Neutralization of Nogo-A enhances synaptic plasticity in the rodent motor cortex and improves motor learning in vivo

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Abstract: The membrane protein Nogo-A is known as an inhibitor of axonal outgrowth and regeneration in the CNS. However, its physiological functions in the normal adult CNS remain incompletely understood. Here, we investigated the role of Nogo-A in cortical synaptic plasticity and motor learning in the uninjured adult rodent motor cortex. Nogo-A and its receptor NgR1 are present at cortical synapses. Acute treatment of slices with function-blocking antibodies (Abs) against Nogo-A or against NgR1 increased long-term potentiation (LTP) induced by stimulation of layer 2/3 horizontal fibers. Furthermore, anti-Nogo-A Ab treatment increased LTP saturation levels, whereas long-term depression remained unchanged, thus leading to an enlarged synaptic modification range. In vivo, intrathecal application of Nogo-A-blocking Abs resulted in a higher dendritic spine density at cortical pyramidal neurons due to an increase in spine formation as revealed by in vivo two-photon microscopy. To investigate whether these changes in synaptic plasticity correlate with motor learning, we trained rats to learn a skilled forelimb-reaching task while receiving anti-Nogo-A Abs. Learning of this cortically controlled precision movement was improved upon anti-Nogo-A Ab treatment. Our results identify Nogo-A as an influential molecular modulator of synaptic plasticity and as a regulator for learning of skilled movements in the motor cortex.

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Neutralization of Nogo-A enhances synaptic plasticity in the rat motor cortex and improves motor learning in-vivo.

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The membrane protein Nogo-A has been well described as an inhibitor of axonal outgrowth and central nervous system (CNS) regeneration, whereas its physiological functions remain less well characterized. Here, we investigated the role of Nogo-A on cortical synaptic plasticity and motor learning in the uninjured CNS. We identified synaptic location of Nogo-A and its receptor NgR1 in the motor cortex (M1) and showed that treatment with antibodies against Nogo-A and its receptor increased long-term potentiation of layer 2/3 horizontal fibers. Furthermore, Nogo-A antibody treatment promoted synaptogenesis of pyramidal neurons in M1, as revealed by in vivo two-photon microscopy. More importantly, improved motor learning in rats upon anti-Nogo treatment correlates the changes in synaptic plasticity. Together, our findings reveal a novel role for Nogo-A. It acts as a negative regulator of activity-dependent functional and structural synaptic plasticity in the motor cortex and a crucial factor for motor learning.
Functional synaptic plasticity describes the ability of excitatory synapses to undergo activity-driven long lasting changes in the efficacy of synaptic transmission. This change may be expressed as long-term potentiation (LTP) or long-term depression (LTD) and correlates with structural modifications at dendritic spines (Yu and Zuo 2011). Both functional and structural synaptic plasticity have been suggested to serve as underlying mechanisms for learning and memory in several regions of the brain (Marr 1969; Morris, Anderson et al. 1986; Ito 2001; Kandel 2001; Whitlock, Heynen et al. 2006; Holtmaat and Svoboda 2009; Yang, Pan et al. 2009), including acquisition of novel motor skills (Rioult-Pedotti, Friedman et al. 1998; Rioult-Pedotti, Friedman et al. 2000; Harms, Rioult-Pedotti et al. 2008; Xu, Yu et al. 2009; Fu, Yu et al. 2012).

The membrane protein Nogo-A has originally been identified as a myelin-derived neurite outgrowth inhibitor in the adult CNS (Caroni and Schwab 1988). Neutralization of Nogo-A, Nogo Receptor 1 (NgR1) or inhibition of the downstream signaling cascades via RhoA and Rho-associated protein kinase (ROCK) enhances regenerative and compensatory fiber growth and improves functional recovery after CNS injury or stroke (Schnell and Schwab 1990; Chen, Huber et al. 2000; GrandPre, Li et al. 2002; Fournier, Takizawa et al. 2003; Kim, Liu et al. 2004; Liebscher, Schnell et al. 2005; Gonzenbach and Schwab 2008; Schwab 2010). In addition to its source in oligodendrocytes, Nogo-A is expressed by neurons, especially in highly plastic areas such as the hippocampus, neocortex and subventricular zone / olfactory bulb system (Huber, Weinmann et al. 2002; Grunewald, Kinnell et al. 2009; Raiker, Lee et al. 2010; Rolando, Parolisi et al. 2012). Neuronal Nogo-A and its receptors NgR1 and Paired Ig-like Receptor B (PirB) negatively modulate functional synaptic plasticity (McGee, Yang et al. 2005; Syken, Grandpre et al. 2006; Raiker, Lee et al. 2010; Delekate, Zagrebelsky et al. 2011). Structurally, neutralization or ablation of Nogo-A or NgR1 increases spine turnover rate
and density (Zagrebelsky, Schweigreiter et al. 2010; Wills, Mandel-Brehm et al. 2012; Akbik, Bhagat et al. 2013). Together, these studies reveal a role for Nogo signaling in remodeling of synaptic strength and structure and raise the possibility that this might affect learning and memory. However, whether the Nogo signaling influences learning is currently unknown.

Here, we investigated the influence of Nogo-A signaling on functional and structural synaptic plasticity in the motor cortex and during motor learning in vivo. At an ultrastructural level, we confirmed the presence of Nogo-A and NgR1 at synaptic sites of the motor cortex. Functional neutralization of Nogo-A increased synaptic strength, spine density of pyramidal neurons in the motor cortex and improved motor learning in vivo. Our results identify Nogo-A as an influential molecular modulator of synaptic plasticity and learning.
RESULTS

Distribution and synaptic localization of Nogo-A and NgR1
Nogo-A and NgR1 are expressed in the CNS in neurons and at synapses (Huber, Weinmann et al. 2002; Wang, Chun et al. 2002; Raiker, Lee et al. 2010; Rolando, Parolisi et al. 2012). Immunostaining of cryosections of the adult rat motor cortex showed that Nogo-A and NgR1 are expressed in the primary motor cortex (Fig. 1a, e) and were detected in layer 2/3 and 5 somata (Fig. 1b,c,f,g). Furthermore, Nogo-A is visible in the initial segment of the apical dendrites. Immunogold electron microscopy was used to further examine the synaptic localization of Nogo-A and NgR1. Fixed vibratome sections were immunostained and labeled by a secondary antibody coupled to a gold particle. Opposing localization of ligand and receptor was identified by Nogo-A immunoreactivity along the PSD (Fig. 1d) and presynaptic detection of NgR1 (Fig. 1h). Synaptic sites were confirmed by detection of the presynaptic protein vesicular glutamate transporter 1 (vGlut-1; Supplementary Fig. S2a) and the postsynaptic density protein 95 (PSD-95; Fig. Supplementary Fig. S2b).

Taken together, these findings show juxtaposed organization of Nogo-A and NgR1 at synaptic sites of the motor cortex, which suggests participation of this system in structural and functional synaptic modifications.

Functional neutralization of Nogo-A increases synaptic plasticity in the motor cortex
Blockade or ablation of Nogo-A increases functional synaptic plasticity (Raiker, Lee et al. 2010; Delekate, Zagrebelsky et al. 2011). In the motor cortex, micro-RNA mediated knock-down of Nogo-A in neurons elevated LTP, confirming a role for the protein in activity-dependent synaptic strength (Tews, Schonig et al. 2013). Having observed the synaptic localization of Nogo-A, we next explored how its acute neutralization affects synaptic plasticity in the caudal primary motor cortex (M1). Fresh 500μm slices containing the forelimb M1 area of young adult (5-6 weeks old) rats were treated for 1 hour with the Nogo-A specific function blocking antibody 11C7 (Oertle, van der Haar et al.
2003; Liebscher, Schnell et al. 2005) or control antibody (mouse IgG) at a concentration of 5 µg/ml. We determined synaptic plasticity by stimulating horizontal fibers in cortical layer II/III and measuring the field potential amplitude (FPA). LTP was induced with a TBS protocol and touch application of the GABA_A antagonist bicuculline until saturation was reached according to (Rioult-Pedotti, Friedman et al. 1998; Rioult-Pedotti, Friedman et al. 2000). Anti-Nogo-A treated slices showed a significant increase in synaptic strength during the final 20 minutes of saturated LTP (Fig. 2a; 198.7 ± 22.7%, n = 14 slices from 9 animals) compared to IgG treated control slices (142.3 ± 4.9%, n = 10 slices from 8 animals; p = 0.038, t-test, df: 13.9, t-value: 2.28). To test whether the effect of anti-Nogo upon LTP is NMDA-receptor (NMDAR)-dependent, we blocked NMDARs with AP-5 in the presence of the anti-Nogo-A antibody 11C7 and observed no LTP induction (Fig. 2a; n = 3 slices from 3 animals). FPA values for the final 20 minutes after TBS application in presence of AP-5 were 93.74 ± 0.91. Beside the NMDAR pathway, LTP can be induced via decrease of inhibitory GABAergic activity (Bliss and Collingridge 1993). A possible role of the GABAergic system could not be analysed because, as shown in earlier studies (Hess, Aizenman et al. 1996) and confirmed here, no LTP could be induced in the motor cortex without reducing GABAergic inhibition by local application of bicuculline (Fig 2a; n = 6 slices from 6 animals, 102.4 ± 2.4% during final 20 min).

LTP is one mechanism to adapt synaptic transmission in response to external experience. The opposite event to synaptic strengthening is the weakening of neuronal connections. NgR1 is necessary for LTD induction in the hippocampal Schaffer collateral pathway (Lee, Raiker et al. 2008; Raiker, Lee et al. 2010), whereas the acute neutralization of Nogo-A does not affect LTD expression (Delekate, Zagrebelsky et al. 2011). Since the influence of Nogo-A on synaptic depression has not been investigated in the cortex yet, we wondered whether Nogo-A signaling affects LTD. The experimental conditions were identical as for LTP experiments except for the LTD protocol, which consisted of low-frequency stimulation (LFS; 2 Hz for 15 minutes) that was applied four times as described (Rioult-Pedotti, Friedman et al. 2000). Consistent with previous studies (Raiker, Lee et al. 2010; Delekate,
Zagrebelsky et al. 2011), no effect was observed on LTD between anti-Nogo treated slices and IgG controls (Fig. 2b; 11C7: 50.9 ± 5.9%; n = 12 slices from 10 animals; IgG controls: 55.4 ± 5.6%; n = 9 slices from 9 animals; p = 0.59; t-test; df: 18, t-value: 0.54). Partial involvement of NMDARs in the LTD effect of anti-Nogo was suggested by successful LTD induction in presence of AP-5; however, this did not reach the level of 11C7 experiments with active NMDARs (Fig. 2b; 69.54 ± 2.05; n = 3 slices from 3 animals).

The observed potentiation of synaptic responses could result from alterations in baseline synaptic transmission. To evaluate this possibility, we recorded input-output (IO) curves and found no significant differences between 11C7 and control antibody treated slices (Fig. 2c; n = 15 vs. 15; p-value: 0.74 (between-subjects); 2-way mixed ANOVA, df: 1; F: 0.104). The effect of anti-Nogo-A treatment on baseline synaptic transmission was further characterized by analyzing the kinetics of the field response. In particular, we analyzed different phases of the fast component of the field response as indicated in Fig. 2d and found no significant difference between anti-Nogo treated slices and control experiments (mean values in ms for 11C7 vs. IgG controls for onset latency: 1.29 ± 0.23 vs. 1.57 ±0.13, p = 0.32, t = 1.02; time-to-peak: 4.64 ± 0.37 vs. 4.42 ± 0.35, p = 0.67, t = 0.44; rise time: 0.94 ± 0.08 vs. 0.76 ± 0.1, p = 0.18, t = 1.42; decay time: 3.59 ± 0.12 vs. 2.96 ± 0.43, p = 0.19, t = 1.43; in all cases df = 14; two-tailed t-test). To exclude long-term changes in physiologic basal synaptic transmission, we measured field potential responses for more than 4.5 hours without adding antibodies or applying plasticity protocols. FPAs remained stable throughout the recording duration (Supplementary Fig. S1). Finally, we aimed to confirm the postsynaptic localization of Nogo-A with a functional readout. Paired pulse facilitation (PPF) is a presynaptic form of short-term plasticity characterized by a transient increase in presynaptic [Ca^{2+}]_i and neurotransmitter release induced by two closely separated stimuli (Schulz, Cook et al. 1994). We neutralized Nogo-A to test a possible presynaptic function and found no difference between 11C7 treated slices and IgG controls (Fig. 2e) indicating no presynaptic involvement of Nogo-A (11C7: n = 12, IgG control: n = 11; p-value: 0.83 (between-subjects), 2-way mixed Anova, df: 1; F: 0.046).
In summary, these results demonstrate that functional blockade of Nogo-A leads to an enhancement of the synaptic modification range through increase of LTP and consistent LTD (Fig. 2f,g). Baseline synaptic transmission, presynaptic properties of short-term plasticity and fast kinetics of the field response are not affected by anti-Nogo-A treatment.

**Blockade of Nogo receptor increases synaptic plasticity in the motor cortex**

The Nogo receptor NgR1 negatively modulates synaptic plasticity (McGee, Yang et al. 2005; Lee, Raiker et al. 2008; Raiker, Lee et al. 2010; Delekate, Zagrebelsky et al. 2011). Nep 1-40 is a 40 amino acid peptide from the 66 amino acid extracellular loop region of Nogo-A that binds to the Nogo receptor and acts as a competitive antagonist for Nogo-66 (GrandPre, Li et al. 2002). We evaluated the impact of Nep 1-40 on synaptic strengthening at the layer 2/3 horizontal fiber pathway in the motor cortex. Protocols for incubation and measurements were identical as for the anti-Nogo-A antibody experiments; Nep 1-40 was used at a concentration of 300 nM. Figure 3a shows a significant increase (p = 0.0047, t-test; df: 15, t-value: 3.31) in the magnitude of LTP saturation for Nep 1-40 treated slices (176.4 ± 10.0%, n = 7 slices from 7 animals) compared to controls (141.8 ± 6.3%, n = 9 slices from 6 animals). Also here, no influence of Nep 1-40 was observed on baseline synaptic transmission (Fig 3b, p-value: 0.48 (between-subjects), 2-way mixed Anova, df: 1; F: 0.509) and paired-pulse analysis (Fig 3c; n = 7 vs. 7; p-value: 0.30 (between-subjects), 2-way mixed Anova, df: 1; F: 1.148). Next, we asked whether the Nogo receptor gates synaptic plasticity in the motor cortex. We used a function-blocking antibody against NgR1 and a goat IgG antibody as control at a concentration of 5 μg/ml and applied identical protocols as described above. Treatment with functional antibodies against NgR1 yielded significantly higher levels of LTP saturation compared to IgG controls (Fig. 3d; anti-NgR1: 159.52 ± 7.79%, 8 slices from 8 animals, IgG controls: 126.45 ± 2.07%, 10 slices from 9 animals; p = 0.0032, t-test; df: 7.8, t: 4.19). Influence of NgR1 signaling on baseline synaptic transmission was not observed (Fig. 3e; anti-NgR1: n = 15; IgG: n = 17; p-value: 0.94 (between-subjects), 2-way
mixed Anova, df: 1; F: 0.004) but paired-pulse measurements revealed significantly higher ratios in anti-NgR1 treated slices (Fig. 3f; p-value: 0.029; between-subjects, 2-way mixed Anova; df: 1; F: 6.065), underlining the presynaptic localization of NgR1 shown in Fig. 1f. Thus, blockade of NgR1 increases synaptic gain without affecting baseline synaptic transmission and confirms the presynaptic localization of NgR1 at a functional level.

**Anti-Nogo treatment increases spine formation in vivo**

The results above identify Nogo-A and NgR1 as negative regulators of functional synaptic plasticity. In addition to synaptic strengthening and weakening, adaptations in the number and structure of synapses also participate in learning and memory storage processes (Holtmaat and Svoboda 2009; Fu and Zuo 2011). Structural refinements of synaptic connections are influenced by Nogo-A and NgR1 (Zagrebelsky, Schweigreiter et al. 2010; Wills, Mandel-Brehm et al. 2012; Akbik, Bhagat et al. 2013; Mironova and Giger 2013). To gain insights into the significance of acute Nogo-A neutralization in regulating structural synaptic plasticity in the motor cortex, we administered anti-Nogo-A antibodies intrathecally and used *in vivo* transcranial two-photon microscopy (Xu, Yu et al. 2009) to repeatedly image the apical dendrites of yellow fluorescent protein expressing layer V pyramidal neurons (YFP-H line) (Feng, Mellor et al. 2000). Treatment with anti-Nogo-A antibodies 11C7 for 6 days significantly increased the amount of new spines added compared with mice treated with IgG control antibodies or mice without any treatment (Figure 4c, 11C7: 15.3 ± 4.2%, n = 4; IgG: 6.5 ± 1.2%, n = 3; control: 6.3 ± 0.9%, n = 4; p = 0.018, t = 3.44; df = 5, 11C7 vs. IgG; p = 0.006; t = 4.17; df = 6, 11C7 vs. control; p = 0.820; t = 0.24; df = 5, IgG vs. control). In contrast, spine elimination during the same period was comparable among these three experimental groups (11C7: 9.3 ± 1.1%, IgG: 10.7 ± 1.2%, control: 10.9 ± 1.2%; p > 0.1, all pair comparisons). Together, these results show a negative effect of Nogo-A signaling in regulating spine number in the motor cortex.
Anti-Nogo-A antibody treatment improves motor learning in vivo.

Previous studies have identified a negative modulatory role for Nogo-A and NgR1 on synaptic plasticity (McGee, Yang et al. 2005; Lee, Raiker et al. 2008; Raiker, Lee et al. 2010; Zagrebelsky, Schweigreiter et al. 2010; Delekate, Zagrebelsky et al. 2011; Wills, Mandel-Brehm et al. 2012; Akbik, Bhagat et al. 2013; Petrinovic, Hourez et al. 2013). Whether this activity of Nogo-A influences learning in vivo has not been investigated yet. Since changes in function and morphology of neuronal connections have been proposed to underlie motor learning (Rioult-Pedotti, Friedman et al. 1998; Rioult-Pedotti, Friedman et al. 2000; Rioult-Pedotti, Donoghue et al. 2007; Xu, Yu et al. 2009; Yang, Pan et al. 2009; Yu and Zuo 2011; Fu, Yu et al. 2012), we wondered whether blocking Nogo-A function might improve motor skill acquisition in vivo. To answer this question, we exposed rats to a skilled forelimb-reaching task, which differed in its complexity from the classical reaching paradigm and forced the animal to increase the precision of the learned movement components (Buitrago, Ringer et al. 2004). Animals were trained daily over 6 days, during which they received function blocking anti-Nogo-A (11C7) or control IgG antibodies by continuous intrathecal infusion. Arrival of 11C7 in the motor cortex by the applied pump implantation technique was described previously (Weinmann, Schnell et al. 2006) and confirmed here (Supplementary Fig. S3). To exclude possible effects of the catheter, pump or control antibody on motor learning, we trained an additional sham group. Figure 5b shows the success rate for the three groups. Rats started with low success rates on day 1 (11C7: 17.66 ± 2.65%, IgG control: 17.59 ± 1.5%, sham: 11.27 ± 3.59%) and reached scores around 25% on the second session (11C7: 27.29 ± 2.66%, IgG control: 25.04 ± 2.45%, sham: 26.67 ± 2.23%). On subsequent days, the 11C7 group showed a significantly steeper learning curve and reached a higher level of successful performance between day 4 – 6 compared to both control groups (11C7: 42.80 ± 3.34%, IgG: 31.45 ± 2.20%, sham: 29.10 ± 2.96% average success rates for day 4 – 6). In total, the 11C7 group showed significantly improved motor learning compared to the control groups, whereas IgG controls and sham animals did not significantly differ in their performance (p = 0.0067; 2-way mixed Anova (between-subjects); 95% confidence interval = 6.99; Bonferroni corrected).
Several lines of research suggest that motor skill acquisition consists of a slow phase (between training sessions) and a fast phase (within an individual training session) (Karni, Meyer et al. 1998; Buitrago, Schulz et al. 2004). Improvement in motor learning within a session was assessed by separating the 150 reaches per day in 25-trial bins and dividing successful scores within a bin by the total number of trials on the respective day (Fig. 5c). Anti-Nogo treated rats finished with higher scores than both control groups towards the end of the first two days. From day 3 on, anti-Nogo treated rats started with higher success rates, suggesting advanced memory consolidation and maintained elevated scores throughout all phases of the subsequent daily sessions compared to the control groups. Another form of assessing skilled movement acquisition is to measure the degree of precision and fine-tuning of the learned movement. To determine this component, we analysed exclusively first attempts of successfully grasped pellets in a randomly selected subset of the three experimental groups. In order to be counted as a positive first attempt, the rat had to grasp the pellet in a single monolithic movement execution without disruption, hesitation or repetition of the individual motion components. Anti-Nogo treated animals achieved significantly higher successful first attempt values on day 3 - 6 compared to IgG controls (Fig. 5d; Day 3 - 11C7: 11.33 ± 1.70%, IgG controls: 6.75 ± 0.82%, sham: 8.67 ± 0.67%; Day 4 - 11C7: 17.42 ± 1.84%, IgG controls: 9.50 ± 1.36%, sham: 11.22 ± 0.86%; Day 5 - 11C7: 16.17 ± 2.04%, IgG controls: 7.92 ± 1.33%, sham: 10.67 ± 0.83%; Day 6 - 11C7: 14.50 ± 1.61%, IgG controls: 9.00 ± 1.13%, sham: 10.33 ± 0.80%; p = 0.0089; 2-way mixed Anova (between-subjects); significance obtained for day 3 – 6 (p < 0.05).

In summary, acute neutralization of Nogo-A with function blocking antibodies improve the acquisition of a skilled motor task. These results demonstrate a yet unidentified role for Nogo-A in negatively regulating motor skill learning.

**Anti-Nogo-A antibody treatment modulates spine density upon motor learning**

Finally, we questioned how the acute blockade of Nogo-A influences experience-dependent structural plasticity at motor cortex dendritic spines. Rats received either no infusion, a continuous intrathecal administration of
control (IgG1) or Nogo-A function blocking antibodies while they learned a skilled forelimb-reaching task over 6 days. Post-learning, layer 2/3 spines in the forelimb region of the motor cortex were labeled by diolistics (O’Brien and Lummis 2007; Rauskolb, Zagrebelsky et al. 2010). As previously reported (Kleim, Lussnig et al. 1996; Kleim, Barbay et al. 2002), dendritic spine density was significantly higher in apical dendrites of the trained versus the not trained hemisphere (HS) of untreated control rats (sham) (Fig. 4e; sham untrained HS: 1.41 ± 0.05 (n = 14); trained HS: 1.59 ± 0.04 (n=13); p = 0.01; t = 2.754; df = 25, students t-test). In rats receiving IgG control antibodies a similar increase in spine density could be observed for the trained HS (Fig. 4f; IgG untrained HS: 1.45 ± 0.07 (n = 6); IgG trained HS: 1.65 ± 0.06 (n = 12); p = 0.05; t = 2.117; df = 16). Conversely, rats receiving Nogo-A blocking antibodies did not show increased spine density upon training (Fig. 4g; 11C7 untrained HS: 1.64 ± 0.05 (n = 20); 11C7 trained HS: 1.72 ± 0.05 (n = 15); p = 0.23; t = 1.223; df = 32). Interestingly, spine density in the untrained HS of anti-Nogo treated rats was already significantly higher than under control conditions (Fig. 4h; 11C7 untrained HS: 1.64 ± 0.05 (n = 20); IgG1 untrained HS: 1.45 ± 0.07 (n = 6); p = 0.04; t = 2.131; df = 23) resembling the result shown in Fig. 4c.

In summary, spine density for the apical dendrites of layer 2/3 neurons is equally increased by both, anti-Nogo-A antibody treatment and training. The combination of anti-Nogo treatment and experience-driven plasticity results in indistinguishable spine density levels between trained and untrained hemispheres of the same animals.
DISCUSSION

Blockade or ablation of Nogo-A and NgR1 increases structural and functional synaptic plasticity (Lee, Raiker et al. 2008; Raiker, Lee et al. 2010; Zagrebelsky, Schweigreiter et al. 2010; Delekate, Zagrebelsky et al. 2011; Wills, Mandel-Brehm et al. 2012; Akbik, Bhagat et al. 2013; Petrinovic, Hourez et al. 2013; Tews, Schonig et al. 2013). However, it was so far not known whether Nogo-A signaling affects learning processes. In the present study, we tested the effects of Nogo-A neutralization on cortical synaptic plasticity and motor learning in vivo. At the ultrastructural level, we report a juxtaposed localization of Nogo-A and NgR1 at synaptic sites in the motor cortex. We show that functional blockade of Nogo-A modulates synaptic strength and shapes neuronal architecture by increasing spine formation and density. These findings are correlated with improved motor learning in vivo upon blockade of Nogo-A function.

Synaptic influence and mechanism of action of Nogo-A

Ablation of Nogo-A and NgR1 enhances LTP without affecting LTD (Lee, Raiker et al. 2008; Raiker, Lee et al. 2010; Delekate, Zagrebelsky et al. 2011). In this study we find that interference with Nogo-A signaling leads to enhancement of LTP saturation without altering maximal LTD (Fig. 2a, b). This change expands the limits, which determine the synaptic modification range within which synapses operate (Fig. 2f, g) possibly allowing a larger learning capacity.

The ultrastructural analysis reveals a postsynaptic localization of Nogo-A, which is confirmed by the outcome of functional paired pulse measurements. Thus, one possibility to mediate synaptic plasticity is through cis interaction of Nogo-A with postsynaptic glutamate receptors (Peng, Kim et al. 2011). Conversely, the Nogo receptor NgR1 is identified at the presynapse both by the morphological and functional analysis. This opposing arrangement of receptor and ligand allows trans-synaptic communication of Nogo-A with NgR1 can influence short- and long-term synaptic changes through reverse
signaling as suggested for Ephrin/Eph interactions (Klein 2009). As similar mechanisms have been suggested to underlie synaptic transmission and neuronal outgrowth (Llinas 1979; Schmitt 1979; Kater and Mills 1991; Bloom and Morgan 2011; Skucas, Duffy et al. 2013), it is intriguing to speculate that Nogo-A acts on synaptic function and growth processes in a similar manner as for axonal sprouting. A likely target of Nogo-A signaling is the actin cytoskeleton as it is involved in pre- and postsynaptic control of synaptic transmission (Dillon and Goda 2005) and shows constant rearrangement in response to experience-dependent synaptic plasticity (Fischer, Kaech et al. 1998; Matus 2000). It is well known that Nogo-A mediates growth cone collapse through modulation of the Rho/ROCK pathway and its downstream effectors Lim kinase (LIMK-1) and cofilin (Montani, Gerrits et al. 2009; Nash, Pribiag et al. 2009). Similarly, synaptic Nogo-A may act as an upstream effector of this pathway to negatively regulate plasticity promoting processes by e.g. neurotrophic factors as suggested by the finding that NgR1 counteracts FGF2 action on LTP (Lee, Raiker et al. 2008). A key candidate to link the effects of Nogo-A and neurotrophins is p75NTR as an associated component of the Nogo Receptor complex (Wong, Henley et al. 2002) as well as an affecter of the binding affinity of neurotrophins to the Trk receptor (Segal 2003). This pathway has been suggested to mediate negative synaptic plasticity (Rosch, Schweigreiter et al. 2005; Woo, Teng et al. 2005; Zagrebelsky, Holz et al. 2005).

**Nogo-A mediated changes in structural synaptic plasticity**

Long-term net changes in synaptic efficacy of the cortex can either result via synaptic strengthening or through changes in synapse structure and number, which represents an efficient way to increase the capacity for memory storage (Kleim, Lussnig et al. 1996; Kleim, Barbay et al. 2002; Yu and Zuo 2011). Newly formed spines are mostly transient and rarely survive more than a week (Holtmaat, Trachtenberg et al. 2005). Learning however, stabilizes newly formed synapses (Holtmaat, Wilbrecht et al. 2006; Hofer, Mrsic-Flogel et al. 2009; Xu, Yu et al. 2009). Little is known about molecular cues that convey stabilization of synapses. We find that synapse formation is enhanced with anti-Nogo treatment after 6 days (Fig. 4a-c). Similarly, increased spine
density is observed after blockade of Nogo signaling (Fig. 4j), which, however, vanishes upon training-induced neuronal activity (Fig. 4k). The increase in synaptic plasticity through blockade of Nogo signaling may be beneficial during short temporal windows (minutes – weeks), whereas Nogo-A and NgR1 may be required to stabilize the newly formed synapses over longer time periods (days to weeks). In the absence of Nogo-A training-induced synapses are not stabilized and thus return to control levels. Sequential interplay between anti-Nogo-A treatment to initially increase synaptic plasticity and a later Nogo-A action to stabilize newly formed connections may be a key concept to efficiently exploit the potential of this concept.

**Nogo-A affects motor learning in vivo**

Since LTP and LTD have been suggested as cellular correlates for learning and memory and improvement of the learned motor skill is accompanied by changes in synaptic strength (Rioult-Pedotti, Friedman et al. 1998; Rioult-Pedotti, Friedman et al. 2000), synapse formation (Xu, Yu et al. 2009) and the synaptic modification range (Rioult-Pedotti, Friedman et al. 2000; Rioult-Pedotti, Donoghue et al. 2007), we hypothesized that enhancement of structural and functional synaptic plasticity enlarges the memory capacity per synapse to improve motor learning *in vivo*. In the present study, we have identified Nogo-A as a molecular player, which negatively regulates synaptic plasticity and motor learning. Interestingly, whereas short-term interference with Nogo-A leads to improved learning, chronic inhibition of Nogo-A in combination with activity over several weeks has been shown to result in negative outcomes of the learned task (Maier, Ichiyama et al. 2009; Starkey and Schwab 2012). In line with this concept, interference with NgR1 has been reported not to affect short-term memory but to impair long-term memory (Karlen, Karlsson et al. 2009). Whereas acutely interfering with Nogo-A facilitates synaptic plasticity and motor learning in a short temporal window, chronic application of Nogo-A antibodies *in vivo* may overload the system via a coherent increase of structural and functional plasticity. A challenging assignment for future studies is to determine the optimal window and sequential strategy during which beneficial features of anti-Nogo-A treatment are exploited for CNS regeneration without disturbing basic neuronal
functioning.

**Potentials for CNS Regeneration**

Activity-dependent plasticity is a crucial driving force to enhance functional regeneration after CNS injury (Maier, Ichiyama et al. 2009). Nogo-A and NgR1 are both downregulated in sensory-motor areas during increased activity (Josephson, Trifunovski et al. 2003; Ghiani, Ying et al. 2007), indicating that the Nogo system needs to be decreased physiologically in order to release the brakes and allow plastic changes. Absence of Nogo-A ameliorates this process and thus may increase the potential for CNS regeneration. Selective and sequential enhancement of this strategy may be used to gain clinical benefit and behavioral advantage after CNS injury. Furthermore, the use of function blocking antibodies is a promising clinical feature. Whereas Nogo-A KO mice and knock-down rats show neuropsychiatric phenotypes (Willi, Weinmann et al. 2010; Tews, Schonig et al. 2013), antibodies against Nogo-A are well tolerated (Willi, Weinmann et al. 2010). Our result in Fig. 2d indicates that basic neuronal functioning is not disturbed by anti Nogo-A antibodies.

In summary, we identify Nogo-A and NgR1 at synaptic sites in the motor cortex and show that functional blockade of Nogo-A enhances synaptic gain through strengthening of connections, formation of new synapses and an overall increase of the synaptic modification range. These changes are accompanied by improvement of motor learning *in-vivo* during treatment with the Nogo-A antibody 11C7. The relevance of this mechanism for functional recovery and rehabilitation after CNS injury remains to be investigated.
MATERIALS & METHODS

Up to 3000 words

**Animals.** Adult male Sprague Dawley rats (5-6 weeks, 190-220g, Janvier) were used for LTP, LTD, in vivo motor learning and electron microscopy (EM) experiments. They were housed in standard cages in groups of three animals per cage, in a reversed light/dark cycle (light on 8:00 P.M., light off 8:00 A.M.). All experiments were conducted with the approval of the Veterinarian Office Zurich, Switzerland and in accordance with their guidelines. Thy1-YFP-H line mice (Feng, Mellor et al. 2000) were purchased from Jackson Laboratory for in vivo imaging of dendritic spines. Mice were housed and bred in University of California Santa Cruz (UCSC) animal facilities according to approved animal protocols.

**Immuno Electron Microscopy.** For pre-embedding immunolocalization of PSD-95, VGlut-1, Nogo-A and NgR1, 40μm thick coronal cryostat sections were prepared from M1 layer 2/3, treated with 0.5% NaBH4 in tris buffer saline (TBS) for 10 min and in tris glycin 50mM (pH 8.0) for 30 min to quench the free aldehyde groups. After passing freeze and thaw cycles to enhance the permeability, sections were rinsed several times in TBS and incubated for 2 days with the primary antibody (PSD-95, 1:200; VGlut1, 1:500; Nogo-A/Laura, 1:200; NgR1, 1:100) at 4°C on a shaker. Exposition to biotinylated secondary antibody (1:200; Jackson Immuno Research) was followed by Streptavidin Peroxidase ABC Elite Kit (1:100; Vector). Sections were subsequently incubated with 0.025% 3,3′-diaminobenzidine tetrahydrochloride (DAB) in the presence of 0.006% H2O2 for 5 - 10 min. DAB staining was intensified by the methenamine silver-gold reaction according to (Teclemariam-Mesbah, Wortel et al. 1997; Petrinovic, Hourez et al. 2013). Compared to the conventional DAB product, the high electron density of gold-substituted DAB-silver grains facilitates ultrastructural recognition of stained structures. Sections were osmicated and after uranyl acetate contrast
incubation embedded in epoxy. Ultrathin sections of 100nm were analyzed in a Zeiss EM10 electron microscope. Synaptic sites were identified by detection of the described presynaptic marker vesicular glutamate transporter 1 (v-Glut1) and the postsynaptic marker PSD-95 (El Mestikawy, Wallen-Mackenzie et al. 2011; Sun and Turrigiano 2011).

Blocking reagents. For ex-vivo slice physiology, four different highly purified mouse and goat monoclonal antibodies were used: A Nogo-A specific blocking antibody (11C7), raised against an 18-aa peptide in the most active region of NogoA (NogoΔ20, a gift from Novartis Pharma AG, Basel, Switzerland) and shown to block the Nogo-A mediated neurite outgrowth inhibition in vitro and in vivo (Oertle, van der Haar et al. 2003; Liebscher, Schnell et al. 2005; Maier, Ichiyama et al. 2009), a control antibody (mouse IgG), an antibody against the Nogo receptor subunit NgR1 (mNogo receptor affinity-purified goat IgG; R&D Systems) and a goat IgG control antibody (R&D Systems). Antibody solutions were freshly prepared in carbogenated ACSF at a final concentration of 5 μg/mL. To prevent sticking of the antibody to the tubing and the chamber, silicon tubing was used and washed with ACSF containing BSA (0.1 mg/mL). The slices were pre-incubated for 1 h with the anti-Nogo-A, anti-Nogo Receptor or respective control antibodies in an incubation chamber maintaining a constant flow of the solution. The Nogo-66 (1-40) antagonist peptide (Nep1-40) was used at a concentration of 300nM. For in-vivo experiments, the above-mentioned NogoA-specific blocking antibody 11C7 and mouse IgG control antibody were used at a concentration of 3.0 – 4.2 mg/ml. To exclude possible effects of the catheter, pump or control antibody on motor learning, we trained an additional sham group. Animals belonging to this group received identical steps of the surgical protocol as the two antibody groups but no pump-implantation.

Slice Preparation. Coronal slices containing the forelimb area of M1 (1-2mm anterior to the bregma; Donoghue and Wise 1982), were prepared from adult Sprague Dawley rats (180-220 g) as previously described (Hess, Aizenman et al. 1996; Rioult-Pedotti, Friedman et al. 1998). Animals were anesthetized by intra-peritoneal injection of pentobarbital. After decapitation, the brain was
removed quickly and coronal slices, ~500μm thick, were cut using a vibratome (Leica VT 1000S, Leica Biosystems, Nussloch, Germany).

**Electrophysiological Recordings.** Slices were transferred to a fluid-gas interface chamber and superfused at a constant rate of 1ml/min with ACSF (composition in mM): 126 NaCl, 3 KCl, 1.25 NaH2PO4, 26 NaHCO3, 1MgSO4, 2CaCl2 and 10 glucose, bubbled with a 95% O2, 5% CO2 mixture at 33 ± 0.5°C). The humidified atmosphere over the slices was saturated with 95% O2 and 5% CO2. Slices were left in the chamber to recover for at least 1 hour after dissection. To allow optimal antibody penetration, responses were recorded from the slice surface of layer II/III within the motor cortex. For all experiments, measurements were obtained as previously described (Rioult-Pedotti, Friedman et al. 1998; Rioult-Pedotti, Friedman et al. 2000). For LTD experiments, low-frequency stimulation (LFS) was induced four times (referred to as maximum LTD), all other protocols were identical to (Rioult-Pedotti, Friedman et al. 1998; Rioult-Pedotti, Friedman et al. 2000).

For data analysis, we computed the amplitude of the field potential response because it serves as a measure of the population excitatory synaptic response (Rioult-Pedotti, Friedman et al. 1998; Rioult-Pedotti, Friedman et al. 2000), reflects a monosynaptic current sink (Hess, Aizenman et al. 1996) and correlates well with the intracellular excitatory postsynaptic response evoked in this pathway (Aroniadou and Keller 1995). Measurement of the field potential slope, as routinely used, e.g., in the hippocampus, has not been used for neocortical field potential responses due to the interference of the response’s initial part by variable non-synaptic components (Hess and Donoghue 1994).

Presynaptic properties were investigated through paired pulse measurements, which were performed by applying a pair of two stimuli in different interstimulus intervals (ISI) in a range of 10, 20, 40, 80 and 160 ms. The amplitude of the second response was divided by the amplitude of the first response. The result was expressed as the paired pulse ratio (PPR).

**Analysis of Kinetics.** The kinetics of the field response were characterized by rise time, decay time, and width at half maximum. The rise time, was estimated from the 30/70 time (the time it takes the signal to transition from
30% to 70% of its maximal value or peak amplitude). Decay time constants were estimated by fitting the decaying phase of the field to a sum of two or three decaying exponentials (Levenberg-Marquardt non-linear least squares). Decay time $d$ reported in the results section refers to the fastest decay time constant obtained by the fitting. The onset of the field response was further characterized by its onset latency and time to peak. The onset latency was obtained as the time interval between the stimulation and response onsets (the latter estimated from the intercept of a line fitting of the rising phase of the field response with the baseline immediately before the stimulation). The time to peak was determined as the time interval between stimulation onset and the field response maximum. All time constants were obtained individually for a total of 1148 trials corresponding to $n = 16$ slices (8 treated with anti-Nogo and 8 controls). For each slice the median across trials was computed to reduce the effects of outliers. Each group was finally described by the mean of the individual medians.

**Data Analysis Electrophysiology.** Experimental data were collected with LabView software (National Instruments) and analyzed with LabView, Matlab (MathWorks), Graphpad Prism (Graphpad software) and Excel (Microsoft). Horizontal fibers in layer 2/3 of the motor cortex were stimulated and the amplitude of elicited fEPSPs was measured over time, normalized to baseline values and plotted as average ± SEM.

**Intrathecal pump implantation.** Animals were anesthetized with a subcutaneous injection of Hypnorm (120μl/200gm body weight; Janssen Pharmaceutics, Beerse, Belgium) and Dormicum (0.75mg in 150μl per 200gm body weight; Roche Pharmaceuticals, Basel, Switzerland). Vitamin A–containing eye ointment was applied to protect the eyes from dehydration during the procedure. After partial laminectomy at T3 vertebral level, a fine intrathecal catheter (32G, Recathco) was inserted into the subarachnoid space from the lumbar level L2/L3 and pushed rostrally to the spinal segment C7, delivering the antibodies from an osmotic minipump (10μl/h, Alzet 2ML1, Alzet Osmotic Pumps, Cupertino, CA) into the CSF for 7 days as previously described (Weinmann, Schnell et al. 2006; Willi, Weinmann et al. 2010).
Analgesics (Rimadyl; Pfizer AG, Zürich, Switzerland) were given post-operation by subcutaneous injection. The antibiotic Baytril (5mg/kg body weight, SC; Bayer AG, Leverkusen, Germany) was administered once a day for 2 days starting on the day of operation to prevent bladder infections. Sham animals received all surgical steps and medications except for pump implantation.

**In Vivo Transcranial Imaging and Data Quantification.** YFP-H line mice of both sexes at one month of age were used in all the experiments. The surgical procedure for transcranial two-photon imaging and data quantification have been described previously (Xu, Yu et al. 2009). Intrathecal pumps were implanted as described above immediately following the first time images. The second time images were obtained 6 days later. Percentages of eliminated and formed spines were normalized to total spines counted in the first images.

**Motor Skill Learning.** Animals were handled to get accustomed to the experimenter and the housing environment and were food-restricted for 24 h prior to the first pre-training session. During pre-training and training, animals were kept on a standard laboratory diet to maintain their initial weight (187.8 ± 3.29 g). Water was given *ad libitum*. Training was performed in a plexiglas box (34 × 14 cm) with a vertical window opening, which allowed the animal to retrieve the food pellet. During pre-training, pellets were close enough to be reached with the animal’s tongue. The animal had to learn to run to the rear of the cage in order to access the next pellet at the slit opening. This task allowed the rat to reposition prior to each pellet retrieval. Once >=50 trials of pellets were eaten with the tongue in less than 15 minutes, animals underwent surgery to receive osmotic pumps 16 - 24 h after the last pre-training session. During pre-training, animals did not retrieve food pellets with their forelimb. 48 hours after the pump implantation animals were exposed to the first motor learning session. For the forelimb-reaching task, the pellet board was replaced by a vertical pedestal, on which the pellets were placed at a distance (1.5 cm) at which they could only be retrieved by the rat’s forelimb. During the first 10 trials forelimb preference was determined by positioning the pedestal at the center of the cage’s vertical slot. The pedestal was then
shifted contrary to the rat’s preferred forelimb to allow an optimal reaching angle with the preferred forelimb and remained in that position throughout all remaining trials. The diameter of the pedestal corresponded to that of the pellet, thus, pellets were discarded from the post easily. For a successful grasp, pellets had to be retrieved by a precise, skilled forelimb movement composed of stretching the forelimb toward the box window and pellet, targeting the pellet, pronation, opening of the paw, grasping, pulling the forelimb back while supinating, retrieving the pellets successfully and eating them (Whishaw and Pellis 1990). A trial, defined as a new pellet presented to the animal was classified as “successful” (reach, grasp, retrieve and eat the pellet), drop (reach, grasp and lose pellet during retrieval) or “fail” (discard pellet from the pedestal). A daily session consisted of 150 trials or a maximum time of 1 hour for each animal. The success rate of each daily session (between training sessions) was calculated as the sum of successfully retrieved pellets divided by the total number of trials. Except for the first day of training, no animal reached the maximum time but completed 150 trials in less than an hour. Sessions lasted on average 23.2 ± 1.38 min. To analyse the performance during each daily session (within session analysis), we divided the 150 trials into 25 trial bins and calculated the percentage of successfully grasped pellets for each bin by dividing the number of successes in the respective bin by the total number of trials on the respective day. Precision and fine-tuning of the movement were investigated through first attempt analysis in a subset of animals: First attempts were considered positive when the animal grasped the pellet in a single monolithic movement execution without disruption, hesitation or repetition of individual movement components. All experiments were performed in a double-blind manner: animals were coded with random numbers and the groups were mixed within the cages. Experimenters were blind to the treatments throughout all phases of the experiment until completion of data analysis.

**Statistical Analysis.** For in-vivo motor skill learning, two-way mixed analysis of variance (ANOVA) (between-subjects factor: group of treatment; within-subjects factor: day of training) was applied. If a significant effect was found for a factor, post hoc tests were performed using Bonferroni correction for
multiple comparisons. For all other experiments, two-sampled, two-tailed t-tests with unequal sample size were used. Normality and equal variance hypotheses were tested by Kolmogorov-Smirnov test and F-test, respectively. All values in the results section are given as mean ± SEM.

**Diolistics and dendritic spine imaging in fixed tissue.** Coronal slices containing the forelimb area of M1 were prepared as for electrophysiological experiments. The slices were fixed in 4% PFA overnight at 4°C and washed with 1x Phosphate Buffered Saline (PBS). The Diolistic was performed as previously described (Rauskolb, Zagrebelsky et al. 2010). Dye-coated particles were delivered to the coronal slices using a hand held gene gun (Bio-Rad; Helios Gene Gun System) and kept in PBS for 3 days for the dye to diffuse. The slices were postfixed in 4% PFA, washed and stained with DAPI to enable the identification of the cortical layers. Layer 2/3 neurons from the forelimb area of M1 were imaged with a BX61WI FLUOVIEW 1000 (FV1000) Olympus confocal microscope. Image stacks of defined dendritic stretches from the mid-apical dendritic parts were acquired using a 40x oil objective (NA1.3), z-step of 0.5µm and a zoom of 4.
Fig. 1: Distribution and synaptic localization of Nogo-A and NgR 1 in the motor cortex. (a-c) Immunostaining with anti-Nogo-A revealed expression of the protein in cell bodies of layer 2/3 and 5 of the motor cortex. (e-g) NgR1 was detected in layer 2/3 and 5 somata. (d, h) Nogo-A and its receptor NgR1 are complementary expressed. Strong immunolocalization of Nogo-A is observed in the postsynaptic density, whereas NgR1 labeled immunogold particles are identified in presynaptic terminals.

Fig. 2: Functional blockade of Nogo-A increases synaptic plasticity in the rat primary motor cortex. Slices from the forelimb region of the rat primary motor cortex were treated with the functional Nogo-A blocking antibody (Ab) 11C7 (green) or control Ab (orange). Insets show representative field potential (FP) waveforms obtained from layer II/III horizontal fibers taken at time points indicated by numbers in each figure. Up arrows: LTP induction, down arrows: LTD induction. (a) During the last 20 minutes of saturated LTP, a significant difference between 11C7-treated and IgG control treated slices is observed (p < 0.05; t-test). LTP induction in 11C7 treated slices fails in presence of NMDA antagonist AP-5 (grey). No LTP is induced in 11C7-treated slices in absence of bicuculline (blue). (b) LTD shows no obvious difference between the 11C7 and control group for the last 20 minutes of maximal LTD (p > 0.05, t-test). LTD is successfully induced in 11C7-treated slices in presence of NMDA antagonist AP-5. (c,e) Input-output strength and paired pulse measurements revealed no significant differences between 11C7-treated slices and IgG controls. (d) Kinetics of the fast component of the field response (FR). The rise time $\tau_L$, time-to-peak $\tau_P$, rise time $\tau_R$ and decay time $\tau_D$ of the field response (FR) kinetics were characterized. No significant difference is seen between anti-Nogo and control antibody treated slices.
Inset illustrates phases of the field response used for analysis. (f,g) Expansion of the synaptic modification range. The space between LTP and LTD saturation is defined as the “synaptic modification range”. Treatment with anti-Nogo expands LTP saturation (98%) without altering LTD and by this leads to an enlarged range.

**Fig. 3: Blockade of the Nogo receptor enhances long-term potentiation in the rat primary motor cortex.** Slices from the M1 forelimb region were treated with the Nogo-66 (1-40) antagonist peptide Nep1-40 and a functional antibody against NgR1. Insets show representative field potential waveforms taken at time points indicated by numbers in each figure. Up arrows: LTP induction, down arrows: LTD induction. Protocols were identical as in Fig. 2. (a-c) During the last 20 minutes of saturated LTP, a significant difference between Nep 1-40-treated slices (red) and controls (grey) is observed (p < 0.005; t-test). Input-output strength and paired pulse analysis revealed no significant differences between Nep 1-40 treated slices and controls. (d-f) During the last 20 minutes of saturated LTP, a significant difference between anti-NgR1-treated (blue) and IgG control treated slices (grey) is observed (p < 0.005; t-test). Input-output strength revealed no significant difference between anti-NgR1-treated slices and IgG controls. Paired pulse analysis reveals significantly higher ratios for anti-NgR1 treated slices in comparison to IgG controls (p < 0.05).

**Fig. 4: Functional blockade of Nogo-A increases spine formation in vivo.** (a,b) Representative images of in vivo imaging in mice. Repeated imaging of the same dendritic branches over six-day intervals in the motor cortex in animals treated with treated with IgG control antibodies (a) or Anti-Nogo antibodies (b). Arrows represent newly formed spines. Scale bar represents 2μm. (c) Quantified percentage of spine formation and elimination in untreated (grey), IgG control antibody treated (orange) and anti-Nogo treated animals (green).

**Fig. 5: Anti-Nogo-A antibody treatment improves motor learning in vivo.** Rats were exposed to a complex precision forelimb reaching task while
receiving anti-Nogo-antibodies (11C7; green), IgG control antibodies (orange) or no antibody treatment (sham, grey). (a) Experimental procedure. (b) Motor skill acquisition over six days. 11C7 treated animals show significantly improved motor learning compared to IgG control treated and sham animals (p < 0.05; 2-way mixed Anova). No significant difference was observed between IgG controls and sham animals (p > 0.05; 2-way mixed Anova). (c) Within-day success rates illustrate improvement in motor learning throughout a single session. Anti-Nogo treated animals finish with higher scores towards the end of the first two days and reach higher success rates throughout all phases between day 3 - 6. (d) First attempts are shown as a measure for fine tuned movement learning. 11C7 receiving animals shows significantly higher first attempt values on day 3 - 6 compared to IgG controls (p < 0.05; 2-way mixed Anova, Bonferroni correction).

Fig. 6: Anti-Nogo-A antibody treatment affects spine density upon motor learning. (a-d) Representative images of the apical dendritic regions labeled with DiI, comparison of the trained versus untrained hemisphere for sham control vs. Nogo-A blocking antibodies (11C7). Scale bar represents 2μm. (e-h) Quantified data showing dendritic spine density for the apical dendrites in the trained versus untrained hemisphere in sham animals, IgG control antibody treated animals and 11C7 treated animals. (k) Quantification showing spine density for the untrained hemisphere of IgG control vs. 11C7.
**SUPPLEMENTARY FIGURES**

**Fig. S1: Long-term baseline recording (280 min). (a)** Slices from the M1 forelimb region were prepared and baseline was recorded over 4.5 hours as a negative control to evaluate possible long-term changes in baseline levels.

**Fig. S2: Confirmation of synaptic sites in the rat layer 2/3 motor cortex. (a, b)** Labeling of presynaptic vGlut-1 and postsynaptic PSD-95 markers were used to confirm synaptic sites in the rat motor cortex layer 2/3.

**Fig. S3: IgG antibody uptake in the motor cortex. (a)** Relative optical density measured in slices from the motor cortex. Anti-Nogo antibodies show 22% higher mean grey values compared to control mouse IgG. (b) Representative image taken from adult rat motor cortex.
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