

Research report

Acute reductions in GABA_A receptor binding in layer IV of adult primate somatosensory cortex after peripheral nerve injury

C.L. Wellman, L.L. Arnold, E.E. Garman, P.E. Garraghty*

Department of Psychology, Program in Neural Science, Indiana University, Bloomington, IN 47405, USA

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Abstract

Following peripheral nerve transection, reorganizational plasticity has been reported to occur in two phases, one immediate and one more protracted. GABA (γ -aminobutyric acid) has been implicated in the immediate ‘unmasking’ phase of reorganization. We have used quantitative autoradiography to assess potential changes in GABA_A and GABA_B receptor binding in primate somatosensory cortex following peripheral nerve injury. Here we report reductions in GABA_A receptor binding in layer IV of primate somatosensory cortex deprived of its normal activating inputs for 2–5 h by peripheral nerve transection.

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1. Introduction

Over the past two decades, it has been established that the adult mammalian cortex retains a substantial degree of flexibility with which to respond to alterations in input patterns [11]. One particularly fruitful model for the study of such adult neural plasticity has been to study topographic changes in the primate somatosensory system following various sorts of peripheral deafferentations. In a typical experiment, a well-defined skin surface is disconnected from the ascending somatosensory pathway, and the deprived region of the cortex is studied to determine whether and how it changes in response to the deafferentation. Generally, many of these neurons come to have ‘new’ receptive fields on skin surfaces with intact innervation, either immediately, or within the ensuing days to weeks [12]. We have previously reported that this latter, more protracted phase of reorganizational plasticity does not occur when *N*-methyl-D-aspartate (NMDA) glutamatergic receptors are blocked [8]. In contrast, the immediate,

unmasking phase of reorganization proceeds whether NMDA receptors are blocked or not [13]. We, and a number of others, have suggested that this immediate unmasking is due to a reduction in afferent-driven inhibition [10,13], and reductions in immunostaining for γ -aminobutyric acid (GABA) have been previously reported in monkeys surviving peripheral nerve transections for several months [6]. To more directly evaluate the hypothesis that immediate reorganization results from changes in GABAergic circuits, we have used quantitative autoradiography to assess GABA_A and GABA_B receptor binding in cortical area 3b of adult squirrel monkeys that were sacrificed 2–5 h after paired transections of the median and ulnar nerves. We expected that any changes in binding would most likely involve GABA_A receptors as they are classic ionotropic receptors that would presumably play a larger role in tonic inhibitory circuits than the slower metabotropic GABA_B receptors.

2. Materials and methods

The medial and ulnar nerves were transected in seven adult squirrel monkeys (*Saimiri sciureus*). Monkeys were

*Corresponding author. Tel.: +1-812-855-9679; fax: +1-812-855-4520.

E-mail address: pgarraght@indiana.edu (P.E. Garraghty).

anesthetized with a mixture of ketamine hydrochloride (30 mg/kg) and xylazine (4 mg/kg), with supplemental doses given as necessary to maintain stage III, plane 2 level of anesthesia [4]. A longitudinal incision was made through the skin of the ventral forearm under aseptic conditions. Using a dissection microscope, the median and ulnar nerves were located by blunt dissection, separated from the surrounding tissue, and transected. Animals were then maintained under anesthesia for 2 ($N = 2$) to 5 h ($N = 5$) before processing for quantitative autoradiography. In addition, six unoperated adult squirrel monkeys served as intact controls.

For quantitative autoradiography, all animals were deeply anesthetized with either ketamine/xylazine or sodium pentobarbital and decapitated. Brains were rapidly removed and somatosensory cortex was dissected, frozen in dry ice, and stored at -70°C until sectioning. For each animal, 16 coronal sections through somatosensory cortex were cut at $14\ \mu\text{m}$ on a cryostat and thaw-mounted on chrome–alum gelatin-coated slides. GABA_A receptors were labeled with [³H]muscimol using a procedure similar to that of Xia and Haddad [19]. Sections were rinsed 30 min at room temperature in 50 mM Tris/Citrate (pH 7.0). To assess total binding, sections were incubated 45 min at 4°C in 50 mM Tris/Citrate (pH 7.0) plus 50 nM [³H]muscimol (17.5 Ci/mmol; NEN, Boston, MA); non-specific binding was assessed by incubation with the tritiated ligand plus 100 μM unlabeled GABA (RBI, Natick, MA). After five 2-s rinses in ice-cold 50 mM Tris/Citrate (pH 7.0), sections were dipped once in ice-cold dH₂O.

To label GABA_B receptors, slide-mounted sections were incubated for 30 min at room temperature in 50 mM Tris–HCl, pH 7.4, containing 2.5 mM CaCl₂ and 50 nM [³H]GABA (93.2 Ci/mmol; NEN, Boston, MA, USA). To block binding of GABA to GABA_A receptors, 40 μM unlabeled isoguvacine (RBI, Natick, MA, USA) was added to this buffer. Adjacent sections were incubated with the tritiated ligand, unlabeled isoguvacine, and 200 μM un-

labeled GABA (RBI, Natick, MA, USA) to assess non-specific binding (Pratt & Bowery, 1993, [14]). Sections were rinsed 3 times (3 s each) in 50 mM Tris–HCl, pH 7.4 and then dipped once in dH₂O. All sections were then dried, fixed in paraformaldehyde vapors, and opposed to film (³H Hyperfilm; Amersham Pharmacia Biotech, Little Chalfont) for 4 weeks. Films were developed (Kodak D-19), fixed (Kodak Fixer), and air dried. Slides were stained with thionin.

Density of receptor binding in somatosensory cortex was quantified using a computer-based image analysis system (MCID; Imaging Research, St. Catharines, Ontario, Canada). Histological slides and corresponding autoradiograms were placed on a light box (Imaging Research), digitized, and aligned. Regions of interest were then defined on the histological sections and samples taken from the corresponding areas of the autoradiograms. For each animal, average optical density was measured in layers II–III, IV, and V–VI (identified with standard morphological criteria of neuronal cell type and packing density) of the hand and hindlimb representations in area 3b of somatosensory cortex [18] in each section (see Fig. 1). Samples from the hand representation were taken immediately lateral to the central sulcus and extending no more than 1.5 mm laterally, based on previous electrophysiological data [6,8], while samples from the hindlimb representation were taken immediately medial to the central sulcus and extending no more than 1.5 mm medially. Measures were standardized against autoradiographic microscales (Amersham) included on each film and expressed in fmol/mg wet tissue weight. Specific binding was calculated by subtracting nonspecific from total binding for each pair of sections, and average specific binding in hand and hindlimb representations was computed for each animal. Finally, to control for differences in overall amounts of binding across animals, the ratio of binding in the hand representation to binding in the hindlimb representation was computed for each animal. Ratios in somatosensory cortex contralateral to the nerve transections were

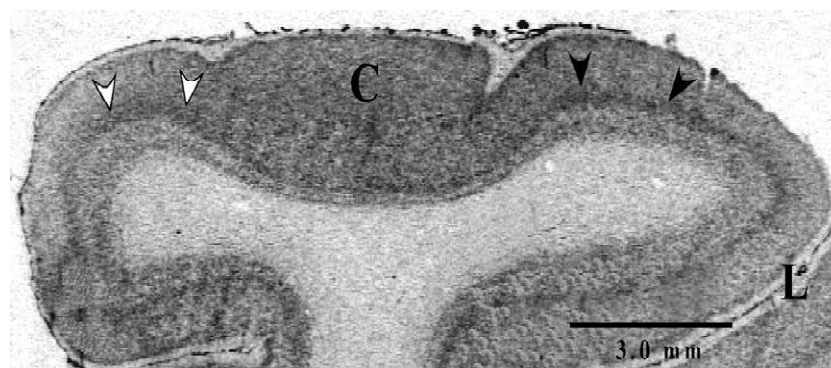


Fig. 1. Samples from the hand representation were taken immediately lateral to the central sulcus (C) and extending no more than 1.5 mm laterally (dark arrows), while samples from the hindlimb representation were taken medial to the central sulcus (white arrows). L indicates lateral sulcus. $5\times$ magnification.

compared to ratios averaged across the two hemispheres of the control monkeys.

3. Results

Consistent with previous reports, nonspecific binding of [^3H]muscimol was minimal, averaging 12% [3], while nonspecific binding to GABA_B receptors averaged 18% [14]. The laminar distribution of binding to both GABA_A and GABA_B receptors was similar to that previously documented in sensory cortical areas of primates [15,16] in that binding for both receptor sub-types was highest in superficial layers of area 3b (Fig. 2a and b). Also consistent with previously reported data [16], we found

GABA_B receptor binding to be substantially lower than GABA_A receptor binding across all cortical layers.

Fig. 3 presents the binding ratios (hand/hindlimb) for GABA_A (Fig. 3a) and GABA_B (Fig. 3b) receptors in layer IV, and in upper and lower cortical layers of area 3b in the nerve-transected and the intact control monkeys. Systematic reductions were found in binding for both receptor sub-types in the nerve-transected monkeys relative to controls (sign test; $P \leq 0.0156$). Individual comparisons, however, find that the only conservatively reliable difference is for the roughly 20% reduction in GABA_A receptor binding in layer IV (t -test = -2.55 ; $P < 0.05$). Because 2 animals survived nerve transection for 2 h while the remaining 5 had 5-h survival durations, they were compared separately with the control animals and with each

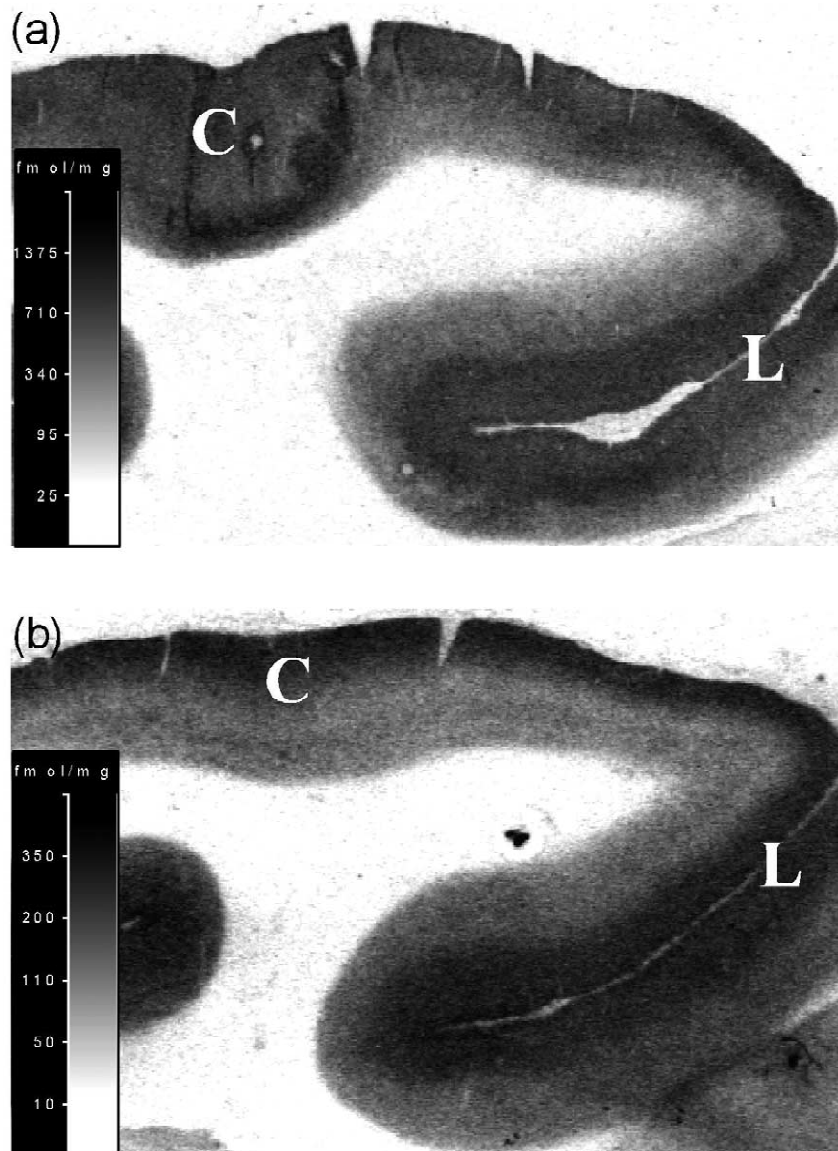


Fig. 2. Digitized autoradiograms of GABA_A (a) and GABA_B (b) receptor binding in area 3b of an intact control monkey. Consistent with previous reports, both GABA_A (a) and GABA_B (b) receptor binding were highest in superficial layers of area 3b. Also consistent with previously reported data, GABA_B receptor binding is substantially lower than GABA_A receptor binding across all cortical layers. C, central sulcus; L, lateral sulcus.

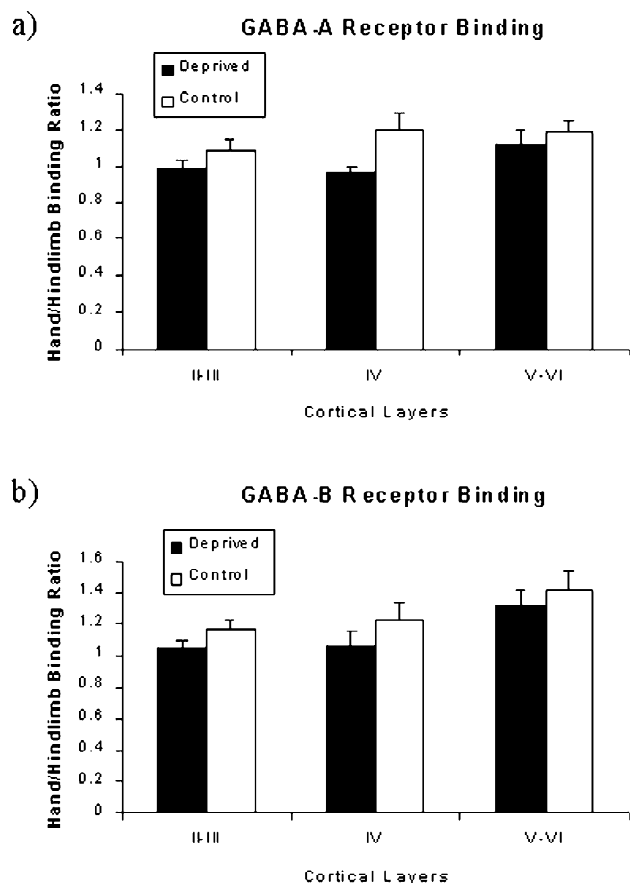


Fig. 3. Mean (\pm S.E.M.) GABA_A (a) and GABA_B (b) receptor binding in layers II–III, IV, and V–VI of the hand representation in cortical area 3b in nerve-transsected and control monkeys, expressed as ratios of hand/hindlimb binding ratios. There is a consistent reduction for both receptor subtypes across all cortical layers, but the reduction in GABA_A binding in layer IV is the most statistically reliable change.

other. The animals surviving nerve injury for 5 h were significantly different from the controls (t -test = -2.58 ; $P < 0.05$), but they did not differ significantly from the animals with 2-h survivals.

4. Discussion

Data from the present experiment reveal a decline in both GABA_A and GABA_B receptor binding in a region of area 3b of squirrel monkey somatosensory cortex that had been deprived of its primary activating inputs for 2–5 h. While this pattern of results might reflect real changes in both receptor subtypes across all layers of somatosensory cortex [7], a more rigorous statistical assessment of these changes suggests that the reduction in GABA_A receptor binding in layer IV is of sufficient magnitude to generate a much higher level of statistical confidence. For this reason, we confine our discussion to layer IV GABA_A receptors.

Several papers have reported reductions in GABA, its

synthesizing enzyme glutamate decarboxylase (GAD), and GABA_A receptors following adult-onset sensory loss [11]. Perhaps the most dramatic demonstrations have been in primary visual cortex of primate, where such changes are evident within 3–4 days of the imposition of deprivation. At least for GAD, it appears that the change is due to post-translational mechanisms, as reductions in GAD are detectable 10–11 days before changes in GAD mRNA levels are demonstrable [11]. The present results represent the first demonstration of significant reductions in GABA receptor binding in primate somatosensory cortex within hours of peripheral nerve transection. As such, they demonstrate quite dramatically the extent to which the somatosensory cortex is sensitive to alterations in the patterns of peripheral sensory inputs, and the rapidity with which this sensitivity can be expressed.

The initial, ‘unmasking’ phase of reorganizational plasticity in central somatotopic representations that follows peripheral sensory manipulations has been assumed to reflect a release from inhibition. This assumption arose originally because of the expansions in the receptive field sizes of many somatosensory cortical neurons found with the blockade of GABA_A receptors [1,5]. The revelation of these presumptive subthreshold inputs with the relaxation of intracortical inhibition clearly mirrored the immediate unmasking of novel receptive fields after peripheral nerve injury [12], and subsequent work demonstrated that the thalamocortical projections are sufficiently broad to support the idea that inputs are available to the cortex that are not typically expressed in the absence of injury or pharmacological manipulation [9]. Moreover, the existence of these putative latent inputs can be revealed with supra-threshold activation of the non-dominant peripheral afferents [17]. These studies together are clearly consistent with the idea that the anatomical and functional infrastructure of the intact somatosensory cortex can account for the immediate unmasking of novel receptive fields without the need to appeal to rapid changes in gross neuronal morphology. The present results demonstrate that reductions in GABA_A receptor number and/or affinity in layer IV of area 3b could well contribute to a relaxation of inhibition in deprived cortex, and, thus, to the immediate expression of novel receptive fields after peripheral nerve injury.

The work of Jones and colleagues [11] implies that a measurable genetic response to nerve injury becomes evident in the cortex only after a number of days of deprivation. If one assumes the same to be the case for area 3b after peripheral nerve injury, the reduction in layer IV GABA_A reported here would necessarily arise from post-translational processes, just as the measurable reductions in GAD in primate visual cortex precede detectable changes in mRNA. In this view, local (i.e., extra-nuclear) regulatory mechanisms, sensitive to the changes in patterns of activity caused by the nerve transections, are responsible for the early reduction in GABA_A receptor binding in layer IV, perhaps via an internalization of the receptors. In any

event, this response is large and rapid, and topographically confined.

5. Summary

Using quantitative autoradiography, we have shown that GABA_A receptor binding in layer IV of adult squirrel monkey area 3b is reduced in deafferented cortex within 5 h of peripheral nerve injury. It has been conjectured by many that the immediate reorganizational changes in cortical topography that follow peripheral nerve transections must be due to a relaxation in inhibition, permitting the expression of tonically-suppressed (i.e., latent) inputs. The present results constitute the first demonstration of a change in cortical inhibitory circuits occurring so quickly after deafferentation, and provide support for the contention that the immediate, unmasking phase of cortical reorganization is due to a release from inhibition of the newly expressed inputs. That receptor function can be so quickly adjusted following an alteration in the patterns of inputs to a region of the cortex underscores the exquisite sensitivity and responsiveness of the cortex to such manipulations. It is interesting to speculate that comparable changes might also be found in primate somatosensory cortex after training-related changes in topography, e.g., see [2].

Acknowledgements

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