



HAL
open science

Disconnection of the entorhinal cortex and the dorsomedial striatum impairs the sensitivity to instrumental contingency degradation

Bjoern Lex, Wolfgang Hauber

► **To cite this version:**

Bjoern Lex, Wolfgang Hauber. Disconnection of the entorhinal cortex and the dorsomedial striatum impairs the sensitivity to instrumental contingency degradation. *Neuropsychopharmacology*, 2010, 10.1038/npp.2010.46 . hal-00522560

HAL Id: hal-00522560

<https://hal.science/hal-00522560>

Submitted on 1 Oct 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Disconnection of the entorhinal cortex and the dorsomedial striatum impairs the sensitivity to instrumental contingency degradation

Bjoern Lex and Wolfgang Hauber

Abteilung Tierphysiologie, Biologisches Institut

Universität Stuttgart, D-70550 Stuttgart, Germany

Address for correspondence:

Prof. Dr. Wolfgang Hauber, Universität Stuttgart, Biologisches Institut
Abteilung Tierphysiologie, Pfaffenwaldring 57, D-70550 Stuttgart, Germany

Phone: ++49-711-685-65003

Fax: ++49-711-685-55000

E-Mail: hauber@bio.uni-stuttgart.de

Abstract

The capacity to detect changes in the causal efficacy of actions is mediated by a number of brain areas including the entorhinal cortex (EC) and the posterior part of the dorsomedial striatum (pDMS). Here, we examined whether interactions between the EC and the pDMS are required to detect changes of the instrumental contingency. Rats that received EC-pDMS disconnection lesions, i.e. unilateral cell body lesions of the EC and contralateral dopamine depletions of the pDMS, were trained to press two levers, one delivering food pellets, the other a sucrose solution. Thereafter, we tested whether rats were sensitive (i) to a selective devaluation of the value of one of two outcomes using a specific satiety procedure, and (ii) to a selective degradation of one of two contingencies controlling instrumental choice behavior. Our results reveal that rats with EC-pDMS disconnection lesions were sensitive to outcome devaluation. However, unlike rats with sham lesions or unilateral EC and pDMS lesions, rats with EC-pDMS disconnection lesions showed a reduced sensitivity to contingency degradation. These findings suggest that the EC and the pDMS may be part of a neural system that supports the detection of changes in the causal relationship between an action and its consequences.

Key words: entorhinal cortex, posterior dorsomedial striatum, instrumental conditioning, dopamine, rat

Introduction

It is well known that animals not only can encode the causal relationship between actions and their consequences but also detect changes in the causal efficacy of their actions. For instance, in instrumental conditioning post-training degradation of one of two action-outcome contingencies selectively reduced performance of the action that is no longer causal to the delivery of a particular reward (see Balleine and O'Doherty, 2009 for an excellent recent review).

A flexible control of actions in the pursuit of goals is crucial for adapting goal-directed behavior to changing environments. Recent studies suggest that the capacity to detect changes in the causal efficacy of actions is mediated by a number of brain areas including the dorsomedial striatum. For example, using functional magnetic resonance imaging in humans, Tanaka et al. (2008) revealed that neural responses in the caudate nucleus, i.e. the human homologue of the rat dorsomedial striatum, were modulated as a function of the contingency between the rate of button pressing and the amount of money earned. Furthermore, Yin et al. (2005) implicated a particular subregion of the dorsomedial striatum in mediating the sensitivity to changes in the instrumental contingency. The authors demonstrated in rats that cell body lesions of the posterior but not of the anterior part of the dorsomedial striatum produced an insensitivity to contingency degradation. Likewise, Lex and Hauber (2009) recently revealed that 6-hydroxydopamine (6-OHDA) lesions in the posterior part of the dorsomedial striatum produced an insensitivity to contingency degradation as well. In addition, another lesion study in rats indicated that among the major components of the hippocampal formation, the entorhinal cortex seems to be critical for the detection of changes in the instrumental

contingency (Corbit *et al*, 2002). It has been suggested that deficits in context conditioning rendered animals with entorhinal lesions insensitive to contingency degradation because they have deficits in forming or representing associations between context and reward. Therefore, contextual cues did not compete with other predictors of reward during contingency degradation (Corbit and Balleine, 2000; Corbit *et al*, 2002). Notably, behavioral studies using maze tasks (Mulder *et al*, 2004; Ragozzino *et al*, 2002a; Yin and Knowlton, 2006) provided support to the notion that the dorsomedial striatum and the hippocampal formation may form a functional circuit that mediates goal-directed behavior based on a representation of the context (see Yin *et al*, 2006 for a review). In addition, anatomical studies revealed that the dorsomedial striatum including its posterior part receives direct projections from the EC (McGeorge and Faull, 1987, 1989). Therefore, we wondered whether in instrumental conditioning the capacity to detect changes in the causal efficacy of actions depends on interactions between the entorhinal cortex (EC) and the posterior dorsomedial striatum (pDMS).

Here we tested rats that received disconnection lesions of the unilateral EC and contralateral pDMS for their sensitivity to contingency degradation. Specifically, rats subjected to disconnection lesions received unilateral cell body lesions of the EC and contralateral dopamine depletion of the pDMS. A pDMS dopamine depletion was used because dopamine in the pDMS has been implicated in the detection of causal relationships between an action and its consequences (Lex *et al*, 2009). Furthermore, reward-directed behavior critically depends on a striatal integration of hippocampal inputs mediated by dopamine (Goto and Grace, 2005). If interactions between EC and pDMS are important for monitoring action-outcome contingencies underlying goal-directed behavior, then, lesions that disconnect EC and pDMS should render rats insensitive to contingency degradation. In instrumental conditioning, rats also encode the contingency between their actions and the incentive value of the outcomes (e.g. Balleine and Dickinson, 1998; Dickinson and Balleine, 1994). Therefore, we also examined lesion effects on the sensitivity to outcome devaluation, i.e. whether post-training

devaluation of the value of one of two outcomes contingent upon distinct actions selectively reduced performance of the action that leads to the devalued outcome.

Materials and Methods

All animal experiments were conducted according the German Law on Animal Protection and approved by the proper authorities.

Subjects and apparatus

Subjects were 40 naive male Lister-Hooded rats (Harlan-Winkelmann, Borchon, Germany) weighing between 200-230g upon arrival. The rats were housed in groups of four in transparent plastic cages (55x39x27cm, Ferplast, Nürnberg, Germany) in a temperature- and humidity- controlled room ($20\pm 2^{\circ}\text{C}$, 50-60%) on a 12:12-h light-dark cycle (lights on at 7.00 a.m). Throughout the experiment the rats had *ad libitum* access to water. Standard laboratory maintenance chow (Altromin, Lage, Germany) was given *ad libitum* for 2 days after arrival, after which food was restricted to 15g per animal per day to maintain them at $\sim 85\%$ of their free-feeding weight.

Training and testing took place in identical operant chambers (24x21x30cm; Med Associates, St. Albans, VT, USA) housed within sound-attenuating cubicles. Each operant chamber was equipped with a pellet dispenser that delivered 45mg Noyes Pellets (formula A/I; Sandown Scientific,

Hampton, Middlesex, UK) into a dual pellet/liquid cup receptacle which was positioned in the middle of the right wall, and a syringe pump that delivered 0.1 ml of a 20% sucrose solution into the same receptacle. Each chamber also contained two retractable levers located on either side of the dual pellet/liquid cup receptacle. A 24V / 3W houselight mounted on the top-centre of the opposite wall illuminated the chambers and an electric fan integrated into the cubicle provided a constant background noise (~70dB).

Surgery

Before surgery animals were assigned to four treatment groups (sham lesion, disconnection lesion, unilateral pDMS lesion, unilateral EC lesion). Animals of the sham lesion group (n=11) received unilateral vehicle infusions into the pDMS of one hemisphere and unilateral vehicle infusions into the EC of the contralateral hemisphere. Animals of the disconnection lesion group (n=9) received unilateral 6-OHDA infusions into the pDMS of one hemisphere and unilateral N-methyl-D-aspartic acid (NMDA) infusions into the EC of the contralateral hemisphere. Animals of the unilateral pDMS group (n=11) were subjected to a unilateral 6-hydroxydopamine (6-OHDA) infusion into the pDMS of one hemisphere and a unilateral vehicle infusion into the EC of the contralateral hemisphere; animals of the unilateral EC group (n=9) received unilateral NMDA infusions into the EC of one hemisphere and vehicle infusions into the pDMS of the contralateral hemisphere. The sides of the lesions were balanced in each group, such that there were approximately equal numbers of rats with drug/vehicle infusions in the left or right hemispheres.

After pre-treatment with atropine (0.2mg/kg i.p.; WDT, Garbsen, Germany), the animals were anaesthetized with sodium pentobarbital (60mg/kg i.p.; Medial GmbH, Hallbergmoos, Germany)

and xylazine (4mg/kg i.m.; Bayer AG, Leverkusen, Germany) before being placed in a stereotaxic frame (Kopf Instruments, Tujunga, USA) with the toothbar -3.3 mm below the interaural line. The skull surface was exposed and small holes were drilled above the respective sides. Infusions were made using a 1µl Hamilton syringe at the following coordinates as determined from the atlas of (Paxinos and Watson, 2007): pDMS = AP -0.4; ML \pm 2.6; DV -5.0; EC = AP -7.5; ML \pm 4.6; DV -7.0 and at AP -8.2; ML \pm 4.6; DV -5.5; 6-OHDA hydrobromide (6 µg; Sigma Aldrich, Steinheim, Germany; in 0.4 µl saline containing 0.01% ascorbic acid; Sigma Aldrich, Steinheim, Germany) was infused into the pDMS, NMDA (8 µg, Sigma Aldrich, Steinheim, Germany; in 0.4 µl saline) was infused into the EC. Intra-pDMS vehicle infusions consisted of 0.4 µl saline containing 0.01% ascorbic acid, intra-EC vehicle infusions of 0.4 µl saline.

Procedure

The behavioral procedure was similar to a protocol used previously by Corbit et al. (2003a).

Magazine and lever press training. On the first day all animals received two magazine training sessions for 20 min. in which both outcomes (pellets and 20% sucrose solution) were delivered on independent random-time schedules (RT-60). Thereafter, lever-press training was started; for half of the animals of each group pressing the left lever earned one pellet and pressing the right lever earned 0.1 ml 20% sucrose solution. The other half received the opposite action-outcome pairings. All animals received two daily 20 min. lever-press training sessions in which only one lever and one outcome was available. After the first training session, there was at least a two-hour break before the second training session with the other lever-outcome pairing began. The order of the pellet and sucrose sessions was alternated each day. Lever-press training was conducted for 8 consecutive days with

progressively leaner random ratio (RR-) schedules of reinforcement, except for the first two days in which a continuous reinforcement schedule (CRF) was used (Day 1-2 = CRF, $P(O|A) = 1.0$ / Day 3-5 = RR-5, $P(O|A) = 0.2$ / Day 6-8 = RR-10, $P(O|A) = 0.1$)

Outcome devaluation test. On the day after the last instrumental training session, all animals were given 1 hour *ad libitum* access to one of the two outcomes in the feeding cages (1 animal per cage). Half of the animals of each group received pellets in a glass bowl and the other half received the sucrose solution in a drinking bottle. Immediately after pre-feeding, the rats were placed into the operant chambers and a 10 min. choice extinction test was conducted, in which both levers were inserted but no outcomes were given. On the next day the rats received two retraining session (RR-10; one session on each lever) after which they were given a second choice extinction test. This second devaluation test was identical to the first one except that those animals that had received free access to the pellets on the first test were given free access to the sucrose solution and *vice versa*.

Contingency degradation training. Rats received three days of retraining under the RR-10 schedule (two sessions/day, one session on each lever), after which they were trained for another three days on an RR-20 schedule of reinforcement ($P(O|A) = 0.05$; two sessions/day, one session on each lever). Thereafter the contingency training started. To assess whether the rats were sensitive to a degradation of the instrumental contingency, a protocol was applied in which the same outcome earned by pressing one of the two levers was given additionally in a non-contingent manner with the same probability in each second without a response, i.e. the probability of outcome delivery by pressing a lever was $P(O|A) = 0.05$ and the probability of outcome delivery by not pressing a lever was $P(O|no A) = 0.05$. Thus the experienced probability of outcome delivery was the same regardless if the animal performed that action or not, a protocol which degrades this action-outcome association (see Corbit *et al*, 2003a). The animals received two training sessions each day, one with

each action-outcome pairing. The outcome given non-contingently was the same in both sessions, therefore, one action-outcome association was degraded while the other action-outcome association was not degraded. For half of the animals of each group the lever-pellet contingency was degraded (i.e. pellets were the outcome given non-contingently), whereas for the other half the lever-sucrose contingency was degraded. The rats received two 30min. training sessions each day (one on each lever) and had a break of at least two hours between the two sessions. The order of the two sessions was alternated each day and training continued for 6 days.

Contingency degradation test. On the day after the last contingency training session a 10min. choice extinction test was given. Both levers were inserted but no rewards were given.

Statistical analysis

Lever training was given for 8 days, contingency degradation training for 6 days. Respective data were subjected to repeated-measures ANOVA with two within-subject factors (days of testing and degradation, i.e. degraded *vs.* non-degraded) and one between-subject factor (treatment groups: sham lesion, disconnection lesion, unilateral EC lesion, unilateral pDMS lesion). Outcome devaluation and contingency degradation effects were tested in extinction in a single test day, respectively. The data were subjected to *a priori* comparisons to assess the sensitivity to outcome devaluation and contingency degradation of each treatment group. Presses for the devalued *vs.* valued lever and the degraded *vs.* not-degraded lever in each treatment group were analyzed separately using planned

contrasts. All statistical computations were carried out with STATISTICA™ (version 7.1, StatSoft®, Inc., Tulsa, OK, USA).

Histology

After the behavioral testing animals were killed by an overdose of isoflurane (cp-pharma, Burgdorf, Germany) and perfused transcardially as described previously (Lex *et al*, 2009).

Tyrosine-hydroxylase-Immunohistochemistry in the pDMS: Coronal brain sections were cut (40µm; HM550, Microm GmbH, Walldorf, Germany) in the region of the pDMS. Tyrosine-hydroxylase staining was performed as described previously (Calaminus and Hauber, 2009; Lex *et al*, 2009). To determine the size and placement of the lesions the TH-immunoreactivity was analyzed under a microscope with reference to the atlas of Paxinos and Watson (2007).

Nissl-staining in the EC: Coronal brain sections were cut (40µm; Microm HM550, Microm GmbH, Walldorf, Germany) in the region of the EC, mounted on coated slides and stained with cresyl violet. Size and placement of lesions was analyzed under a microscope with reference to the atlas of Paxinos and Watson (2007).

Results

Histology

Fig. 1 and 2 provide photomicrographs of brain sections with lesions and schematic representations of the minimum and maximum extent of NMDA-induced cell body lesions of the EC and 6-OHDA-induced DA depletions of the pDMS across all lesion groups in one hemisphere, respectively.

In animals with EC-pDMS disconnection lesions we observed a marked reduction of neuronal density in the unilateral EC accompanied by gliosis and an atrophy of neurons in the target area. Damage was mostly restricted to the caudomedial, medial and ventral intermediate entorhinal cortex with occasional minimal damage to the parasubiculum. Unilateral damage of the EC seen here was comparable to damage observed in single hemispheres of bilaterally EC lesioned animals in studies using similar infusion parameters (Corbit *et al*, 2002; Coutureau *et al*, 2000; Majchrzak *et al*, 2006; Traissard *et al*, 2007). In the contralateral side, TH-positive fibers in the pDMS were markedly reduced. There was no evidence of damage outside the pDMS, i.e. to areas more anterior than +0.2mm from bregma and to areas more laterally than 3.2mm from the midline. The volume and concentration of 6-OHDA in this experiment (6µg/0.4µl) was the same as in previous studies from our lab in which we found a marked reduction of TH-immunoreactivity in the pDMS (Calaminus *et al*, 2009; Lex *et al*, 2009). A number of earlier studies reported that intrastriatal infusion of solutions

with lower concentrations of 6-OHDA ($<7\mu\text{g}/\mu\text{l}$) as used here ($15\mu\text{g}/\mu\text{l}$) profoundly reduced tissue concentrations of DA ($>85\%$) (Baunez and Robbins, 1999; Blandini *et al*, 2007; Brown and Robbins, 1991; Faure *et al*, 2005; Moukhles *et al*, 1994). Likewise, intrastriatal infusions of solutions containing $3.5\mu\text{g}/\mu\text{l}$ 6-OHDA caused a significant DA denervation ($\approx 90\%$) as assessed by DA transporter autoradiography (Winkler *et al*, 2002). Together these findings provide support to our observation that rats subjected to a 6-OHDA infusion had an almost complete unilaterally pDMS DA depletion. **One caveat to note however, is that we did not quantify the exact extent of the DA depletion, e.g. by measuring striatal tissue levels of DA (Hauber *et al*, 1994). Thus, we can not exclude that the extent of DA depletion induced in our present study differed from those quantified in previous studies using lower concentrations of 6-OHDA (Baunez and Robbins, 1999; Blandini *et al*, 2007; Brown and Robbins, 1991; Winkler *et al*, 2002).**

In animals with control lesions, i.e. unilateral pDMS and unilateral EC lesions, neuronal density in the EC and TH-immunoreactivity in the pDMS was reduced to a similar extent as observed in animals with disconnection lesions. By contrast, in animals subjected to sham lesions we observed only minimal damage along the injection cannulae tracks with no damage to the respective target area. We did not include a further control group with combined unilateral lesions of the pDMS and EC in the same hemisphere as numerous studies using disconnection designs demonstrated that ipsilateral lesions of two interconnected structures in the same hemisphere do not impair behavior relative to the effect of crossed disconnection lesions (Chudasama *et al*, 2003; Dunnett *et al*, 2005; Hauber and Sommer, 2009; Olton *et al*, 1982; Warburton *et al*, 2000).

Lever-press training

All animals acquired the instrumental task during training (Fig. 3). First, we analyzed within each group, whether the side of the lesions had an effect on performance. Separate repeated measures ANOVA from data of each group (sham lesion, disconnection lesion, unilateral EC and unilateral pDMS lesion) showed no significant effect of lesion side on lever pressing during training (sham lesion: lesion side $F_{(1,9)} = 0.55$, n.s.; day $F_{(7,63)} = 32.73$, $p < 0.001$; unilateral pDMS lesion: lesion side $F_{(1,9)} = 0.02$, n.s.; day $F_{(7,63)} = 49.15$, $p < 0.001$; unilateral EC lesion: lesion side $F_{(1,7)} = 0.15$, n.s.; day $F_{(7,49)} = 33.28$, $p < 0.001$; disconnection lesion: lesion side $F_{(1,7)} = 0.03$, n.s.; day $F_{(7,49)} = 51.97$, $p < 0.001$; no lesion side x day interactions were observed) as well as in other tests (data not shown). Therefore, possible effects of the lesion side were not considered further in subsequent statistical analyses. A repeated measures ANOVA of lever pressing during training revealed a significant effect of day ($F_{(7, 252)} = 171.58$, $p < 0.001$), but no effect of group ($F < 1$, n.s.) and no group x day interaction ($F < 1$, n.s.).

Outcome devaluation

A clear outcome devaluation effect was observed in all treatment groups. Planned contrast analysis revealed that lever press rates in each treatment group were significantly different for the valued *versus* devalued reward (Fig. 4).

Contingency degradation

A selective degradation of the instrumental contingency over 6 days of degradation training had distinctive effects in treatment groups as shown in Fig. 5. A repeated measures ANOVA of the contingency training data revealed a significant effect of degradation ($F_{(1, 34)} = 13.53, p < 0.001$), no effect of group ($F < 1, n.s.$) and no group \times degradation interaction ($F < 2, n.s.$). Although it is possible that the failure to find a reliable interaction reflects no difference between groups, an analysis of simple effects can be warranted even in the face of a non-significant interaction (Howell, 2007). Accordingly, an explorative simple effects analysis revealed that the sham lesion group ($F_{(1, 10)} = 5.93, p < 0.05$), the unilateral pDMS lesion group ($F_{(1, 9)} = 5.54, p < 0.05$) and the unilateral EC lesion group ($F_{(1, 8)} = 6.06, p < 0.05$) showed a degradation effect; this was not the case for the disconnection lesion group ($F < 2, n.s.$). Hence, animals with a disconnection of EC and the pDMS seemed to be less sensitive to the contingency degradation training procedure.

The critical contingency degradation test performed in extinction confirmed this observation and indicated that the sensitivity to contingency degradation was impaired in animals with a disconnection of the EC and the pDMS. Planned contrast analysis of the data from the degradation test in extinction revealed that, except for the disconnection lesion group, animals performed significantly less responses on the lever for which the contingency had been degraded (Fig. 6).

Discussion

Our results reveal that rats subjected to a disconnection of the EC and the pDMS were sensitive to outcome devaluation, but showed a reduced sensitivity to the degradation of the instrumental contingency. Previous studies already demonstrated that bilateral cell body lesions of the EC (Corbit

et al, 2002) and the pDMS (Yin *et al*, 2005) as well as a bilateral DA depletions of the pDMS (Lex *et al*, 2009) impaired the sensitivity to contingency degradation. Therefore, our present data suggest that interactions between the EC and the pDMS support the detection of changes in the causal relationship between an action and its consequences.

Animals with EC-pDMS disconnection lesions responded selectively in the outcome devaluation test indicating that their ability to discriminate between the two actions and the two outcomes was not compromised. Furthermore, lesioned rats were sensitive to changes in the outcome value and could integrate such a change in value into an association between a specific action and the related outcome. Hence, encoding the current value of an outcome as well as encoding and retrieval of action-outcome associations that guide selective responding does not depend on an interaction between the EC and the pDMS. Given the fact that the prelimbic region of the prefrontal cortex is critical for instrumental learning (Balleine *et al*, 1998; Balleine *et al*, 2009; Corbit *et al*, 2003a; Killcross and Coutureau, 2003), interactions between prefrontal cortex and pDMS may play a crucial role in encoding actions and outcomes.

By contrast, animals with a disconnection lesion of the EC and the pDMS pressed the levers as if reward delivery was still dependent on their actions, even if one action was no longer causal to the delivery of a specific reward. This impairment was pronounced if animals with disconnection lesions were tested in extinction, but moderate if tested during contingency degradation training. Conversely, animals with sham lesions were already sensitive to the effects of the contingency degradation on the first day of training, which is in accordance with earlier observations (Corbit *et al*, 2001, 2002, 2003). It is important to note that in the contingency degradation procedure the critical test is the extinction test, as it examines previously learned associations without contamination by new learning (Dickinson *et al*, 1994; Niv *et al*, 2006). Thus, lesions that impair the sensitivity to contingency degradation tested in extinction can have no (Corbit *et al*, 2003a) or moderate effects (e.g. Corbit *et al*, 2000; Corbit *et al*, 2002; Lex *et al*, 2009) on contingency degradation training. The impaired sensitivity

might not reflect an impairment in encoding action-outcome associations *per se* as the same animals showed an outcome devaluation effect that relies on an intact action-outcome association. It has been argued that in normal rats reduced instrumental performance associated with contingency degradation results from a comparison of the validity that an action is predictive of reward and the validity that the background or context is predictive of reward (e.g. Colwill and Rescorla, 1986; Corbit *et al*, 2000). Previous studies demonstrated that bilateral EC lesions impaired context conditioning (e.g. Majchrzak *et al*, 2006; Maren and Fanselow, 1997) and produced an insensitivity to a degraded instrumental contingency, but left intact the sensitivity to outcome devaluation (Corbit *et al*, 2002). Therefore, Corbit *et al.* (2002) suggested that animals with bilateral EC lesions are insensitive to the contingency degradation procedure because they have deficits in forming or representing associations between context and reward. As a result, contextual cues did not compete with other predictors of reward during contingency degradation. Importantly, anatomical studies in rats using retrograde labeling revealed that the pDMS receives significant direct projections from the EC (McGeorge *et al*, 1987, 1989). Thus, the sensitivity to contingency degradation as tested here could rely on the contextual information flow from the EC to the pDMS. Accordingly, it is conceivable that the reduced sensitivity to contingency degradation in animals with EC-pDMS disconnections was due to an impaired processing of reward-related contextual information.

Our previous study demonstrated that a bilateral DA depletion of the pDMS impaired the sensitivity to contingency degradation, but left the sensitivity to outcome devaluation intact. Therefore we suggested that DA in the pDMS contributes to instrumental conditioning by supporting the detection of the causal relationship between an action and its consequences (Lex *et al*, 2009). Consistent with this notion, the present disconnection study that involved pDMS DA depletions produced a similar pattern of impairments. Furthermore, our present results point to the possibility that DA signaling in the pDMS is important in enabling the integration of contextual information within the pDMS. In a similar vein, electrophysiological studies demonstrated that reward-directed

behavior depends on the interaction between hippocampal inputs and neurons in the ventral striatum that is modulated by DA (Goto *et al*, 2005). Specifically, it has been suggested that a phasic DA release evoked when an animal encounters unexpected reward facilitates the hippocampal drive of nucleus accumbens neurons via action on D1 receptors. By contrast, a suppression of DA release could decrease tonic DA stimulation of D2 receptors and, in turn, input integration in the nucleus accumbens is shifted favoring prefrontal over hippocampal signals (Goto and Grace, 2008). Correspondingly, contingency degradation as tested here represents a situation involving an unexpected transition of the predictive value of the context. Such a change of the instrumental contingency could entail a prediction error signaled by a phasic DA release (Schultz, 1997, 1998) that facilitates the integration of entorhinal information by activation of D1 receptors. As phasic DA signaling was impaired in animals with EC-pDMS disconnection lesions, striatal processing of entorhinal signals might have been compromised such that the context did not become a predictor for the reward during contingency degradation. However, it is also evident that a large variety of behavioral processes simply rely on an appropriate level of tonic DA receptor stimulation (see Schultz, 2007 for a review). Given this enabling function of tonic DA transmission, a striatal integration of entorhinal signals underlying the sensitivity to contingency degradation could merely depend on tonic DA release. Thus, from our results it is not clear whether tonic or phasic DA release or an interaction between these DA states could be involved in the striatal regulation of EC input. Notably, studies that examined spatial navigation in mazes provided support to the notion that the DMS and the hippocampal formation may form a functional circuit (Mulder *et al*, 2004; Ragozzino *et al*, 2002b). For instance, unlike sham controls, animals with pDMS cell body lesions tested in a cross maze task used a response, instead of a place strategy, possibly due to an impaired representation of contextual cues (Yin and Knowlton, 2004). Remarkably, bilateral DA depletions of the pDMS produced a similar strategy shift in the same task (Lex and Hauber, unpublished results). Therefore, the pDMS might represent a key component of a cortico-striatal circuit that mediates flexible goal-

directed behavior based on contextual representation (Balleine *et al.*, 2009; Yin *et al.*, 2006). Consistent with this notion, our present findings suggest that, possibly by processing contextual signals, direct pathways between EC and pDMS provide flexibility to goal-directed behavior if the context becomes a reliable predictor of reward. However, goal-directed behavior tested in operant tasks as used here and spatial navigation tasks might involve different forms of learning (see Yin *et al.*, 2008 for a recent review), therefore, respective inferences have to be done with caution.

Notably, studies using functional magnetic resonance imaging in humans demonstrated an involvement of the caudate nucleus in contingency learning. For instance, Tricomi *et al.* (2004) showed that caudate activity is modulated by the action-outcome contingency as a strong striatal activation occurred when subjects perceived a contingency between a button press response and an outcome. Likewise, Tanaka *et al.* (2008) revealed that neural activity in the caudate nucleus was modulated as a function of the contingency between the rate of button pressing and the amount of money earned. In addition, Delgado *et al.* (2005) demonstrated that during contingency learning the activity in the caudate nucleus was particularly robust during the early phases of learning but decreased as learning progressed. However, it is yet unknown whether a compromised striatal DA transmission, e.g. patients in Parkinson's disease or in patients treated with dopamine antagonists, impairs the sensitivity to a contingency degradation.

To conclude, a number of brain areas such as the prelimbic region of the medial prefrontal cortex (Balleine *et al.*, 1998; Killcross *et al.*, 2003), the basolateral amygdala (Balleine *et al.*, 2003), the pDMS (Yin *et al.*, 2005), the mediodorsal thalamus (Corbit *et al.*, 2003b) and the EC (Corbit *et al.*, 2002) have been implicated to mediate the sensitivity to contingency degradation. In addition, DA in the pDMS (Lex *et al.*, 2009), but not in the prelimbic subregion of the prefrontal cortex (but see Naneix *et al.*, 2009) mediates the sensitivity to contingency degradation. However, interactions between these brain areas underlying sensitivity to contingency are largely unknown so far. The present results suggest that interactions between the EC and the pDMS are involved in the detection of changes in the

causal relationship between an action and its consequences. Furthermore, our findings suggest that DA in the pDMS is critical to adapt goal-directed behavior to changing environments, possibly by modulating the contextual information flow from the EC to the pDMS.

Disclosure/Conflict of Interest

Wolfgang Hauber: I declare that, beside the income received from my primary employer, I have received an honorarium for consulting from Merz Pharmaceuticals GmbH (Frankfurt, Germany) within the last three years. Furthermore there are no personal financial holdings that could be perceived as constituting a potential conflict of interest

Bjoern Lex: I declare that, except for income received from my primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

Acknowledgements:

This research was supported by the Deutsche Forschungsgemeinschaft (HA2340/8-2)

References

- Balleine BW, Dickinson A (1998). Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. *Neuropharmacology* **37**(4-5): 407-419.
- Balleine BW, Killcross AS, Dickinson A (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. *J Neurosci* **23**(2): 666-675.
- Balleine BW, O'Doherty JP (2009). Human and Rodent Homologies in Action Control: Corticostriatal Determinants of Goal-Directed and Habitual Action. *Neuropsychopharmacology*.
- Baunez C, Robbins TW (1999). Effects of dopamine depletion of the dorsal striatum and further interaction with subthalamic nucleus lesions in an attentional task in the rat. *Neuroscience* **92**(4): 1343-1356.
- Blandini F, Levandis G, Bazzini E, Nappi G, Armentero MT (2007). Time-course of nigrostriatal damage, basal ganglia metabolic changes and behavioural alterations following intrastriatal injection of 6-hydroxydopamine in the rat: new clues from an old model. *Eur J Neurosci* **25**(2): 397-405.
- Brown VJ, Robbins TW (1991). Simple and choice reaction time performance following unilateral striatal dopamine depletion in the rat. Impaired motor readiness but preserved response preparation. *Brain* **114** (Pt **1B**): 513-525.
- Calaminus C, Hauber W (2009). Modulation of behavior by expected reward magnitude depends on dopamine in the dorsomedial striatum. *Neurotoxicity research* **15**(2): 97-110.
- Chudasama Y, Baunez C, Robbins TW (2003). Functional disconnection of the medial prefrontal cortex and subthalamic nucleus in attentional performance: evidence for corticosubthalamic interaction. *J Neurosci* **23**(13): 5477-5485.
- Colwill R, Rescorla R (1986). Associative structures in instrumental learning. In: Bower G (ed). *The psychology of learning and motivation*. New York: Academic Press. Vol 20, pp 55-104.
- Corbit LH, Balleine BW (2000). The role of the hippocampus in instrumental conditioning. *J Neurosci* **20**(11): 4233-4239.
- Corbit LH, Balleine BW (2003a). The role of prelimbic cortex in instrumental conditioning. *Behav Brain Res* **146**(1-2): 145-157.
- Corbit LH, Muir JL, Balleine BW (2003b). Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. *Eur J Neurosci* **18**(5): 1286-1294.
- Corbit LH, Ostlund SB, Balleine BW (2002). Sensitivity to instrumental contingency degradation is mediated by the entorhinal cortex and its efferents via the dorsal hippocampus. *J Neurosci* **22**(24): 10976-10984.
- Coutureau E, Galani R, Jarrard LE, Cassel JC (2000). Selective lesions of the entorhinal cortex, the hippocampus, or the fimbria-fornix in rats: a comparison of effects on spontaneous and amphetamine-induced locomotion. *Exp Brain Res* **131**(3): 381-392.
- Delgado MR, Miller MM, Inati S, Phelps EA (2005). An fMRI study of reward-related probability learning. *NeuroImage* **24**(3): 862-873.
-

- Dickinson A, Balleine BW (1994). Motivational control of goal directed action. *Anim Learn Behav* **22**(1): 1-18.
- Dunnett SB, Meldrum A, Muir JL (2005). Frontal-striatal disconnection disrupts cognitive performance of the frontal-type in the rat. *Neuroscience* **135**(4): 1055-1065.
- Faure A, Haberland U, Conde F, El Massioui N (2005). Lesion to the nigrostriatal dopamine system disrupts stimulus-response habit formation. *J Neurosci* **25**(11): 2771-2780.
- Goto Y, Grace AA (2005). Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nat Neurosci* **8**(6): 805-812.
- Goto Y, Grace AA (2008). Limbic and cortical information processing in the nucleus accumbens. *Trends in neurosciences* **31**(11): 552-558.
- Hauber W, Bubser M, Schmidt WJ (1994). 6-Hydroxydopamine lesion of the rat prefrontal cortex impairs motor initiation but not motor execution. *Exp Brain Res* **99**(3): 524-528.
- Hauber W, Sommer S (2009). Prefrontostriatal Circuitry Regulates Effort-Related Decision Making. *Cereb Cortex*.
- Howell D (2007). *Statistical Methods for Psychology*, 6th. edn. Thomson Wadsworth: Belmont.
- Killcross S, Coutureau E (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cereb Cortex* **13**(4): 400-408.
- Lex B, Hauber W (2009). The Role of Dopamine in the Prelimbic Cortex and the Dorsomedial Striatum in Instrumental Conditioning. *Cereb Cortex*.
- Majchrzak M, Ferry B, Marchand AR, Herbeaux K, Seillier A, Barbelivien A (2006). Entorhinal cortex lesions disrupt fear conditioning to background context but spare fear conditioning to a tone in the rat. *Hippocampus* **16**(2): 114-124.
- Maren S, Fanselow MS (1997). Electrolytic lesions of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. *Neurobiol Learn Mem* **67**(2): 142-149.
- McGeorge AJ, Faull RL (1987). The organization and collateralization of corticostriate neurones in the motor and sensory cortex of the rat brain. *Brain research* **423**(1-2): 318-324.
- McGeorge AJ, Faull RL (1989). The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience* **29**(3): 503-537.
- Moukhles H, Amalric M, Nieoullon A, Daszuta A (1994). Behavioural recovery of rats grafted with dopamine cells after partial striatal dopaminergic depletion in a conditioned reaction-time task. *Neuroscience* **63**(1): 73-84.
- Mulder AB, Tabuchi E, Wiener SI (2004). Neurons in hippocampal afferent zones of rat striatum parse routes into multi-pace segments during maze navigation. *Eur J Neurosci* **19**(7): 1923-1932.
- Naneix F, Marchand AR, Di Scala G, Pape JR, Coutureau E (2009). A role for medial prefrontal dopaminergic innervation in instrumental conditioning. *J Neurosci* **29**(20): 6599-6606.
- Niv Y, Joel D, Dayan P (2006). A normative perspective on motivation. *Trends in cognitive sciences* **10**(8): 375-381.
- Olton DS, Walker JA, Wolf WA (1982). A disconnection analysis of hippocampal function. *Brain research* **233**(2): 241-253.
- Paxinos G, Watson C (2007). *The Rat Brain in Stereotaxic Coordinates*, 6th edn. Elsevier Academic Press, San Diego.
-

- Ragozzino ME, Jih J, Tzavos A (2002a). Involvement of the dorsomedial striatum in behavioral flexibility: role of muscarinic cholinergic receptors. *Brain research* **953**(1-2): 205-214.
- Ragozzino ME, Ragozzino KE, Mizumori SJ, Kesner RP (2002b). Role of the dorsomedial striatum in behavioral flexibility for response and visual cue discrimination learning. *Behavioral neuroscience* **116**(1): 105-115.
- Schultz W (1997). Dopamine neurons and their role in reward mechanisms. *Current opinion in neurobiology* **7**(2): 191-197.
- Schultz W (1998). Predictive reward signal of dopamine neurons. *Journal of neurophysiology* **80**(1): 1-27.
- Schultz W (2007). Multiple dopamine functions at different time courses. *Annu Rev Neurosci* **30**: 259-288.
- Tanaka SC, Balleine BW, O'Doherty JP (2008). Calculating consequences: brain systems that encode the causal effects of actions. *J Neurosci* **28**(26): 6750-6755.
- Traissard N, Herbeaux K, Cosquer B, Jeltsch H, Ferry B, Galani R, *et al* (2007). Combined damage to entorhinal cortex and cholinergic basal forebrain neurons, two early neurodegenerative features accompanying Alzheimer's disease: effects on locomotor activity and memory functions in rats. *Neuropsychopharmacology* **32**(4): 851-871.
- Tricomi EM, Delgado MR, Fiez JA (2004). Modulation of caudate activity by action contingency. *Neuron* **41**(2): 281-292.
- Warburton EC, Baird AL, Morgan A, Muir JL, Aggleton JP (2000). Disconnecting hippocampal projections to the anterior thalamus produces deficits on tests of spatial memory in rats. *Eur J Neurosci* **12**(5): 1714-1726.
- Winkler C, Kirik D, Bjorklund A, Cenci MA (2002). L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. *Neurobiology of disease* **10**(2): 165-186.
- Yin HH, Knowlton BJ (2004). Contributions of striatal subregions to place and response learning. *Learn Mem* **11**(4): 459-463.
- Yin HH, Knowlton BJ (2006). The role of the basal ganglia in habit formation. *Nat Rev Neurosci* **7**(6): 464-476.
- Yin HH, Ostlund SB, Balleine BW (2008). Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. *Eur J Neurosci*.
- Yin HH, Ostlund SB, Knowlton BJ, Balleine BW (2005). The role of the dorsomedial striatum in instrumental conditioning. *Eur J Neurosci* **22**(2): 513-523.

Titles and legends to figures

Fig.1. Schematic representations of the extent of neuronal damage in the EC (left hemisphere) and the areas devoid of TH-immunoreactivity in the pDMS (right hemisphere) across all lesion groups. Animals received either a disconnection lesions using NMDA infusion into the ipsilateral EC and 6-

OHDA infusion into the contralateral pDMS, unilateral NMDA infusions into the EC or unilateral 6-OHDA into the pDMS. Grey areas represent the smallest extent, black areas the largest extent of lesions. Numbers indicate distance from bregma in mm.

Fig.2. Representative photomicrographs of an NMDA induced cell body lesion of the EC and a 6-OHDA induced loss of tyrosine-immunoreactive fibers in the pDMS. The panels on the left side show an EC lesion (middle) and an EC sham lesion (bottom), the panels on the right side a pDMS DA depletion (middle) and a pDMS sham lesion (bottom). The boxed regions in the top panel are shown at high magnification in the middle and bottom panels. Plates are adaptations from the atlas of Paxinos and Watson (2007); scales are relative to bregma.

Fig.3. Lever training. Mean lever presses \pm standard error of the mean per minute averaged for both actions from animals of all four groups. Ratio schedules increased across training days as follows Day 1-2 = CRF, Day 3-5 = RR-5, Day 6-8 = RR-10.

Fig.4. Outcome devaluation. Mean lever presses per minute \pm standard error of the mean on the devalued and not-devalued lever during the 10 min choice extinction test, after one of the outcomes was devalued using a specific satiety procedure. Both levers were inserted but no outcomes were given. Planned contrast analysis revealed that lever press rates in each treatment group were significantly different for the valued *versus* devalued reward ($\#p < 0.01$).

Fig.5. Contingency degradation training. Mean lever presses per minute \pm standard error of the mean during contingency degradation training. **(A)** Animals with sham lesions, **(B)** animals with unilateral pDMS lesions, **(C)** animals with unilateral EC lesions, **(D)** animals with EC-pDMS

disconnection lesions. One action-outcome contingency was degraded by unpaired delivery of an outcome that was the same as that which was earned by pressing the degraded lever (RR-20).

Fig.6. Contingency degradation test. Mean lever presses \pm standard error of the mean per minute during the 10 min. choice extinction test from animals of all treatment groups. Both levers were inserted but no outcomes were given. Planned contrast analysis revealed that, except for the disconnection lesion group, animals performed significantly less responses on the lever for which the contingency had been degraded ($\#p < 0.01$).

Fig. 1

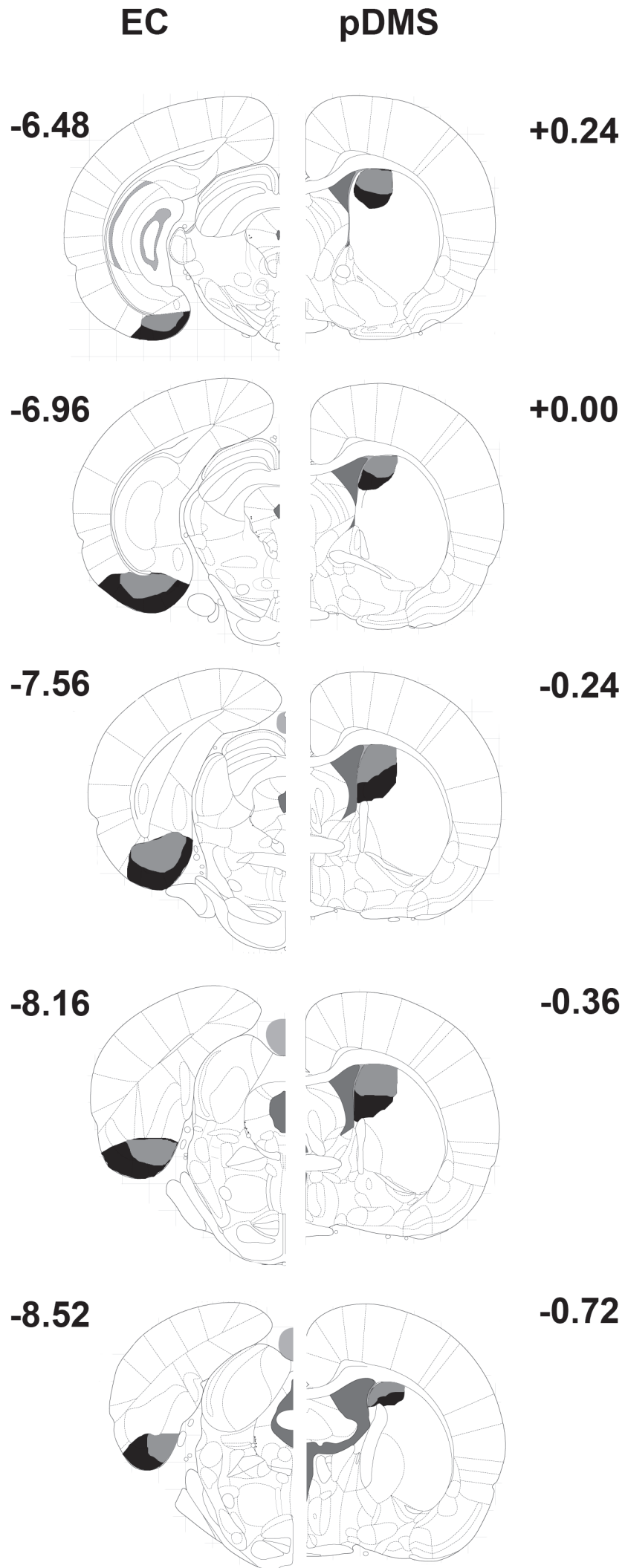
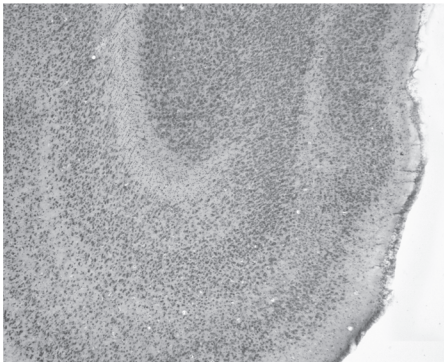
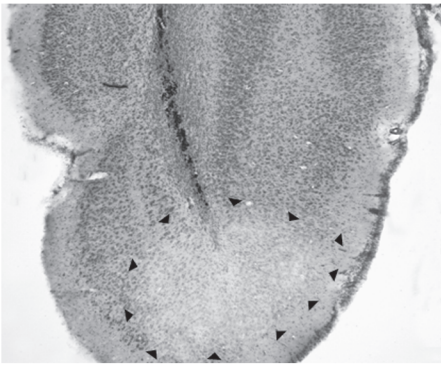
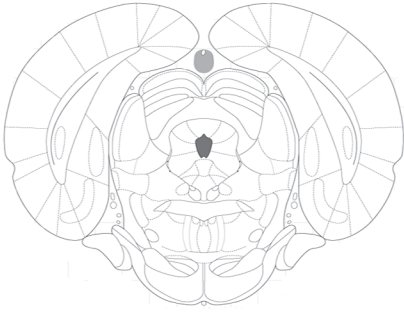


Fig. 2

EC



pDMS

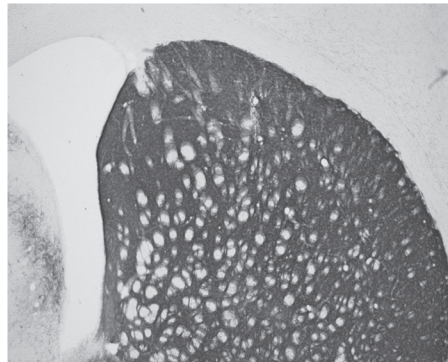
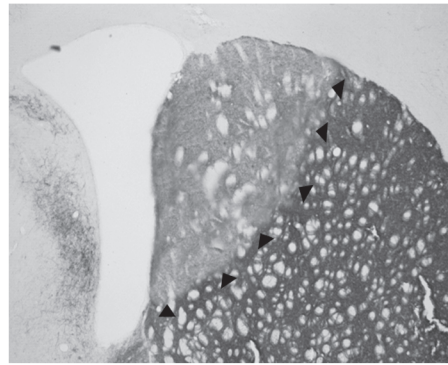
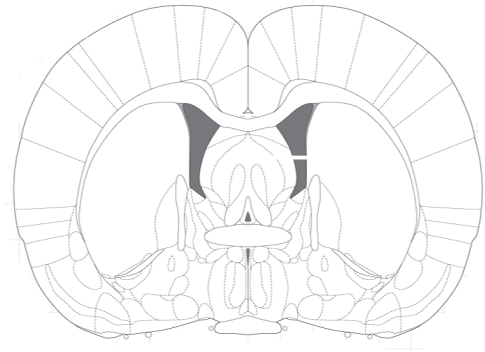


Fig. 3

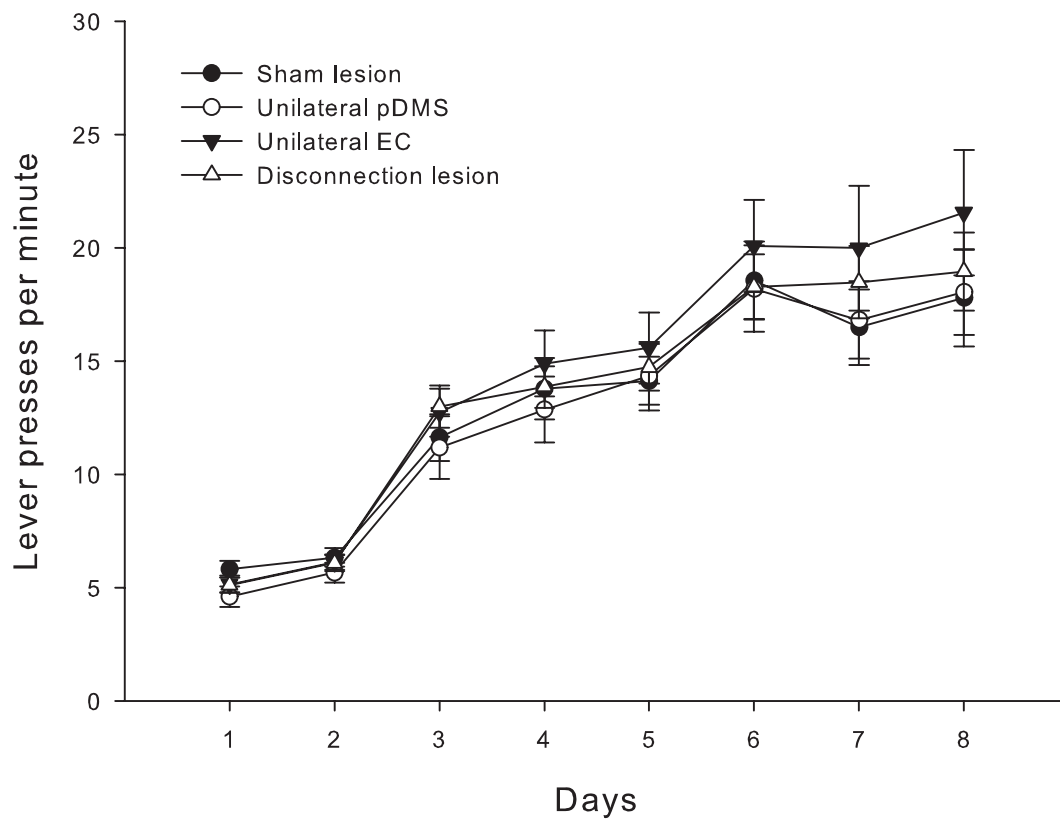


Fig. 4

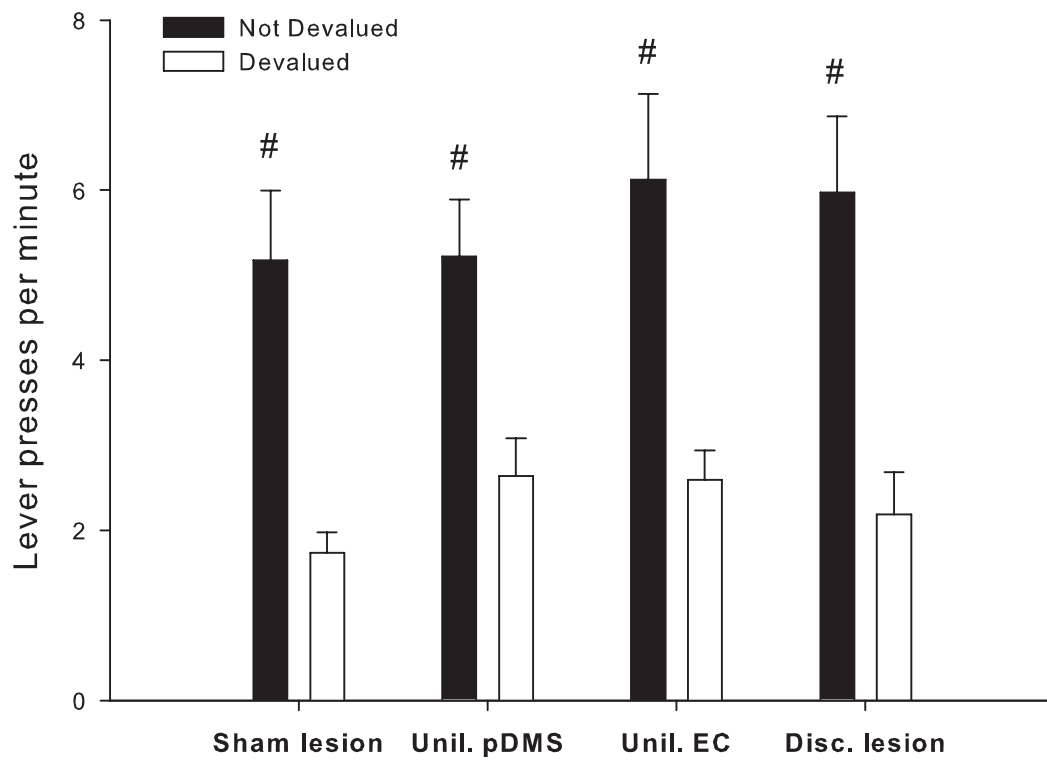


Fig. 5

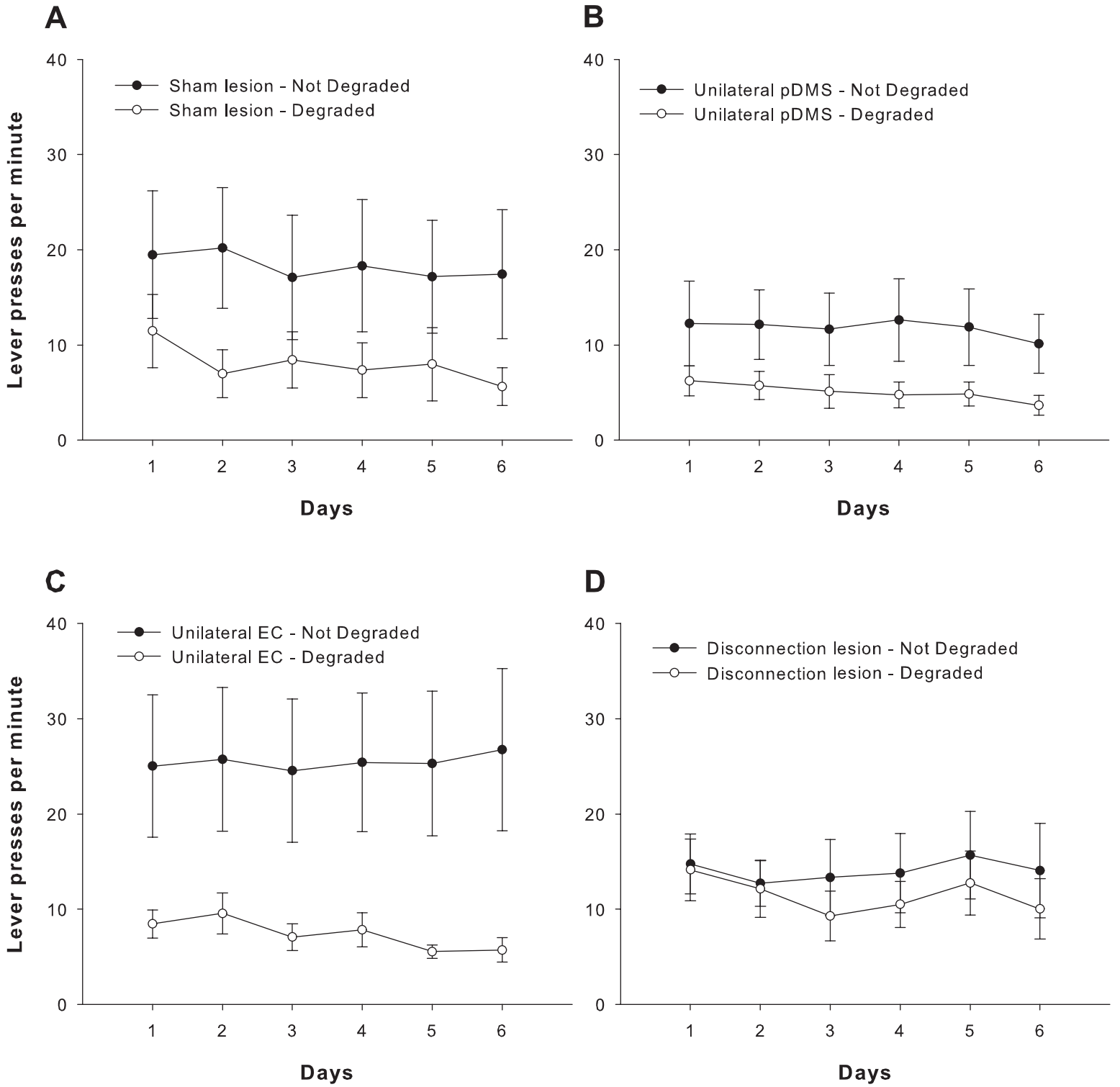


Fig. 6

