Cortical dopaminergic transmission and motor learning:

Studies in a rodent motor learning model

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to my parents

In spite of all the memories that held me down, all the unspoken truths and a past that haunted me. For doing things as best they could and showing me the meaning of family: forgiveness, acceptance, perseverance, faith and eventually understanding.

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Last, but certainly not least, I would like to thank my siblings Leo and Paty. Your presence has been the motive for perseverance, both as a copy-kat and example. With all my love and in the assurance of your forthcoming success and happiness!

Declaration

I hereby declare that I have produced the work entitled: 'Cortical dopaminergic mechanisms and motor learning: studies in a rodent motor learning model', submitted for the award of a doctorate , on my own (without external help), have used only the sources and aids indicated and have marked passages included from other works, whether verbatim or in content, as such. I swear upon oath that these statements are true and that I have not concealed anything. I am aware that making a false declaration under oath is punishable by a term of imprisonment of up to three years or by a fine.

Katiuska Molina-Luna

Tübingen, July 2007

Statement of Authorship Contribution

The works described throughout this thesis are all papers in progress. I contributed in all stages of the work; I designed the experiments and performed the data analysis together with Dr. A. Luft. I wrote the drafts, and revised the final manuscripts.

The PET imaging study was conducted in cooperation with the imaging group in Tübingen in the group of Dr. B. Pichler.

LTP studies were conducted in cooperation with Dr. M. Rioult-Pedotti in Brown Univeristy, Rhode Island.

Animal Care Statement

During the experimental part of my thesis, all precautions were taken to minimize pain and suffering of the animals and to provide the best care possible during the experimentation period. Without the use of these animals as models, all studies conducted concerning basic mechanisms in the brain would have not been possible. All procedures have been reviewed and approved by the animal care committee and by the responsible state agencies of Baden-Württenberg and the University of Tübingen.

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SUMMARY

Motor learning is thought to play a crucial role for the adaptation of organisms to their environment. It has been shown in animal models as well as in humans, that the motor cortex is a key structure for movement learning. In this PhD thesis, electrophysiological and pharmacological approaches were used to investigate motor skill acquisition and motor memory consolidation in a rodent model from two different aspects: (1) learning effects in structural representation change and (2) mediation of skill acquisition by aminergic neurotransmission. In both studies a surface electrode array was used to stimulate motor cortex and produce maps of cortical representations of different body areas and specifically to identify forelimb clusters. In the first study, the change in forelimb representation was followed over time in animals that were subjected to a forelimb reach training paradighm and was compared to animals that exercised the arm, but did not acquire the reaching skill. In the first study, differences between activity (exercise) and learning-dependent motor map representations were demonstrated as modeled by muscle twitches in response to electrical stimulation. This study demonstrated that structural changes in the motor cortex mediate the acquisition of skills but not the storage of the motor memory. The second study was based on previous findings of our group that dopamine receptors are up-regulated during learning. Dopamine receptor antagonists were intracortically (i.c.) administered to rats that were trained in the reaching task and the effects of transient vs. permanent dopamine depletion were compared. Furthermore, animals that were permanently depleted of dopaminergic terminals in the motor cortex were administered dopamine i.c. by means of an osmotic pump. The learning capacity in these animals was recovered and reached levels comparable to those of controls. Overall, the thesis highlights the role of structural plasticity in motor cortex for skill acquisition and the importance of dopaminergic neurotransmission for the functional capacity of M1 neurons.

INTRODUCTION

The learning and the memory of motor skills are unique: after slow acquisition requiring repeated training, skills are retained very long often even for life. This amazing storage capacity renders motor memories different from other forms of memory and an interesting topic for investigation. Acquisition and storage of a novel motor skill is mediated by a series of functional adaptations and structural growth processes in different brain regions that likely occur in sequence (Luft & Buitrago 2005). These processes are summarized by the term "neuroplasticity".

Neuroplasticity in the motor system also occurs after brain injury, such as a stroke. These neuroplastic processes are likely similar to the ones that occur during motor skill learning. Therefore, motor skill learning can be used as a model to uncover the basic events that lead to functional restoration. Given that stroke is leading cause of disability in Europe and North America, understanding the basic neurophysiology of recovery is of eminent importance and may help to individualize and devise future therapies.

While other brain areas, especially cerebellum and basal ganglia have a role, motor cortex is a key structure for movement learning. It undergoes functional and structural modifications during learning of a skill including strengthening of lateral connections and reorganization of neural representations (Rioult-Pedotti et al. 1998; Kleim et al. 1998). Our laboratory focuses on motor cortex plasticity during skill learning. This thesis focuses on two phenomena in the motor cortex that are necessary for successful motor learning: *motor map reorganization* (Chapter 1) and *dopaminergic neurotransmission* (see Chapter 2).

CHAPTER 1. MOTOR MAP REORGANIZATION

1.1 INTRODUCTION AND HYPOTHESES

Skill learning is associated with an enlargement of representations of trained body parts in monkey (Nudo et al., 1996), rat (Kleim et al., 1998) and human (Pascual-Leone et al., 1995; Karni et al. 1998). Monkeys trained to grasp with their forefingers show an enlargement of the finger representation in the motor cortex (Nudo et al. 1996). In rodents trained to reach and grasp for a food pellet similar enlargement has been observed for distal forelimb representations after at least 8 training sessions (Kleim et al. 2003). Such enlargements are not present in controls that merely use the limb but do not learn (activity control). Map reorganization depends on cholinergic input to motor cortex; if this input is removed, motor learning is significantly impaired (Conner et al. 2003). This led to the hypothesis that motor maps may be one substrate for the motor memory trace (Monfils et al. 2005). In concordance with this assumption are findings in musicians and sport professionals who show enlarged representations after long term training (Tyc et al. 2005, Elbert Science 1995).

If this assumption is true, one would expect map changes to persist after learning ended for as long as the skill is retained. Therefore, we designed an experiment with longitudinal measurements of motor cortex somatotopy ("motor cortex mapping") during and after acquisition of a precision forelimb reaching movement. Acquisition here means learning until a performance plateau is reached, a state that is sometimes called "overlearned" state. The control condition was designed to exclude the possibility that changes in somatotopy are related to activity, i.e., forelimb use, as opposed to learning.

Longitudinal assessments require a mapping technique with high consistency over time and minimized confounding factors induced by the technique itself. Conventional intracortical mapping with protruding electrodes damages cortical integrity with unpredictable consequences for behavior and subsequent mappings. We therefore developed a novel surface stimulation technique, using a 64-electrode thin-film electrode array. This array can be implanted epidurally or subdurally over the motor cortex without inducing motor cortex damage (Molina-Luna et al. 2007). Additionally, an automated mapping procedure reduces the time required for each measurement and – by randomizing the sequence in which the contacts across the array are stimulated – systematic errors related to sequential stimulation or to stimulation of neighboring areas. These technical developments together with their application in the learning model are described in the following.

The hypotheses tested were:

- Epidural microstimulation (EMS)
    - does not induce cortical injury,

  - can reproducibly and reliably measure motor cortex somatotopy, and
  - is comparable to the reference standard technique, i.e., intracortical microstimulation.
- Motor cortex somatotopy
  - changes with learning but not simple use (activity) and
  - persists after training ended for as long as the skill is retained.

1.2 EPIDURAL MICROSTIMULATION (EMS)

1.2.1 METHODS & EXPERIMENTAL DESIGN

1.2.1.1 ELECTRODE ARRAY

The thin-film microelectrode array technology was derived from the German Retina Implant project (Stett et al. 2000). The geometry of the retina implant electrode was modified to cover the rat's motor cortex which is approximately 4.5 mm wide and 6 mm long (Neafsey et al. 1986). The array consisted of a 14 µm thin polyimide foil, 6 x 7 mm² in size (42 mm²) with 72 titanium nitrite contacts (diameter 100 µm) distanced 640 µm in medio-lateral and 750 µm in antero-posterior direction (*Figure* 1).Each contact had a central indentation to increase surface and thereby current transfer. The impedances of the contacts ranged between 5-10 kΩ at 1kHz. A flat cable (polyimide) connected the electrode array to a connector piece. Perforations of 100 µm diameter in the polyimide structure between the contacts allowed for diffusion of body fluids. Because the array's materials and design were the same as for the retina implant, studies confirming the biocompatibility of the implant for up to 12 months are valid (Hammerle et al. 2002).

1.2.1.2 IMPLANTATION

Arrays were implanted in twenty-seven Long Evans rats (8-10 week old, 250-300 gm body weight) between *dura* and skull over the left motor cortex. All experimental procedures were approved by the Animal Care Committee of the University of Tübingen and the responsible State Agencies. Surgeries were performed with the animal placed in a stereotactic frame under ketamine/xylazine anesthesia (70/10 mg/kg i.p.). Body temperature was supported by a heating pad. Bone cement (FLOWline cement, Heraus Kulzer, Dormagen, Germany), two screws (2 mm diameter) anchored in the occipital and ethmoid bone and a plastic frame were used to fixate the two adapter plugs to the skull (figure 1). A craniotomy of 7 x 8 mm² (coordinates relative to Bregma: 4 mm posterior, 5 mm anterior, 5 mm lateral) was performed. The array was laid onto the dura over the motor

cortex using a micropositioner. Antibiotic prophylaxis was given (Ceftriaxone 80 mg/kg i.p. single dose). The entire procedure limited exposure of *dura* to less than 5 min. Implants were maintained up to 6 weeks (in 3 animals) before the animal was euthanized by an overdose of Pentobarbital.

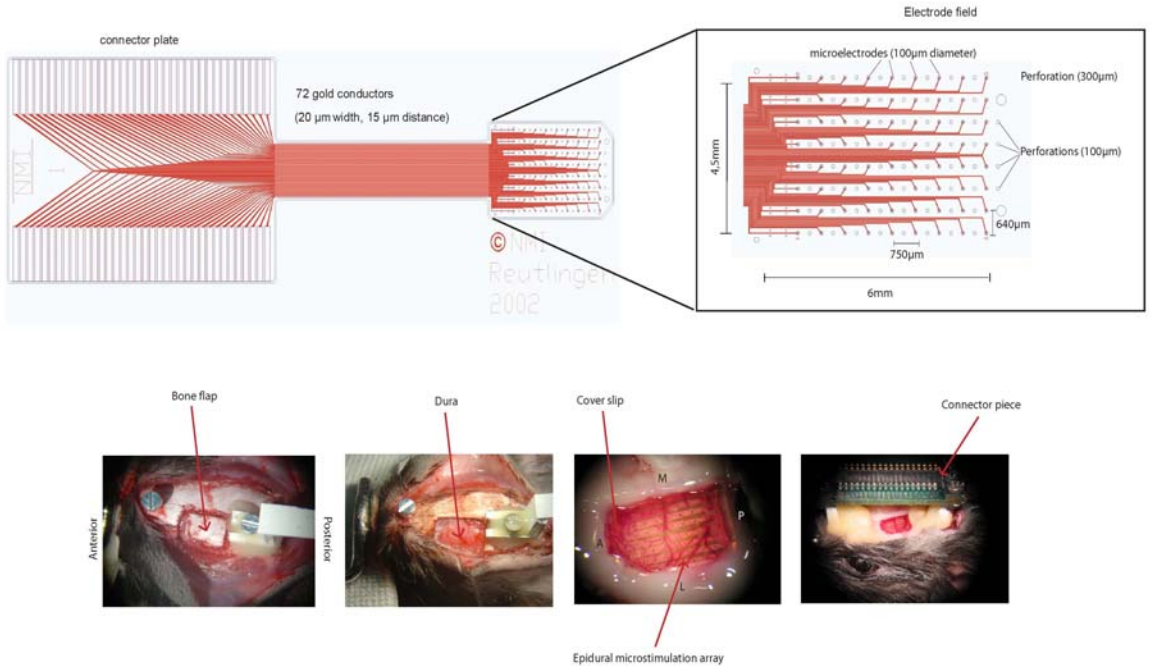

Figure 1. Epidural microstimulation (EMS), electrode array & implantation. A The polyimide-based electrode foil contains an electrode array with 72 electrodes (1 of 64 used as cathode, 8 as anode). The geometry is adapted to the rat's motor cortex (Neafsey et al. 1986). Perforations (100 µm diameter) between each electrode pair allow for fluid diffusion. Each electrode has a central indentation to increase surface area thereby improving current transfer. B Top and middle: electrode array placed epidurally (*in situ*) onto the brain of a rat. Lower: Head stage adapter (black) on the rat's head affixed by a plastic scaffold (white).

1.2.1.3 STIMULATION MAPPING PROCEDURE

Brain stimulation was performed using constant current pulses (100 stimuli in 300 Hz, pulse width 1 µs, constant current of 1 – 5 mA, per channel). Animals were sedated (ketamine 10 mg/kg; xylazine 70 mg/kg) for mapping and had one-dimensional acceleration sensors taped to each limb to detect movements (ADXL202JE, Analog Devices, Munich, Germany). Movement responses were quantified by the peak-to-peak amplitude of the calibrated acceleration sensor signal. Additionally, evoked movements were recorded for off-line inspection using a digital video camera. Off-line inspection was used to verify the exact location of the evoked movement within each limb (shoulder, elbow, wrist, hip, knee, foot). It was also determined whether the movement was 'complex', i.e. composed of several twitches involving more than one joint.

Cortical mapping was performed by routing trains of biphasic stimuli to one of 61 'active' contacts using a custom-designed optically decoupled multiplexer and software based on LabView (National Instruments, Austin, TX, USA). Routing was performed in random order to prevent confounds due to stimulation of neighboring contacts, e.g., due to refractoriness. Three contacts (positions 1, 33 and 64) were not connected for technical reasons (wiring layout of the connecter plug). The remaining 8 contacts were short-circuited to form the 'reference' electrode.

A contact was deemed responsive, if a movement was evoked and confirmed by two series of stepwise increments in stimulation current (1 - 5 mA, steps of 0.2 mA). The motor threshold (MT) was defined as the current at which a response was confirmed. To assess the reproducibility of the method 2 mapping procedures were performed (8 days apart) in 6 animals.

1.2.1.4 COMPARISON WITH INTRACORTICAL MICROSTIMULATION

In four animals, intracortical microstimulation (ICMS) was performed before EMS array implantation. Previously described procedures were used (Haiss and Schwarz 2005). ICMS electrodes were pulled and ground glass-coated (80 µm shank diameter; 23 µm diameter of the metal core; free tip length, 8 µm; impedance, 1 MΩ; Thomas Recording, Giessen, Germany). The free ends of the electrodes were soldered to Teflon-insulated silver wires (Science Products, Hofheim, Germany). Animals were anaesthetized initially with isofluorane (1–3%) for implantation of a tail vein cannula and craniotomy. At least 25 min before micro-stimulation, anaesthesia was switched to continuous intravenous ketamine S infusion (2 mg·kg^{-1}·min^{-1}). The dura was removed, and a single electrode was lowered into the motor cortex perpendicular to its surface using a hydraulic micropositioner (Kopf 650; David Kopf Instruments, Tujunga, CA, depth: 1200 µm). Initial needle penetrations were made to locate the caudal motor cortex. Once a motor response occurred, neighbouring sites in distances of approximately 500 µm (circumventing overlying vessels) were stimulated. Stimuli were delivered in stepwise increments of current (20, 40, to 60µA, trains of 18 biphasic pulses in 57 Hz, cathodal first, pulse width: 200µs, inter-train interval 1.5s, programmable stimulator STG 1008; Multichannel Systems, Reutlingen, Germany). Electrode penetration sites were documented by digital photography. After completion of ICMS with the animal still being anaesthetized, the EMS electrode array was placed and covered with thin glass (microscopy cover-slip). A digital photograph was taken for later reference to the ICMS penetration sites. EMS stimulation was performed as described above. Sites responsive to stimulation using either technique were identified and marked for visual comparisons of spatial overlap (*Figure 5B*).

1.2.1.5 ASSESSMENT OF CORTICAL INJURY

Whether array implantation or mapping induced a behavioral deficit was tested by training 4 animals in a precision forelimb reaching task (see Appendix for detailed description of the reaching paradigm) after implantation and comparing these rats to control animals without implants.

The brains of 3 animals were examined for signs of injury after two mapping procedures (8 days apart) and a total implantation time of 2 weeks. Histological assessment of 50 µm thick sections included screening for cytoarchitectonic evidence of tissue damage using Cresyl Violet and screening for gliosis (chronic tissue damage) using immunohistochemistry for glial fibrillary acidic protein (anti-GFAP rabbit antibodies, Vector Laboratories, Burlingham, CA, USA; biotin-conjugated secondary anti-rabbit antibodies visualized with the avidin-biotin-peroxidase reaction and DAB). For detection of neuronal cell death TUNEL staining was used (i*n situ* Cell Death Detection Kit, Roche Diagnostics, Mannheim, Germany), which detects DNA fragmentation as an indicator of delayed apoptotic and necrotic cell death (Striedinger et al. 2005; Yang et al. 2006).

1.2.2 RESULTS

Implantation, stimulation and brain mapping was successfully carried out in all animals. A median of 1.5 contacts (4.33%) per array were dysfunctional due to manufacturing defects. In 3 animals, the array was maintained for 6 weeks; the implants remained functional without complications. Histological assessment after 2 weeks and 2 mapping procedures showed no signs of injury (*Figure 2*).

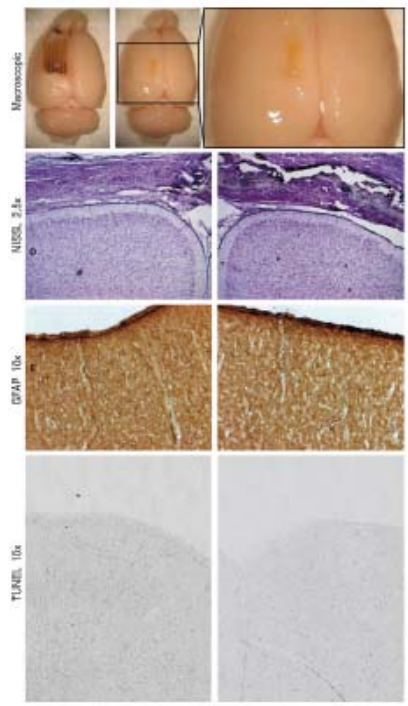

Figure 2 Assessment of brain damage. (1st row) macroscopic appearance of a brain after removal of chronically implanted array. (2nd row) coronal sections Nissl stained for cortical cytoarchitecture (2.5x). (3rd row): GFAP (glial fibrillary acid protein, 10x) immunohistochemistry from same rat for reactive tissue gliosis (left un-implanted right implanted). (4th row) TUNEL staining shows no reactivity providing no indication of incumbent cell death (10x).

Motor performance or learning of a precision forelimb task with the contralateral limb was not affected by implantation or repeated mapping (repeated measures ANOVA effect of group: F(5,55)=0.55, p=.74; *Figure* 3).

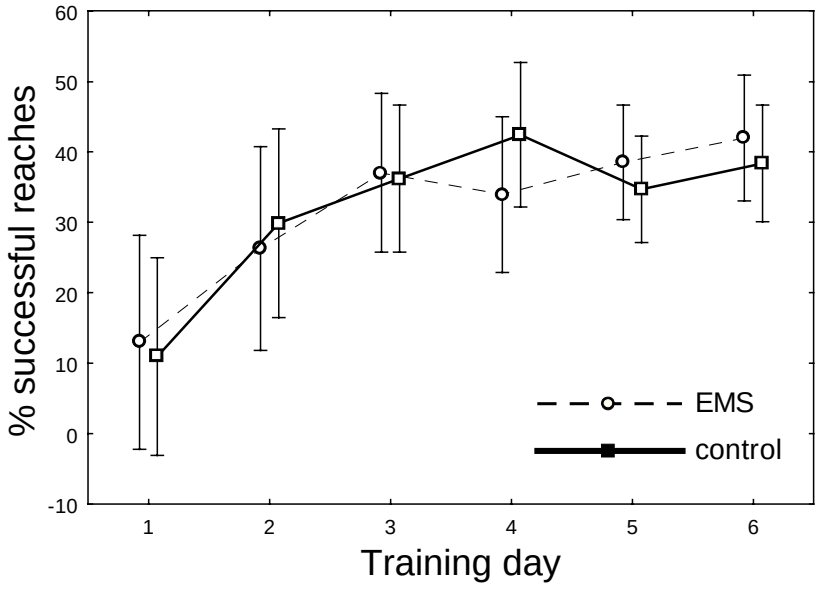

Figure 3. Effects on behavior. No impairment in performance or learning (performance improvement over time) was observed in animals implanted with EMS arrays undergoing one cortical mapping session before training began (effect of group on performance: p=.74).

On average, currents of 2.13 mA, SD .88 mA were required to evoke movements. Charge densities were between 12 and 61 µC/cm². Of all responses observed during the initial stepwise augmentation of current, <1% (0.57%) could not be reproduced. With the array positioned 5 mm anterior and 0.2 mm lateral to bregma (anterolateral corner), more contacts accessed the forelimb area (36.9%, SD19.5%, percent values are relative to the number of functional contacts) than hindlimb (7.2%, SD 8.2%) or neck areas (6.5%, SD 7.2%) [Friedmann nonparametric ANOVA: $\chi^2 = 15.17$; $p < .0005$].

Most responses were contralateral to the instrumented hemisphere (72.6%, SD 14.6% vs. 27.4%, SD 14.6% ipsilateral; nonparametric sign test: Z=3.02; p<.003). 'Complex' motor responses comprising sequential or parallel movements across several joints within one limb were observed in 7.7%, SD 12.6% of all forelimb and in 2.8%, SD 8.3% of all hindlimb responses. Simultaneous movement of forelimb and hindlimb was observed in 4.8%, SD 4.8%. Motor thresholds to elicit forelimb (2.75 mA, SD 1.50 mA) or hindlimb movements (1.84 mA, SD 0.76 mA) were not different (paired t-test: p=.66).

The size of elbow, wrist and shoulder representations in motor cortex remained constant between two mapping procedures eight days apart, confirming the reproducibility of the procedure (n=6, intraclass correlation coefficient 85%, *Figure* 4).

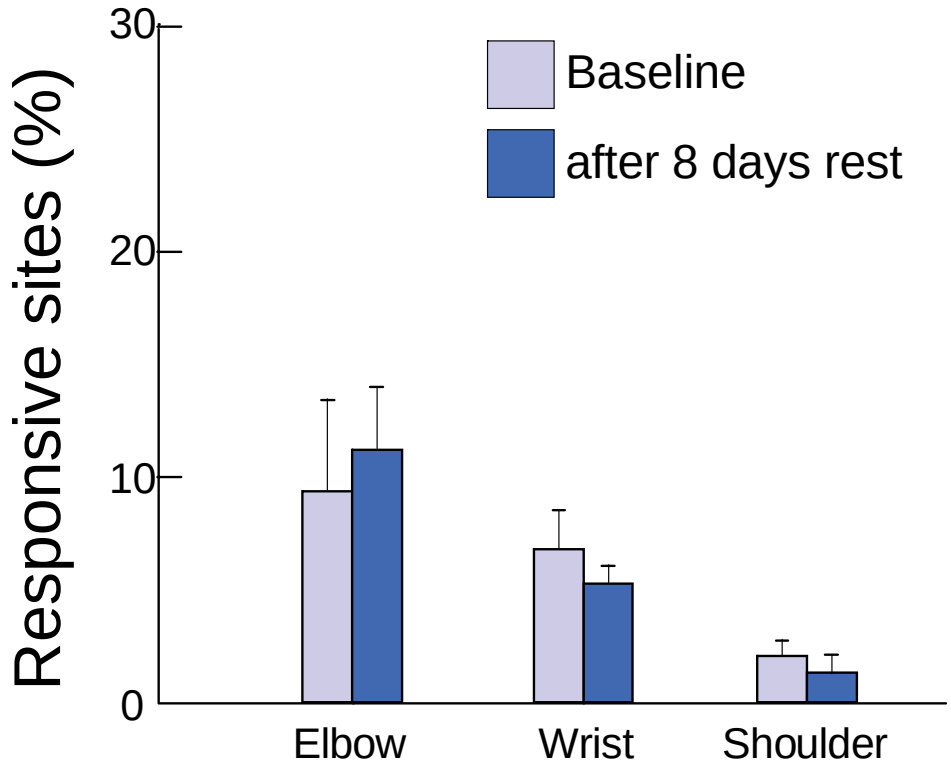

Figure 4. Reproducibility. Repeated cortical mapping before and after an 8-day period shows the reproducibility of elbow, wrist and shoulder representation areas (n=6; intraclass correlation coefficient 85%, difference were not significant, p>0.05).

The average map geometry is shown in

Figure 5*A*. As compared to published data, these maps slightly overestimate the representation size for forelimb and hindlimb (Neafsey et al. 1986). We therefore compared EMS with conventional intracortical microstimulation (ICMS) within four animals (

Figure 5*B*). EMS overestimated the representation areas (forelimb .05 cm^2, SD 1.30 cm^2 vs. .02 cm^2, SD 1.31cm^2 for ICMS; independent sample t-test: $t(6) = 4.76$, $p < .003$). EMS responses had longer latencies than ICMS responses (20.82 ms, SD 2.69 ms vs. 8.27 ms, SD 0.88 ms; independent sample t-test: $t(19) = 10.19$, $p < .0001$).

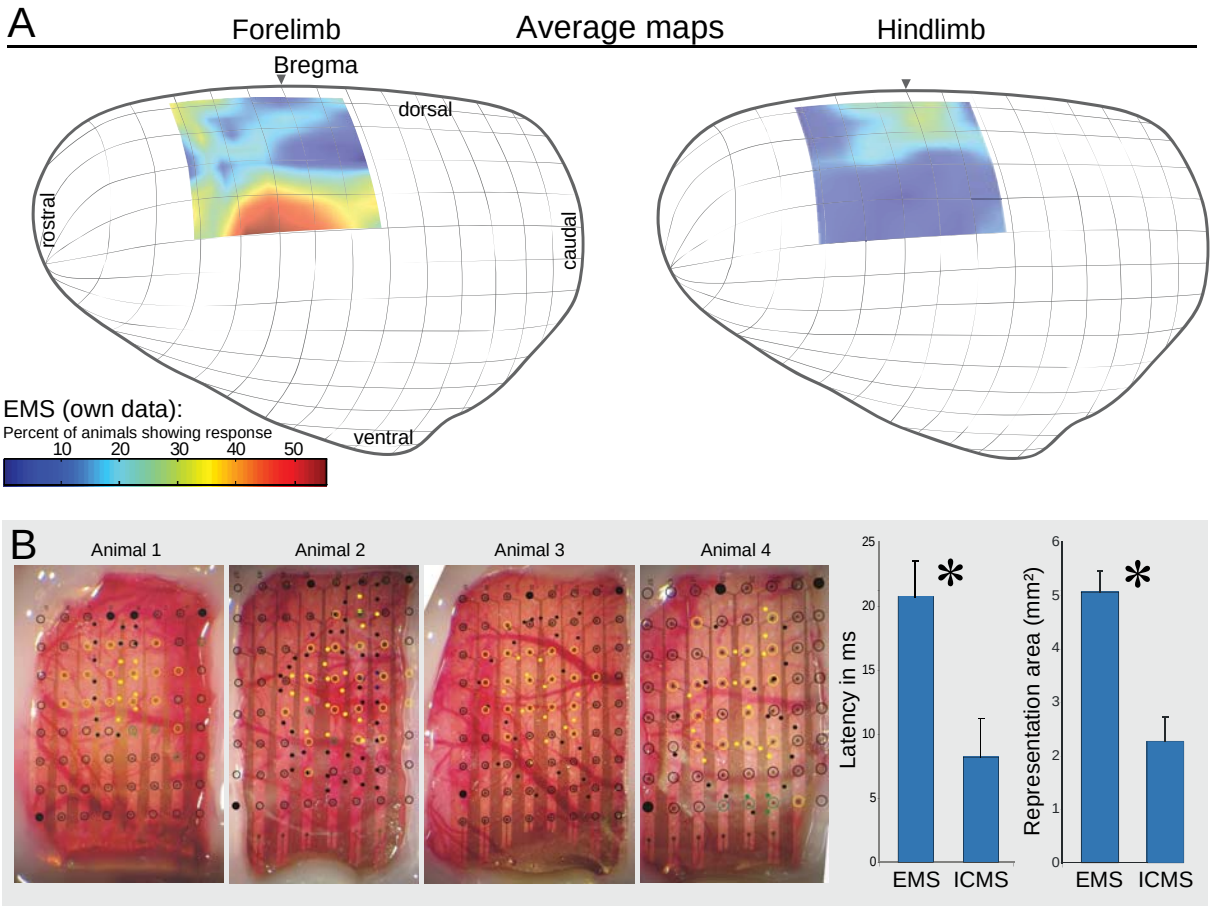

Figure 5. Cortical mapping using the EMS array. (A) Average cortical stimulation maps for forelimb (elbow and wrist) and hindlimb (knee & foot) are shown. These maps were constructed by coding responsive sites as '1', unresponsive as '0', then averaging the 8 by 8 map of 23 animals (all except those animals in which ICMS and EMS was combined, see B) and interpolating the result by the factor 4. (B) The intraindividual comparison of EMS and ICMS in four animals confirms substantial overlap between representations identified by either method (ICMS: dots, EMS: circles; yellow: forelimb, green: hindlimb). The comparisons also show a systematic overestimation of the forelimb representation size (elbow, wrist and shoulder) by EMS. Also, ICMS responses have a shorter latency than EMS responses (* $p<0.05$).

1.2.3 DISCUSSION

These findings confirm our hypotheses that EMS does not induce cortical injury while being able to reproducibly measure motor cortex somatotopy. Map geometries obtained by EMS are comparable to the reference method *intracortical microstimulation* (ICMS), but EMS overestimates the size of representations.

Overestimation of size reflects the reduced focality of EMS. EMS electrodes are in greater distance to target neurons than ICMS electrodes, thereby requiring higher currents to surpass motor thresholds. Higher currents spread to larger volumes of tissue. EMS responses were found to have longer latencies than ICMS responses. This may be the consequence of EMS predominantly exciting cortical interneurons, which transsynaptically activate motor

cortex output neurons. In contrast to output neurons with vertical fiber trees, the fibers of interneurons mainly traverse the cortex parallel to its surface; EMS induces electric fields in that same orientation. A similar mechanism is hypothesized for transcranial magnetic stimulation (Cracco and Cracco 1999). In contrast, ICMS likely stimulates interneurons as well as output cells. Because activation of an output cell does not need to pass a synapse, the evoked response has a shorter latency. Due to these different stimulation mechanisms, ICMS and EMS may provide complimentary information.

As compared to ICMS, EMS has several advantages. The implantation procedure is minimally invasive, i.e., it leaves the dura intact and maintains cortical integrity. EMS is less time consuming and allows for an automatized mapping in which electrode positions are stimulated in true random order. Random order is necessary to avoid systematic confounds induced by stimulation of neighboring electrode positions. The embedding of the array between skull and dura assures that the position of stimulation sites remain relatively constant. Additionally, permanent implantation allows for recording in freely moving rats.

Disadvantages include higher currents required due to the greater distance between electrode and stimulated neuron (Hall and Lindholm, 1974). Although still below the critical charge density limit, these currents may induce cortical damage, particularly with long stimulus trains (Donoghue and Wise, 1982; Neafsey et al., 1986). However, no indication of damage even after repeated stimulation procedures was observed in different histological assessments of cortical tissue. EMS theoretically has lower spatial resolution (inter-electrode distances: 640µm mediolateral, 750 µm anteroposterior) than ICMS. Due to the greater distance between electrode and target neurons, larger populations of cells are excited, thereby reducing stimulus focality. However, the focality of the responses suggests that this resolution is sufficient for motor cortex mapping in rat. Also, enlarging the resolution in ICMS greatly increases procedure time. A small number of electrode positions in the EMS array were found to be faulty. This is a consequence of the complex manufacturing process that is a subject to refinement if the array is to be produced commercially in larger quantities.

Polyimide-film based array technology has been used for intracortical electrode arrays with multiple contacts along the shaft of the electrode (Michigan Probe, Kipke et al. 2003). Polyimide is chosen because of its good biocompatibility. It also allows for incorporation of bioactive compounds such as dextran gels with nerve growth factor (NGF) that promote neural growth onto the electrode probe (Rouche et al. 2001). In comparison to the Michigan probe, our electrode substrate is too thin, hence too flexible for insertion into cortex, because it was designed for surface application. It could, however, be fortified with other materials. A device similar to ours was developed by Hollenberg et al. (2006). They used a polyamide (Katpon) film with 64 gold electrodes for epidural recording of surface field potentials from rat barrel cortex. Contacts (diameter 150 µm), intercontact spacing (750 µm) and thickness (75 µm) was larger as compared with our array. Increased thickness likely

resulted in less flexibility allowing for implantation through a slit burr hole. Although our probe could be inserted through a slit hole, the thin and narrow cable connecting the array to the connector piece did easily buckle. We therefore removed and reimplanted a rectangular bone flap after probe insertion. This procedure allowed for better spatial navigation with respect to anatomically constant vessels on the cortical surface but may have induced more brain damage than slit hole insertion. Chronic recording using the Kapton-gold array has not been tested and – as the authors discuss – may be limited by the bulky circuit board that the rat would have to carry on its head. Although we show the feasibility of chronic implantation and repeated mapping, recording from our array in the behaving animal would face the same difficulties as preamplifier plugs are bulky (2.5 x 2.8 x 0.5 cm^3, two 32 channel plugs required, MPA32I, Multichannel Systems, Reutlingen, Germany).

A flexible microelectrode array consisting 16 platinum contacts on a silicone surface embedded into a polydimethylsiloxane (PDMS) matrix was reported by Kitzmiller et al. (2006). Good biocompatibility and subdural recording ability from the visual cortex of a pig were shown. Contacts were 200 μm^2 squares with an intercontact distance of 400 µm. Small distances and large contacts (compared to our array with 100 µm diameter contacts) was possible because wires connecting each contact to an interface pad were not patterned on the silicone substrate but were bonded to each contact extending into the third dimension (later embedded into PDMS). Bonding was achieved using an ultrasonic vibration device. In contrast, dense patterning of contacts and wires in our array required larger pitch and smaller contacts. Another 8x8 grid of silver contacts with 500 µm spacing has been used for vibrissae cortex but no further details about the array were reported (Jones & Barth 1999).

In conclusion, we demonstrated that thin-film polyimide-based microelectrode arrays can be successfully used to repeatedly stimulate and record from rat motor cortex in sufficient spatial resolution. Despite repeated stimulation procedures, no cortical damage was induced. Therefore, EMS is best suited for serial measurements because we combined stimulation with histological and molecular assessments, for which tissue damage would be a confounding factor.

1.3 MOTOR CORTEX SOMATOTOPY DURING AND AFTER LEARNING

1.3.1 METHODS AND EXPERIMENTAL DESIGN

1.3.1.1 ANIMALS AND EXPERIMENTS

Sixteen male Long-Evan rats from our own stock (8-10 weeks, 250-350g) were included in this study. Animals were housed individually in a 12/12-hr light/dark cycle. All experiments were performed at the beginning of the active phase. Animals were fasted for 24hr before

the first pre-training session (see below). During training animals were kept at 105% of their initial weight (332.1 ± 29.4 g) by providing approximately 50mg/kg of standard lab chow after each training session. Water was provided *ad libitum*. All procedures were approved by the Animal Care Committee of the University of Tübingen and the responsible State Agencies.

Stimulation mapping of cortical somatotopy was performed repeatedly, during and after eight days of training a forelimb reaching task (see Appendix for a detailed description). The experimental timeline is depicted in *Figure 1A*. Mapping sessions were performed either on the day prior to training (maps day 0, d15, d20) or on the day of training 3-5 hours after the session ended (maps d8, d27). Eight animals were assigned to each group (1 control animal had to be excluded due to electrode defects). Each group of animals was subjected to a Skilled Reaching Task or an Activity Control Task (see Appendix for details). Eight of these animals (5 reaching, 3 controls) were subjected to the respective task for a second time between days 21 and 27.

1.3.1.2 DATA ANALYSIS

Analyses were performed using Statistica version 7.0 (StatSoft Inc., Tulsa OK, USA). Reaching performance was quantified as the percentage of successful reaches per session. First, linear regression models were used to test for effects of *training day* on *reaching performance*:

$$performance = \text{m} * \text{training Day} + \text{performance}_{\text{offset,}}$$

where session is experimental session number, *m* is the learning slope, and $\text{perfomance}_{\text{offset}}$ is the initial performance. Additionally, learning curves were approximated using nonlinear regression procedures based on the exponential model

$$performance = \frac{p}{1 + e^{k(a - session)}}$$

with parameter *p* indicating the performance plateau, parameter *k* the maximum steepness of the curve (learning rate) and parameter *a* the inflection point (time of maximum learning).

The size of a representation was quantified as the number of responsive contacts per limb or joint. Shapiro-Wilk tests were used to test for normality and Levene's test for homogeneity of variance. Repeated measures analysis of variance with *group* (SRT, ACT) and *limb* (forelimb, hindlimb) as between subjects factors, *time* as within-subjects factor and the interaction of *group* x *time* x *limb* tested for effects of group on map changes and motor thresholds. Baseline map size was entered as a continuous covariate in the model. Fisher's least significant difference tests were used in post hoc analyses. Two-tailed probability less or equal to 5% was considered significant. Numerical results are expressed as mean and SEM.

1.3.2 RESULTS

All animals acquired the motor task reaching a performance plateau by session 3 (linear model, effect of *training day*: $F(6,36)=5.01$; $p<0.001$, non-linear model performance = $65/(1+e^{1.76 \cdot (1.06\text{-session})})$,

Figure 6) as observed in previous studies (Buitrago et al. 2004; Luft et al., 2004a). The skill was then maintained over the 8-day rest period. At baseline the size of the forelimb representation in caudal motor cortex was similar in SRT and ACT animals ($t(13)=-0.95$, $p=0.36$). Repeated measures ANOVA revealed a significant interaction between *group* x *time* x *limb*, $F(2,36)=6.0$, $p=0.006$. Post-hoc testing using Fisher's least significant difference test revealed a significant increase in the number of forelimb-responsive contacts in the SRT group during training (map 0 – map 8: $p=0.020$) followed by a decrease after training (map 8 – map 15: $p<0.001$); in ACT animals, the representation decreased during training ($p<0.001$, *Figure* 6).

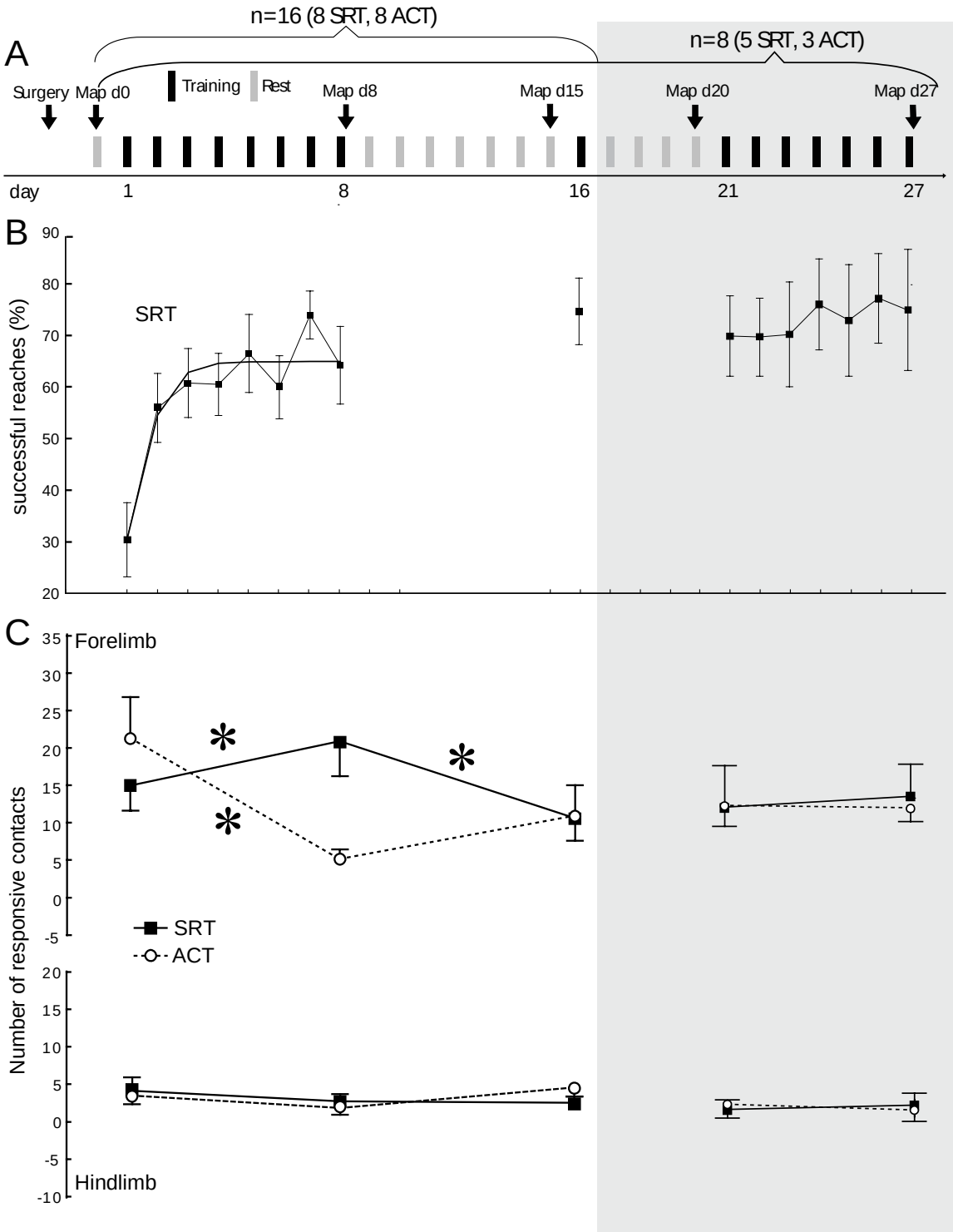

Figure 6 (A) Experimental timeline (white background: n=15, gray background: n=8 animals trained for a second 8-day period). **(B) Reaching performance in SRT.** The learning curve (successful reaches in percent of all trials per session over time) shows an exponential improvement in skill performance before it plateaus (n=8 SRT animals, ACT has no measure of performance, therefore this group is not included in this graph). Five SRT animals were trained for a second 8-day period but show no further learning. Error bars represent SEM. **(C) Map changes in SRT and ACT.** In SRT (n=8), forelimb representation size increases during learning and

decreases thereafter whereas hindlimb representation size remains largely unchanged. Opposite forelimb changes are observed for ACT (n=8, one animal excluded because of technical failure of the electrode). Representation size is measured by the number of contacts evoking a given limb movement. The ordinate plots the response variable of the general linear model which includes baseline performance as a covariate. Error bars represent SEM, * indicates post-hoc Fisher's LSD p<0.05.

Exemplary stimulation maps are shown in *Figure 7*. Average motor thresholds to evoke a forelimb motor response were smaller in ACT (1.8 mA) than in SRT (2.4 mA) at baseline ($t(13)=2.36$; $p=0.034$), but did not change over time (repeated measures ANOVA, effect of *time*: $F(2,18)=0.17$; $p=0.84$; *group* x *time* interaction: $F(2,18)=0.93$; $p=0.41$).

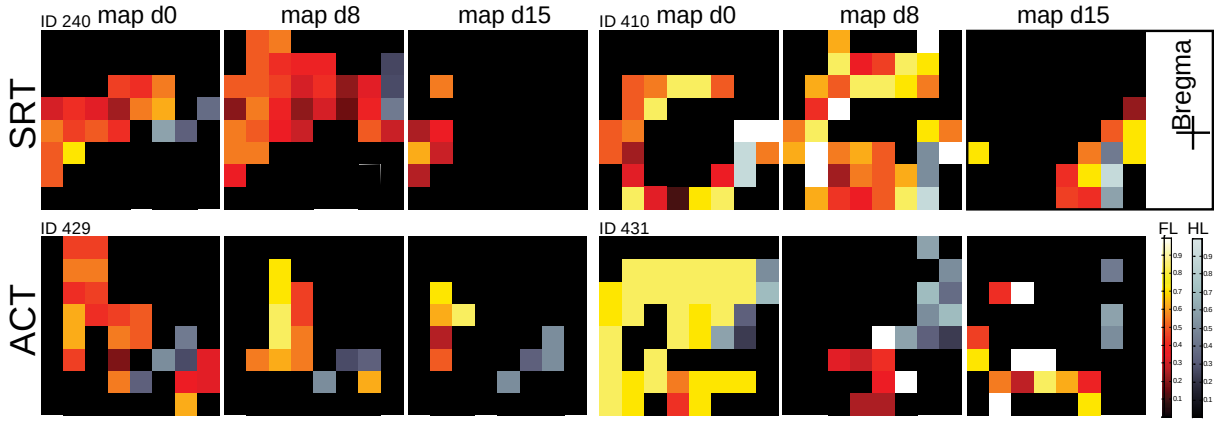

Figure 7 Exemplary motor cortex maps of 2 SRT and 2 ACT animals. The colour represents the inverse of the motor threshold at a given position (1/threshold in mA). Forelimb responsive sites are shown in red to yellow, hindlimb sites in gray to blue. Sites responsive to both forelimb and hindlimb were not included in these maps. For the upper right map, its relative position to Bregma is indicated by the cross. Each square represents one contact site (100µm).

The enlargement of the forelimb representation (map 8 – map 0) in the SRT group correlated with the improvement in reaching performance during the training period, i.e., the difference between average performance over the last three sessions and baseline ($r=0.82$; $p=0.012$; n=8, *Figure* 8). The subsequent decrease showed no correlation with performance changes occurring between training days 8 and 16 ($r=-0.31$, $p=0.62$).

The size of the entire motor map, i.e., all contacts where motor responses in any body part were evoked, neither changed over time ($p=0.16$) nor was different between groups ($p=0.30$). Post-hoc testing (which has to be considered with caution because the omnibus F is insignificant) showed a decrease in motor map size in the SRT group between maps 8 and 15 ($p=0.040$).

To test whether motor map changes were specific for motor learning and were not a consequence of SRT related motor activity, 8 animals (5 SRT, 3 ACT) were trained again for 8 days. They started this second training with similar performance (paired t-test, $p=0.56$) and

did not improve further (linear model, effect of *training day*: p=0.8). ACT animals performed the same task like before. No changes in forelimb area were observed in either group (effect of *time*: p=0.80).

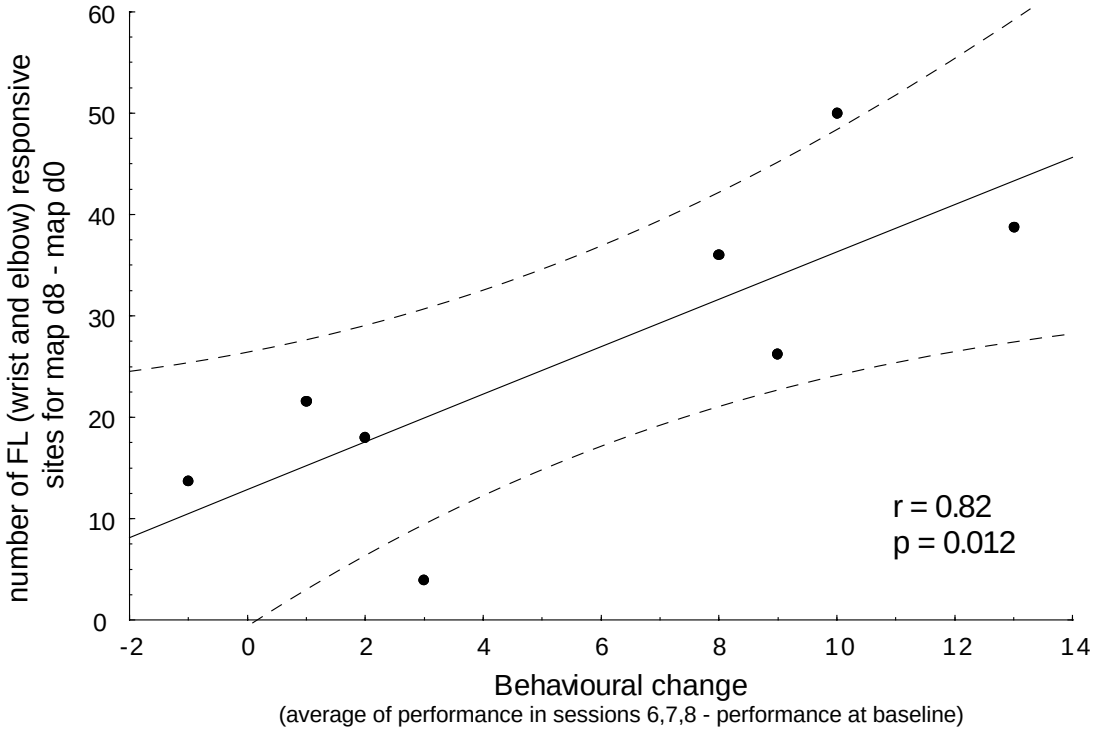

Figure 8 Correlation between learning success and representation change. A direct correlation was found between learning (average reaching success for sessions 6, 7 and 8 minus success at baseline) and change in the size of the forelimb representation (number of forelimb responsive sites in map d8 minus map d0, n=8 SRT animals). Dotted lines represent 95% confidence intervals.

1.3.3 DISCUSSION

These findings show that learning a novel movement (1) is associated with changes in the output organization of the motor cortex, (2) that these changes correlate with performance gains, i.e., with the efficacy of learning, and (3) that these changes are transient and quickly reversed after training ends. Using the forelimb without skilled learning is associated with shrinkage of the forelimb area.

Previous cross-sectional studies using intracortical microstimulation (ICMS) showed larger representations of distal movements in the motor cortex of rats that underwent skill learning versus those that performed activity control tasks (Kleim et al., 1998; Remple et al. 2001). Proximal movement representations became smaller. These changes were only seen late (day 10) but not early (day 3) in training (Kleim et al., 2004). These results are largely congruent with our longitudinal data, although we do not differentiate between proximal and distal responses. Longitudinal studies have also been carried out in monkeys and have

confirmed these observations (Nudo et al., 1996). In the rat, one study using repeated mapping failed to show enlargement, however it was not designed to test for learning-related map changes, but rather for the effects of protein synthesis inhibition on motor maps (Kleim et al., 2003). Taken together, there seems to be agreement between different authors that representations of trained body parts enlarge during learning. If such enlargement is avoided by reducing cholinergic inputs to cortex, learning is reduced (Conner et al., 2003). The causal relationship between motor cortex map and behavioral changes helps to explain our observation of a correlation between the degree of forelimb map enlargement and learning success.

In ACT animals, representations did enlarge, but shrunk. Shrinkage contributes to between group differences observed in the cross-sectional studies cited above. Little evidence for map shrinkage with activity exists in the literature: in monkeys unskilled activities produce an unsystematic pattern of motor map changes (Plautz et al., 2000); in rats, one experiment – again conducted for a different purpose – shows insignificant shrinkage (Kleim et al. 2003). Shrinkage may be a consequence of habituation or automation when a movement is repeated but not continuously modified between trials. Automation may lead to stronger involvement of sub cortical regions, a classical concept that may be applicable to certain movement sequences (Ioffe, 2004). Noteworthy, the decrease does not occur during a second training phase indicating that first-time exposure to ACT has an effect on cortical input-output organization.

Cortical map changes regardless of task were transient, i.e., maps reverted to baseline after a rest period, while the skill was retained. In the SRT group, the reversal of the enlarged forelimb representation was accompanied by a reduction of the entire motor map – statistical caveats weaken this finding as pointed out above. If true, this finding may indicate a broad increase in cortical inhibition across the entire motor cortex. An overall increase in motor cortex inhibition may serve as a mechanism to terminate or revert learning-specific shifts, such as the enlargement of the elbow in our case. This may reset the motor cortex to allow for acquisition of new movement sequences. What may be the role of transient map changes? It may be that transient enlargement of representations during learning renders the cortex apt to lasting modifications (consolidation), i.e., shifts the system into a “learning mode”. This can be reversed once learning has taken place.

The transient nature may seem incompatible with the hypothesis that new movements are encoded in motor cortex maps (Monfils et al., 2005). But this study only allows for the conclusion that new movements are not encoded in forelimb representation size. The motor cortex may still be a candidate brain region for the motor engram (Monfils et al. 2005). It may be that motor engrams for complex tasks are formed by distributed circuits of interacting neurons within motor cortex (Huntley and Jones, 1991; Maynard et al., 1999) that cannot be captured by the gross representational mapping performed here.

Other studies have shown learning-related lasting changes in motor cortex. Karni and colleagues (Karni et al., 1995) asked human volunteers to train a finger sequence movement daily for several weeks. Using functional magnetic resonance imaging they observed slow enlargement of motor cortex activation; in a subset of 2 subjects, they report that enlarged activation persisted after training was stopped. Long-term training may also explain persistently enlarged representations in skilled musicians (Elbert et al., 1995). The disparities to our study likely are related to different training schedules or methods to assess cortical physiology.

Several methodological issues related to stimulation mapping in general and specifically our technique warrant consideration. Stimulation mapping overestimates representation size if excitability is increased and the same neurons can, hence, be excited from a greater distance. This confound seems unlikely to have affected our results, because average motor thresholds that directly reflect excitability remained constant despite map changes. As discussed in the previous chapter, ´we have previously compared EMS with ICMS in the same animal and found a good correlation between the two methods for map location and geometry. Representation size and motor response latencies were larger with EMS (Molina-Luna et al., 2007). These differences do not affect our conclusions here which are based on relative comparisons. Longer latencies may indicate indirect stimulation of output neurons through cortical interneurons. Hence, the changes in motor maps reported here may reflect a modification to cortical circuits as opposed to output neurons only.

In conclusion, this longitudinal study showed that motor cortex representations of trained body parts enlarge during learning of a complex fine motor skill. The enlargement of the representation correlates with learning success suggesting the possibility of a causal relationship between representational changes and motor learning. Representation changes are transient suggesting that consolidation of the skill does not require the motor map to persist and that motor skills are not encoded through gross representational modifications.

CHAPTER 2. DOPAMINERGIC NEUROTRANSMISSION

2.1 INTRODUCTION & HYPOTHESES

In the previous chapter, we have shown that reorganization of motor cortical maps is a feature of motor learning processes but motor maps unlikely represent the storage mechanism for movements. Nevertheless, such transient adaptations may help to acquire and/or store motor information. Another transient process that our group has previously demonstrated and that occurs during the acquisition phase of a novel movement is protein synthesis (Luft et al. 2004a, 2004b).

These experiments showed that protein synthesis inhibition within motor cortex during the interval period between training sessions impaired successful skill acquisition; once the skill was learned, protein synthesis inhibition had no effect on performance. Experiments addressing the question which proteins might be synthesized in motor cortex by using gene expression profiling (Buitrago et al. 2005, abstract SfN) revealed, among others, the dopamine receptor 2 (D2R).

Although it is known for many years that the primary motor cortex (M1) of various species contains dopaminergic receptors, little is known about their physiological role. In an autoradiography study published in 1989, Lidow and coworkers demonstrated a rostro-caudal gradient of D2 receptors across the cortex of monkeys and rats: highest concentrations were found in prefrontal and temporal areas, lowest in occipital cortex; M1 of rats has as many D2 receptors as prefrontal cortex, M1 of primates has about 60%. In another study these authors further characterized D1 and D2 receptor distribution in the primate M1 (Lidow et al. 1991). These findings were confirmed by in situ hybridization of D1 and D2 mRNA in primate and cat M1 (Huntley et al. 1992).

The physiological role of dopamine in the motor cortex may be the inhibition of pyramidal neurons. Intracortical application of dopamine lead to a progressive decrease in spontaneous firing rates of pyramidal tract neurons in rat M1 (Awenowicz J Neurophysiol 2002). D1 and D2 antagonists blocked this effect. In another study, systemic application of the D2 antagonist haloperidole lead to a similar decrease in firing rate that was accompanied by bradykinesia (Parr-Brownlie et al. 2005). Systemic application of the D2 antagonist sulpiride prevented changes in human motor cortex excitability induced by 22 min of transcranial direct current stimulation (Nitsche et al. 2006). The interpretation of studies using systemic administration of dopamine agonists or antagonists is difficult, because of the effect on dopaminergic neurotransmission in other parts of the brain, especially in striatum.

In the prefrontal cortex, D1 and D2 receptor function is much better understood. It is known that these receptors have a role in memory mechanisms, such as those related to reward

learning and addiction (Hyman et al. 2006), as well as in plasticity mechanism in prefrontal neurons (Huang et al. 2004).

Considering the evidence outlined above, a role of dopaminergic signaling in motor cortex physiology seems likely and its involvement in learning mechanisms at least possible. We therefore hypothesized (1) that dopaminergic nerve terminals exist in M1 of rats, (2) that dopaminergic signaling in M1 is relevant for motor learning and (3) it is necessary for functional plasticity of M1 neurons.

2.2 EVIDENCE FOR DOPAMINERGIC SIGNALING IN MOTOR CORTEX

2.2.1 METHODS & EXPERIMENTAL DESIGN

2.2.1.1 TYROSINE HYDROXYLASE WESTERN BLOTTING

Treated and control animals were anesthetized with ketamine/xylazine (1 ml/kg) and perfused through the ascending aorta with ice-cold 0.1 M potassium phosphate buffer (PBS) followed by ice-cold 0.1 M PBS at pH 7.4, containing 4% (wt/vol) paraformaldehyde. The brains were removed, post-fixed in the same fixative for 24 hr at 4°C, soaked for an additional 48 hr in phosphate buffer containing 30% (wt/vol) sucrose, and rapidly frozen in isopentane. Free floating coronal sections (50 µM) were incubated with monoclonal anti-tyrosine-hydroxylase antibody (1:500, Chemicon) overnight at 4°C. Then, slices were incubated in PBS containing the CY3-conjugated secondary antibodies for 1 h at room temperature. Finally, the sections were mounted with mowiol (CalBiochem) and stored in the dark at 4°C. Fluorescence was visualized by using a Zeiss Axiovert 200M system.

Total protein extracts of motor cortex were resolved on SDS/PAGE, transferred to nitrocellulose membrane, and incubated with mouse monoclonal anti-tyrosine hydroxylase (1:1000, Chemicon) over night at 4°C. A secondary horseradish peroxidase-conjugated antibody (1:2,000, Santa Cruz Biotechnology) and enhanced chemiluminescence (ECL) Western blotting reagents (Pierce) were used for band detection.

2.2.2 RESULTS

To prove the existence of presynaptic elements of a dopaminergic system in motor cortex, we used quantitative Tyrosin hydroxylase (TH) Western Blotting. TH is the first enzyme of the catecholamine synthesis cascade converting L-tyrosine to dihydroxyphenylalanine (DOPA), the precursor of dopamine. Norepinephrine, another neurotransmitter present in motor cortex, is synthesized by b-oxidation of dopamine by the dopamine beta hydroxylase. Therefore, TH exists in dopaminergic as well as noradrenergic neurons. By pharmacologically depleting either dopaminergic or noradrenergic neurons in motor cortex, TH binding

reflected the remaining neuronal population. Depleting dopaminergic neurons by injection of 6-OHDA intracortically and desipramine systemically led to a significant reduction in TH Western blot signal relative to the contralateral hemisphere not treated with 6-OHDA (paired t-test t=-5.42, p=0.03). By contrast, after unilateral depletion of noradrenergic neurons no difference between the hemispheres was observed (t=-0.92, p=0.45, *Figure 9*).

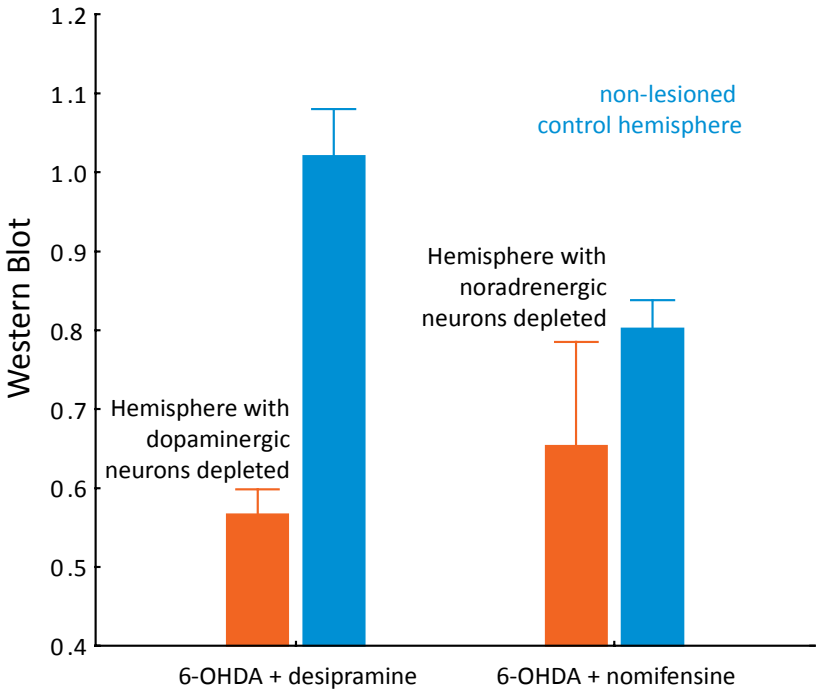

Figure 9 Dopaminergic terminals in motor cortex. Reduction of Tyrosine hydroxylase positive neurons after depletion of dopaminergic or noradrenergic neurons using 6-OHDA plus desipramine or nomifensine respectively, provide evidence for the existence of presynaptic dopaminergic terminals in motor cortex.

Immunohistochemistry of 6-OHDA treated versus control animals showed that dopaminergic terminals are present in layers II/III and V of motor cortex (*Figure 10*). These findings provide indirect evidence for the existence of dopaminergic neurons or terminals in motor cortex.

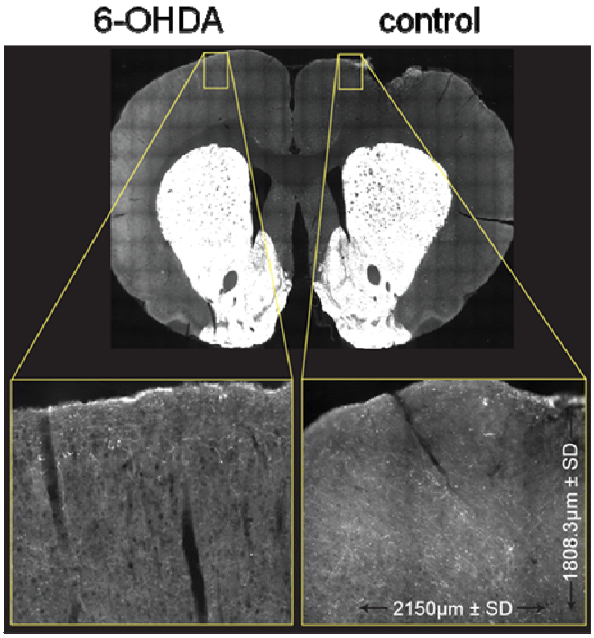

Figure 10 Tyrosine Hydroxylase (TH) staining for visualization of dopaminergic terminals. TH staining showed successful lesioning of dopaminergic terminals confirming the involvement of DA in learning. Lesion area was evaluated by in 4 brains by 3 blinded raters (93% intraclass correlation coefficient, means±SD shown).

2.3 BEHAVIORAL RELEVANCE: DOPAMINERGIC SIGNALING AND MOTOR LEARNING

2.3.1 METHODS & EXPERIMENTAL DESIGN

Please refer to the Appendix for a detailed description of animals, surgical procedures, behavioral tasks and statistical procedures.

2.3.1.1 EXPERIMENT 1: D2-ANTAGONIZATION IN MOTOR CORTEX DURING LEARNING

Raclopride n=7, sulpiride n=6, control n=8.

S(-)- Raclopride (+)- tartrate salt (Sigma-Aldrich, Inc. Munich) prepared in a vehicle solution of physiological saline 0.9% in a concentration of 10µg/µl

(S)-(-)-sulpiride [20µg/µl] and SCH23390 [600µg/µ] were obtained from Tocris Biosciences (Avonmouth UK) and dissolved in 0.01 M PBS, pH 7.4

5µl Hamilton syringe 85 RN for injection (Hamilton Bonaduz AG, Switzerland). One end of a 10cm long tube (PE40 Plastics one, Roanoke, VA USA) was attached to the syringe needle and the other end was attached to a 34 gauge (15mm/pst 3) injection needle to allow the animal unrestricted movement

64-channel thin-film electrode arrays (NMI, Reutlingen) were used for all surgical procedures. Animals were anaesthetized i.p. with a mixture of ketamine, xylazine, and temgesic [70_10_0.01mg/kg]. For mapping without pharmacological interventions, the surgical procedure has been described in Molina-Luna et al. 2007. Procedures for forelimb identification prior to cannula implantation were *in situ* microinfusion and cannula implantation. A craniotomy was performed over contralateral motor cortex of the preferred

limb. An area of skull approximately the size of motor cortex (Neafsey et al. 1986) was drilled (4.5mm x 6mm) and bone scalp was carefully removed leaving dura intact (coordinates with respect to Bregma: 5mm/4mm/1mm anterior, posterior, and medial, respectively). Brain area was exposed, and a thin-film microelectrode array designed for in-vivo applications (Molina-Luna et al. 2007), was placed epidurally on motor cortex. Mapping of motor cortex revealed two distinct forepaw clusters, one anteromedial and one posterolateral. For both procedures the anteromedial (rostral) cluster was taken as reference as this is where the most activity for digit is found (Neafsey & Sievert 1982). For cannula implantation two screws (2mm in diameter) were placed above frontal and occipital cortex for fixation, the bone scalp was drilled in two pieces and placed around the cannula to avoid infection. Skull and cannula were cemented in place using FlowLine cement (company….). Animals were allowed 3 – 5 days recovery period and were then introduced in the reach training paradigm as described previously by Buitrago et al. (2004). Infusion volume and rate was 0.5µl/40s for all drugs

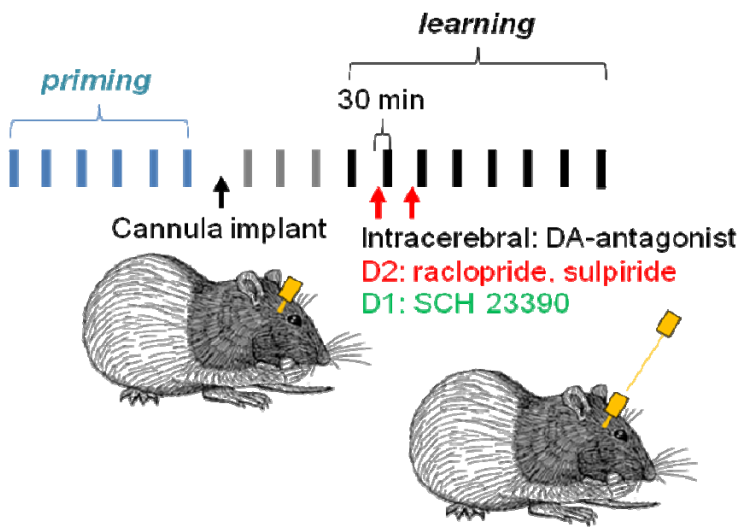

Figure 11 Timeline of Experiments 1 and 2. After a 6-day priming period (blue bars), animals were mapped in order to locate the forelimb area and determine the coordinates for cannula implantation accordingly (cannulas are represented as yellow squares). Three days were allowed for recovery (gray bars) before commencing the training phase (black bars). Freely behaving animals were injected locally with either a D2 receptor antagonist (raclopride or sulpiride) or a D1 receptor antagonist (SCH23390).

2.3.1.2 CONTROL EXPERIMENTS 1A-C

Control Experiment 1A: Spread of raclopride injected into cortex measured using positron emission tomography (PET). N=1

Control Experiment 1B: D2-antagonization in basal ganglia and motor learning. N=10 raclopride, n=10 control

Control Experiment 1C: D2-antagonization in motor cortex and motor function. N=5

2.3.1.3 EXPERIMENT 2: D1-ANTAGONIZATION IN MOTOR CORTEX DURING LEARNING

SCH23390 n=6, control n=8.

2.3.1.4 EXPERIMENT 3: DEPLETION OF CORTICAL DOPAMINERGIC TERMINALS

6-hydroxidopamine hydrobromide (6OHDA) was obtained from Sigma-Aldrich, Inc. (Munich) was infused in 12µg per injection in a concentration of [6µ/µl], diluted in 0.1% ascorbic acid. Seventy-two hours were allowed before experimentation.

Desipramine was given in the concentration of 20 mg/kg and nomifensine 10 mg/kg, both IP, 30 minutes before the injection with 6-ohda.

2.3.1.5 CONTROL EXPERIMENTS 3A-C

Control Experiment 3A: Effects of surgical intervention on motor function. Rotarod n=4 6-OHDA, n=4 Vitamin C

To exclude the possibility that motor learning deficits could be explained by a general motor deficiency in the injected animals, 8 animals were randomly assigned to either a placebo or a 6-OHDA group and were tested on a rotarod before training. Animals were tested on a rotarod rod (7cm diameter) with acceleration capacity elevated 40cm above the floor of the cage. Rats were placed on the rod and allowed to accommodate 2 mins before each session. Inter-trial interval was 15secs. The rod had a constant acceleration of 1cm/s² and the end of one trial was marked by the animals' fall onto the floor and subsequent disruption of a light beam. Baseline sessions consisted of 20 trials, and took place on two consecutive days before surgical procedure. After end of microinfusion 30 mins for recovery from anaesthesia were allowed and from then on, rats were required to run on the rod for 10 trials 3, 6, 9, 24 and 48hrs post-infusion before starting the training protocol.

Control Experiment 3B: Depletion of cortical dopaminergic terminals and motor function

N=10, lesion on day 11

2.3.1.6 EXPERIMENT 4: DEPLETION AND RESCUE WITH LEVODOPA

Control n=10, levodopa n=7, saline n=7

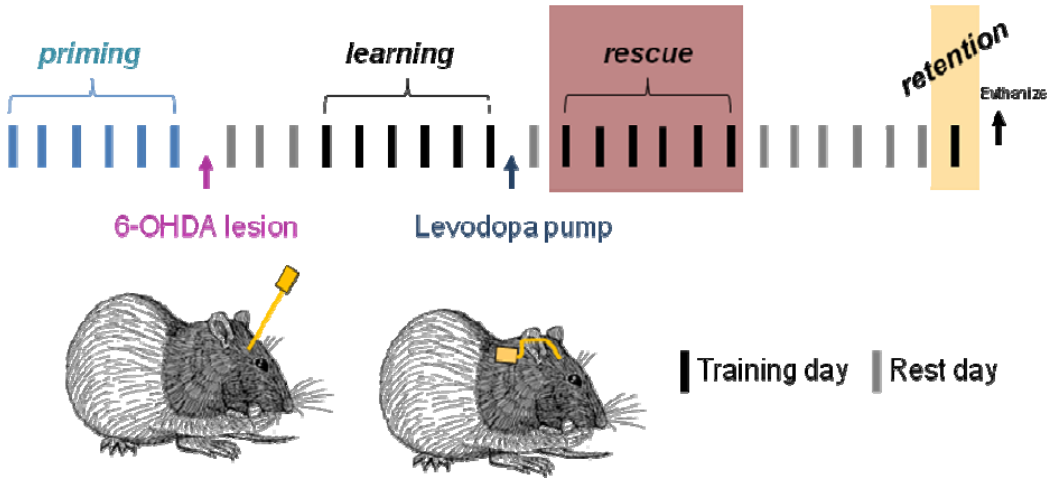

Figure 12 Timeline of experiment 4. After priming, rats received one session of training in order to establish a baseline performance and hand laterality. The day after the first training session, motor cortex dopaminergic neurons were lesioned with 6OHDA and 72hrs were allowed for recovery. Either desipramine or nomifensine was administered i.p. 20mins prior to 6-OHDA. After 6 days of training an osmotic pump was implanted to deliver levodopa directly into motor cortex. This was followed by 6 more days of training, 6 days of rest and a final training session testing for retention of the skill.

2.3.1.7 EXPERIMENT 5: LEVODOPA DURING LEARNING

Control n=6, levodopa n=8.

2.3.2 RESULTS

Experiment 1. D2-receptor antagonization by intracortical injection of raclopride temporarily impaired learning of the reaching skill as long as the drug was given (*time x group* interaction: F(7,91)=2.58, p=0.018, *Figure* 13). Once the drug was discontinued, animals quickly learned to finally reach the performance level of controls. To test whether this phenomenon was truly an effect of D2 antagonization and not related to potential side effects of raclopride, an alternative D2-antagonist, sulpiride, was applied. Sulpiride produced a similar learning impairment (*time x group* interaction: F(7,84)=3.06, p=0.006, Figure 13)

Latencies between pellet removal and subsequent door opening were not different between groups across time (*group x time* interaction: p=0.98).

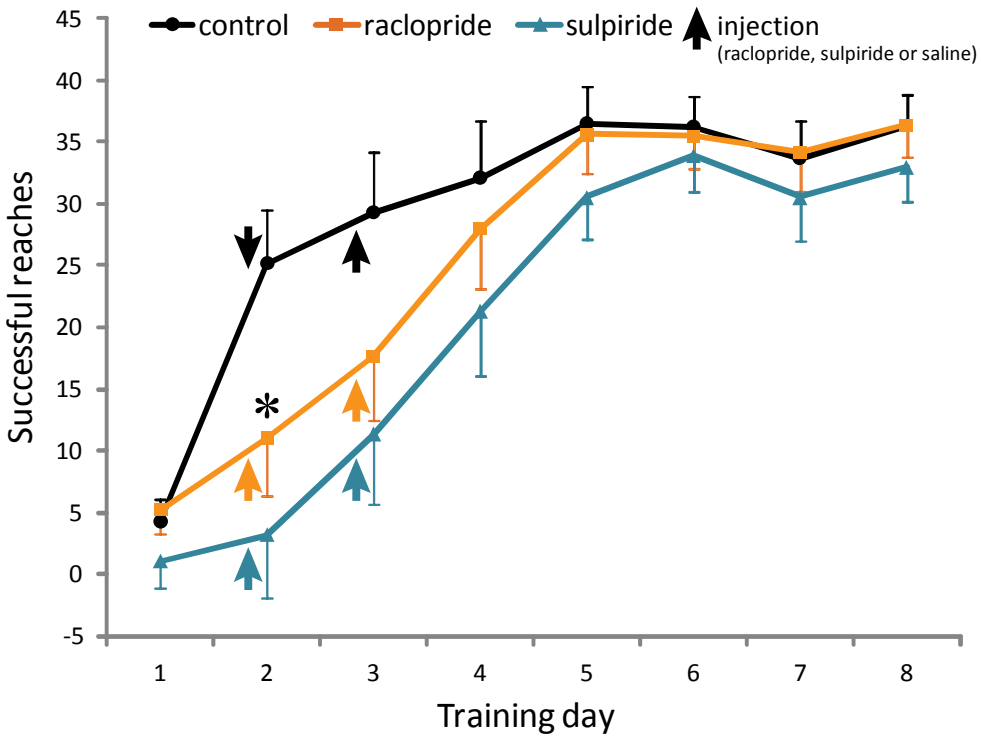

Figure 13. Experiment 1. Animals treated with D2 antagonists in motor cortex performed worse than controls during the days of injection, Learning recovered when the drug was discontinued. (error bars represent SE, * post hoc $p<0.05$, Bonferroni corrected)

Control Experiments 1A & B: To assess whether the D2-antagonist had spread to basal ganglia after intracortical injection and had thereby produced learning impairments we conducted two control experiments. In the first, the spread of radioactively labeled [11C]raclopride was measured in vivo using positron emission tomography (PET). Twenty min after injection the drug was detected in only a small volume (~0.5 mm^3) around the needle tip (*Figure 14*)

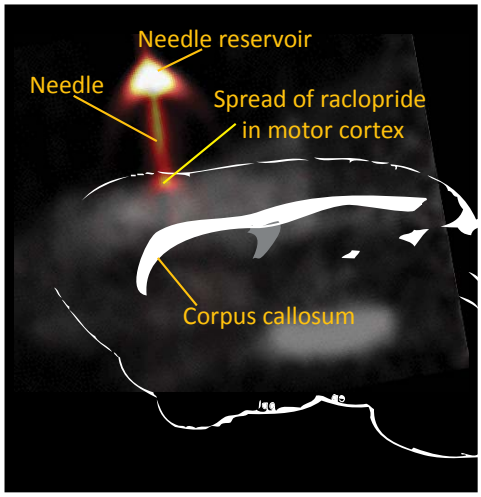

Figure 14. Control experiment 1A. Tissue spread of intracortically injected raclopride measured using positron emission tomography

While the same fluid volumes were injected in Experiment 1 and in the PET study, concentrations differed substantially: In PET, raclopride was used as a tracer and therefore injected 1000 fold more diluted than in pharmacological experiments. With higher concentrations substantial amounts of raclopride could have diffused to basal ganglia producing a learning deficit. We therefore injected raclopride directly into the posterior striatum using volume, concentration and protocol identical to intracortical applications. The learning curves of these rats did not differ from control (group x time interaction F(,98)=0.57, p=0.78, *Figure* 15).

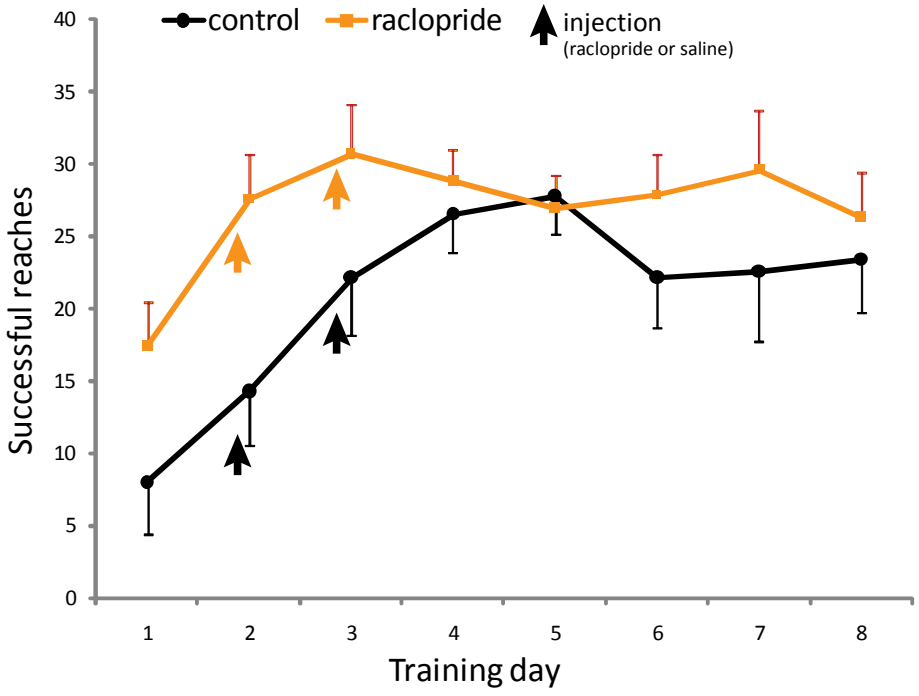

Figure 15. Control experiment 1B (Reaching). Motor learning curves of animals injected with raclopride into basal ganglia (orange) are not different from control. (error bars represent SE)

In contrast to reaching success, latencies between removal of the pellet and subsequently triggering the door opening sensor were affected by raclopride in basal ganglia. Treated

animals performed slower during training and mainly on day 2 (*time x group* interaction, F(7,98)=2.49, p=0.022).

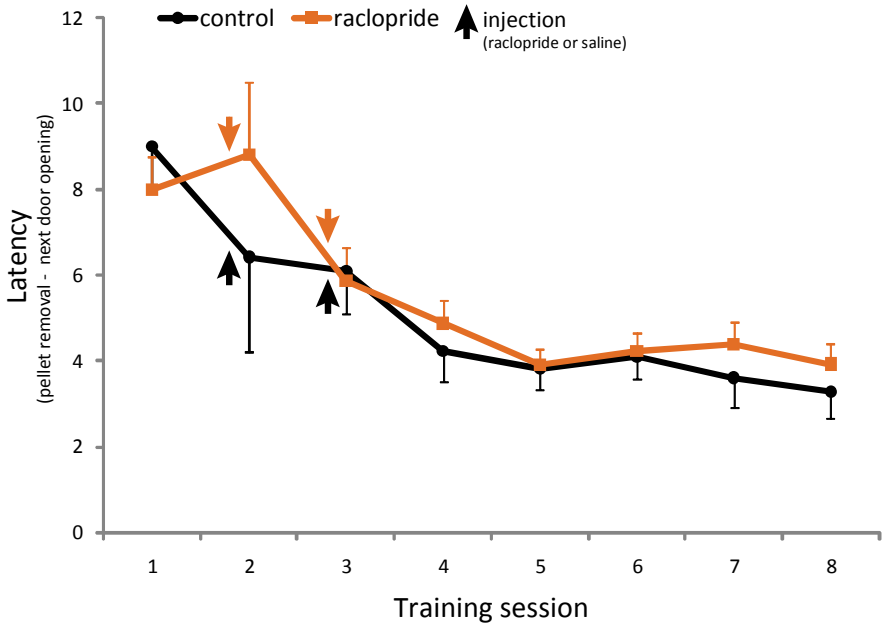

Figure 16. Control experiment 1B (Latency). Animals injected with raclopride into basal ganglia move slower between subsequent reaching trials. (error bars represent SE)

Control experiment 1C. Since movement ability is a prerequisite to motor learning, any impairment of motor function would dampen the learning curve, e.g., an injury to the rat's forepaw may flatten the learning curve while the neural machinery for acquiring a skill remains intact. To test whether D2-mediated signaling affects motor function in the reaching task, we injected raclopride intracortically in overtrained animals which had long reached a performance plateau. Raclopride did not cause a deterioration of performance in overtrained rats (*Figure* 17).

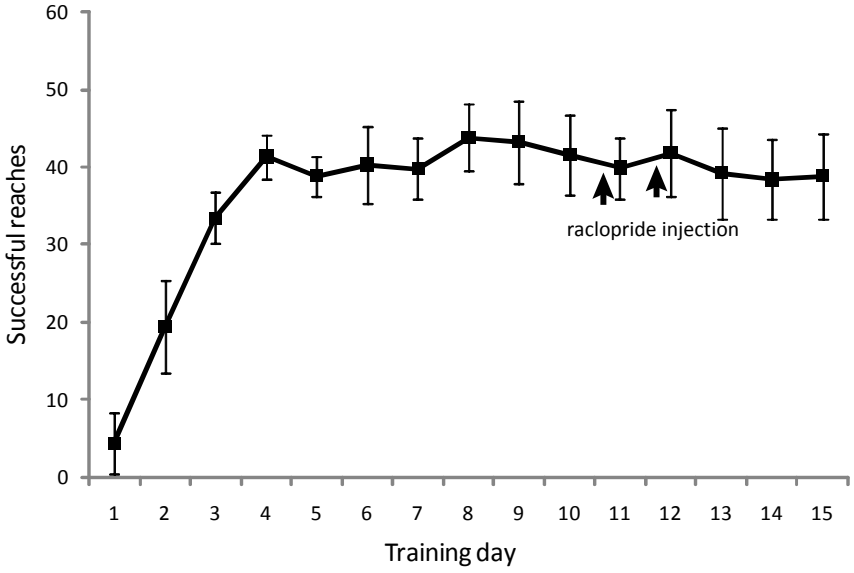

Figure 17. Control experiment 1C. D2 antagonization by raclopride injection into motor cortex does not affect reaching performance in overtrained animals that had learned the task before. (error bars represent SE).

These findings demonstrate that blocking dopaminergic signalling postsynaptically by D2-antagonization in motor cortex specifically impaired motor learning but not motor function.

Experiment 2. In a similar experiment, we tested whether D1 antagonization affected motor learning. Animals injected with the selective D1-antagonist SCH 23390 learned significantly worse than controls receiving vehicle injections (group x time interaction: $F(7,77)=7.16$, $p<0.001$, *Figure* 18). After the drug was discontinued animals learned and finally reached the performance level of controls. Latencies were not different between groups across time (*group x time* interaction: $p=0.99$).

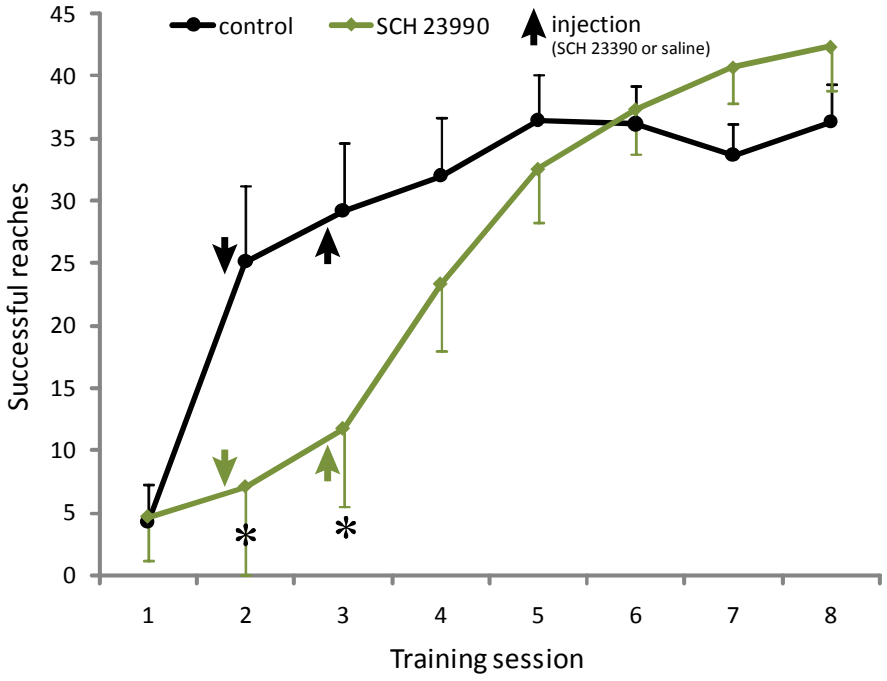

Figure 18. Experiment 2. D2 antagonization in motor cortex by local application of SCH 23390 impairs learning. Learning recovery when the drug is discontinued (error bars represent SE).

Experiment 3. Based on these findings we hypothesized that presynaptic depletion of dopaminergic terminals in motor cortex would lead to a permanent learning deficit. To explore this possibility, either dopaminergic or noradrenergic terminals were depleted by intracortical injection of 6-OHDA plus desipramine or nomifensine i.p., respectively. Desipramine and nomifensine selectively inhibit norepinephrine or dopamine reuptake thereby protecting the respective neuronal population from the effects of 6-OHDA. Including a group treated with 6-OHDA+nomifensine served a dual purpose: to test (1) whether noradrenergic neurons contribute to motor learning and (2) whether effects of 6-OHDA other than its toxicity for dopaminergic neurons contribute to an expected learning impairment.

To minimize additional motor cortex damage from the injection procedure, 6-OHDA was not applied through a pre-implanted cannula – like dopamine antagonists in experiments 1 and 2 – but was given by direct needle insertion under general anaesthesia. To exclude potential deficits in general motor function related to this procedure or to 6-OHDA that would have confounded the learning curve, accelerated rotarod tests were performed at different time points before and after injection of 6-OHDA or vehicle (**Control Experiment 3A**, *Figure* 19).

There was no difference between groups (group x time interaction: p=0.81). There was an overall effect of time in the model without group as independent variable (F(6,30)=3.51, p=0.008). Post-hoc testing showed significantly reduced rotarod performance at 3, 6 and 9h after injection (p<0.05). By 24 hours there was a statistical trend for reduced performance (p=0.069) and performance returned to pre-injection levels by 48h (p=1.00). Therefore, in all training protocols a 48h pause was maintained after injection procedures.

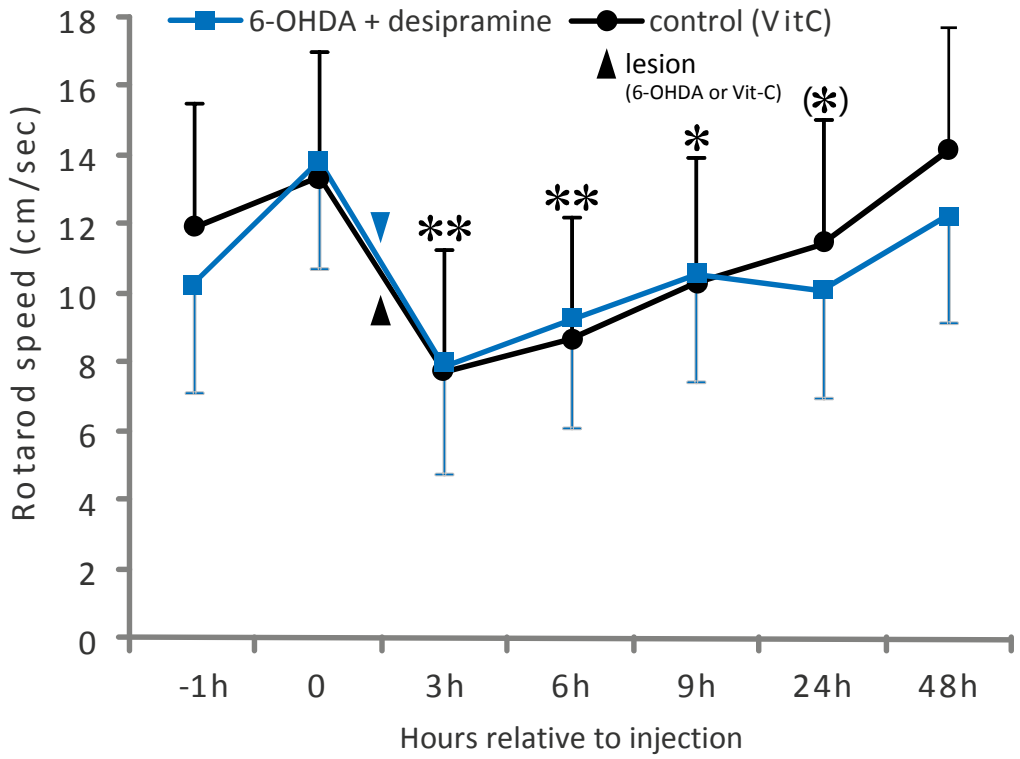

Figure 19. Control experiment 3A. General motor function deteriorates for about 9h after general anesthesia and injection of a drug into motor cortex as tested using an accelerated rotarod test. There is no additional detrimental effect of 6-OHDA plus desipramine versus vehicle (Vitamin C). (error bars represent SE, * p<0.05, ** p<0.01 difference to time point 0, Bonferroni corrected)

There was a significant overall difference in learning between the groups (group x time interaction: F(12,252)=4.37; p<0.001, Figure 20). Post hoc testing revealed significant differences between the dopamine-depleted group and controls (p<0.001) as well as between dopamine-depleted and norepinephrine-depleted animals (p=0.042).

Latencies between pellet removal and door opening were slightly higher in both 6-OHDA-treated groups than in control but there was no significant difference (*time x group* interaction: p=0.08).

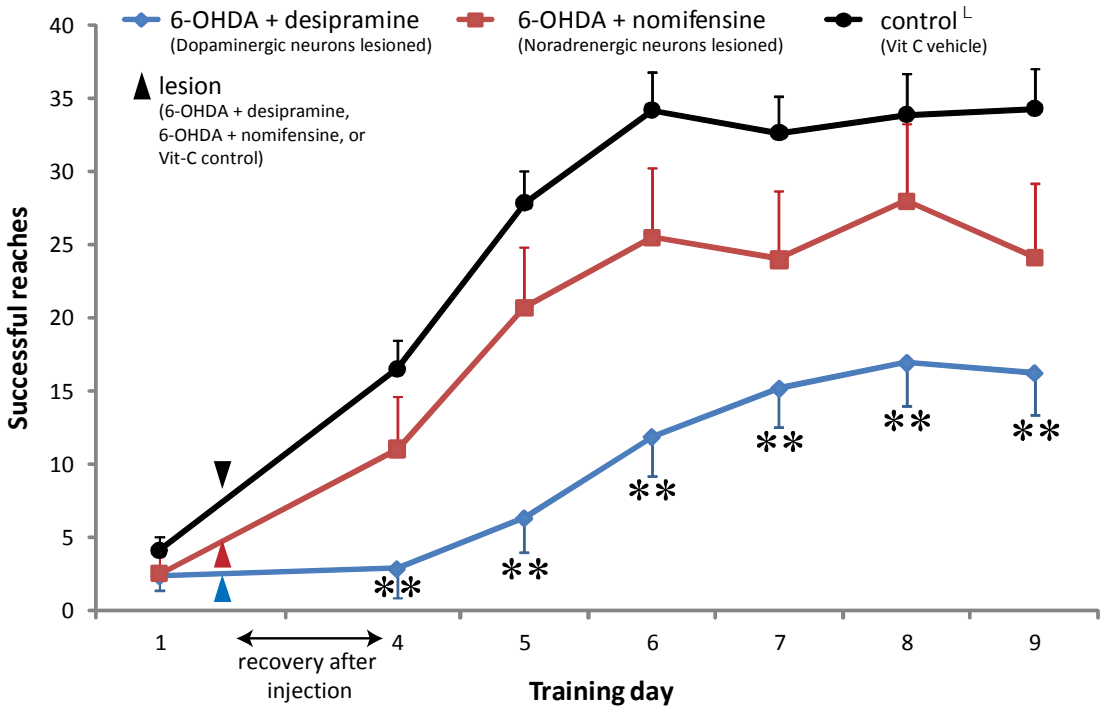

Figure 20. Experiment 3. Depletion of cortical dopaminergic terminals using a combination of 6-OHDA intracortically and desipramine systemically attenuates the learning curve significantly. In contrast, noradrenergic terminals (depleted using 6-OHDA plus nomifensine) contribute only minimally to the learning paradigm (error bars represent SE, ** post hoc p<0.1, Bonferroni corrected).

To exclude potential detrimental effects of 6-OHDA + desipramine on motor function we injected the drugs in overtrained animals that had reached plateau performance. No decline in performance was observed (Figure 21) there was a significant effect of time ($F(14,126)=19.4$, $p<0.001$); post-hoc testing revealed no significant differences between pre-injection (session 11) performance and post-injection performance in sessions 12-15 ($p>0.99$).

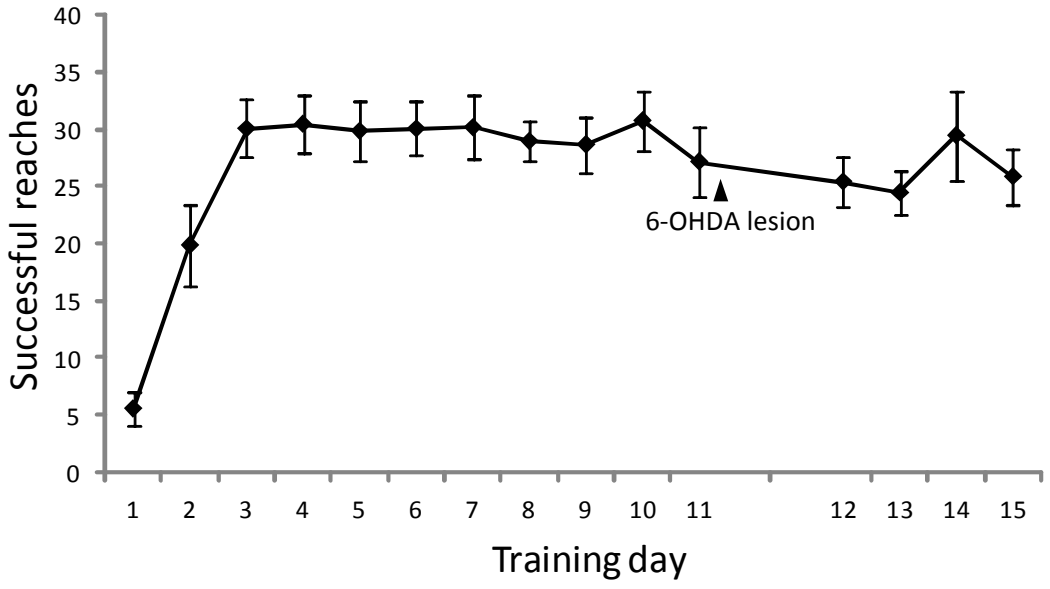

Figure 21. Control experiment 3B. Depletion of dopaminergic terminals in motor cortex during the plateau phase of the learning curve (overtrained animals) does not affect performance of the skill indicating that dopamine may not be necessary for motor function per se. (error bars represent SE).

Experiment 4. Depletion of presynaptic dopaminergic terminals removes the dopaminergic drive within motor cortex. Supplementation of levodopa (l-dopa), the precursor of dopamine, can theoretically rebuild this drive assuming that the enzymatic machinery for converting l-dopa to dopamine (aromatic amino acid dopa decarboxylase, AADC) that still exists after depletion of dopaminergic terminals. This has been shown in the 6-OHDA lesioned striatum of rats and is explained by extracellular or non-neuronal occurrence of AADC (Sarre et al. 1994). To prove that a loss of dopamine is underlying the effects 6-OHDA lesions in motor cortex, we supplemented l-dopa intracortically – a rescue strategy. The experiment consisted of 3 phases. In phase 1, dopaminergic terminals in motor cortex were lesioned in two groups of animals with 6-OHDA plus desipramine. As compared to Vitamin C treated controls both groups learned significantly slower (

Figure 22 days 1-8: *group x time* interaction: $F(10,95)=2.98$, $p=0.003$; post-hoc different to control for groups $p<0.01$). In phase 2 after training of day 8, both lesioned groups were implanted with osmotic mini-pumps delivering either l-levodopa or saline into motor cortex for 7 days, i.e., the duration of post-surgical recovery plus training. L-dopa treated animals started learning and quickly reached the performance level of controls with intact cortical dopaminergic terminals (*Figure* 22 days 10-15: *group x time* interaction: $F(10,90)=3.16$, $p=0.002$; post-hoc difference control vs. lesion + l-dopa: $p=0.71$, control vs. lesion + saline: $p=0.022$, lesion + l-dopa vs. lesion + saline: $p=0.15$). Phase 3 was a test of motor skill retention after 6 days without training and l-dopa supplementation (the pumps remained implanted but ran out of l-dopa). Lesioned animals that had learned under l-dopa supplementation retained their skill (lesion + l-dopa vs. lesion + saline t-test: $p=0.049$, difference to control non-significant).

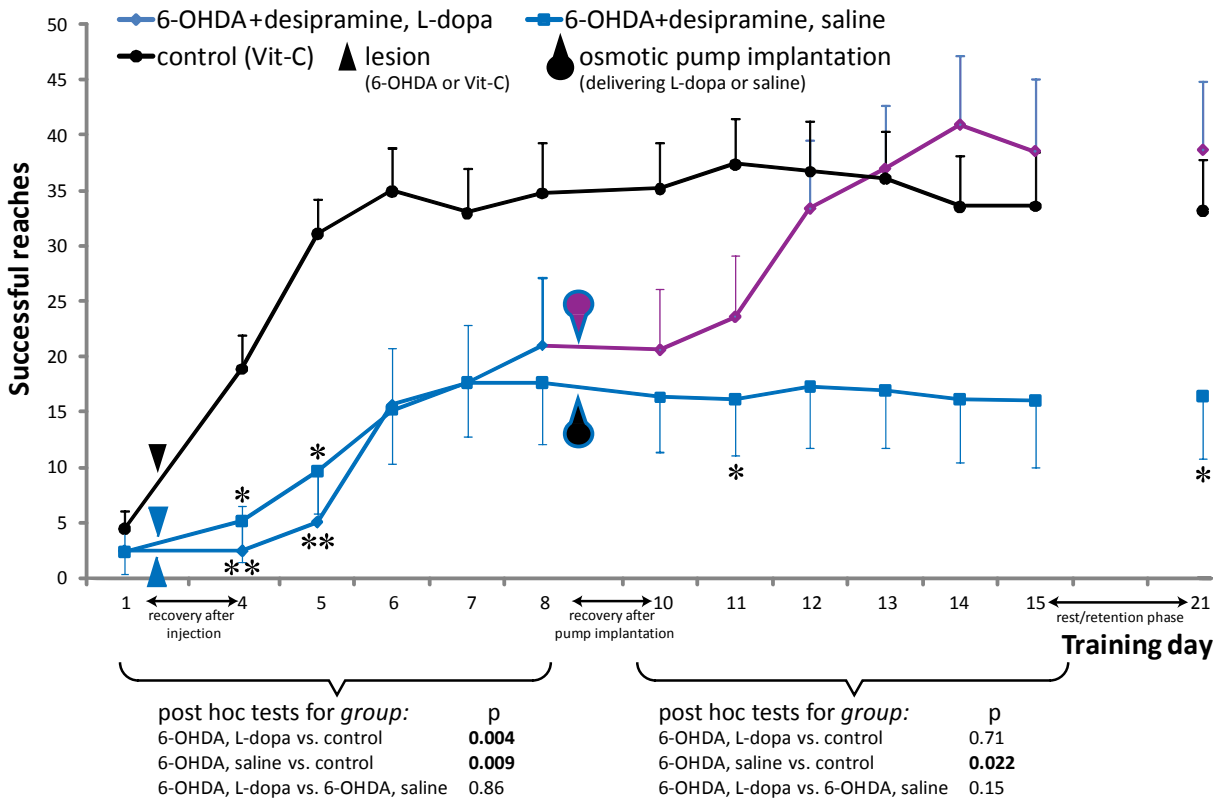

Figure 22 Experiment 4 (Reaching). As shown in Experiment 3, depletion of dopaminergic terminals in motor cortex significantly impairs motor learning: animals learn slower and reach a lower plateau (days 1-8). Subsequent supplementation of l-dopa into motor cortex by an osmotic pump significantly improves performance versus saline supplementation. L-dopa animals quickly reach the performance of control animals with an intact cortical dopaminergic system (days 10-15). This performance is maintained during a rest period even in the absence of l-dopa supplementation (day 21) (error bars represent SE, * post hoc $p<0.05$, ** $p<0.1$, Bonferroni corrected).

Latencies slowly decreased over the all three phases (effect of *time*: $F(12,252)=3.06$, $p<0.001$) of the experiment with any differences between groups (*group x time* interaction: $F(24,168)=0.94$, $p=0.55$*, Figure 23).*

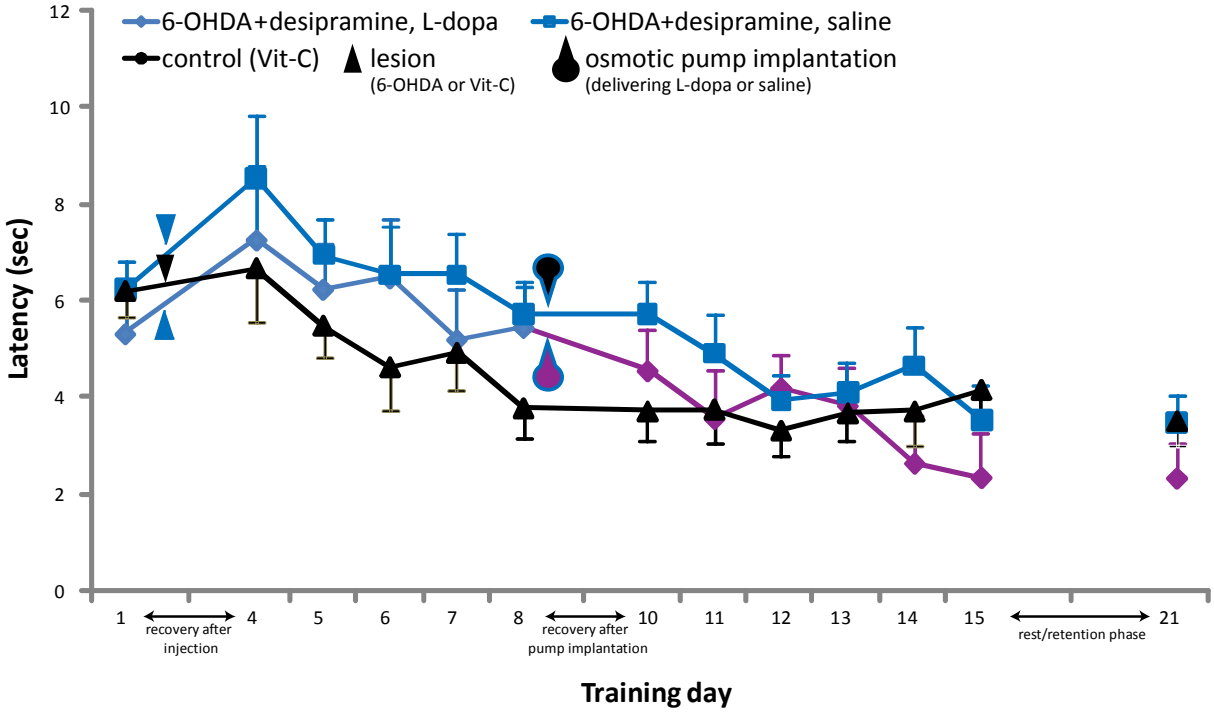

Figure 23. Experiment 4 (Latency). Latencies decrease during training without any differences between the groups (error bars represent SE).

This experiment demonstrates that exogenous l-dopa supplementation can reverse the learning deficits induced by depletion of dopaminergic terminals within motor cortex. It also shows that dopamine is specifically required for learning but neither for other elements of the task measured by latencies (motivation, conceptual knowledge) nor for the maintenance of the skill nor for motor function to carry out the learned movement.

Experiment 5. If dopamine is necessary for successful motor learning, one may speculate that increasing dopaminergic drive within motor cortex may enhance learning. This was tested by continuously infusing l-dopa into motor cortex using osmotic mini-pumps. As compared to vehicle-treated controls the l-dopa group learned significantly faster but reached a similar plateau performance (group x time interaction: $F(7,77)=4.62$, $p<0.001$, Figure 24).

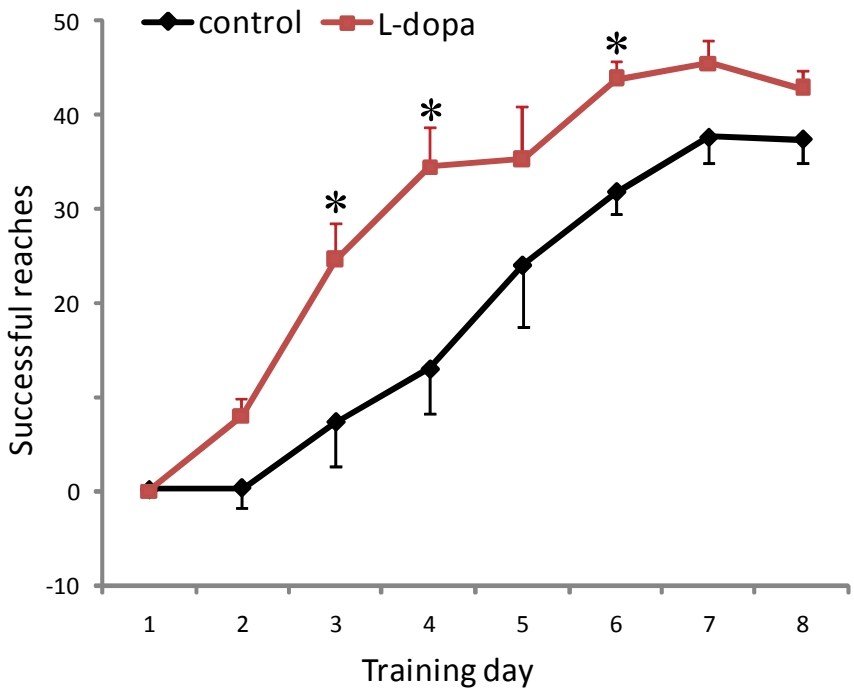

Figure 24. Experiment 5. Continuous infusion of l-dopa into motor cortex during learning accelerates learning (error bars represent SE, * post hoc p<0.05, Bonferroni corrected).

2.4 MECHANISMS: DOPAMINERGIC SIGNALING AND LTP

2.4.1 METHODS & EXPERIMENTAL DESIGN

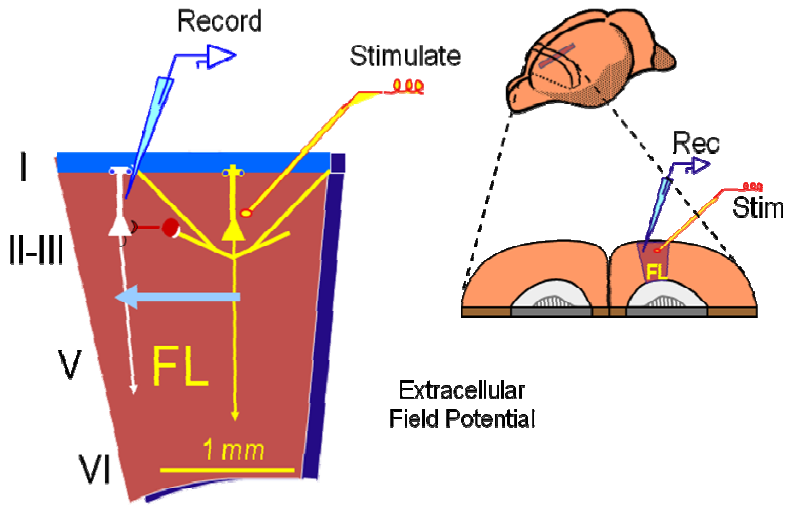

Figure 25. LTP experiment (Method). Experimental procedure for LTP induction in motor cortex rat slices.

2.4.2 RESULTS

Motor learning is associated with long term potentiating (LTP) induction in motor cortex (Rioult-Pedotti et al. 2000), therefore, motor cortex LTP has been suggested as a storage mechanism for skill memories. To determine whether dopaminergic signaling in motor cortex affects LTP, we recorded extracellular field potentials (FP) of layer II/III horizontal

connections in slices including the forelimb area. LTP was induced by theta burst stimulation following transient bicuculline application. LTP was induced repeatedly (saturated) to assess the maximum synaptic strength. LTP was significantly reduced in the presence of raclopride (109.12±1.64) compared to control (139.01±6.17; N=7, $p<0.001$, Figure 26). LTP was usually saturated after a single LTP attempt in raclopride while 2-4 attempts were required in control conditions. Hence, synaptic plasticity in M1 depends on D2 receptor activation.

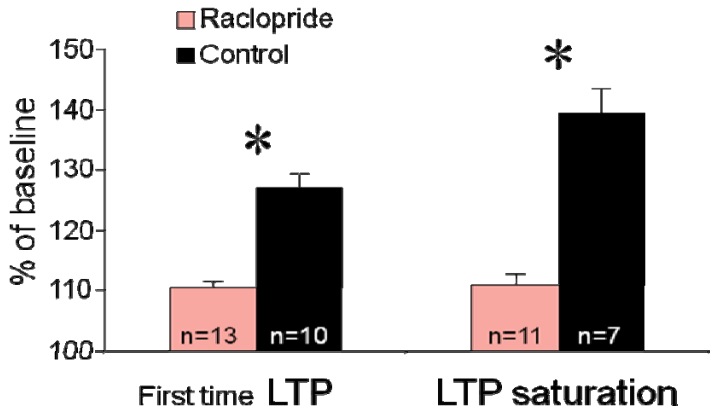

Figure 26 LTP experiment (Results). Raclopride treatment of cortical slices results in a significant reduction in cortical first time and saturation LTP.

2.5 DISCUSSION

These findings demonstrate that dopaminergic neurotransmission within motor cortex is specifically important for successful learning but not performance of a movement sequence. Dopamine may help learning by allowing LTP in motor cortex neurons; LTP has been suggested as a mechanism of motor learning.

It has been known for almost 20 years that dopamine receptors are expressed on motor cortex neurons. Not much emphasis has been given to the relevance of these receptors for cellular function and behavior. Our findings show that presynaptic as well as postsynaptic elements of a dopaminergic system in motor cortex are important for motor learning and motor cortical LTP. Both learning and LTP are not completely suppressed under the influence of dopamine antagonists. Although antagonism is never complete in pharmacological experiments in vivo, the presence of dopaminergic neurotransmission in motor cortex may not be an absolute requirement for learning or its blockade may be compensated for by other neurotransmitter systems.

Our findings show flattened learning curves under dopamine blockade or depletion. Retention of the skill over a period without training does not require a dopaminergic drive and is unaffected in animals with depleted cortical dopaminergic terminals. Learning processes are often segregated into acquisition and consolidation phases. During motor learning these may occur in an interleaved arrangement (Luft & Buitrago 2005). Therefore, from the dampening of the learning curve if cannot be discerned whether acquisition or consolidation is affected.

Dopaminergic neurotransmission occurs predominantly in striatum, nucleus accumbens and prefrontal cortex. In dorsal striatum, dopaminergic drive is necessary for movement initiation and regulation of muscle tone. Its destruction leads to Parkinson's syndrome. Could our results be an artifact of dopamine antagonism in striatum? This is unlikely, because spread of raclopride in the PET study was restricted to cortex. Additionally, direct delivery of raclopride to basal ganglia did not affect the motor learning curve. As expected based on the physiology of striatal dopamine, rats injected with raclopride in the striatum showed higher latencies, i.e., they moved slower. Dopamine release in the ventral striatum is thought to mediate a putative reward mechanism (Schultz et al. 1998). According to Hernandez et al. (2005), dopamine receptors in the ventral striatum are necessary only during acquisition of an operant skill rather than for memory consolidation, which supports the temporary need for reward valuation.

Dopaminergic signaling in the PFC is thought to encode evaluations of goals that direct behavior. If the drugs applied here would have affected PFC, motivation to obtain the goal 'food pellet' would expectedly change. Since latencies between pellet removal and subsequent door opening provide a measure of motivation, an effect on latencies would be expected. Also, coordinates for injections targeting PFC are typically 3.2-3.5 mm rostral to bregma. We injected about 1mm rostral to bregma. Assuming a spread of the drug in a diameter of 1mm around the injection site (results of the PET experiment), interference with PFC D2 receptors seems unlikely. Therefore, we do not think that our results are an artifact of interference with the dopaminergic system in PFC.

Where does the dopaminergic projection to motor cortex originate and what are its effects on motor cortex neurons. Due to close proximity, the dopaminergic projections to PFC that are well characterized in anatomy and function may serve as a source for hypotheses. The PFC is involved in working memory, that is, to keep information available which is necessary for ongoing behavior. It is hypothesized that the PFC stores the goal of an action together with its value, such as receiving a food reward. Distractions that occur during the goal-seeking behavior are undervalued and, therefore, do not affect the behavior. The PFC received dopaminergic projections from the ventral tegmental area (VTA). Phasic release of dopamine is thought to be necessary for encoding and valuation of new goals. Addictive drugs affect dopamine release and thereby interfere with the valuation mechanism such that the drug is preferred over other goals (Montague et al 2004, Drevets et al. 2001). On the cellular level, dopamine receptor D1 and D5 activation induces protein synthesis in the hippocampus via activation of protein kinase A (PKA). Most evidence suggest a crucial role of D1 receptors as mediators of plasticity, but there is also evidence for D2 receptors as forming associations between visual cues and rewards in the rhinal cortex (Liu et al. 2004). D1 receptor mediated enhancement of LTP in hippocampus-PFC synapses may account for this encoding process (Gurden et al 2000). In the hippocampus dopamine antagonists

prevent the late phase of LTP (>3h), while dopamine agonists induce the maintenance of LTP (Huang & Kandel 1995).

In analogy to studies in PFC, we have shown that dopamine supports the development of LTP in motor cortex neurons. Like in PFC, one could hypothesize that dopamine helps to select elements of the movement sequence that are relevant for the behavioral goal (reaching food) over those that are not. One can further hypothesize that the dopaminergic projection to motor cortex originates in VTA.

Dopaminergic involvement in LTP may be explained by its facilitation of NMDA receptor responses. Besides that, D2 receptors modulate sodium currents (Gorelova & Yang 2000) and are involved in the regulation of gene expression via activation of protein kinase C that promotes Ca2+ influx. Therefore numerous other effects of dopamine on motor cortex neurons may be speculated.

It will also be important to discern the molecular mechanisms of dopamine receptor action in motor cortex. Dopamine interacts with 5 subtypes of G-protein coupled receptors and depending on which protein they are attached to, they are subdivided into D1 and D2 families. Receptor binding induces dissociation of Gα and Gβγ subunits of the G-protein that are responsible for further downstream mechanisms in the cell. Regulators of G-protein signaling (RGS) are a family of proteins that modulate G-protein function and most importantly, accelerates, the self-inactivating activity of the Gα subunit (Cabrera-Vera et al.2004, Burchett 2005).

Previous studies from our lab have revealed that during learning there is an up regulation of two RGS proteins; RGS2 and RGS9. In the striatum, RGS2 gene expression is induced by application of D2 antagonists and D1 agonists (Burchett 2005) and similar results are shown by Rahman and colleagues (2003) in RGS9. RGS2 can also deactivate p38-mitogen activated protein kinase (p38-MAPK) involved in plasticity-related gene expression (Ingi et al. 1998, Adams & Keefe 2000). RGS9 has two variants, one of which is involved in D2 receptor mediated locomotor and rewarding responses to cocaine and being present in reward-based learning (Rahman et al. 2003). Kovoor et al. (2005) have shown that mice lacking the RGS9 protein develop dyskinesia when D2 receptors are activated and are similar to those movements observed during treatment with antipsychotic drugs of dopamine receptor antagonists. It is therefore hypothesized that RGS9-2 has an additional role in limiting the extrapyramidal side effects of these treatments.

In interpreting these results one has to consider that they pertain only to the task investigated. Motor learning tasks are complex requiring a number of different brain functions to be successfully mastered. It may be that other functions are being affected by dopaminergic modulation. But, by obtaining dual readouts from out task, i.e. reaching

success and latencies, we were able to discern movement related from other functions that are reflected in latencies. Such functions are likely motivation, attention, conceptual and spatial knowledge, although the exact influence of these cognitive functions on latency has not been systematically investigated.

The finding of a cortical dopaminergic system relevant for motor learning has broad clinical implications. It provides a mechanistic explanation for enhancement of motor learning after systemic application of dopamine in healthy humans (Floel et al. 2005) and of motor recovery after stroke (Scheidtman et al. 2001). It might also help to explain motor learning deficits in patients with Parkinson's syndrome where the dopaminergic terminals in motor cortex may degenerate like other dopaminergic projections. The motor cortex should be investigated as a brain region of Parkinsonian pathology.

CHAPTER 3. CONCLUSIONS

Motor skill learning likely is mediated by a series of events that lead to acquisition, consolidation and long term storage of an optimized sequence of movement elements. A motor task can only be learned by repeated training. Acquisition is likely occurring during training. There is evidence that consolidation happens between early training sessions (Walker et al. 2003, Luft et al. 2004, Luft & Buitrago 2005). Whether this consolidation is sufficient to produce the almost permanent storage that is observed with most skills, or another long term storage process is necessary after the skill is long mastered (over-trained).

Motor control is an ability of the brain that requires the close interaction of different brain regions, such as motor cortex, basal ganglia and cerebellum (Hikosaka et al. 1999). While it is still unclear where (and how) motor skills are stored, these regions have different roles during acquisition: The cerebellum may coordinate agonist/antagonist activity and the timing of the movement, it may also integrate feedback and feedforward information about the ongoing movement to optimize motor execution. The basal ganglia may provide the motivation for skill learning by emotionally valuating a reward for a successful movement. Corticostriatal input may be reinforced if it coincides with dopaminergic input representing award expectations (Hikosaka et al. 1999). An interaction between sensory and motor cortices is necessary for motor learning likely representing the need for integrating somatosensory signals into ongoing and future actions. Asanuma and colleagues have shown that neuronal plasticity during motor learning occurs at synapses of somatosensory and motor cortex neurons (Iriki et al. 1989, review).

As discussed above, the primary motor cortex undergoes various modifications during learning, including changes in somatotopic maps (Kleim 1998, 2004), alterations in the ability of neurons to undergo LTP (Rioult-Pedotti et al. 2000), synthesis of proteins (Luft et al. 2004) such as the immediate early gene c-fos (Kleim 1996) that may eventually mediate the structural changes reflected by increased synaptic density (Kleim 2002, 2004). In bird song learning that may be seen as a special form of movement learning, the genes and proteins regulated are better characterized (Jarvis & Nottebohm 1997).

It is unknown whether any of these “plasticity phenomena” that seem to be related to motor learning represent the *motor memory trace*. By definition, this trace should persist as long as the skill is “remembered”, even over long periods of rest. Except for the modification of the LTP/LTD range where preliminary evidence exists (Rioult-Pedotti, personal communication), persistence during rest had not been shown for any of the above mentioned phenomena. My first study (Chapter 1) demonstrates that gross modifications in somatotopy do not persist and therefore, as one might expected, do not reflect the memory trace.

Somatotopy is known to be influenced by continuing protein synthesis (Kleim et al. 2003) and by aminergic projections that reach motor cortex. Connor and colleagues showed that cholinergic projections mediate somatotopic map changes during learning. Norepinephrine has an influence on motor cortical excitability (Plewnia et al. 2004).

In the second part of my thesis (Chapter 2) I have conducted experiments that show for the first time that dopaminergic neurotransmission within motor cortex is a necessary requirement for successful motor learning. Dopamine is involved in various learning and cognitive processes (see El-Ghundi et al. 2007 for review). Because the dopaminergic system is of high clinical importance, in the diagnosis of Parkinson's and related syndromes and as a target of various drugs, its involvement in motor learning could open new research perspectives.

APPENDIX

ANIMALS

Male adult Long Evans rats weighing 250g – 350g (between 7 and 10 weeks old) from our own stock were used for these experiments. Rats were housed in a standard animal facility under a 12h light/dark cycle. Animals were allowed *ad libitum* access to water and had a restricted diet of 50mg/kg of food. After surgery, animals were caged individually in a constant ambient temperature.

REACHING TASK

The reaching task for animals used in the mapping experiment has been previously described in our group (Buitrago et al. 2004). A variation of the reaching task was used for the pharmacological experiments, as follows. Three – five days before cannula implantation, animals completed a pre-training phase. Animals were accustomed to handling, the new environment (training chamber) and exercise of the forepaw. Summarized, forelimb preference was defined after a period of 100 reaching - but not grasping - trials in less than 30 min. Animals were allowed 3 days of pause before the surgical procedure took place. The acquisition of a fine motor skill (reach training) was conducted over 8 consecutive days. Thirty minutes prior to the session of interest, the drugs were intracranialy injected into the motor cortex of the preferred forelimb. Substances were infused (unilaterally) in preferred motor cortex of awake, behaving animals. For 6-OHDA injection (see below) and corresponding vehicle), animals were trained for one day (training day 1) and on the following day were infused with the substance. Seventy-two hours were allowed for recovery before proceeding with training on day 2.

ACTIVITY CONTROL TASK

Control animals were subjected to an activity control task that required a similar number of repetitions of arm reaching movements as compared with the skilled reaching task (activity control training, ACT). Control animals reached through the window to touch a sensor in 1.5cm distance; the pellet was then delivered directly onto their tongue by forceps. ACT required no precision grasping or pellet retrieval (Kleim et al., 2004). All animals were allowed to choose laterality except those that were implanted with the array. Array implanted animals were trained to use the forelimb contralateral to the implanted left hemisphere. By moving the pellet pedestal 5mm to the left (relative to the rat), animals could not reach the pellet with their left forelimb and started using the right (or vice versa). Daily training sessions consisted of 100 trials (115.8 ± 5.7 reaches).

SURGICAL PROCEDURES

ANAESTHESIA

For all procedures requiring the use of anesthetics, animals were anaesthetized with a ketamine/xylazine mixture (10% i.p., 70-100mg/kg bw; 2% i.p. 5-10mg/kg bw). Mapping was performed under ketamine anaesthesia of superficial depth as determined by slight whisker tremor (Kisley & Gerstein 1999, Brecht et al. 2004).

INTRACORTICAL GUIDE CANNULAS & INJECTION

Guide cannulas for drug infusion into motor cortex (Unimed, Lausanne, Switzerland) were implanted after pre-training of the animals in pellet retrieving with the tongue as described by Luft et al. (2004). In short, Three-five days were allowed for recovery after surgery. The system consisted of an obturator and external guide cannula (400µm/15mm). A 34 gauge needle was used at the time of injection (pst3/15mm). Drug delivery was done with a Hamilton syringe (5µl, 85 RN). One guide cannula was implanted through a burr hole into the forelimb area of motor cortex (coordinates relative to Bregma: anterior 1mm, lateral 3mm, depth 900µm) (Neafsey et al. 1986). The hemisphere of cannula implantation was decided according to the animals' paw preference, defined as laterality. Laterality was determined during the last day of pre-training where the animals were encouraged to reach 3-5 times with the same paw consecutively. The cannula, two screws (one over the frontal skull, one on the occipital skull; 2mm diam), and the burr hole were covered with dental cement (FLOWline) to ensure implant stability in the freely behaving animal.

STATISTICAL PROCEDURES

Analyses were performed using Statistica version 7.0 (StatSoft Inc., Tulsa OK, USA). Reaching performance was quantified as the percentage of successful reaches per session. General linear models were used to test for effects of *training day* on *reaching performance* including *group* and *group x time* (session) as independent variables. In cortical mapping studies, the size of a representation was quantified as the number of responsive contacts per limb or joint. Repeated measures analysis of variance with *group* and *limb* (forelimb, hindlimb) as between subjects factors, *time* as within-subjects factor and the interaction of *group* x *time* x *limb* tested for effects of group on map changes and motor thresholds.

The baseline parameter was entered as a continuous covariate in all models. Scheffe's test or Fisher's least significant difference tests (were indicated) were used in post hoc analyses. Dependent variables were tested for normal distribution using Shapiro-Wilk tests and for homogeneity of variance using Levene's test. Two-tailed probability less or equal to 5% was considered significant. Numerical results are expressed as mean and SEM.

HISTOLOGICAL PROCEDURES

Sixty minutes after the end of the last training session animals were deeply anaesthetized with an overdose of ketamine/xylazine for intracardial perfusion. Brains were extracted, fixated for 24hrs, cryoprotected with 30% sucrose and frozen at -80°C before coronal sections were made.

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Publications

Molina-Luna K, Buitrago MM, Hertler B, Schubring M, Haiss F, Nisch W, Schulz JB, Luft AR. 'Sampling motor cortex physiology using epidurally implanted thin-film micro-electrode arrays'. J Neurosci Methods. 2007 Mar 30; 161(1):118-25.

Molina-Luna K, Hertler B, Buitrago M, Schulz JB, Luft AR. 'Motor cortex somatotopy changes transiently during motor skill learning'. Submitted Learn Mem.

Molina-Luna K, Pekanovic A, Hertler B, Schubring M, Luft AR 'Acquisition of complex motor skills is mediated by dopaminergic mechanisms in motor cortex'. In preparation for Neuron.

Schubring-Giese M, **Molina-Luna K**, Buitrago MM, Hertler B, Luft AR. 'The speed of motor recovery after experimental skill depends on prior skill' In press Exp Brain Res. 2007 Mar 27.

Hosp J*, **Molina-Luna K***, Pekanovic A, Luft AR 'Dopamine receptors in the motor cortex modulate neuronal excitability'. In preparation (*both authors contributed equally).

Selected Conferences

Molina-Luna K, Hertler B, Schubring-Giese M, Buitrago MM, Luft AR. *'Motor map enlargement during learning of a motor skill reverts after practice ends'*. 36th Ann. Meeting of the Society for Neuroscience, Oct 2006. Atlanta GA, USA. Poster No. 161.5

Molina-Luna K, Buitrago MM, Hertler B, Schulz J.B, Luft AR. *'Changes in the motor cortex during learning of a motor skill'*. 35th Annual Meeting of the Society for Neuroscience, Nov 2005. Washington DC, USA. Poster No. 775.2

Molina-Luna K, Buitrago MM, Hertler B, Schulz JB, Luft AR. *'Structural and plasticity changes in the motor cortex during learning of a novel movement'*. 1st Neuroplasticity and Rehabilitation Workshop. Sep 2004. Ftan, Switzerland Talk.

Molina-Luna K, Buitrago MM, Schulz JB, Luft AR.. '*Thin film microelectrode array for motor cortex mapping'*. 34th Ann. Meeting of the Society for Neuroscience, Oct 2004. San Diego CA, USA. Poster No. 190.24

Molina-Luna K., Buitrago MM, Hertler B, Schulz JB, Luft AR. *'Motor cortex gene expression during learning of a motor skill'*. DGN (German Neurological Society). Sep 2005. Wiesbaden

Selected Schools, Courses & Workshops

Professional skills Workshop. Society for Neuroscience. Oct 2006. Atlanta GA, USA

Statistical Analysis of Neuronal data 3. Carnegie-Mellon University. May 2006. Pittsburgh, USA

Neuroinformatics Summer School. Marine Biological Laboratories. Aug 2005. Woods Hole MA, USA

Neurobiology of Disease Workshop. Society for Neuroscience. Oct 2005. Washington DC, USA

Visualizing large-scale patterns of activity in the brain. Optical and Electrical signals. Society for Neuroscience. Oct 2004. San Diego CA, USA

1st Neuroplasticity and Rehabilitation Research Workshop. Sep 2004. Ftan, Switzerland

2nd Neuroplasticity and rehabilitation Research workshop. University of Tübingen. Oct 2006

Ethics in animal experimentation. Feb 2006. University Hospital, Tübingen

German grammar for advanced speakers. Summer Semester 2005. University of Tübingen

Introduction to Matlab for the biological Sciences. June 2005. University of Tübingen

Successful fundraising for Research projects. Feb 2005. University of Tübingen

Training for Research Project Leaders in the Biological Sciences. Nov 2004. University of Tübingen

2nd Summer school Assessment of Functional Recovery in Animal Models of Neurological Disease. July 2004. Friedrich-Schiller University Jena

Cognitive and Computational Psychophysics Workshop. Max-Planck-Institute for Biological Cybernetics in Tübingen. March 2004

Molecular Cell Biology Workshop. Feb 2004. University of Tübingen

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Academic award & Scholarships

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