Denervation facilitates neuronal growth in the motor cortex of rats in the presence of behavioral demand

Scott D. Bury, DeAnna L. Adkins, Jay T. Ishida, Chris M. Kotzer, Alethea C. Eichhorn, Theresa A. Jones

Abstract

This study tests the hypothesis that degeneration of a neocortical pathway may facilitate behaviorally-induced growth of neurons in a connected region of the cortex. Degeneration of transcallosal afferents to the motor cortex and changes in forelimb use were independently manipulated in adult rats. The combination of degeneration and behavioral change resulted in the growth of layer V pyramidal neuron dendrites which was not found as a result of either denervation or behavioral manipulation alone. These results indicate that mild degeneration in the adult brain can facilitate neuronal growth when accompanied by appropriate behavioral demand, a finding which has implications for rehabilitative therapy after brain damage.

Keywords: Denervation; Motor cortex; Recovery of function; Behavioral compensation; Collosotomy; Dendritic arborization; Structural plasticity; Layer V pyramidal neurons

Previous research has indicated that injury in the adult brain can lead to dramatic and long-lasting neuronal changes distant, but connected to, the site of damage [2,6]. Denervation resulting from damage to an afferent structure has been found to trigger a cascade of growth promoting processes including increases in neurotrophic factors, reactive changes in glial cells, neuronal cytoskeletal restructuring and reactive synaptogenesis [2,4,6,12,14]. These changes are typically considered in terms of their capacity to enhance a brain region’s adaptation to a partial loss of input. However, these changes may also provide an exceptionally fertile template on which behavioral change might shape new patterns of connections. If so, this raises new possibilities for the use of behavioral manipulations as therapy for the treatment of functional impairments following brain damage.

Recent research has provided indirect evidence in support of the hypothesis that lesion-induced degenerative events may provide a permissive substrate for behaviorally-induced change. Unilateral lesions of the forelimb representation area of the sensorimotor cortex (FLsmc) in adult rats lead to sensorimotor impairments of the contralateral forelimb and a hyper-reliance on the ‘intact’ (ipsilateral-to-the-lesion) forelimb [8,9]. The FLsmc opposite the intact forelimb showed time-dependent increases in cortical volume, layer V dendritic arborization and synapse number per neuron [7,8]. These changes were found to be dependent on the forelimb behavioral changes induced by unilateral FLsmc lesions [9] and were enhanced by post-operative training on a complex motor skills task [5]. However, manipulations in intact rats that peripherally mimicked the events of the lesion were not sufficient to reproduce the neuronal changes found after cortical damage [9]. Thus, it appears that behavioral demand is necessary, but not sufficient to reproduce the neuronal growth observed opposite unilateral FLsmc lesions. This raises the possibility that lesion-induced degeneration together with relevant changes in behavior is sufficient to enhance neuronal growth.

To directly test this hypothesis that degeneration together with changes in behavior is sufficient to enhance neuronal growth, degeneration and behavioral change must be independently manipulated. In the present study, degeneration of transcallosal afferents was produced by transections of the rostral two-third’s of the corpus callosum (Fig. 1). This damage can be expected to cause degeneration of a moderate portion of the axonal terminals supplied to the sensor-
imotor cortex but does not result in behavioral asymmetries nor overt impairments in the use of the forelimb. Changes in forelimb use were then induced in transected- and sham-operated rats using vests which forced reliance on a single forelimb or, as a control, permitted unrestricted use of both forelimbs (Fig. 2). After 18 days, the dendritic arborization of layer V pyramidal neurons was measured in the motor cortex opposite the forced-use or unrestricted limb. To assess the specificity of the effects, dendritic changes were also quantified in the granular insular cortex, which is responsive to thermal stimulation of the tongue [10] and has no known motor function related to the forelimb.

Thirty-five adult (3–4.5 month-old) male Long–Evans hooded rats were used. Rats were housed in pairs, given food and water ad libitum, and kept on a 12:12 h light-dark cycle. Animal use was in accordance with a protocol approved by the Animal Care and Use Committee of the University of Washington. The following conditions were used: (1) Callosal transections + forced limb use (Degen + Use), (2) Callosal transections + no forced limb use (Degen + Cont), (3) Sham operation + forced limb use (Sham + Use), (4) Sham operation + no forced limb use (Sham + Cont).

Corpus callosum transections were produced using an epoxy-coated platinum wire with an exposed tip as described previously [1]. The electrode was positioned 1.5 mm posterior to Bregma at midline, the sagittal sinus was gently retracted to one side (opposite the ‘side of approach’) and the electrode was lowered into the corpus callosum to a depth of 4.7 mm below dura. 0.5 mV anodal current was applied for 9 s while the electrode was moved rostrally towards Bregma. The electrode was then raised 1.0 mm and moved 1.0 mm rostrally during 6 s of current application. Selection of a side of approach minimized cortical damage associated with the electrode tract in the measured cortex. Subjects with lesions that extended lateral from midline into motor cortex (n = 1) or that failed to damage corpus callosum (n = 1) were removed from the study.

To test for possible confounding forelimb asymmetries resulting from the lesion or the control vests, rats were videotaped pre and post-operatively (days 1, 7 and 18) during exploratory movements in a transparent cage. Forelimb-use observations were recorded during slow-motion playbacks, including use of forelimb(s) for upright support against cage walls, to push off the floor during rearing, and to land on when descending from a reared position, as described previously [9]. Preoperatively, there were no clear asymmetries in forelimb use in these behaviors for any group.

At day 18, animals were perfused and brains were impregnated using Golgi–Cox to visualize dendritic processes. Day 18 has previously been found to be a time point of forelimb behavior-linked increases in dendritic branching following injury to the opposite sensorimotor cortex [8]. Dendritic increases in motor cortex revealed using this Golgi–Cox impregnation procedure have been found to correspond to increases in dendritic volume and membrane surface area per neuron, revealed using electron microscopy, and to increases in synapses [7]. Golgi staining provides much more sensitive analyses of dendritic growth than electron microscopy because it permits the dendrites of identified neuronal populations and subsets of the dendritic tree to be selectively analyzed. Animals were anesthetized with sodium pentobarbital and perfused intracardially with 400 ml 0.1 M phosphate buffer (PB) and 50–100 ml 4% paraformaldehyde in PB. Brains were removed and placed in Golgi–Cox solution for 5 weeks, developed en bloc and resin embedded. Coronal sections (200 μm) were visualized at 785X magnification.

The motor cortex (forelimb region) was located using cytoarchitectural and macrostructural landmarks and ten...
layer V pyramidal neurons per brain were randomly chosen as previously described [9]. Within the same sections in which motor cortex was sampled, five layer V neurons from the granular insular cortex were sampled. Measurements were taken from neurons that were not near the outer faces of the section and that were not overly obscured by astrocytes or blood vessels. Neurons were judged to be completely impregnated if there were no signs of beading or gaps in dendrites or incompletely labeled cell bodies. Brains that showed signs of poor impregnation were removed from the study (n = 3). Centrifugal ordering was used to count basilar branch points (Fig. 3A) where the first branches arising from the soma are designated as first order, the next branch points arising from this process is order 2, and so forth, as described previously [16]. SAS general linear model procedure for Contrasts was used to perform planned comparisons of the following: Degen + Use vs. Degen + Cont, Degen + Use vs. Sham + Use, Degen + Cont vs. Sham + Cont and Sham + use vs. Sham + Cont.

Anterograde tracers were used to verify that transections effectively severed transcallosal afferents of the sensorimotor cortex. Rats received either a transection or sham operation (n = 4, each group) as described above. Four days later, rats were reanesthetized with equithesin. A micropipette filled with the anterograde tracer biotinylated dextran amine (BDA, 10% in 10% sterile saline, Molecular Probe) was lowered to a depth of 1.5 mm below dura into the approximate center of the FLsmc. One microlitre of BDA solution was slowly injected with a microsyringe and allowed to diffuse for 5 min before pipette withdrawal. Animals were anesthetized 14 days later and perfused intracardially with 0.1 M PB and 4% paraformaldehyde in PB (approximately 400 ml each solution). Brains were post-fixed overnight and 50 μm sections were cut using a vibratome. Reaction product was visualized using 3-3′ diaminobenzidine (DAB) with cobalt intensification.

Fig. 3C shows the mean number of basilar dendritic branch points per neuron per rat for each condition. Subjects with callosal transections plus behavioral demand (Degen + Use) had a significant increase in the total number of layer V pyramidal dendritic branch points in comparison to both Degen + Cont (F(1, 26) = 3.93, P < 0.001) and to Sham + Use (F(1, 26) = 4.43, P < 0.001). This increase occurred primarily in topologically higher-order branches (3rd through 6th; Fig. 3D). In contrast, animals with degeneration of transcallosal fibers but no change in forelimb behavior (Degen + Cont) showed no significant increase in branch points in comparison to control animals (Sham + Cont). Furthermore, forced forelimb use in the absence of transcallosal degeneration (Sham + Use) did not result in a significant increase in arborization of this population of dendrites in comparison to Sham + Cont. Within the granular insular cortex, no significant group differences in total branch points were found. The mean ± SEM branch points per neuron were 17.97 ± 0.51 for Degen + Use, 17.72 ± 0.36 for Degen + Cont, 16.92 ± 0.56 for Sham + Use and 19.06 ± 1.13 for Sham + Cont.

Behavioral measurements of forelimb use verified that callosal transections and control vests did not result in forelimb asymmetries. For the first 2 weeks post-injury the mean % use of the forelimb ipsilateral to the side of approach of the transections was 48.03 ± 17.11 for Degen + Cont and 48.24 ± 3.53 for Sham + Cont. There were no significant differences between Degen + Cont and Sham + Cont in forelimb-use. For subjects in forced-use vests, the forelimb used was always the unrestricted forelimb.

BDA tract tracing verified that corpus callosum transections resulted in a depletion of transcallosal projections between the two hemispheres (Fig. 1B,C). In sham-operated animals many darkly labeled fibers descended from the injection site, traveled through the corpus callosum and appeared to terminate in the deep through superficial layers of the contralateral homotopic cortex. Following corpus callosum transections, only a few labeled fibers were seen in the contralateral motor cortex.

In summary, degeneration of transcallosal afferents induced by transections of the corpus callosum in combination with increased behavioral demand resulting from forced forelimb use was sufficient to produce extensive increases in the basilar dendritic arborization of layer V pyramidal neurons in the
foredritic branches of the primary motor cortex. The increase in dendritic branches resulted primarily from a sprouting of new, topologically distal from the soma, dendritic segments consistent with previous findings of the pattern of behaviorally-related dendritic growth [15]. In contrast, forced-use alone and denervation alone did not result in a quantitative change in this population of dendrites in comparison to sham-controls. Finally, the failure to find dendritic increases in the granular insular cortex of the same sections suggests that the effects of the behavioral manipulation may be specific to regions involved in the behavioral change. These data suggest that mild axonal degeneration, resulting from severing of a subset of neocortical afferents, can facilitate dendritic growth in the neocortex in the presence of relevant changes in behavior.

Numerous cellular changes follow denervation, including increases in neurotrophic factors, reactive changes in astrocytes, synapse addition (‘replacement’) and restructuring of dendritic processes [2,4,6,12,14]. Corpus callosum transections have previously been found to result in increased neocortical astrocytic expression of basic fibroblast growth factor (FGF-2) [1,3]. In developing cortex, neurotrophin induced dendritic growth requires appropriate coincident neural activity [11]. Recently, Bury et al. [1] have found that the astrocytic responses to denervation in the motor cortex can be influenced by behavioral demand. The surface density of glial fibrillary acidic protein (GFAP) immunoreactive (IR) astrocytic processes and density of FGF-2-IR astrocytes in layer V of the motor cortex was increased 8 days following either corpus callosum transections or the onset of forced-use of the opposite forelimb in intact animals. The combination of denervation and behavioral demand lead to further increases in GFAP-IR, but not FGF-2-IR. These data suggest that the astrocytic reactions to axonal degeneration can be shaped by relevant behavioral demand, and it is possible that these changes facilitate the neuronal growth seen in the current study.

The present findings raise the need to understand the interaction between degeneration and behavioral change at the cellular level and to assess the role of other injury-induced changes, such as excitotoxicity, in these effects. It seems plausible to hypothesize that the cellular changes initiated by denervation create a fertile environment which, when coincident with behaviorally-induced changes in neural activity, can lead to a neuronal growth and restructuring of a magnitude which is, perhaps, not normally possible in the adult neocortex.

These results could have important implications for physical rehabilitation in persons suffering from stroke or other degenerative disorders, where understanding the interactions between degeneration, cellular restructuring and behavioral influence could lead to more effective rehabilitative treatments. Because denervation induced changes, such as reactive synaptogenesis, have a time course [2,13], capitalizing on these processes by using behavior as therapy may require that manipulations be done during appropriate time-windows following injury.

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