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AMPA Receptor Subunit Expression in the Cuneate Nucleus of Adult Squirrel Monkeys After Peripheral Nerve Injury

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Abstract

The primate somatosensory system provides an excellent model system with which to investigate adult neural plasticity. Here, we report immunohistochemical staining data for the GluR1 and GluR2/3 AMPA receptor subunits in the cuneate nucleus of adult squirrel monkeys one week after median nerve compression. These data are compared to subunit changes in the area 3b cortex of the same animals. We report differences between control and deprived brainstem implying that deprivation induced changes in subunit expression mirror those reported in the cortex. There are significant increases in GluR1 receptor subunit staining intensity and significant decreases in GluR2/3 receptor subunit staining intensity. This pattern of expression resembles receptor configurations reported in developing sensory systems. Taken together, these results suggest that the brainstem and the cortex initially progress through a phase of developmental recapitulation prior to the onset of NMDA mediated adult somatosensory reorganization.

Keywords

GluR1; GluR2/3; Somatosensory; Reorganization; Developmental Recapitulation; Adult Plasticity

Introduction

Beginning with the seminal work of Merzenich, Kaas and colleagues (14, 15), the adult primate somatosensory system has provided a fertile model for the study of adult neural plasticity and the heuristics that govern it. At the simplest level, the search for neural mechanisms of plasticity have involved attempts to explain the immediate unmasking of novel receptive fields that occur subsequent to nerve transection (18, 12), and the more protracted phases of reorganization that then ensue (14, 11, 2).

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In 1983, Merzenich, Kaas and colleagues reported that cortex that was initially silenced by peripheral nerve injury progressively responded to stimulation of adjacent skin surfaces with intact innervation in the days to weeks following the nerve transection (14,15). Following these studies the vast majority of adult plasticity research conducted in non-human primates focused on the cortex (2, 10,11,16,17,18, 20), with much less attention directed toward subcortical structures (1, 9,17, 23, 24). Regardless the limited number of studies have provided evidence that reorganization is found at all levels of the sensory neuroaxis.

As the field of molecular neuroscience grew, we began to employ the use of these techniques to further enhance the exploration of the mechanisms governing somatosensory reorganization. Autoradiography studies from our lab (12) indicated that while there was an increase in AMPAR density at acute survival durations (~3 days) these trends were not significant. More sensitive immuno-histochemical experiments were then designed to reveal receptor subunit specific changes at survival durations associated with the various phases of reorganization. The first experiment in this series of studies was designed to investigate peripheral nerve injury induced changes to cortical and brainstem AMPAR and GABAR subunit expression just prior to the wide scale onset of reorganization.

We have recently reported that the pattern of AMPA glutamatergic receptor subunit expression changes in cortical area 3b after peripheral nerve injury (16). Specifically, there is an increase in GluR1 subunits and a concomitant decrease in GluR2/3 subunits. We noted that this pattern was comparable to that found early in development, and suggested that deprivation induced cells to transition to a pseudo-developmental state. In a continuation of that study we reported a similar induction of pre-critical period plasticity for the GABA_A, GABABR1a, and R1b subunits for both cortex and cuneate nucleus of the same animals (17).

In the present experiment, we measured the levels of AMPA receptor subunits in the cuneate nucleus of brainstem one week after median nerve compression. This survival duration represents a period of plasticity prior to the prominent activity dependent reorganization of somatosensory cortex ~ 11 days (see 14, 15). The data from the cuneate nucleus are presented in the context of the cortical data (16). We find that the pattern of subunit expression in cuneate nucleus one week after injury parallels the pattern of developmental recapitulation in the cortex of the same animals.

Experimental Methods

We report data from 3 adult squirrel monkeys (*Saimiri sciureus*). Methods for nerve injury, immunohistochemical staining, and data quantification have been previously described in detail (16,17). The median nerve innervating one hand underwent compression injury one week prior to the sacrifice of the subjects (see 22). All procedures were approved by the Indiana University Institutional Animal Care and Use Committee. After seven days of recovery, animals were anesthetized with isoflurane gas and transcardially perfused with cold 0.9% saline solution followed by 400 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brainstem was dissected out and coronal sections were cut (40µm) using a freezing microtome. Sections containing the cuneate nucleus at the level of the pars

rotunda, which contains the glabrous inputs (7,25), were kept for immuno-histochemical staining. Alternating tissue sections were stained using antibodies against GluR1 (1:1000 Chemicon) or GluR2/3 AMPA (1:1000 Affinity Bio Reagents) receptor subunits. These brainstem slices were stained contemporaneously with cortical slices prepared from the same animals. Therefore ratios derived from this brainstem study have been directly compared with ratios derived from the supragranular, granular, and infragranular data from our previous cortical study (16).

The entire hand representation is present in each nucleus, and digit/palm representations were visible to the trained observer (see 7; also see figure 1A/D). Within the region corresponding to the injured median nerve, inputs from the intact nerve representations (ulnar, radial) were in close proximity. In the control region all inputs remained intact. Digit 1 and 3 and proximal regions of all digits remained close to intact inputs. Therefore soma staining intensity quantification was carried out in the more distal regions of the median nerve representation digit 2 in deprived and homologous intact regions of cuneate nucleus pars rotunda (7; also see figure 1D). A 100 μ m by 100 μ m bounding box was placed within the region of interest at low magnification (4 $\times$) and then cell contours were traced at higher magnification (40 $\times$) using the Stereo Investigator software (MBF Bioscience; Williston, VT, USA). Staining intensity measurements were generated using the luminance function (densitometry) in Control and deprived cuneate nuclei. These were present on the same tissue sections and therefore quantification always occurred under the same lighting conditions during a single user session ($\sim$ 30 per section $\times$ 3 sections). A detailed description of quantification procedures is described in a previous study (17).

Results

Figure 1 displays the percentage difference in AMPA receptor subunit staining in the deprived and control regions approximate to digit 2 in the cuneate nucleus of animals that received median nerve compressions 1 week earlier. There were significant increases ($t = 5.69$ $p < .05$) in expression levels for GluR1 in the deprived region of cuneate nucleus (mean intensity $\pm$ SEM, 53.04 ± 1.6) relative to the homologous representation in contralateral cuneate nucleus (mean intensity $\pm$ SEM, 43.71 ± 2.8). Alternatively there were significant decreases ($t = -3.98$ $p < .05$) in the expression levels of GluR2/3 subunit in the deprived region of cuneate nucleus (mean intensity $\pm$ SEM 15.76 ± 4.7) compared to the homologous region in contralateral cuneate nucleus (mean intensity $\pm$ SEM 32.42 ± 2.5).

This significant increase in GluR1 receptor subunit expression was increased by $21.7 \pm 5.0\%$ in the deprived regions of the cuneate nucleus compared to the control cuneate nucleus, which is comparable in magnitude to that found in deprived regions of area 3b 1 week after nerve compression ($26.0 \pm 4.9\%$; see Fig. 2). In contrast, we find a decrease in GluR2/3 receptor subunit expression on the order of $48.7 \pm 11.5\%$ relative to measurements in the control cuneate nucleus. This significant decrease is also in general agreement with our findings in area 3b ($37.4 \pm 1.2\%$; see Fig. 2).

Discussion

AMPA receptor subunit binding data in the cuneate nucleus are presented for 3 squirrel monkeys that survived median nerve compression for one week. We report a significant increase in binding for GluR1 AMPA receptor subunits and a significant decrease in binding for GluR2/3 subunits. Because activity dependent processes are thought to progressively drive reorganization as the animal recovers use of the affected limbs (over 1 to 2 months), these data strongly suggest that the changes we report in the brainstem are not merely a result of top-down influence from the cortex. Rather, the data support the notion that there are mechanisms governing this pre-reorganizational phase of plasticity that are intrinsic to the brainstem.

The pattern of change in binding for GluR1 and GluR2/3 receptor subunits in the cuneate nucleus is quite comparable to that reported previously for area 3b of somatosensory cortex (16). In reporting the cortical data, we suggested that the pattern of change in these receptor subunits resulted in proportions that are reminiscent of early development. Subsequently, we have reported a significant increase in staining of the GABA_BR1a receptor subunit, and a significant decrease in staining of the GABA_BR1b receptor subunit for both brainstem and cortex of the animals used in the current study (17). That shift also resulted in proportions of these two subunits being more characteristic of early development. Developmental recapitulation following nervous system injury has been previously reported following central injury (3) and neuronal plasticity resembling critical period plasticity states has recently been reported in the primary auditory cortex of juveniles and adults (26).

In sum, we find changes in the relative proportions of particular AMPA and GABA_B receptor subunits in the cortex and the brainstem that are consistent with a reversion of the deprived tissues to a pseudo-developmental state of plasticity. This reported developmental stage displays an increased number of calcium-permeable GluR1 AMPA receptors (5,13), decreased levels of mature AMPA receptor subunits, such as GluR2/3, low levels of inhibition achieved by lowered expression of GABA_A (21) and increases in pre-synaptic GABA_B levels (8). This state is also characterized by low levels of the NMDA receptor-inhibiting post-synaptic GABA_B receptors (19).

Conclusion

Because the pattern of changes observed in the cuneate nucleus are similar to those seen in the cortex one week after nerve injury, it is likely that the same mechanisms govern early stages of injury-induced reorganization in both structures. It is important to note that it is impossible to rule out the degree of influence that cortico-fugal inputs might have in this brain region in the current study, but the reorganizing potential from the perspective of intact inputs is greater in the brainstem. Immediate unmasking of latent inputs in the brainstem and thalamus (~ 75% and 50% respectively; 1,9,23) is much greater than that in the cortex (~ 25%; 23) according to the percentage of neurons that express latent receptive fields after nerve injury. The point remains that the brainstem and cortex share a common form of plasticity at this time.

This survival duration is approximate to the onset of wide scale NMDA-mediated somatosensory reorganization. As such, a return to such a pseudo-developmental state leaves the system primed for plasticity in two major ways. First, the inhibitory tone is largely reduced, as evidenced by the lowered levels of GABA_A, decreased expression of post-synaptic GABA_B receptor binding and increased levels of pre-synaptic GABA_B. A system-wide reduction in inhibition affords a higher probability of neuronal depolarization. Second, increased activation of calcium-permeable AMPA receptors allow for synaptic maturation by facilitating the un-silencing of NMDA receptors; the activation of which is necessary for reorganization (Garrahy and Muja, 1996). Together, the pattern of subunit expression in both the cortex and the brainstem seem to indicate that a return to earlier stages of development is a general feature of injury-induced plasticity. Furthermore the use of non-human primates in our study provides a more translational observation concerning central response to peripheral nerve injury. Taken together with our other reports (16,17), it seems that there is a short time window between nerve injury and the pronounced onset of reorganization (~2 weeks) where the system becomes highly plastic. This temporal window would be an ideal target for treatment (eg, 4) and the etiology of neuropathic states that emerge during recovery from peripheral injury (eg phantom pain/sensation) are parsimoniously accounted for by any inappropriate synaptic connections that are primed during this state of heightened plasticity. Finally, whether reorganization is passed forward or happens contemporaneously along the sensory neuroaxis, the cuneate nucleus would provide an easily accessible target for therapeutic intervention.

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Highlights

- GluR1 subunits are significantly increased suggesting increased Homomeric AMPAR
- GluR2/3 subunits are significantly decreased suggesting lower GluR2/3 AMPAR
- This pattern of subunit expression was also found in the somatosensory cortex

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Figure 1.

The changes to GluR1 and GluR2/3 receptor subunit staining within the cuneate nucleus 1 week after nerve injury. A: Photomicrograph of a control section indicating the region of interest for immunohistochemical quantification of GluR1 staining intensity: scale bar .25 mm; cn *cuneate nucleus*; gn *gracile nucleus*. B: Qualitative examples of somatic staining in deprived and control regions of distal digit two. C: Chart showing the quantified differences in GluR1 receptor subunit staining intensity in the control and the deprived regions of the cuneate nucleus for all three animals. Pentagon is centered on the mean. Size of the pentagon indicates SEM; *sig < .05. Shapes represent the individual means for each animal: denoted by animal number. D: Photomicrograph of a control section indicating the region of interest for immuno-histochemical quantification of GluR2/3 staining intensity cn *cuneate nucleus*; gn *gracile nucleus*; Cartoon adapted from (7). E: Qualitative examples of somatic

staining in deprived and control regions of distal digit two: scale bar 25 μm . F: Chart showing the quantified differences in GluR2/3 receptor subunit staining intensity within the control and the deprived regions of the cuneate nucleus. Pentagon is centered on the mean. Size of the pentagon indicates SEM; *sig < .05., Shapes represent the individual means for each animal: denoted by animal number.

Figure 2.

A comparison of changes in receptor subunit staining intensity between the cuneate nucleus and area 3b of the cortex. A: Percent differences of control supragranular, granular, and infragranular cortical layers from deprived layers are compared to the average percent difference of staining intensity between control and deprived brainstem regions. Error bars (SEM).