relatively straightforward. Neonatal sensory deprivation has been shown to alter both excitatory and inhibitory neurotransmitter systems in the rodent somatosensory cortex. For example, neonatal vibrissal follicle lesions result in a reduction in GABA (Kossut et al., 1991; Micheva and Beaulieu, 1995) and glutamate (Stewart et al., 1993) immunoreactivity in the barrel cortex. In addition, a transient decrease of approximately 10–20% in $GABA_A$ receptor binding is observed in the deprived barrels following unilateral lesions of all vibrissal follicles at postnatal day 2 (P2; Skangiel-Kramska et al., 1994). In another study, although continuous whisker trimming initiated at birth did not appear to affect the levels of GAD immunoreactivity, it did eliminate the ability to modulate transmitter enzyme levels by activity (Akhtar and Land, 1991). These results suggest that, in the rodent somatosensory system, activity-dependent regulation of neurotransmitters is likely to play a role in development and plasticity.

Although glutamate and GABA are the primary neurotransmitters in the developing mammalian neocortex, a number of studies suggest that acetylcholine (ACh) may also play a role during cortical development (Bear and Singer, 1986; Höhmann et al., 1988, 1991). Studies in the kitten suggest that ACh facilitates experience-dependent alteration in neuronal response properties in the developing visual cortex through activation of muscarinic ACh receptors (Gu and Singer, 1993). Reports of high levels of expression of α-bungarotoxin ([^{125}I]α-BTX) binding in sensory cortices of rats during early postnatal development (Fuchs, 1989; Broide et al., 1995), in conjunction with studies showing that [^{125}I]α-BTX binds to nicotinic acetylcholine receptors (nAChRs) composed of α7 nAChR subunits (Alkondon and Albuquerque, 1993; Seguela et al., 1993), suggest that synaptic responses that are mediated/modulated by α7 nAChRs may also be important in the development of cortical circuitry. In addition, in chick ciliary ganglion neurons, this receptor has been implicated in regulating neuritic outgrowth (Pugh and Berg, 1994). We have shown that high levels of α7 mRNA and [^{125}I]α-BTX-binding receptor protein are expressed transiently in a barrel-like pattern in layer IV of the somatosensory cortex of early postnatal mice (Bina et al., 1995). The presence of high levels of α7 nAChR expression in the barrel centers, where the dendrites of the cortical neurons and thalamic afferent fibers are closely intermingled, during the initial phases of functional synaptic transmission (Agmon and O'Dowd, 1992; Agmon et al., 1996), led us to investigate whether reduced activity in the sensory pathway could influence the pattern of expression of these neurotransmitter receptors in the mouse somatosensory cortex. In the studies presented here, we employed two experimental paradigms to alter sensory input from the periphery: one in which there were no apparent changes in the barrel morphology (daily whisker plucking beginning at P0) and a second, in which the normal barrel pattern was disrupted (whisker follicle cautery at P0). Our findings demonstrate that manipulations of the periphery that have different effects on the morphological organization of the barrel field both regulate levels of [^{125}I]α-BTX binding in layer IV of somatosensory cortex in a similar direction. However, neither of these manipulations appears to disrupt the localization of these receptors in a pattern that reflects the cellular organization of the barrel field during the first postnatal week, nor does it prevent the normal developmental down regulation in [^{125}I]α-BTX binding that is seen by the end of the second postnatal week.

MATERIALS AND METHODS

Data were obtained from a total of 55 ICR mice. Timed pregnant HSD:ICR mice (Harlan Sprague-Dawley, San Diego, CA) were housed under normal light-dark cycles (LD 12:12 hours) with food and water ad libitum. The day of birth was designated as P0. The procedures employed in these experiments were approved by the University of California Irvine Committee on Animals Research, protocol 94–1607.

Vibrissae removal

All of the large mystacial vibrissae from the right snout of experimental animals were removed by one of two methods. In the first group, all of the vibrissae on the right snout were removed by daily plucking (plucked) using a sharp pair of tweezers, beginning at P0 and continuing until the day of killing. In the second group, all of the vibrissae on the right snout were removed at P0 by applying heat to the vibrissal follicle (cauterized) with an electrocautery tool and removing the vibrissa with the follicle attached. A second cautery was performed if there was any evidence of vibrissal regrowth at P7. All experimental manipulations were carried out in animals that were anesthetized by cooling on ice (for animals P0–P5) or by inhalation of halothane (animals older than P5). Unoperated litter mates of both experimental groups served as controls.

Perfusion and tissue processing

Control and experimental mice were killed by decapitation under halothane anesthesia at P6 or at P14, and their brains were removed rapidly. The cortices were separated from the rest of the brain, flattened between two glass slides, wrapped in parafilm, rapidly frozen by dipping in 2-methylbutane for 1 minute, and stored at −20°C until all brains for a given experiment were ready for sectioning. Serial tangential sections of each cortex from the pia to the white matter, 20 μm thick, were cut on a cryostat. The first and second of every three sections from a single cortex were processed for total [^{125}I]α-BTX binding and nonspecific binding, respectively. Every third section was processed for cytochrome oxydase (CO) staining. In one to three sections from each cortex, approximately 300–400 μm from the pial surface, we observed CO barrel staining, thereby positively identifying the location of layer IV barrel cortex (Wong-Riley and Welt, 1980). Total and nonspecific binding densities were always measured from the two sections adjacent to those exhibiting CO barrel staining.

Receptor binding with [^{125}I]α-BTX

Sections from four to six animals through both the left and right cortices of each individual animal were pro-

cessed in a single binding experiment, eliminating the possibility of systematic changes in binding conditions affecting the outcome of the experiment. Cortices from at least one unoperated litter mate were also included in each experiment. The tissue from 55 mice, experimental and control, were processed in a total of 11 separate binding experiments.

Total [^{125}I]α-BTX binding in each experiment was determined by preincubating the slide-mounted sections in a buffer containing 0.12 M NaCl and 0.05 M Tris-HCl, pH 7.4, allowing them to air dry, and then incubating the sections in the same buffer containing 5 nM iodo-α-BTX ([^{125}I]α-BTX; Amersham, Arlington Heights, IL) for 2 hours in darkness at room temperature. The sections were subsequently washed twice for 10 minutes in ice-cold buffer, rinsed for 30 seconds in distilled water, and then air dried. Nonspecific binding in each experiment was determined as described above, except these sections were incubated in a radiolabeled ligand solution to which 10^{-4} M nicotine were added. All slides were exposed for 1 day at 4°C on tritium-sensitive Hyperfilm (Amersham).

Histology

Sections processed for CO staining were incubated overnight at 4°C in phosphate buffer (0.1 M), pH 7.2, containing 0.06% cytochrome C and 0.04% diaminobenzidine. All slides were dried, dehydrated in ascending series of alcohol, and coverslipped in Permount. Digitized images of the sections were obtained by using a video-based image-analysis system, Microcomputer Image Device (MCID; Imaging Research Inc., St. Catherines, Ontario, Canada). The area occupied by CO staining in four identified barrels (B2, B4, D2, and D4) in each section was used to determine the average barrel area at P6 and P14 in control animals and in those that had undergone chronic whisker plucking or cautery. No barrel areas are reported for animals that underwent cautery, because this manipulation causes distortions in barrel boundaries and, in some cases, fusion of adjacent barrels with no septae seen between them.

Quantitative analysis of [^{125}I]α-BTX binding

The density of [^{125}I]α-BTX binding in the somatosensory cortex was quantified from digitized images of the autoradiographic film obtained by using the MCID video-based image-analysis system. All cortical sections from a single animal were processed in parallel and exposed to the same piece of autoradiographic film. Calibration curves of optical density vs. ligand concentration (dpms) were constructed for each film by using [^{14}C] brain paste standards of known radioactivity. For all of the films, an exposure time of 1 day was chosen, because this yielded optical density (OD) readings in the tissue sections that were within the linear range of detection of the film. Although there was some variability in the plane of section between individual cortices, OD measurements from a single animal were made from the sections processed for total binding and nonspecific binding that were adjacent to CO-stained sections that revealed the most complete barrel pattern. In each case examined, the OD readings were higher in these sections compared with the adjacent sections, which contained less complete representations of the barrel field. Specific binding was determined by subtracting the mean OD measurement for the section processed for nonspecific binding from the adjacent section processed for total binding. The average OD in each cortex was calculated from OD values obtained at four defined locations in the barrel cortex (B2, B4, D2, and D4) by using a circular window 80 μm in diameter. The mean OD value obtained from the denervated cortex (contralateral to the manipulation) was expressed as a ratio of the control cortex (ipsilateral to the manipulation) in a single animal. Paired comparisons of experimental and control cortices from the same animal were chosen, because the predicted magnitude of change in the density of [^{125}I]α-BTX binding, based on the effect of activity on other neurotransmitter receptors in this system (Skangiel-Kramska et al., 1994), was small (20–50%). This comparison minimizes the contribution of parameters, such as handling, nutrition, tissue removal and processing, etc., that vary between animals and may obscure or attenuate differences due to the experimental manipulation. All measurements were made blind with respect to experimental condition and were decoded only after all of the data were obtained from a single experiment. Ratios obtained for experimental animals at P6 and P14 were compared with ratios obtained for unoperated, age-matched control animals that were processed in the same binding experiment.

RESULTS

Effect of manipulation of sensory periphery on CO staining in the barrel cortex

Comparisons of organizational features of the PMBSFs, as revealed by CO staining, were made between unoperated control animals and the two groups of experimental animals. In one group of experimental animals (plucked), all of the large mystacial vibrissae from the right snout were removed by daily plucking beginning at P0. The overall CO staining pattern was similar in the contralateral cortices of the experimental group that was subjected to continuous vibrissae plucking from P0 to P6 (Fig. 1C) and from P0 to P14 (Fig. 1D) compared with their age-matched, unoperated controls (Fig. 1A,B). The area described by CO in four individual barrels in two rows (B2, B4, D2, and D4) was determined in both left and right cortices obtained from control and plucked animals processed at P6 and P14. There was no significant difference in barrel area between right and left sides or between experimental and control groups in either age group (Fig. 1G). In addition, a similar increase in the mean barrel area between P6 and P14 was observed in both the experimental and control groups (Fig. 1G).

In the second group of experimental animals, unilateral cautery of all mystacial vibrissae at P0 resulted in alterations in the CO staining pattern characterized by a lack of discernible barrels in the PMBSF contralateral to the deprived snout at both P6 (Fig. 1E) and P14 (Fig. 1F). In some animals, individual barrel boundaries were distorted, and, in other cases, fusion of adjacent barrels resulted in a row-like pattern of staining. The lack of distinct barrel boundaries precluded measurement of barrel areas. On average, the level of CO staining appeared lower in the contralateral barrels following cautery in both P6 and P14 animals. However, due to the variability in CO staining in our study, this was not examined quantitatively.

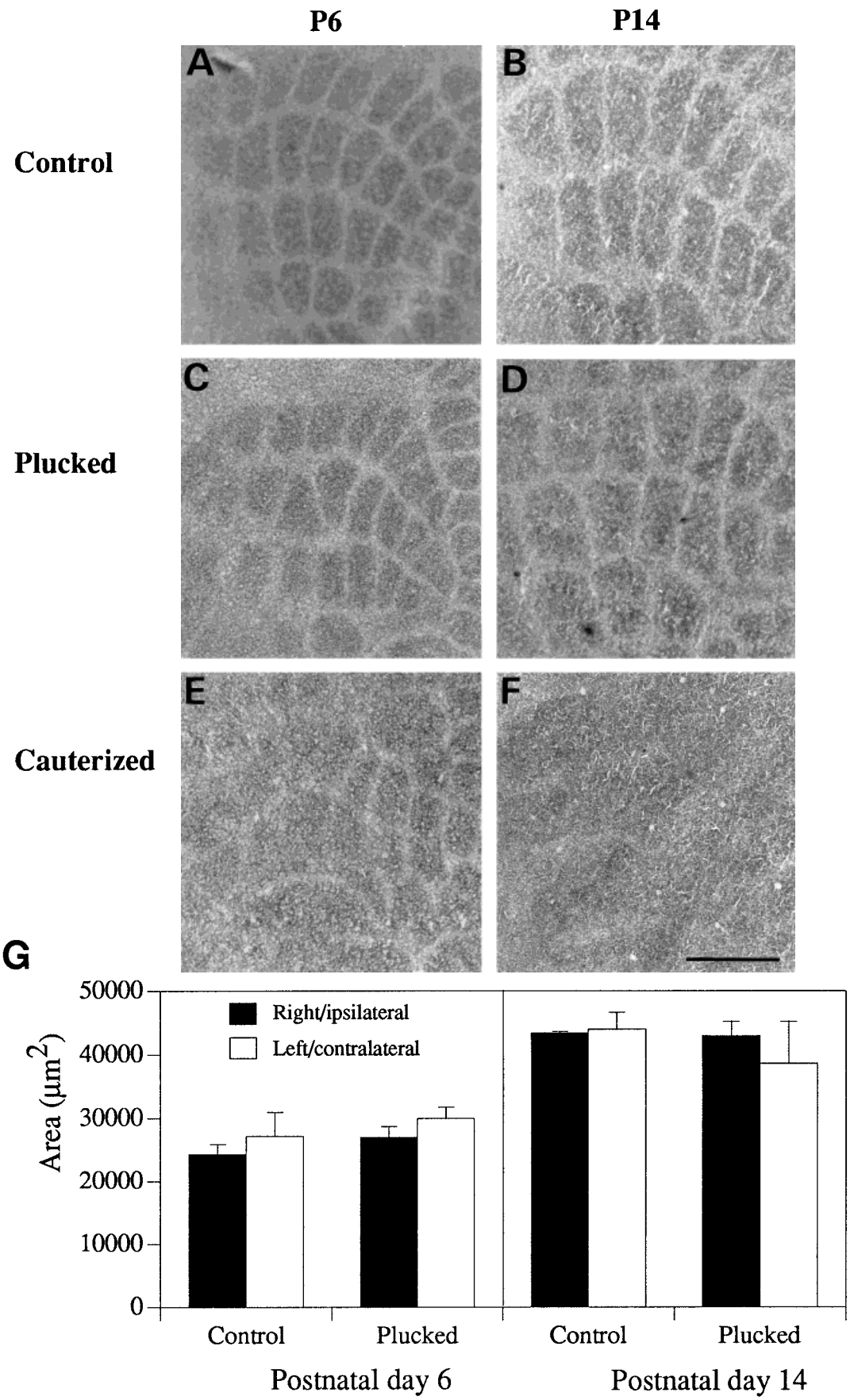
P6
P14
Control
Plucked
Cauterized
A
B
C
D
E
F
G
Right/ipsilateral
Left/contralateral
Area (µm2)
50000
40000
30000
20000
10000
0
Control
Plucked
Control
Plucked
Postnatal day 6
Postnatal day 14

Figure 1

Daily vibrissae plucking (plucked) initiated at P0 alters levels of [^{125}I]α-BTX binding in P6 mouse barrel cortex

To determine whether daily whisker plucking altered the distribution or levels of α7 nAChRs, [^{125}I]α-BTX binding in the PMBSF of experimental animals was compared with control animals at P6, an age at which high levels of [^{125}I]α-BTX binding describe a barrel-like pattern (Bina et al., 1995). The distinct barrel pattern of [^{125}I]α-BTX binding was present in both the contralateral and ipsilateral PMBSF in experimental animals, similar to that seen in control animals (Fig. 2). However, the increased incidence of red and yellow pixels in the pseudocolor images of the contralateral cortices suggested that there were higher levels of binding in these sections. To examine this issue quantitatively, OD measurements were obtained by using an 80-μm circular transcept tool placed in the central region of barrels B2, B4, D2, and D4 on both the contralateral (Fig. 2, Contra) and the ipsilateral (Fig. 2, Ipsi) section through the barrel cortex of each animal. The histogram of the average paired contralateral vs. ipsilateral OD ratios obtained from 17 animals indicates that the contralateral side was significantly higher (23 ± 7%) than the ipsilateral side (double asterisks, $P < 0.01$; Student's t-test, paired). In contrast, there was no difference in the paired left/right ratio histogram, determined in P6 naive control animals in which no experimental manipulation was performed (Student's t-test, paired; n = 9).

Cautery of mystacial vibrissae at P0 alters levels of [^{125}I]α-BTX binding in P6 mouse barrel cortex

The distribution of [^{125}I]α-BTX binding in the contralateral somatosensory cortex of cauterized animals examined at P6 was altered compared with the ipsilateral cortex (Fig. 3). A normal, barrel-like pattern of [^{125}I]α-BTX sites can be seen on the ipsilateral side (Fig. 3, Ipsi), whereas the [^{125}I]α-BTX binding on the contralateral side (Fig. 3, Contra) does not define a clear barrel pattern. Analysis of adjacent sections stained for CO suggested that the distribution of [^{125}I]α-BTX sites corresponds with the disruption of barrel boundaries that result in the appearance of CO staining in fused rows rather than barrels. Because the barrel boundaries were not readily apparent, it was not possible to compare directly the binding densities within barrels on the contralateral and ipsilateral sides. Therefore, on the side contralateral to cautery, the average [^{125}I]α-BTX binding density was determined by measuring density at positions within the PMBSF using a circular window 80 μm in diameter, roughly corresponding to the location of barrels B2, B4, D2, and D4, as indicated (Fig. 3, Contra). The average paired contralateral/ipsilateral OD ratio of 1.14 ± 0.03 shown in the histogram (mean ± S.E.M.; n = 11) indicated that the binding on the contralateral side was significantly higher (14%) than the ipsilateral side ($P < 0.001$; Student's t-test, paired). There was no difference in the left/right ratio histogram for control animals (Fig. 3), as illustrated previously.

Neither cautery nor daily plucking alters the developmental change in [^{125}I]α-BTX binding seen by the end of the second postnatal week

Analysis of the CO pattern of staining in P14 animals following P0 cautery or daily plucking revealed, as expected, either a disruption of barrel boundaries in the case of cautery (Fig. 1F) or no difference in the barrel pattern following whisker plucking (Fig. 1D). Similar to the observation we reported in a previous study (Bina et al., 1995), a dramatic developmental decline in the levels of [^{125}I]α-BTX (binding density/unit area) between P6 (Fig. 4A), and P14 (Fig. 4B) was observed in the control cortices. Interestingly, a similar decline was observed in both experimental groups (Fig. 4C–F). At P14, although the levels of [^{125}I]α-BTX binding were slightly higher in the PMBSF than in surrounding cortical areas, there was no significant difference between the ipsilateral and contralateral sides in the experimental animals (paired Student's t-test) nor between the control and experimental animals (analysis of variance; ANOVA). To quantify the developmental decline, the levels of binding within barrels (position determined by comparison with adjacent CO-stained sections) in individual P14 cortices were expressed as a ratio of the average levels in barrels from P6 cortices that were processed in the same experiment for each of the groups (Fig. 4G). In all three groups, the ratio was significantly less than 1 (two-sided t-test of individual means). However, there did not appear to be a significant difference in the decrease in binding between the three groups (ANOVA), suggesting that neither of the manipulations blocked the normal developmental reduction in mean [^{125}I]α-BTX binding level/unit area between P6 and P14.

DISCUSSION

Our findings demonstrate that altered sensory input from the facial vibrissae, begun at P0, affects the expression of α7 nAChRs in mouse somatosensory barrel cortex during the first postnatal week. These data are consistent with the hypothesis that reduced activity in the somatosensory pathway during early postnatal development can regulate the numbers of α7 nAChRs in cortical neurons. Manipulation of the periphery, however, does not appear to affect the normal developmental decrease in receptor density (mean binding/unit area) in the barrel cortex during the second postnatal week.

Manipulation of sensory input alters [^{125}I]α-BTX binding during the first postnatal week

The two distinct peripheral manipulations, whisker plucking and cautery, both resulted in significantly higher levels of [^{125}I]α-BTX binding in the cortex deprived of sensory input compared with the nondeprived side at P6.

Fig. 1. Barrel pattern revealed by cytochrome oxydase (CO) staining. Tangential sections through the posteromedial barrel subfield (PMBSF) of animals killed at postnatal day 6 (P6) and P14 and subsequently processed for CO staining. **A,B:** CO staining in control animals reveals the stereotypic pattern of barrel organization. **C,D:** Daily whisker plucking, from P0 until day the of killing (plucked), does not alter the barrel pattern of CO staining. **E,F:** Cautery of the whiskers on the day of birth causes a disruption of the barrel boundaries delineated by the CO staining. **G:** Average area described by CO staining in four individual barrels in two rows (B2, B4, D2, and D4) was determined in both left and right cortices obtained from control and plucked animals processed at P6 and P14. The control and experimental groups were not significantly different (Student's t-test) in either age group. Scale bar = 400 μm.

The changes were not only in the same direction, but the magnitude of the change was similar, 23 ± 7% and 14 ± 3% in the case of plucking and cautery, respectively. The magnitude of the difference in [^{125}I]α-BTX binding reported in this study as a result of cautery is similar to the reduction (12 ± 2%) in $GABA_A$ receptor binding seen in the deprived cortices following unilateral lesioning of vibrissae in neonatal mice (Skangiel-Kramska et al., 1994). Immunohistochemical studies have also shown a 25–40% decline in the number of glutamate-immunoreactive neurons within layer IV following neonatal lesioning of the whiskers (Stewart et al., 1993) and a 50% reduction in the density of GABA-immunoreactive neurons (Micheva and Beaulieu, 1995) compared with control animals.

Although the electrophysiological consequences of whisker plucking and vibrissal follicle cautery have not been examined directly during early development, several lines of evidence suggest that these manipulations result in a reduction of activity in the somatosensory cortex. The lesioning of whisker follicles on P0 destroys the peripheral nerve endings and has been shown to reduce activity in the deprived cortex of adult rodents (Durham and Woolsey, 1978). In addition, previous studies have demonstrated reductions in levels of CO staining following cauterization (Wong-Riley and Welt, 1980) and whisker trimming (Akhtar and Land, 1991) during the early postnatal period. A reduction in CO staining is consistent with these manipulations causing a decrease in neuronal activity (Wong-Riley, 1989). Our data showing a higher level of [^{125}I]α-BTX binding in the cortex contralateral to the plucked or cauterized snout, in conjunction with the knowledge that sensory input from the vibrissae to the barrel cortex is entirely crossed, are consistent with the suggestion that a decrease in activity in the direct somatosensory pathway increases expression of [^{125}I]α-BTX binding sites. Interestingly, most of the previous studies examining the effect of decreasing sensory activity, both during development and in the adult, report a decrease in the expression of neurotransmitters and their receptors in the cortex. However, up-regulation in immunocytochemically detectable levels of type II calcium-calmodulin-dependent protein kinase (CaM II kinase; Hendry and Kennedy, 1986) and CaM II kinase mRNA (Benson et al., 1991) in response to a decrease in activity has been reported in the adult monkey visual cortex.

The very low levels of α7 mRNA and [^{125}I]α-BTX binding observed in the ventrobasal complex of the mouse thalamus reported in our previous study suggested that the [^{125}I]α-BTX sites, which are present at high levels in the barrel hollows, are localized primarily on the dendrites of layer IV cortical neurons (Bina et al., 1995). In the present study, whisker plucking did not disrupt the localization of binding sites, which remained densely clustered in the barrel centers. In contrast, cautery, a manipulation that induces the spread of dendrites of barrel neurons into neighboring septal areas and a fusion of barrel boundaries (Harris and Woolsey, 1981), resulted in a more homog-

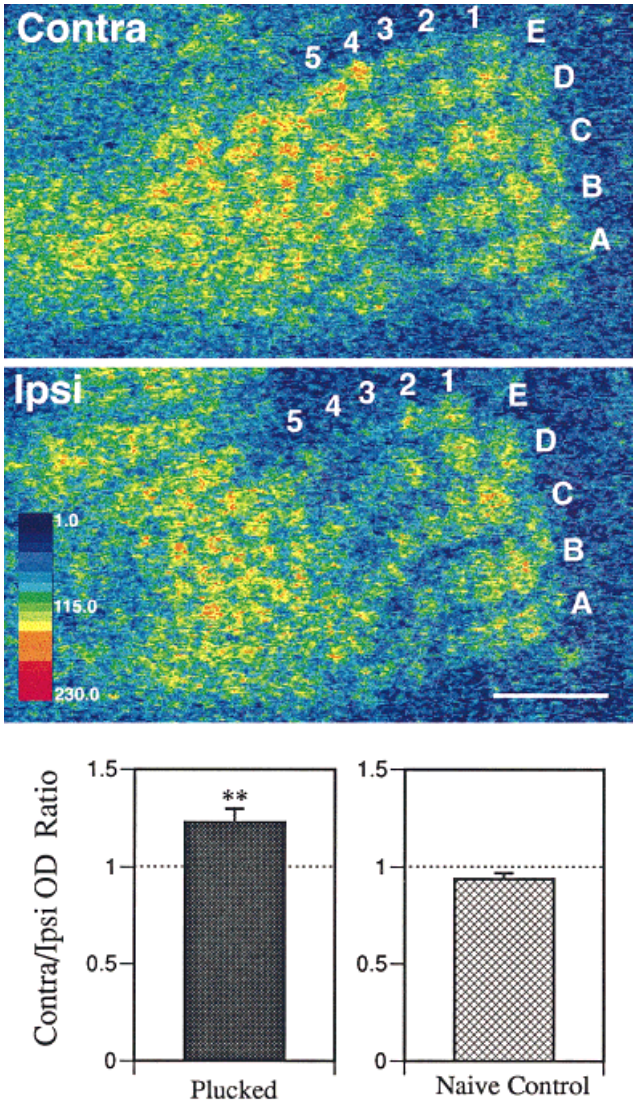

Fig. 2. Levels but not distribution of [^{125}I]α-bungarotoxin (α-BTX) binding in P6 cortices are altered by daily plucking of vibrissae. Digitized pseudocolor images of film autoradiograms of two tangential sections through layer IV of the somatosensory cortex of a P6 animal in which all of the large mystacial vibrissae from the right snout were removed by daily plucking beginning at P0. The upper section is from the left cortex that was contralateral (Contra) to the peripheral manipulation, and the lower section is from the right cortex that was ipsilateral (Ipsi) to the manipulation. These sections were processed in parallel for [^{125}I]α-BTX binding and were exposed to the same piece of autoradiographic film. The pseudocolor scale is identical for the two images, in which red represents the highest optical density (OD), and blue represents the lowest OD. The barrel pattern is seen in both the cortex contralateral and ipsilateral to the side of whisker removal. The rows and arcs are indicated by letters and numbers, respectively. In this example, the increased incidence of yellow and red pixels in the PMBSF in the image from the contralateral cortex indicates increased level of [^{125}I]α-BTX binding. Medial is up, posterior is to the right. For quantitation, OD measurements were obtained using an 80-μm circular transcept tool placed in the central region of barrels B2, B4, D2, and D4 on both the contralateral and the ipsilateral sections. The average paired contralateral vs. ipsilateral OD ratios (histogram) indicate that the contralateral sides were significantly higher than the ipsilateral sides (double asterisks, $P < 0.01$; Student's t-test, paired; n = 17). In contrast, there was no difference in the left/right ratios, determined in P6 naive control animals (Student's t-test, paired; n = 9) in which no experimental manipulation was performed. Bars indicate S.E.M. Images were obtained with NIH Image Software (Bethesda, MD), gray levels were converted to a color scale, and the composite figure was created in Adobe Photoshop (Adobe Systems, Mountain View, CA). All settings for capture and processing were identical for the two images. Scale bar = 500 μm.

enous distribution of [^{125}I]α-BTX binding sites. This suggests that, in both manipulation paradigms, [^{125}I]α-BTX sites are localized primarily on the dendrites of neurons in layer IV of the barrel cortex. However, our level of resolution is not sufficient to determine whether binding sites may change from synaptic to extrasynaptic locations or vice versa.

Peripheral manipulations do not alter the normal developmental change in [^{125}I]α-BTX binding seen at the end of the second postnatal week

There is a dramatic developmental change in [^{125}I]α-BTX binding that occurs during the second postnatal week (Bina et al., 1995). The high levels of binding localized in the barrel centers seen at P6 are replaced by lower levels that are distributed homogeneously throughout layer IV of the barrel cortex at P14. This normal developmental change is likely to reflect a down-regulation in expression of receptors in the dendrites of the layer IV cortical neurons, because the dendrites remain anatomically aggregated in the barrel centers throughout the life of the animal. We were unable to detect any difference in the level of [^{125}I]α-BTX at the end of the second postnatal week between animals subjected to chronic, unilateral sensory deprivation and naive control animals. This suggests that these manipulations did not alter the normal developmental decline in [^{125}I]α-BTX that is apparent by the end of the second postnatal week. The transient nature of this change is reminiscent of activity-dependent regulation of $GABA_A$ receptor binding in the mouse somatosensory cortex (Skangiel-Kramska et al., 1994). Unilateral lesioning of all vibrissae in neonatal mice resulted in a 15% reduction in the levels of [^{3}H] muscimol binding in the deprived cortices at P10. However, when assayed 60 days later, the levels of binding were not different in control and deafferented cortices.

Functional implications

Changes in functional organization of the neocortex, including alteration in the response properties of cortical neurons, following a number of different neonatal sensory deprivation paradigms suggest that activity-dependent plasticity is important in development of cortical circuitry. Neonatal sensory deprivation has been shown to affect neurotransmitter content and receptor number in the rodent somatosensory cortex (for review, see Fuchs, 1995). Components of both the principal excitatory and inhibitory neurotransmitter systems, mediated by glutamate and GABA, respectively, appear to be either permanently or transiently down-regulated in response to decreased activ-

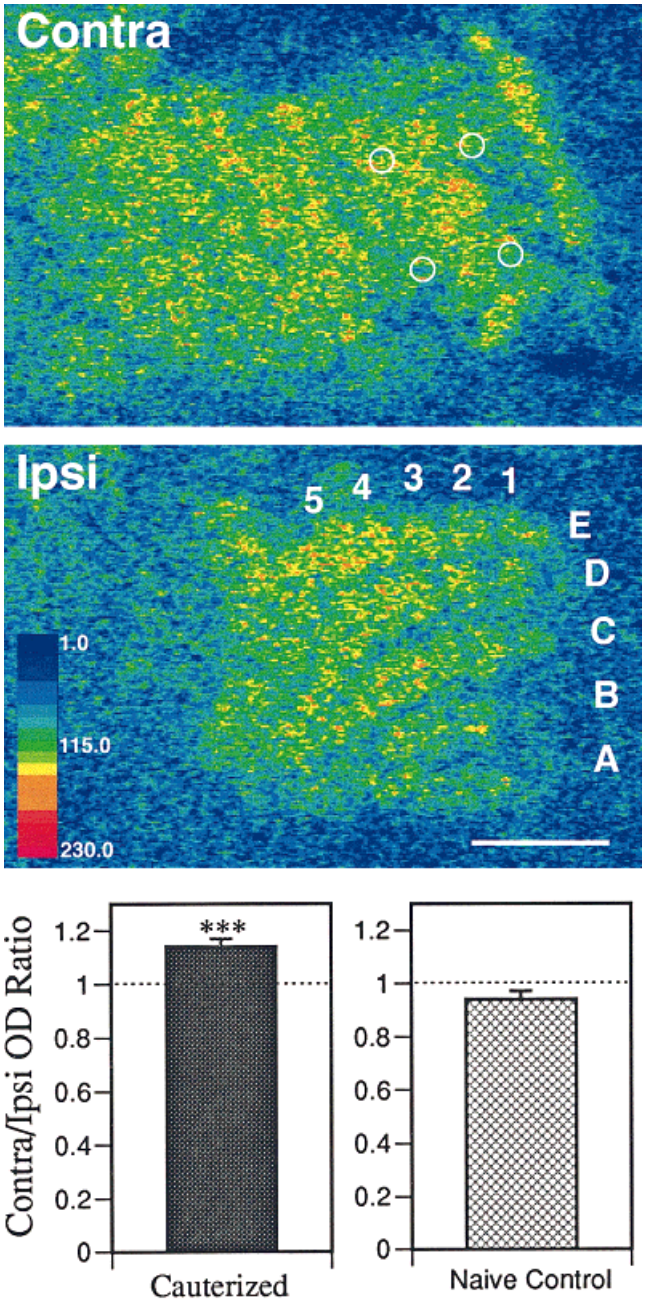

Fig. 3. Levels of [^{125}I] α-bungarotoxin (α-BTX) binding in P6 cortices are altered by cautery at P0. Digitized pseudocolor images of film autoradiograms of two tangential sections through layer IV of the somatosensory cortex of a P6 animal in which all of the large mystacial vibrissae on the right snout were removed by cautery at P0. The top section is through the left cortex that was contralateral (Contra) to the lesion, and the lower section is from the right cortex that was ipsilateral (Ipsi) to the lesion. These sections were processed in parallel for [^{125}I]α-BTX binding, and the pseudocolor scale is identical for the two images, in which red indicates the highest optical density (OD), and blue represents the lowest OD. A barrel pattern is seen in the cortex ipsilateral to the lesioned side, and the rows and arcs are indicated by the letters and numbers, respectively. The [^{125}I]α-BTX binding in the cortex contralateral to the lesion did not outline a clearly defined barrel pattern but showed a distribution that was consistent with the disruptions in barrel boundaries visualized with cytochrome oxydase (CO) staining. Medial is up, posterior is to the right. For quantitation, OD measurements were obtained by using an 80-μm circular transcept tool placed in the central region of barrels B2, B4, D2, and D4 on the ipsilateral sections and in regions roughly corresponding to these same areas in the contralateral sections as indicated by the location of the white circles. The average paired contralateral vs. ipsilateral OD ratios demonstrates that the contralateral side was significantly higher than the ipsilateral side (triple asterisks, $P < 0.01$; Student's t-test, paired; n = 11). The left/right ratios, determined in naive control animals at P6 (n = 9), in which no experimental manipulation was performed, were not significantly different than 1. Error bars indicate S.E.M. Images were obtained with NIH Image Software, gray levels were converted to a color scale, and the composite figure was created in Adobe Photoshop. All settings for capture and processing were identical for the two images. Scale bar = 500 μm.

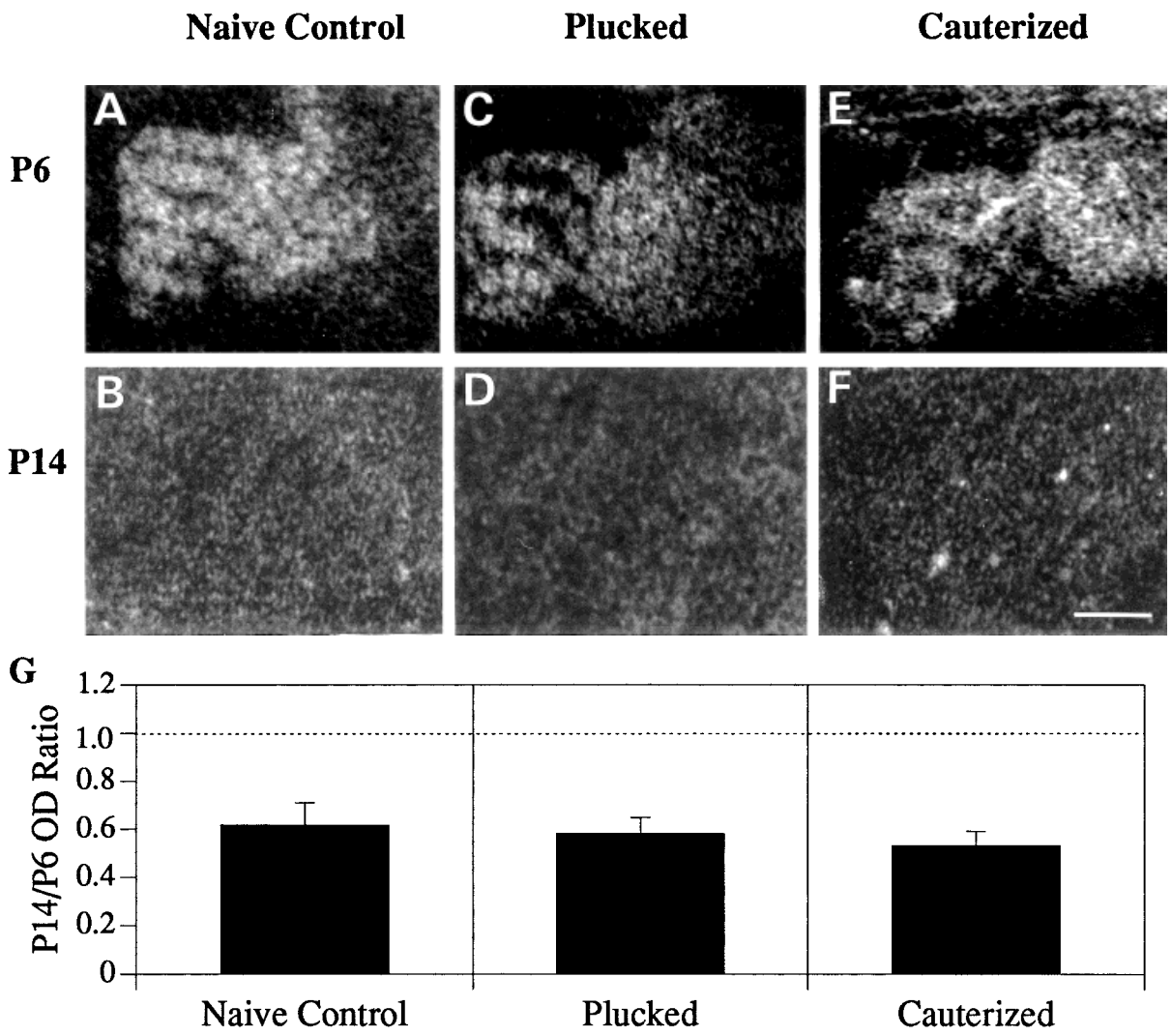

Fig. 4. **A–G:** Developmental regulation of [^{125}I] α-bungarotoxin (α-BTX) binding is not altered by vibrissectomy or cautery. Negative images of film autoradiograms of tangential sections through the left posteromedial barrel subfield (PMBSF) of control animals (A,B) and the contralateral PMBSF of experimental animals (C–F) processed for [^{125}I]α-BTX binding. The distinct barrel-like pattern seen at P6 in control animals (A) and those that had undergone vibrissectomy (C) is replaced by a relatively homogeneous distribution of binding at P14 in both the control (B) and the plucked (D) PMBSF. Although there is not a clear barrel pattern in the PMBSF at P6 following cautery (E), this region is clearly outlined by high levels of [^{125}I]α-BTX binding. The homogeneous distribution of binding sites seen in cauterized animals at P14 (F) is similar to that seen in the control and plucked groups. G: The P14/P6 [^{125}I]α-BTX binding ratio was calculated for control (n = 5), vibrissectomized (n = 6), and cauterized (n = 4) animals. The P14/P6 [^{125}I]α-BTX binding ratio in each of the groups was significantly less than 1 (two-sided t-test of individual means; $P < 0.05$). No significant difference was observed between the three different groups (analysis of variance). Bars indicate S.E.M. Scale bar = 500 μm.

ity in the afferent pathway during early postnatal development. These data suggest that developmental plasticity in the neocortex may be mediated, at least in part, by activity-dependent modulation of these neurotransmitter systems. Although ACh clearly plays a role in modulation of synaptic transmission in the rodent central nervous system (Howard and Simons, 1994; McGehee et al., 1995), the role of ACh mediated by α7 nAChR during early development is less clear. A number of studies have demonstrated that receptors that modulate intracellular calcium levels play a significant role in mediating developmental plasticity (Lipton and Kater, 1989). Data demonstrating that activation of α7 nAChR can lead to an increase in intracellular calcium levels (Vijayraghavan et al., 1992) and altered neurite outgrowth (Pugh and Berg, 1994) in the chick ciliary ganglion lend support to the hypothesis that this receptor may be involved in the regulation of normal developmental changes and in sensory deprivation-induced plasticity. Our previous studies documenting transient expression of α7 nAChRs at high levels on cortical neurons in layer IV of the somatosensory cortex during early postnatal development and the present study showing that the expression of these binding sites are modulated by activity suggest that plasticity mediated by α7 nAChRs may also be important in the development of functional organization in the mammalian neocortex. Future studies utilizing transgenic mice or antisense technology to regulate expression of nAChR in the develop-

ing nervous system will be important for understanding the role of these receptors in cortical development.

ACKNOWLEDGMENTS

We thank Drs. Frances Leslie and Ron Broide for providing expert technical advice on α-BTX-binding protocols. We also thank Drs. A. Agmon and M.A. Smith for critical comments on the paper. D.K.O. was supported by RCDA award NS01854.

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