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Cortical sensory map rearrangement after spinal cord injury: fMRI responses linked to Nogo signalling

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Cortical sensory maps can reorganize in the adult brain in an experience-dependent manner. We monitored somatosensory cortical reorganization after sensory deafferentation using functional magnetic resonance imaging (fMRI) in rats subjected to complete transection of the mid-thoracic spinal cord. Cortical representation in response to spared forelimb stimulation was observed to enlarge and invade adjacent sensory-deprived hind limb territory in the primary somatosensory cortex as early as 3 days after injury. Functional MRI also demonstrated long-term cortical plasticity accompanied by increased thalamic activation. To support the notion that alterations of cortical neuronal circuitry after spinal cord injury may underlie the fMRI changes, we quantified transcriptional activities of several genes related to cortical plasticity including the Nogo receptor (NgR), its co-receptor LINGO-1 and brain derived neurotrophic factor (BDNF), using *in situ* hybridization. We demonstrate that *NgR* and *LINGO-1* are down-regulated specifically in cortical areas deprived of sensory input and in adjacent cortex from 1 day after injury, while *BDNF* is up-regulated. Our results demonstrate that cortical neurons react to sensory deprivation by decreasing transcriptional activities of genes encoding the Nogo receptor components in the sensory deprived and the anatomically adjacent non-deprived area. Combined with the BDNF up-regulation, these changes presumably allow structural changes in the neuropil. Our observations therefore suggest an involvement of Nogo signalling in cortical activity-dependent plasticity in the somatosensory system. In spinal cord injury, cortical reorganization as shown here can become a disadvantage, much like the situation in amblyopia or phantom sensation. Successful strategies to repair sensory pathways at the spinal cord level may not lead to proper reestablishment of cortical connections, once deprived hind limb cortical areas have been reallocated to forelimb use. In such situations, methods to control cortical plasticity, possibly by targeting Nogo signalling, may become helpful.

Keywords: functional magnetic resonance imaging; spinal cord injury; Nogo receptor; plasticity; cortical reorganization

Abbreviations: BDNF = brain-derived neurotrophic factor; BOLD = blood oxygenation level-dependent; fMRI = functional magnetic resonance imaging; MAP = mean arterial pressure; NgR = Nogo receptor

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Introduction

Structural rearrangements in CNS neuropil (Raisman, 1969; Cotman *et al.*, 1973) underlie reorganization of cortical sensory maps in the adult brain in response to sensory deafferentation (Florence and Kaas, 1995; Florence *et al.*, 1998). Following peripheral nerve injury, cortical receptive fields with intact inputs expand into deprived territory in primary somatosensory cortex very soon after deafferentation (Merzenich *et al.*, 1983; Kolarik *et al.*, 1994). To allow such efficient cortical reorganization, reduction of γ-aminobutyric acid (GABA)-mediated inhibition has been proposed (Hendry and Jones, 1986) because of the role of GABAergic inhibition in shaping receptive fields in somatosensory cortex (Dykes *et al.*, 1984).

Recently, we demonstrated down-regulation of Nogo receptor (NgR) to be in register with increased neuronal activity, including motor learning (Josephson *et al.*, 2003), implicating NgR as an important regulator restricting experience-dependent plasticity in hippocampus and cortex. In agreement with this, it has been shown that ocular dominance shift plasticity remains into adulthood in NgR knockout mice (McGee *et al.*, 2005). Expression of

both Nogo and NgR mRNA in adult neurons (Fournier *et al.*, 2001; Josephson *et al.*, 2002), and NgR protein in axons (Wang *et al.*, 2002) also suggest a potential role for NgR in synaptic plasticity.

To test a possible link between functional plasticity and regulation of Nogo signalling in the adult mammalian CNS, we studied cortical responses to spinal cord injury in rats. Since this will cause sensory deprivation from levels caudal to injury, a process of reorganization was expected, in which cortical responses to forelimb stimulation would expand into deafferented hind limb territory of primary somatosensory cortex following thoracic spinal cord injury. To monitor such sensory reorganization in somatosensory cortex, we performed functional magnetic resonance imaging (fMRI) using blood oxygenation level-dependent (BOLD) contrast to map changes of cortical neuronal activation in response to forelimb stimulation (Ogawa *et al.*, 1990; Kwong *et al.*, 1992; Mukamel *et al.*, 2005).

Having established plastic changes of cortical BOLD responses to forelimb stimulation after spinal cord injury, we next monitored transcriptional activity of NgR regionally in somatosensory cortex following the selective silencing of input to hind limb sensory cortex using quantitative *in situ* hybridization, and compared such changes to the changes of BOLD responses. To obtain further evidence for an involvement of changes of Nogo signalling in cortical plasticity, we similarly monitored LINGO-1 (leucine-rich repeat and Ig domain-containing, Nogo receptor-interacting protein), an essential component of the Nogo receptor complex (Mi *et al.*, 2004). We hypothesized that down-regulation of the Nogo receptor components should correlate in space and time with cortical plasticity, and that increased transcriptional activity of brain-derived neurotrophic factor (BDNF), a key signalling molecule in synaptic plasticity events, should occur simultaneously (McAllister *et al.*, 1999; Bramham and Messaoudi, 2005).

Materials and methods

Complete transection of the spinal cord

Adult female Sprague-Dawley rats (Scanbur BK, Sollentuna, Sweden) weighing 230 ± 10 g, received complete transection of the spinal cord at T9. The spinal cord was completely cut and a 1 mm segment of the spinal cord was removed. Immediately after the cut, gelfoam (Spongostan®, Johnson & Johnson AB, Sollentuna, Sweden) was inserted into the lesion and left in the gap to prevent further bleeding. Muscle and skin were sutured separately. Urinary bladders were manually emptied twice daily during the first week and once daily thereafter. Experiments were approved by the Stockholm Animal Ethics Committee.

Behavioural testing

All animals were tested once a week in the first month and every other week thereafter for open field walking using the Basso, Beattie and Bresnahan (BBB) locomotor rating scale (Basso *et al.*, 1995). Hind limb function was scored from 0 to 21(flaccid paralysis to normal gait). An average of BBB scores for the animals used in this study was 0.39 ± 0.10 ($n = 61$, mean ± SEM) at end points.

Functional MRI

Rats subjected to spinal cord transection underwent brain fMRI 3 days ($n = 13$), 1–2 weeks ($n = 13$), 1–2 months ($n = 14$) and 3–6 months ($n = 13$) after injury. We started fMRI at 3 days, since spinally transected animals were considered too unstable physiologically to undergo fMRI experiments at 1 day after injury. In 5 of 53 animals, fMRI experiments failed due to technical problems, and data were excluded. The remaining 48 injured animals were categorized into four groups named 3 days, 1–2 weeks, 1–2 months and 3–6 months after the injury ($n = 12$ each). Age-matched uninjured rats were also selected as controls ($n = 12$). Functional MRI was performed as described previously (Spenger *et al.*, 2000; Lilja *et al.*, 2006). A pair of 28 G′ needle electrodes (Grass Telefactor, Slough, UK) connected to an electric pulse generator (Medres—medical research GmbH, Cologne, GE, Germany) was used to deliver pulsed currents of 1 mA to the left forelimb of each animal. Four out of the 12 normal animals also underwent fMRI with left hind limb stimulation (1 mA) to describe the representation area of the hind limb in primary somatosensory cortex. Anaesthesia was maintained with α-chloralose (20 mg/kg/h) and pancuronium bromide infusion (4 mg/kg/h). The rectal temperature was monitored to maintain body temperature at $37 \pm 0.5^{\circ}$C by a warm-air system during the experiment. Blood pressure of normal ($n = 6$) and spinally injured animals (3 days or 6 months after injury; $n = 5$ each) under the same anaesthetic protocol and electrical stimulation, was monitored separately from fMRI experiments. Mean arterial pressure (MAP), recorded through a femoral artery (Model 1025, SA Instruments, Inc. NY, USA) remained at 139.3 ± 1.1 mmHg (mean ± SEM). After fMRI experiments and blood pressure monitoring, animals were killed using an overdose of pentobarbital (120 mg/kg, i.v.).

Data processing

Functional MRI data sets were first co-registered in a rat template (Schweinhardt *et al.*, 2003) using the normalization function in SPM2 (the Wellcome department of Cognitive Neurology, London, UK). A critical *t*-value for each voxel was calculated for the significance level of $P < 0.001$. In individual animals, the cluster volumes were recorded from significant clusters located in contralateral primary somatosensory cortex and thalamus in response to forelimb stimulation. In these clusters, the voxel with the highest significance was also located and percentage of BOLD signal change was calculated from the estimated contrast difference. The results were averaged and compared between groups using Kruskal–Wallis ANOVA followed by Mann–Whitney U-test. All values are given as mean ± SEM. For group analysis of fMRI data, second-level random effect analysis using the function of 'one-sample *t*-test' in SPM2 was performed. fMRI obtained by hind limb stimulation were analysed individually.

In situ hybridization

Separate animals from those used for fMRI were decapitated 1 and 3 days, 1 week and 1 and 6 months after spinal cord injury ($n = 7$, each). A total of seven control animals were also sacrificed from 3 to 7 days after sham operations. Tissues were prepared and *in situ* hybridization carried out as previously described (Josephson *et al.*, 2003) with oligonucleotide DNA probes labelled with α-^{33}P-dATP and complementary to rat NgR nucleotides 223–268, rat LINGO-1 nucleotides 1198–1247, rat BDNF nucleotides 577–624 or mouse GAD_{67} nucleotides complementary to rat GAD_{67} 377–418 except one mismatch. Sections were exposed for 7 days to X-ray film (Kodak Biomax MR film, Rochester, NY, USA) for quantification using an image analysis system (Image J 1.36, U.S. National Institutes of Health, Bethesda, MD, USA). Measurements were performed for NgR, LINGO-1, BDNF and GAD_{67} in the right primary somatosensory cortex of the hind limb, medial and lateral forelimb areas and secondary somatosensory cortex. NgR and LINGO-1 mRNA expressions were also assessed in the superficial versus the deep half of the cortical mantle in the same areas, and in different areas of thalamus, including the ventral posterolateral nucleus, the ventral posteromedial nucleus and medial thalamus. A mean value was calculated for each animal. Data are presented as mean ± SEM and were statistically analysed with ANOVA followed by Fisher's *post hoc* test.

Results

FMRI localization of forelimb and hind limb representation area in primary somatosensory cortex in response to electrical stimulation

Figure 1 demonstrates representative fMRI results in a normal rat obtained during successive forelimb and hind limb stimulations. As reported previously (Spenger *et al.*, 2000), hind limb and forelimb territories are adjacent to each other, with the hind limb area located medially and caudally to the forelimb area. Coronal sections at the level of Bregma −0.5 mm allow hind limb and forelimb areas to be detected in the same coronal plane, confirming the medial localization of the hind limb area in relation to forelimb area. This anatomical relationship between hind limb and forelimb territories was consistently demonstrated by fMRI throughout the cases in this study. Centre of the clusters representing hind limb was located 2.3 ± 0.13 mm lateral to the midline and 1.7 ± 0.13 mm caudal to Bregma, whereas the one for the forelimb was located 4.0 ± 0.22 mm lateral and 1.0 ± 0.17 mm rostral to Bregma ($n = 4$ each, mean ± SEM). Coordinates match those published in the Paxinos atlas (Paxinos and Watson, 2005), which verified our co-registration method for fMRI data processing (Schweinhardt *et al.*, 2003).

Rapid and long-term cortical plasticity evidenced by fMRI

Group analysis of fMRI signals ($n = 12$ in each group) demonstrated a striking biphasic cortical response with

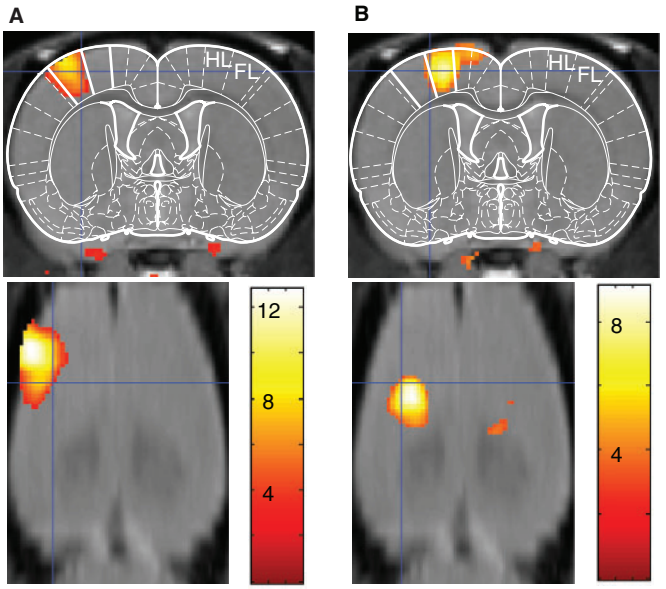

Fig. 1 Representative fMRI signals demonstrating forelimb (**A**) and hind limb (**B**) areas in primary somatosensory cortex of one normal rat. Coronal sections (*top*) at Bregma −0.5 mm, in which horizontal blue lines show the positions of horizontal sections (*bottom*). Crossed points of the blue lines indicate the same coordination in both panels, at the border between forelimb and hind limb territories. Note that the hind limb area (**B**) is located medially and caudally to the forelimb territory (**A**) in primary somatosensory cortex. The colour scale indicates *t*-statistics calculated by SPM2. The coronal sections were overlapped with a schematic brain section from the same level (with permission from the Paxinos and Watson atlas) (Paxinos and Watson, 2005). HL = hind limb territory of primary somatosensory cortex; FL = forelimb territory.

rapid (3 days) and chronic (1 month and thereafter) increases of BOLD signals in primary somatosensory cortex in response to stimulation of the unaffected forelimb (Fig. 2A–E). Three days after spinal cord injury, the fMRI signal had extended medially and caudally to occupy the deprived hind limb area, in addition to the original forelimb territory of primary somatosensory cortex (Fig. 2B and F). One to 2 weeks after injury, BOLD responses to identical forelimb stimulation were decreased and again confined to the original forelimb area in contralateral somatosensory cortex (Fig. 2C). Thereafter, BOLD responses gradually increased again, such that over 1–6 months after injury, the hind limb territory developed permanent responsiveness to contralateral forelimb stimulation (Fig. 2D and E). When individually analysed, both volumes and percentages of BOLD signal changes of activated clusters in contralateral primary somatosensory cortex significantly increased at 3 days, 1–2 months and 3–6 months compared to normal control levels (volume: $P = <0.001$, 0.001 and <0.001, respectively; percentage of BOLD signal change: 0.021, 0.013 and 0.002, respectively), which supported the observation of the group analysis

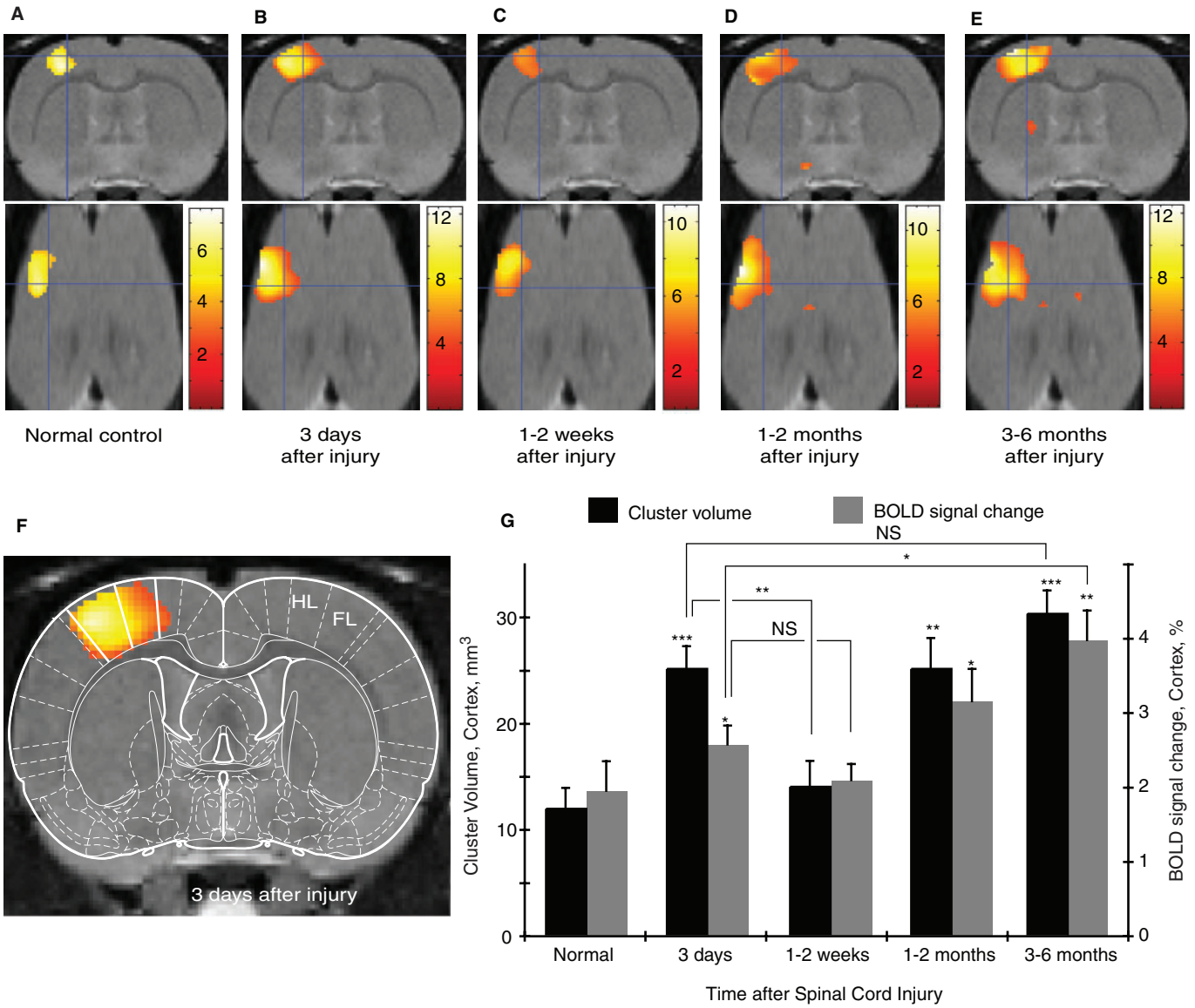

Fig. 2 Rapid and chronic increase of fMRI signals in primary somatosensory cortex in response to contralateral forelimb stimulation after spinal cord transection. (**A–E**), Group analysis of fMRI results ($n = 12$ in each group) of normal controls (**A**) and in injured animals different times after spinal cord injury as indicated (**B–E**). Coronal sections (*top*) are from Bregma −0.5 mm. Horizontal sections (*bottom*) and the colour scale is as defined in Fig. 1. Crossed points of the blue lines indicate the same coordination in all panels (**A–E**) at the medial border of the forelimb area in normal rats (**A**). Note that fMRI signals extended medially and caudally from the original forelimb territory in all periods tested except 1–2 weeks after injury (**B–E**). (**F**) The same coronal section of grouped data 3 days after injury overlapped with a schematic brain section from the same level showing medial but not lateral extension of the fMRI signal (Paxinos and Watson, 2005). HL = hind limb territory of primary somatosensory cortex; FL = forelimb territory. (**G**), Cluster volumes and percentage of BOLD signal changes of activations in primary somatosensory cortex at different times after injury compared to those of normal controls ($n = 12$ each). Significant differences were also noted in volumes of activated areas between 3 days and 1–2 weeks after injury and in percentage of BOLD signal changes between 3 days and 3–6 months after injury. Data represent mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS = non-significant.

(Fig. 2G). There were also significant decreases in volume, when BOLD signals at 1–2 weeks were compared to those at 3 days ($P = 0.004$). When fMRI signals in the 3–6 months period were compared to those at 3 days, it was noted that the volumes of activation were similar but the percentage of BOLD signal change was significantly higher ($P = 0.012$) (Fig. 2G).

Chronic increase of thalamic fMRI signals

There was no rapid increase of activations in thalamus, when fMRI signals in response to contralateral forelimb stimulation were analysed. However, BOLD responses increased 1–2 and 3–6 months after injury (volume: $P = 0.02$ and <0.001, respectively; percentage of BOLD signal change: $P = 0.02$ and <0.001, respectively) (Fig. 3).

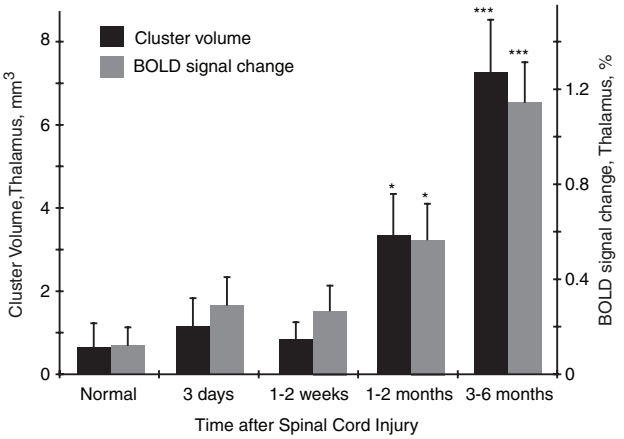

Fig. 3 Chronic increase of fMRI signals in thalamus in response to contralateral forelimb stimulation after spinal cord transection. Cluster volumes and percentage of BOLD signal changes of the activations in the thalamus at different times after injury as compared to normal controls ($n = 12$ each). Data represent mean ± SEM. $^{*}P < 0.05$; $^{***}P < 0.001$.

Specific down-regulation of *NgR* in the sensory-deprived primary somatosensory cortex and its adjacent cortical territory

In situ hybridization revealed a significant decrease of NgR mRNA in the deprived hind limb territory and importantly, also in the anatomically adjacent part of the medial forelimb area of primary somatosensory cortex as soon as 1 day after injury ($P < 0.001$ in both areas) (Fig. 4A and B, Table 1). The down-regulation of NgR remained 3 days ($P = 0.011$) and 1 week ($P = 0.001$) after injury in the deprived hind limb territory as well as in the neighbouring part of the forelimb territory ($P = 0.008$ and <0.001 at 3 days and 1 week, respectively). NgR mRNA in these cortical areas then increased slowly, but remained lower 1 and also 6 months after injury with a significant decrease remaining in the hind limb territory at 1 month ($P = 0.044$) and at both 1 and 6 months in the medial forelimb area ($P = 0.003$ and 0.043, respectively). Notably, similar down-regulations of NgR were observed neither in the lateral part of the forelimb area in primary somatosensory cortex nor in secondary somatosensory cortex, both of which are located anatomically further away from the deprived cortex (Fig. 4A and B, Table 1). Similarly, there was no down-regulation in thalamus (Fig. 5A and B, Table 1). Thus, NgR gene activity was specifically down-regulated in those cortical areas directly affected by sensory deafferentation as well as in the immediately adjacent medial forelimb territory.

Down-regulation of NgR transcription is layer-specific

Given the overlap between primary somatosensory and motor cortex in rats (Donoghue and Sanes, 1988), it remained possible that the regulation of NgR transcriptional activity in cortex might reflect local responses to distant axonal damage of those corticospinal motor neurons that project to levels below the site of the spinal cord injury. To address this issue, we quantified NgR mRNA expression separately in superficial (II/III and IV) and deeper cortical layers (V and VI), where corticospinal pyramidal neurons reside (Gao and Zheng, 2004) (Fig. 6A–C, Table 1). In superficial cortical layers, NgR mRNA was significantly decreased in both the deprived hind limb and the adjacent medial forelimb territories 1 and 3 days, 1 week and 1 month after injury (hind limb area: $P =< 0.001$, 0.002, <0.001, and 0.033, respectively; medial forelimb area: $P = 0.001$, 0.015, <0.001 and 0.008, respectively), whereas the down-regulations of NgR in deep cortical layers were modest and restricted to the hind limb territory with significant decreases only at 1 day and 1 week after injury ($P = 0.005$ and 0.004, respectively) (Fig. 6D). The absence of NgR mRNA reduction in the medial forelimb territory ($P = 0.095$) and the modest down-regulation in the hind limb area of deep cortical layers compared to the strong effects seen in superficial layers, suggest that distant axotomy of a subpopulation of hind limb motor neurons is not the main cause of the observed NgR mRNA down-regulations. Rather, the down-regulations of NgR mRNA seen in the whole cortical thickness and the superficial layers reflect responses to sensory deafferentation and cortical reorganization.

Matched regulation of LINGO-1 and NgR

The above characteristics of NgR mRNA regulation after spinal cord injury, including its time-course, the synchronicity between the deprived hind limb and the adjacent medial forelimb territories, the cortical layer specificity, as well as rather stable levels in thalamus, were faithfully paralleled by changes of the levels of mRNA encoding LINGO-1 (Figs 4C, 5C and 6E, Table 1). When LINGO-1 mRNA was quantified across the whole cortical thickness, there were significant decreases 1 day and 1 week after injury in the deprived cortex ($P = 0.001$ and 0.007, respectively) and 1 and 3 days, 1 week and 1 month in the adjacent forelimb area of primary somatosensory cortex ($P =< 0.001$, 0.011, 0.001 and 0.006, respectively) (Fig. 4C). Like NgR, LINGO-1 mRNA changes were more pronounced in superficial cortical layers with significant decrease 1 and 3 days, 1 week and 1 month after injury both in the hind limb ($P < 0.001$ in all except $P = 0.002$ at 1 month) and in the medial forelimb area ($P < 0.001$ in all), in comparison to deep layers with significance only at 1 day and 1 week in the hind limb area ($P = 0.002$ and 0.011, respectively) (Fig. 6E). This matched NgR and LINGO-1 gene regulation in somatosensory cortex further supports the idea of their functional coupling in the Nogo receptor complex (Mi *et al.*, 2004).

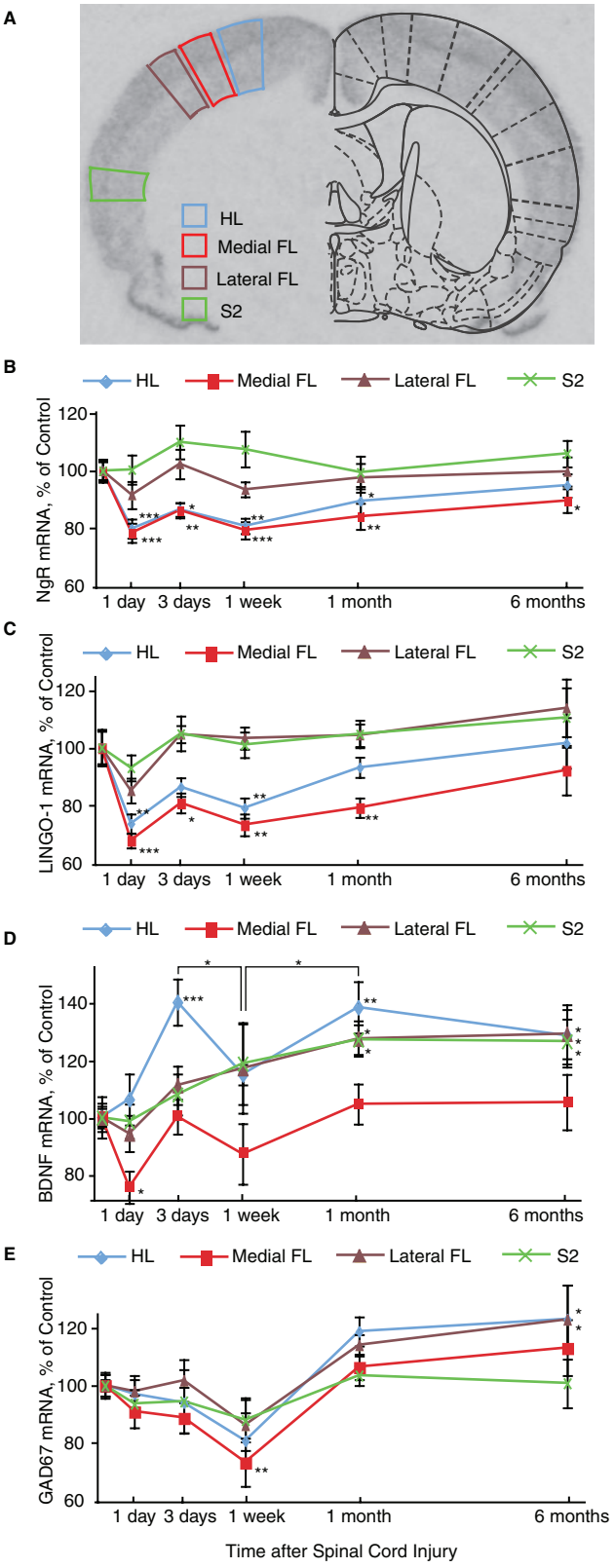

Fig. 4 Regulations of NgR, LINGO-1, BDNF and GAD_{67} mRNA in somatosensory cortex after spinal cord transection. **(A)** Representative autoradiogram of NgR mRNA hybridization, indicating measured areas. Section approximately at Bregma −0.5 mm with a schematic brain section from the same level superimposed (Paxinos and Watson, 2005). **(B–E)** Relative levels of NgR mRNA (**B**), LINGO-1 mRNA (**C**), BDNF mRNA (**D**) and GAD_{67} mRNA (**E**) compared to controls in the designated brain areas **(A)** at different times after injury ($n = 7$ each). HL = hind limb territory of somatosensory cortex; FL = forelimb territory; S2 = secondary somatosensory cortex. Data represent percentages of means ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Inverse up-regulation of BDNF

To estimate activity levels of cortical neurons following spinal cord injury, mRNA encoding BDNF, the levels of which are regulated by neuronal activity (Wetmore *et al.*, 1994), was also quantified. We found BDNF mRNA levels to be up-regulated in the deprived hind limb territory of primary somatosensory cortex 3 days, 1 month and 6 months after injury ($P = < 0.001$, 0.001 and 0.015, respectively) (Fig. 4D, Table 1). This inverse regulation of BDNF versus NgR mRNA is in line with previous findings (Josephson *et al.*, 2003). Importantly, BDNF levels at 1 week were significantly lower than those at 3 days and 1 month after injury ($P = 0.029$ and 0.040, respectively).

No rapid down-regulation of GAD mRNA

To assess the possible contribution of inhibitory systems to the rapid plastic responses in cortex, we quantified mRNA encoding the GAD_{67} isoform of the GABA synthesizing enzyme (Kaufman *et al.*, 1991). In contrast to the rapid regulations observed of NgR, LINGO-1 and BDNF mRNA levels, GAD_{67} mRNA levels were maintained almost unchanged until 1 week after injury. At this time, GAD_{67} mRNA levels had decreased in the medial forelimb area of primary somatosensory cortex ($P = 0.007$, Fig. 4E, Table 1). In the deprived hind limb territory, a similar, although not significant trend was noted. GABA and GAD immunoreactivities in GABAergic neurons have been reported to decrease 4 days after monocular deprivation (Hendry and Jones, 1986), while transcriptional activity of GAD_{67} was rather maintained in our case. Post-transcriptional regulation of GAD enzyme levels is one potential explanation for this apparent discrepancy, as suggested elsewhere (Benson *et al.*, 1991).

Discussion

Spinal cord injury served as a model for cortical plasticity in which to silence sensory input to one area of somatosensory cortex, while leaving the input to neighbouring areas intact. Our findings therefore have important implications for spinal cord injury *per se*, but also suggest possible mechanisms involved in cortical plasticity and learning.

Functional MRI provided clear evidence of functional plasticity in the model. The BOLD signal evoked by forelimb stimulation was very significantly increased 3 days after injury, underwent a temporary decrease, and then became permanently increased. The activation expanded

Table 1 Regulation of different mRNA species in cortex and thalamus after injury

mRNA	Cortical layers/thalamus	Area	Control	Time after spinal cord injury				
				1 day	3 days	1 week	1 month	6 months
NgR	Whole cortical thickness	HL	46.6 ± 1.5	37.2 ± 1.6	40.3 ± 1.1	37.7 ± 1.3	41.7 ± 2.4	44.2 ± 1.9
		Medial FL	45.9 ± 1.4	36.0 ± 1.5	39.6 ± 1.2	36.4 ± 1.4	38.6 ± 2.1	41.2 ± 1.9
		Lateral FL	42.8 ± 1.7	39.2 ± 2.0	43.8 ± 2.2	40.0 ± 1.1	41.8 ± 2.1	42.7 ± 2.2
		S2	41.1 ± 1.5	41.2 ± 2.1	45.2 ± 2.4	44.2 ± 2.6	40.9 ± 2.3	43.5 ± 1.9
LINGO-1	Whole cortical thickness	HL	135.5 ± 8.6	99.5 ± 4.6	116.8 ± 4.3	106.9 ± 4.8	126.2 ± 4.7	137.7 ± 12.0
		Medial FL	130.6 ± 8.0	88.2 ± 3.4	105.3 ± 4.5	95.4 ± 4.9	103.2 ± 4.4	120.2 ± 11.3
		Lateral FL	119.3 ± 6.7	101.4 ± 5.2	124.9 ± 3.6	123.3 ± 4.7	124.4 ± 4.7	135.8 ± 12.0
		S2	127.3 ± 8.0	118.3 ± 5.8	133.5 ± 7.7	128.5 ± 5.6	133.3 ± 6.1	140.6 ± 13.2
BDNF	Whole cortical thickness	HL	6.3 ± 0.2	6.7 ± 0.6	8.9 ± 0.5	7.3 ± 0.2	8.8 ± 0.6	8.1 ± 0.7
		Medial FL	5.5 ± 0.3	4.2 ± 0.3	5.6 ± 0.3	4.8 ± 0.6	5.8 ± 0.4	5.8 ± 0.5
		Lateral FL	5.2 ± 0.2	4.9 ± 0.3	5.8 ± 0.3	6.1 ± 0.8	6.6 ± 0.3	6.7 ± 0.4
		S2	5.9 ± 0.4	5.8 ± 0.4	6.3 ± 0.4	7.0 ± 0.8	7.5 ± 0.3	7.4 ± 0.5
GAD_{67}	Whole cortical thickness	HL	17.9 ± 0.6	17.4 ± 0.9	16.8 ± 0.9	14.5 ± 1.7	21.1 ± 0.9	21.8 ± 2.0
		Medial FL	20.9 ± 0.8	19.1 ± 1.1	18.7 ± 1.1	15.6 ± 1.7	22.2 ± 0.9	23.6 ± 2.0
		Lateral FL	19.9 ± 0.4	19.5 ± 1.1	20.2 ± 1.4	17.3 ± 1.7	22.6 ± 0.7	24.3 ± 2.3
		S2	21.7 ± 1.0	20.4 ± 1.0	20.6 ± 2.3	19.2 ± 1.6	22.5 ± 0.7	21.9 ± 1.8
NgR	Thalamus	VPL	22.2 ± 1.3	19.9 ± 1.4	23.5 ± 2.3	20.5 ± 1.4	18.2 ± 1.2	20.5 ± 2.4
		VPM	25.3 ± 1.7	23.0 ± 2.2	28.1 ± 2.1	23.2 ± 1.7	23.0 ± 2.1	24.6 ± 3.2
		MT	22.7 ± 1.1	19.4 ± 1.2	25.3 ± 2.2	19.7 ± 1.2	19.1 ± 1.5	20.5 ± 2.3
LINGO-1	Thalamus	VPL	80.6 ± 4.4	68.0 ± 2.8	75.4 ± 4.7	77.1 ± 5.0	93.8 ± 2.7	81.4 ± 8.2
		VPM	84.0 ± 5.2	75.8 ± 3.6	82.5 ± 4.8	82.7 ± 5.8	94.2 ± 3.0	80.8 ± 7.9
		MT	93.9 ± 4.1	89.8 ± 5.7	90.5 ± 2.0	80.4 ± 5.7	103.3 ± 3.4	95.6 ± 14.0
NgR	Superficial cortex	HL	53.9 ± 2.2	41.7 ± 1.9	44.0 ± 1.3	41.7 ± 2.2	47.2 ± 2.7	50.6 ± 2.4
		Medial FL	53.3 ± 2.7	40.8 ± 1.6	44.8 ± 1.9	40.2 ± 1.9	43.8 ± 3.4	48.1 ± 2.3
		Lateral FL	49.8 ± 2.0	45.9 ± 2.2	52.2 ± 2.8	46.7 ± 1.5	51.6 ± 2.2	52.8 ± 2.0
		S2	50.1 ± 2.1	49.2 ± 2.8	53.3 ± 3.1	51.9 ± 1.5	53.3 ± 2.8	53.4 ± 1.4
NgR	Deep cortex	HL	39.7 ± 1.3	33.8 ± 1.7	36.8 ± 1.1	33.6 ± 1.2	36.3 ± 1.7	39.1 ± 1.4
		Medial FL	37.7 ± 1.4	32.8 ± 1.4	37.4 ± 1.1	34.2 ± 1.2	33.9 ± 1.9	37.1 ± 1.8
		Lateral FL	36.2 ± 1.3	34.4 ± 1.8	36.5 ± 1.8	34.4 ± 1.2	35.7 ± 2.0	36.0 ± 1.7
		S2	38.6 ± 1.4	38.5 ± 1.9	41.7 ± 2.3	40.0 ± 2.2	39.6 ± 2.4	38.2 ± 1.7
LINGO-1	Superficial cortex	HL	156.2 ± 9.0	100.7 ± 5.2	114.4 ± 6.9	102.0 ± 3.9	119.3 ± 6.4	142.0 ± 12.1
		Medial FL	153.7 ± 8.9	94.2 ± 5.4	107.8 ± 4.5	104.6 ± 6.4	112.0 ± 4.8	149.2 ± 12.8
		Lateral FL	139.6 ± 4.8	116.5 ± 9.9	141.9 ± 6.7	145.4 ± 3.0	146.4 ± 7.6	160.4 ± 14.0
		S2	150.7 ± 4.9	139.8 ± 12.1	142.9 ± 7.0	145.0 ± 5.1	154.6 ± 5.4	168.1 ± 13.6
LINGO-1	Deep cortex	HL	111.1 ± 8.4	81.0 ± 4.6	96.7 ± 2.3	87.0 ± 3.9	95.1 ± 3.6	115.6 ± 10.8
		Medial FL	105.7 ± 7.8	90.6 ± 4.4	105.2 ± 3.4	99.9 ± 6.4	103.9 ± 4.9	119.8 ± 12.2
		Lateral FL	96.2 ± 6.9	91.1 ± 5.7	102.1 ± 2.3	99.3 ± 4.5	103.9 ± 5.0	114.5 ± 11.4
		S2	118.1 ± 9.6	110.3 ± 8.0	127.8 ± 7.0	117.7 ± 6.9	118.9 ± 8.1	137.7 ± 14.9

Data represent mean ± SEM (nCi/g). $n = 7$ for each mean.
Abbreviations: HL = hindlimb area of primary somatosensory cortex; FL = forelimb area; S2 = secondary somatosensory cortex; VPL = ventral posterolateral nucleus; VPM = Ventral posteromedial nucleus; MT = medial thalamus.

medially to sensory deprived hind limb territory but not laterally from the forelimb representation area. This is in line with previous observations of sensory deprived territories that become responsive to spared inputs (Buonomano and Merzenich, 1998; Kaas, 2002; Wall *et al.*, 2002). Notably, there are also reports in which cortical plasticity was not seen in response to dorsal column sections in adult or developing rats (Jain *et al.*, 1995, 2003). One major difference between their methods and ours, which could explain the discrepancy, is degree of spinal cord injury. While we used complete transections, Jain *et al.* left the spino-thalamic tracts intact. This suggests that cortical reorganization in primary somatosensory cortex may depend on type and/or degree of spinal cord injury. The nature of the sensory stimulation may also influence results. In the reports showing no reorganization, responses to tactile stimuli were mainly evaluated. Electrical stimulation, as used in the present fMRI experiments, can be more intense and ascend through both the spino-thalamic and dorsal column pathways (Chang and Shyu, 2001; Lilja *et al.*, 2006).

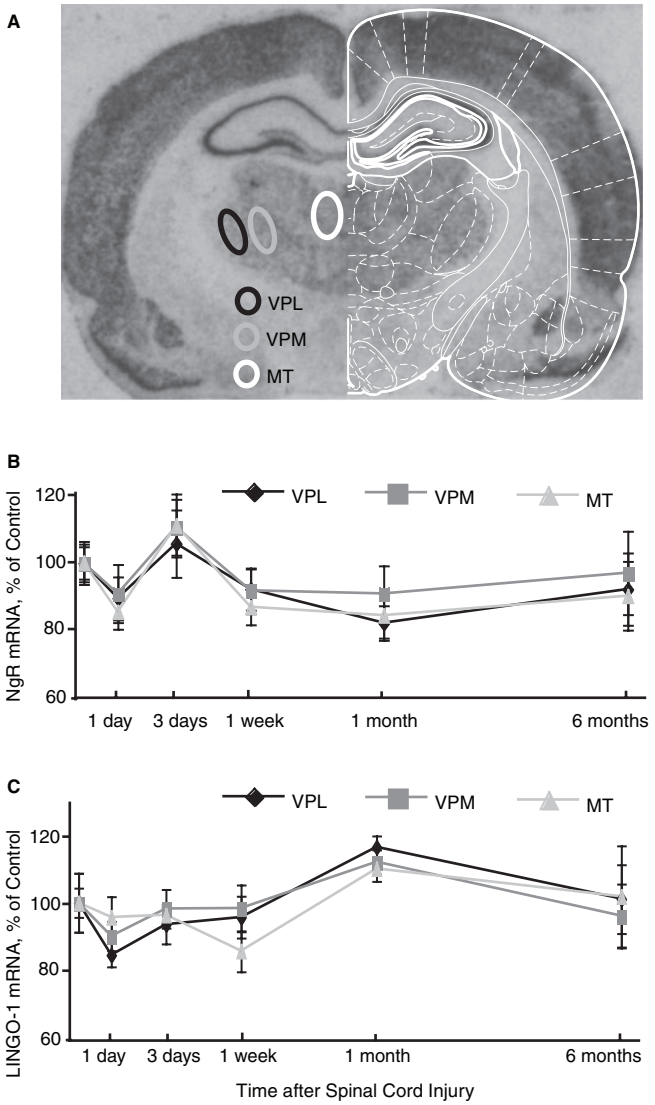

Fig. 5 Regulation of NgR and LINGO-1 mRNA in different parts of thalamus after spinal cord transection. (**A**) A representative autoradiogram hybridized for LINGO-1 mRNA indicating areas used for quantification of *in situ* hybridization. The section is approximately at Bregma −2.8 mm and is shown with a superimposed schematic brain section (Paxinos and Watson, 2005) from the same level. (**B** and **C**) No statistical difference was observed in relative levels of NgR mRNA (**B**) or LINGO-1 mRNA (**C**) as compared to controls in the designated thalamic areas **(A)** at different time points after injury ($n = 7$ each). VPL = ventral posterolateral nucleus; VPM = ventral posteromedial nucleus; MT = medial thalamus.

BOLD responses evoked by forelimb stimulation did also extend caudally after injury. However, caudal extension of the signals was less apparent compared to the clear expansion of fMRI signals in the medial direction. This can be because other non-deprived cortical territories, for instance, corresponding to face, barrel fields, shoulder or trunk, located more caudal to the forelimb area (Paxinos and Watson, 2005), might have contributed to the reorganization.

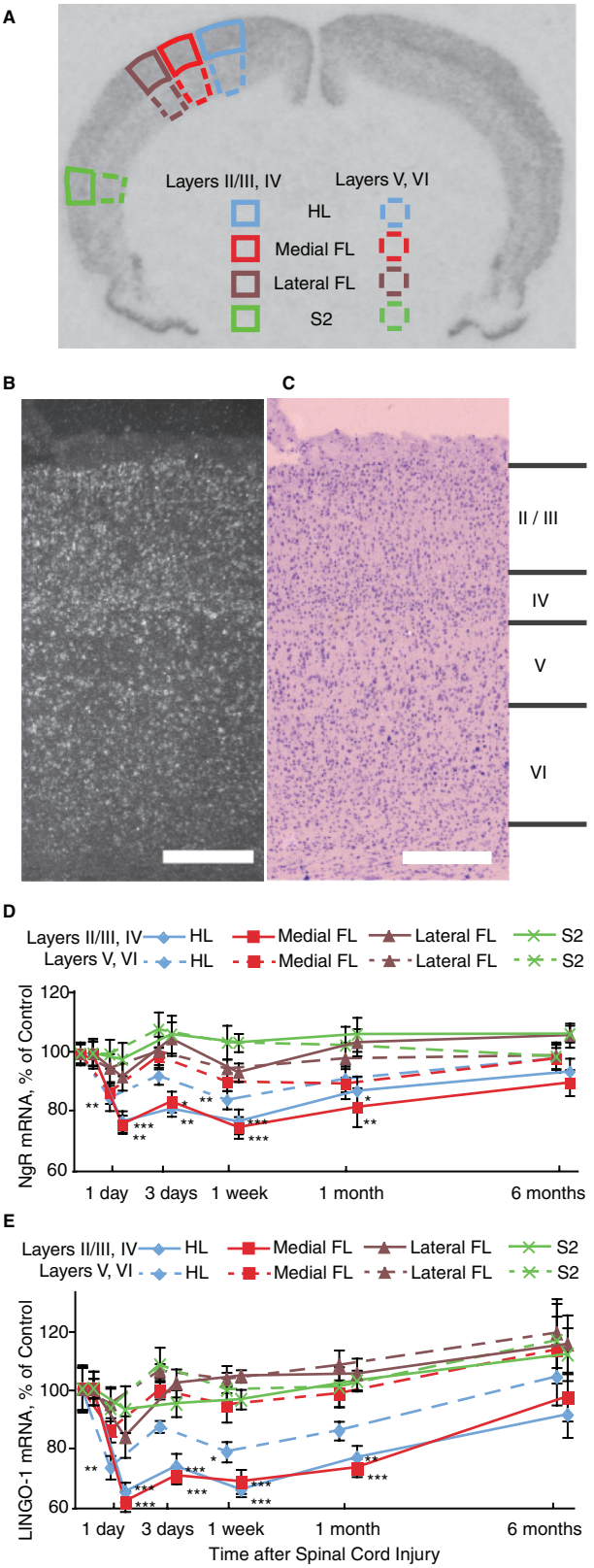

Fig. 6 Regulation of NgR and LINGO-1 mRNA in different layers of primary somatosensory cortex after spinal cord transection. **(A)** A representative autoradiogram (Bregma −0.5 mm) hybridized for NgR mRNA showing the areas selected for quantification of *in situ* hybridization signals. Superficial (Layers II/III, IV) and deep

We have previously reported an increase of BOLD signals representing forelimb areas in rats after thoracic spinal cord injury (Hofstetter *et al.*, 2003). In the previous report, increased fMRI signals were more extensive than in the current study. This difference is explained by the fact that in the current study, we increased the significance threshold to 0.001 in detecting positive voxels for BOLD contrasts from 0.05 in the previous study. Using the current more stringent criteria we were indeed able to restrict observations to the primary somatosensory areas in which BOLD signals are seen in normal rats as shown in Fig. 1.

The temporo-spatial resolution of the averaged BOLD signals also allowed detailed comparisons of accompanying changes of Nogo, LINGO-1, BDNF and GAD_{67} gene activities. Our results from *in situ* hybridization demonstrate that somatosensory cortex mounts rapid responses to sensory deafferentation. Up-regulation of BDNF indicated increased neuronal activity specifically in the sensory-deprived somatosensory cortex, as it did in the case of experience-dependent plasticity in visual cortex (Lein and Shatz, 2000). This may represent an innate neuroprotective response for survival and maintenance of deprived neurons and neuronal circuits (Barde, 1989).

Strikingly, rapid cortical plasticity demonstrated by fMRI was coupled to down-regulation of NgR and LINGO-1 transcriptional activity, specifically in the sensory deprived and anatomically adjacent cortical territories that would also include dysgranular cortex (Dawson and Killackey, 1987). These observations are in line with a suggested inhibitory role of Nogo signalling in synaptic plasticity (Josephson *et al.*, 2003; McGee *et al.*, 2005) and neurite outgrowth (Fournier *et al.*, 2001; Simonen *et al.*, 2003; Schwab, 2004). Importantly, down-regulations of NgR and LINGO-1 were observed in superficial rather than deep cortical layers. This is in agreement with the fact that strengthening of horizontal synaptic connections from non-deprived to deprived cortex occurs in layers II/III after sensory loss (Finnerty *et al.*, 1999), which may lead to increased axonal sprouting in reorganized cortex (Darian-Smith and Gilbert, 1994; Florence and Kaas, 1995; Florence *et al.*, 1998). Synapses of neurons in layer III, rather than those in layer V, appear critical for synaptic plasticity (Kirkwood and Bear, 1994). Thus, down-regulation of Nogo receptor complex enhances intracortical horizontal connections between non-deprived and deprived cortex and increases synaptic plasticity.

Our fMRI results demonstrate a rapid expansion of the receptive field for forelimb stimulation into the deprived cortex followed by a temporal decrease of the BOLD signal 1–2 weeks after injury. The biphasic development of the BOLD response paralleled the changes of NgR, LINGO-1 and BDNF gene activity. The mRNA changes included initial decreases (NgR and LINGO-1) or increase (BDNF) followed by a brief partial return towards normal levels before the mRNA levels again increased their deviation from normal levels, followed by a slow phase of normalization. The most parsimonious explanation for the biphasic events at both the gene expression and BOLD response levels appears to be that the initial sudden and complete lack of sensory input from the hind limb is replaced by a brief period of reactivation of the sensory input to hind limb cortex caused by degeneration release (Garrett and Thulin, 1975) of glutamate from cut sensory axons projecting to thalamus, reactivating the thalamo-cortical pathway to the hind limb somatosensory cortex, only to be followed by permanent silence of this input. Indeed, the long-lasting changes of NgR and BDNF transcriptional activity in the affected cortical areas, are not typical for a motor learning event, but probably reflect the fact that the increased forelimb use is permanent.

As the BOLD signal increased again, we noted a difference between the early (3 days) and chronic (3–6 months) increased responses in percentage of BOLD signal change. Because BOLD signals in thalamus only increased chronically, thalamo-cortical pathways might contribute to long-term cortical BOLD changes and thus the partly different character of early versus long-term changes. The involvement of thalamo-cortical and/or subthalamic pathways in cortical reorganization has been associated with the formation of new anatomical circuits in the brainstem in adult monkeys after cervical spinal cord dorsal transection, and also viewed as a long-term event (Jain *et al.*, 1997, 2000).

Synaptic rearrangements allowing cortical tissue to process forelimb sensory input at the expense of hind limb input when the latter is lost, is a rational mechanism in the adult mammalian CNS, given that axon regeneration does not occur. However, physiological consequences of cortical plasticity are not always beneficial, and may include phantom sensations or pain (Flor *et al.*, 1995; Moore *et al.*, 2000). In the visual system, transient monocular deprivation induces cortical reorganization so that neurons in visual cortex only process information from the non-deprived eye, leading to monocular amblyopia (Wiesel and Hubel, 1963). In experimental spinal cord injury, neutralizing Nogo signalling by anti-Nogo antibodies or other signal blocking agents is promising as a way to enhance regeneration and functional recovery (Schwab, 2004; Liebscher *et al.*, 2005; Freund *et al.*, 2006). Our present findings showing that down-regulation of Nogo receptor

(Layers V, VI) layers of the cortex were separately quantified as indicated. (**B** and **C**) Dark- (**B**) and bright-field (**C**) photomicrographs of medial forelimb territory of the primary somatosensory cortex, an area defined by the red lines in (**A**), confirming different levels of NgR expressions between the cortical layers II/III, IV versus layers V, VI. Scale bars: 500 μm. (**D** and **E**), Relative levels of mRNA encoding NgR (**D**) and LINGO-1 (**E**) as compared to controls in cortical layers II/III, IV (*solid lines*) and layers V, VI (*dotted lines*) in the designated brain areas (**A**) at different times after injury ($n = 7$ each). HL = hind limb territory of somatosensory cortex; FL = forelimb territory; S2 = secondary somatosensory cortex. Data represent mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

components is coupled to plasticity events in somatosensory cortex following spinal cord injury, should facilitate the planning of directed Nogo-inhibition-based treatment strategies for spinal cord injury.

Our results provide support for the hypothesis that functional input-driven plasticity in the adult mammalian cerebral cortex is regulated by concerted opposite changes of Nogo and BDNF signalling. While several other signalling systems undoubtedly contribute, the weight of the evidence suggests that changes of Nogo and BDNF signalling are necessary key events allowing and driving structural modulations in the neuropil of areas undergoing plastic changes. Conversely, it may be assumed that upholding NgR transcription is a prerequisite for maintenance of established synaptic circuits underlying very long-term memory.

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