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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.

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1. Introduction

Beginning with the seminal work of Merzenich et al. [13,14], 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 [11,17], and the more protracted phases of reorganization that then ensue [2,10,13].

In 1983, Merzenich et al. 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 [13,14]. Following these studies the vast majority of adult plasticity research conducted in non-human primates focused on the cortex [2,9,10,15–17,19], with much less attention directed toward subcortical structures [1,8,16,22,23]. 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 [11] indicated that while there was an increase in AMPAR density at acute survival durations (~3 days) these trends were not significant. More sensitive immunohistochemical 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 [15]. 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 [16].

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 [13,14]). The data from the cuneate nucleus are presented in the context of the cortical data [15]. We find that the pattern of subunit expression

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in cuneate nucleus one week after injury parallels the pattern of developmental recapitulation in the cortex of the same animals.

2. 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 [15,16]. The median nerve innervating one hand underwent compression injury one week prior to the sacrifice of the subjects (see [21]). 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 .9% saline solution followed by 400 ml of 4% paraformaldehyde in .1 M phosphate buffer (pH 7.4). The brainstem was dissected out and coronal sections were cut (40 μm) using a freezing microtome. Sections containing the cuneate nucleus at the level of the pars rotunda, which contains the glabrous inputs [6,24], 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 [15].

The entire hand representation is present in each nucleus, and digit/palm representations were visible to the trained observer (see [6]; also see Fig. 1A and 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. Digits 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 ([6]; also see Fig. 1D). A 100 μm by 100 μm bounding box was placed within the region of interest at low magnification (4×) and then cell contours were traced at higher magnification (40×) 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 (~30 per section × 3 sections). A detailed description of quantification procedures is described in a previous study [16].

3. Results

Fig. 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

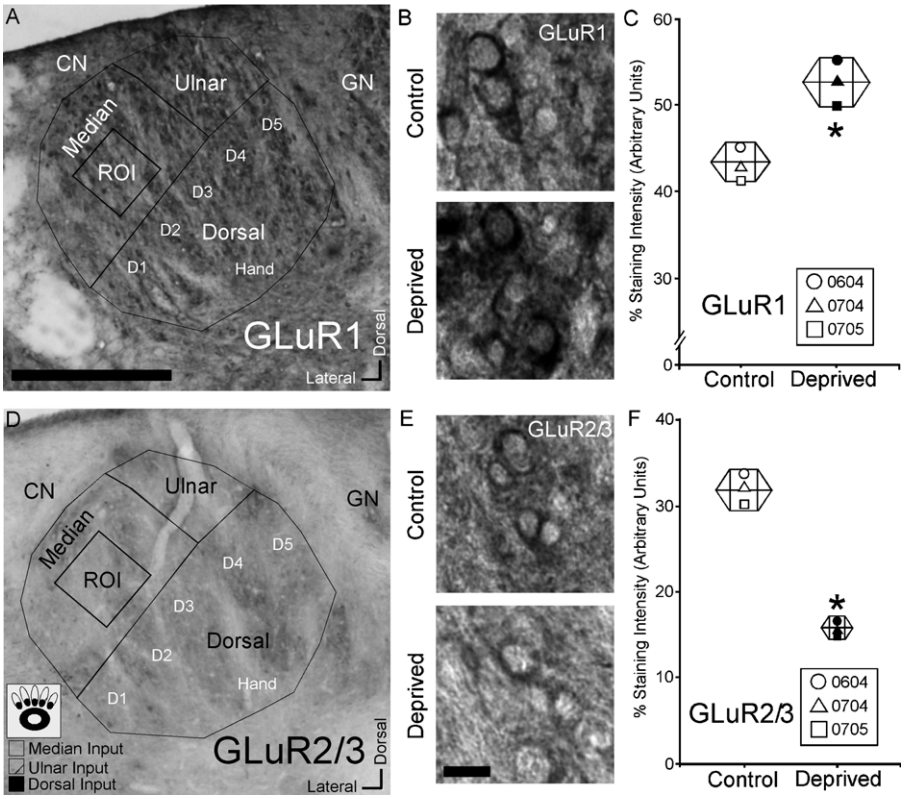

Fig. 1. The changes to GluR1 and GluR2/3 receptor subunit staining within the cuneate nucleus 1 week after nerve injury. (A) Photomicrograph of a control section indicating the region of interest for immunohistochemical quantification of GluR1 staining intensity: scale bar 25 mm; cn *cuneate nucleus*; gn *gracile nucleus*. (B) Qualitative examples of somatic staining in deprived and control regions of distal digit 2. (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 2: 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.