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Title: Learning deficits in an odor reward-task induced by parafascicular thalamic lesions are ameliorated by pretraining d-cycloserine in the prelimbic cortex.

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Abstract: We investigated whether the N-methyl-D-aspartate (NMDA) receptor partial agonist D-cycloserine (DCS) infused into the prelimbic cortex (PLC) would reverse the learning deficits caused by bilateral excitotoxic lesions of the parafascicular nucleus (PFn) in an odor discrimination task (ODT). Rats with PFn lesions received a bilateral infusion of DCS (10μg/side) into the PLC 20min before ODT acquisition. The task retention was evaluated in a drug-free test carried out 24h later. DCS significantly attenuated the PFn lesion-induced deficits as measured by both latency to nose-poke the rewarded odor and number of errors committed during ODT acquisition and retention. Therefore, DCS may be an enhancing memory treatment in animal models of cognitive impairment, such as PFn-lesioned rats. The PFn contribution to learning and memory may possibly be linked to its role in the modulation of glutamatergic PLC activity.

Highlights:

- Pre-training excitotoxic lesions of parafascicular nucleus disrupted an odor discrimination task
- D-cycloserine in the prelimbic cortex reversed memory impairments induced by parafascicular lesions
- D-cycloserine acts as an enhancing memory treatment in animal models of cognitive impairment

Learning deficits in an odor reward-task induced by parafascicular thalamic lesions are ameliorated by pretraining d-cycloserine in the prelimbic cortex.

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Abstract

We investigated whether the N-methyl-D-aspartate (NMDA) receptor partial agonist D-cycloserine (DCS) infused into the prelimbic cortex (PLC) would reverse the learning deficits caused by bilateral excitotoxic lesions of the parafascicular nucleus (PFn) in an odor discrimination task (ODT). Rats with PFn lesions received a bilateral infusion of DCS (10µg/side) into the PLC 20min before ODT acquisition. The task retention was evaluated in a drug-free test carried out 24h later. DCS significantly attenuated the PFn lesion-induced deficits as measured by both latency to nose-poke the rewarded odor and number of errors committed during ODT acquisition and retention. Therefore, DCS may be an enhancing memory treatment in animal models of cognitive impairment, such as PFn-lesioned rats. The PFn contribution to learning and memory may possibly be linked to its role in the modulation of glutamatergic PLC activity.

The partial agonist at the glycine site of NMDA receptors (NMDARs) D-cycloserine (DCS) has gained considerable attention for its potential beneficial effect on cognitive processes and treatment of neuropsychiatric disorders in which persistent maladaptive memories play an important role [1]. It has also been considered as a cognitive enhancer, as it is able to reverse learning and memory deficits due to aging, brain damage, behavioral and pharmacological manipulations [2], [3], [4],[5]. Nevertheless, in animals, research on DCS mainly focused on the beneficial effects of acute systemic injections [6] or intracerebral infusions into the basolateral amygdala (BLA) on extinction of fear conditioning [7]. Additionally, injections of DCS into the hippocampus and prelimbic cortex (PLC) improved radial maze learning [8] and an odor discrimination task (ODT) relearning [9].

The ODT is an olfactory task involving a rapidly acquired association between an odor and a palatable food reward, which has been previously characterized as sensitive to different pharmacological [10], [11] and lesion manipulations [12], especially those affecting the prelimbic cortex (PLC). It has been shown that NMDARs in the PLC are critical for ODT memory [10] and that pre-training excitotoxic lesions of the thalamic parafascicular nucleus (PFn) disrupted ODT performance [12].

The PFn in rodents is a posterior component of the intralaminar nuclei and is an essential structure in the feedback circuits of basal ganglia-thalamo-cortical systems [13]. This intralaminar nucleus is the major thalamic source of glutamatergic projections to the striatum [13] and it also projects to prefrontal regions such as the cingulate cortex and PLC [14]. Consequently, its effects on cognitive function are thought to arise from its influence on such targets [12]. Lesion studies addressing the behavioral role of the PFn have revealed impairments on aversive tasks and spatial relational memory paradigms, appetitive olfactory memory tasks and also object recognition (for a review see [14], [15]). Thus, a likely interpretation of such findings is that the loss of glutamatergic afferents from the PFn at the PLC level may have increased vulnerability to behavioral interferences [12]. As PFn lesion-induced cognitive deficits have been improved by treatments based on deep brain stimulation in structures

projecting to the PLC, such as the lateral hypothalamus [16], we evaluated whether the glutamatergic agonist DCS infused directly into the PLC would reverse the negative effects of PFn excitotoxic lesions on ODT, supporting its potential role as a cognitive enhancer.

Forty-nine *Wistar* rats (mean weight 393.50 g, SD 34.73g; mean age 91.41d, SD 4.37) were used. All procedures were carried out in compliance with the European Community Council Directive for care and use of laboratory animals (86/609/ECC) and with *Generalitat de Catalunya* authorization (DOGC 2073 10/7/1995, DARP protocol number 3211). Rats were randomly assigned to three experimental groups prior to surgical procedures: Lesion-PBS (PFn Lesion and PBS infusion into PLC), Lesion-DCS (PFn Lesion and DCS infusion into PLC) and VEH (PBS infusion into PFn and PLC). Before surgical procedures were carried out, all the rats were submitted to a food restriction schedule and habituation sessions to the behavioral box (for procedures see [12]). During surgery, the animal was placed in a stereotaxic apparatus and Bregma-lambda were made horizontal at the same DV. All subjects underwent implantation of a bilateral chronic double-guide cannula into the PLC and were bilaterally infused with NMDA (0.15 M in sterile phosphate-buffered saline, pH 7.4) or PBS into the PFn. A volume of 0.8µl (DV: -7.1 mm) and 0.4µl (DV: -6.3 mm) of NMDA (0.15 M in sterile PBS, pH 7.4) was infused in each hemisphere at a rate of 0.2µl/min using a microinjector (Model 5000, David Kopf Instruments, Tujunga, CA, USA) in the PF-lesioned groups (Lesion-DCS and Lesion-PBS). The VEH group underwent the same procedure, but with PBS infusions. The stereotaxic coordinates for the PLC were: AP, +3.5 mm from bregma; ML, ±0.6 mm from midline; and DV, -2.9 mm from cranium surface; and for the PFn: AP, -4.2 mm from bregma; L, ±0.8 mm from midline; and DV, -7.1 and -6.3 mm from the cranium surface [17]. Four days after surgery, the rats were again food-restricted (12 g/day), submitted to an additional habituation session, and adapted to a mock infusion protocol (no solutions injected).

On the training day, 20min before the ODT session, Lesion-DCS rats received a bilateral intracerebral infusion of 10µg of DCS (0.5µl/hemisphere for 2min; Sigma–Aldrich, Madrid, Spain) into the PLC, and the remaining groups (Lesion-PBS and VEH) received a

bilateral infusion of PBS. The drug dosage was based on a previous study [9]. ODT learning involved the discrimination of three odors in three different sponges and was carried out in a three-trial session. Behavioral protocols have been described in detail elsewhere [12]. Essentially, the sponges were impregnated with an odor on each of their four corners. Odors used were vanilla (0.2 mL), orange (0.6 mL) and anise (0.3 mL) (Vahiné). The reinforcement (chocolate rice cereal) was associated with the same odor across trials. The intertrial interval was 1 min. The target odor was randomly assigned to each rat in a counterbalanced manner for the three groups and sponges with the non-reinforced odors did not contain any food. A 6-min limit behavioral criterion was established for each rat to find and consume the reinforcement. Latency (sec.) before making a correct response (a nose-poke into the reinforced sponge) and number of errors were scored across sessions. Two different kinds of errors were combined: errors of commission (a nose-poke into non-target sponge) and omissions (sniffing the target odor and no nose-poke). The retention level of the learned response was measured in a DCS-free test 24-h after acquisition. The procedure was the same as in acquisition with the exception that the first trial was not reinforced to measure memory of the previous training. To rule out olfactory alterations due to the DCS infusions, an additional olfactory perception test was conducted at the end of the experiment (for procedures see [12]). Histological analyses of cresyl violet-stained sections were conducted following procedures explained elsewhere [11].

Figure 1A shows the maximum and minimum extents of successful PFn lesions for each atlas plate [17]. A rat was included in the final sample if the bilateral lesion affected at least half of the PFn in three of the four following AP coordinates: 3.80, 4.16, 4.30 and 4.52 mm posterior to bregma. For PLC cannulae implantation, we only considered rats with their cannula tips in the PLC within the area delimited by the anterior cingulate and infralimbic cortices and in which no tissue damage resulting from the rate or volume of the infusions was detected. Specifically, the cannulae were located along different brain coordinates from 3.70 to 3.20 mm anterior to bregma (Fig. 1B). The rats that did not accomplish histological (n=15) or behavioral

criteria (n=6) were excluded from the main behavioral analyses. The final sample was made up of 28 subjects distributed into Lesion-PBS (n = 7), Lesion-DCS (n=9) and VEH (n=12) groups.

Data were submitted to a mixed analysis of variance (ANOVA; PASW v17) in which the between-factor was Group (Lesion-PBS, Lesion-DCS and VEH) and the within-factor was Session (acquisition: the average scores for 3 trials; 24h test: the average scores for 4 trials). The post-hoc tests (latencies and errors) were performed to compare means of all the groups in every session to determine which of all the possible comparisons were statistically significant. The Bonferroni correction was used to counteract the problem of multiple comparisons. The dependent variables were Latencies and Errors. The main behavioral results are depicted in Figure 2, which shows that PFn-lesioned rats receiving a pretraining DCS infusion into the PLC (Lesion-DCS group) and VEH rats displayed shorter latencies and made fewer errors in both ODT sessions than the Lesion-PBS group. ANOVA for latencies indicated a statistically significant effect of Group ($F_{(1,25)}$ =3.507 p=0.045) with differences between Lesion-PBS vs. VEH (p=0.046) and vs. Lesion-DCS rats (p=0.053) in the acquisition session and a trend toward statistical significance between Lesion-PBS vs. VEH (p=0.089) in the retention session. The Session factor ($F_{(1,25)}$=0.129, p=0.723) and the Group × Session interaction ($F_{(1,25)}$ = 0.166 p=0.848) were not statistically significant. As for number of errors (Fig.2B), ANOVA showed that the Group factor was statistically significant ($F_{(1,25)}$ =3.647 p=0.041) with a trend of significance between Lesion-PBS and VEH (p=0.065) in the acquisition session and significant difference (p=0.027) in the retention session. The group Lesion-DCS did not differ from the Lesion-PBS (P=0.128 and p=0.165 in both sessions) or from the VEH group (p=0.777 and p=0.376 in both sessions). The Session ($F_{(1,25)}$=1.480, p=0.235) or the Group × Session interaction factors ($F_{(1,25)}$ = 0.294 p=0.748) were not statistically significant. In terms of food consumption, the analyses did not demonstrate any statistically significant differences between groups in the latency to consume 10 pieces of cereal during the last pre-surgery ($F_{(2,25)}$=1.775 p=0.190) and the post-surgery habituation sessions ($F_{(2,25)}$=0.403 p=0.672). Finally, performance was not related to either deficit in olfactory sensitivity as no significant between-group

differences were observed when the latency to find a buried cookie was analyzed ($F_{(1,25)}$ =2.324 p=0.119).

The aim of the present study was to determine whether the DCS infusion into the PLC could improve the expected memory impairment induced by the bilateral lesion of the PFn, which may cause a remarkable loss of glutamatergic influence on the PLC. As expected from previous studies [12], rats with lesions in the PFn exhibited longer latencies to make the correct responses and more errors when compared to the other groups. The results also indicated that a single pre-acquisition injection of DCS into the PLC reduced the detrimental effects of PFn lesions on ODT acquisition and retention. Our results demonstrated for the first time that direct intracerebral infusions of DCS are able to reverse cognitive deficits due to brain lesions and agree with previous systemic studies [18],[19]. The present findings are also in accordance with numerous studies showing consistent enhancement of many learning and memory paradigms after DCS administration [7]. Although much of the former work showed positive effects of DCS administered before training [20], it also reversed aging- , drug-, and stress-induced memory deficits [5],[21]. In humans, DCS has widely been used as an adjunct to psychotherapy for anxiety-related disorders [22]. Such beneficial effects have been widely interpreted in terms of DCS-induced facilitation of memory consolidation, most probably mediated through modulation of neuroplasticity in the hippocampus and/or basolateral amygdala [23]. In this respect, the NMDA subtype of glutamate receptor plays an essential role in long-term potentiation and depression (LTP and LTD) in cellular models of learning and memory [24].

Since our results showed that the DCS pre-treatment equalized performance of Lesion-DCS and VEH rats in both ODT sessions, it may be argued that DCS influenced brain processes underlying the early codification/storage of new information and also the odor-reward association consolidation. Memory consolidation is a crucial element of adaptive behavior that depends on the early activation of NMDA receptors by glutamate [25]. Accordingly, systemic or intracerebral administration of NMDAR antagonists shortly after training significantly impairs memory in diverse behavioral tasks [25]. Our findings are also consistent with the

hypothesis that DCS may improve cognitive functioning by enhancing the efficacy of glutamatergic transmission via stabilization and strengthening of NMDA receptors during learning processes [23]. In terms of the way it acts, it has been proposed that DCS may facilitate NMDAR functions by elevating the extracellular contents of D-serine , in addition to the direct stimulation of the NMDA glycine site [9].

Current findings also corroborate that NMDA transmission in the PLC is essential for discrimination learning based on odorous stimuli. The PLC has reciprocal connections to olfactory bulbs and piriform cortex [11], which may explain its importance to ODT memory. In agreement with the experiment presented here, Villarejo-Rodríguez et al. (2010) demonstrated that rats injected with DCS in the PLC prior to ODT training exhibited a significant enhancement of performance in such an odor-reward task, especially in the relearning trials evaluated in a drug-free 24-h test. Other studies also indicated that NMDARs in the PLC are important in the early stages of ODT memory consolidation, since the NMDA antagonist APV injected into the PLC immediately after training disrupted the retention and slowed down the relearning of the ODT task [10].

The present and previous reports (for a review see [14]) also confirm the PFn involvement in the modulation of different memory tasks, such as ODT. In the current study, PFn damage may have reduced the activation of specific target brain regions, such as the PLC region, necessary for coping with task demands and critically involved in executive cognitive functions [14]. The PFn may help to modulate cognitive functions by enhancing cortical glutamate, and thereby improve stimuli encoding and sensory-associational information processing. In accordance with such a hypothesis, unpublished results obtained in our laboratory indicate that the intracranial electrical stimulation of PFn evokes glutamate release in the medial prefrontal cortex.

In conclusion, the present research indicates that DCS may be used as a treatment to improve cognitive impairments induced by brain lesions, as DCS infused into the PLC reversed

the detrimental effects of PFn lesions on acquisition and retention of an odor-reward task. Beneficial effects of DCS on cognitive processes may be related to its ability to improve glutamatergic neurotransmission in brain areas critically involved in learning and memory by potentiating the NMDARs function. Further research would be necessary to ascertain the molecular events through which DCS is able to act as a cognitive enhancer, especially when learning deficits have been induced.

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REFERENCES

[1] Lee JLC, Gardner RJ, Butler VJ, Everitt BJ. D-cycloserine potentiates the reconsolidation of cocaine-associated memories. Learning and Memory 2009; 16: 82-85.

[2] Andersen JM, Lindberg M, Myhrer T. Effects of scopolamine and D-cycloserine on non-spatial reference memory in rats. Behavioural Brain Research 2002; 129: 211-216.

[3] Ohno M, Watanabe S. D-cycloserine, a glycine site agonist, reverses working memory failure by hippocampal muscarinic receptor blockade in rats. European Journal of Pharmacology 1996; 318: 267-71.

[4] Silvestri AJ, Root DH. Effects of REM deprivation and an NMDA agonist on the extinction of conditioned fear. Physiology and Behavior 2008; 93: 274-81.

[5] Temple MD, Hamm RJ. Chronic, post-injury administration of D-cycloserine, an NMDA partial agonist, enhances cognitive performance following experimental brain injury. Brain Research 1996; 741: 246-251.

[6] Vervliet B. Learning and memory in conditioned fear extinction: effects of D-cycloserine. Acta Psychologica 2008; 127: 601-13.

[7] Myers KM, Davis M. Mechanisms of fear extinction. Molecular Psychiatry 2007; 12: 120-50.

[8] Kawabe K, Yoshihara T, Ichitani Y, Iwasaki T. Intrahippocampal D-cycloserine improves MK-801-induced memory deficits: radial-arm maze performance in rats. Brain Research 1998; 814: 226-30.

[9] Villarejo-Rodríguez I, Vale-Martínez A, Guillazo-Blanch G, Martí-Nicolovius M. D-cycloserine in prelimbic cortex enhances relearning of an odor-reward associative task. Behavioural Brain Research 2010; 213: 113-116.

[10] Tronel S, Sara SJ. Blockade of NMDA receptors in prelimbic cortex induces an enduring amnesia for odor-reward associative learning. Journal of Neuroscience 2003; 23: 5472-5476.

[11] Carballo-Márquez A, Vale-Martínez A, Guillazo-Blanch G, Torras-Garcia M, Boix-Trelis N, Martí-Nicolovius, M. Differential effects of muscarinic receptor blockade in prelimbic cortex on acquisition and memory formation of an odor-reward task. Learning and Memory 2007; 14: 616-624.

[12] Quiroz-Padilla MF, Guillazo-Blanch G, Vale-Martínez A, Torras-García M, Martí-Nicolovius M. Effects of parafascicular excitotoxic lesions on two-way active avoidance and odor-discrimination. Neurobiology of Learning and Memory 2007; 88: 198-207.

[13] Smith Y, Surmeier DJ, Redgrave P, Kimura M. Thalamic contributions to basal ganglia-related behavioral switching and reinforcement. Journal of Neuroscience 2011; 31: 16102-16106.

[14] Quiroz-Padilla MF, Martí-Nicolovius M, Guillazo-Blanch G. Núcleos intralaminares posteriores del tálamo y procesos cognitivos. Revista de Neurología 2010; 51: 217-225.

[15] Castiblanco-Piñeros E, Quiroz-Padilla MF, Cardenas-Palacio CA, Cardenas FP. Contribution of the parafascicular nucleus in the spontaneous object recognition task. Neurobiology of Learning and Memory 2011; 96: 272-279.

[16] Redolar-Ripoll D, Soriano-Mas C, Guillazo-Blanch G, Aldavert-Vera L, Segura-Torres P, Morgado-Bernal I. Posttraining intracranial self-stimulation ameliorates the detrimental effects of parafascicular thalamic lesions on active avoidance in young and aged rats. Behavioral Neuroscience 2003; 117: 246-256.

[17] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press, 1997.

[18] Aura J, Riekkinen M, Riekkinen P. Tetrahydroaminoacridine and D-cycloserine stimulate acquisition of water maze spatial navigation in aged rats. European Journal of Pharmacology 1998; 342:15-20.

[19] Schuster G, Schmidt. D-Cycloserine reverses the working memory impairment of hippocampal-lesioned rats in a spatial learning task. European Journal of Pharmacology 1992; 224: 97-98.

[20] Nunnink M, Davenport RA, Ortega B, Houpt TA. D-cycloserine enhances conditioned taste aversion learning in rats. Pharmacology, Biochemistry and Behavior 2007; 87: 321-330.

[21] Waddell J, Mallimo E, Shors T. D-cycloserine reverses the detrimental effects of stress on learning in females and enhances retention in males. Neurobiology of Learning and Memory 2010; 93; 31-36.

[22] Hofmann SG, Sawyer AT, Asnaani A. D-cycloserine as an augmentation strategy for cognitive behavioral therapy for anxiety disorders: an update. Current Pharmaceutical Design 2012: 1-4.

[23] Davis M, Ressler K, Rothbaum BO, Richardson R. Effects of D-cycloserine on extinction: translation from preclinical to clinical work. Biological Psychiatry 2006; 60: 369-375.

[24] Kochlamazashvili; Bukalo O, Senkov O, Salmen B, Gerardy-Schahn R, Engel AK et al. Restoration of synaptic plasticity and learning in young and aged ncam-deficient mice by enhancing neurotransmission mediated by glun2a-containing NMDA receptors. Journal of Neuroscience 2012; 32: 2263-2275.

[25] Izquierdo I, Bevilaqua LRM, Rossato JI, Bonini JS, Medina JH, Cammarota M. Different molecular cascades in different sites of the brain control memory consolidation. Trends in Neurosciences 2006; 29:496-505.

FIGURE CAPTIONS

Figure 1. (A) Schematic drawing of the smallest (black dotted area) and the largest (striped area) PF lesions in successive anterior/posterior coronal sections. The extent of the lesions is superimposed on figures modified from Paxinos and Watson (1997). (B) Microinjector tip placements for Lesion-DCS (+), Lesion-PBS (*) and VEH (▪) groups throughout the rostro-caudal extent of the PLC (from 3.20 to 4.20 mm anterior to bregma).

Figure 2. Effects of pre-acquisition DCS injections into the PLC on ODT performance. (A) The behavioral procedure used. (B) Latency to make the correct response (± SEM) and (C) number of errors prior to making the correct response (± SEM) over the acquisition and the 24-h test sessions. (*$p < .05$)

Figure 1

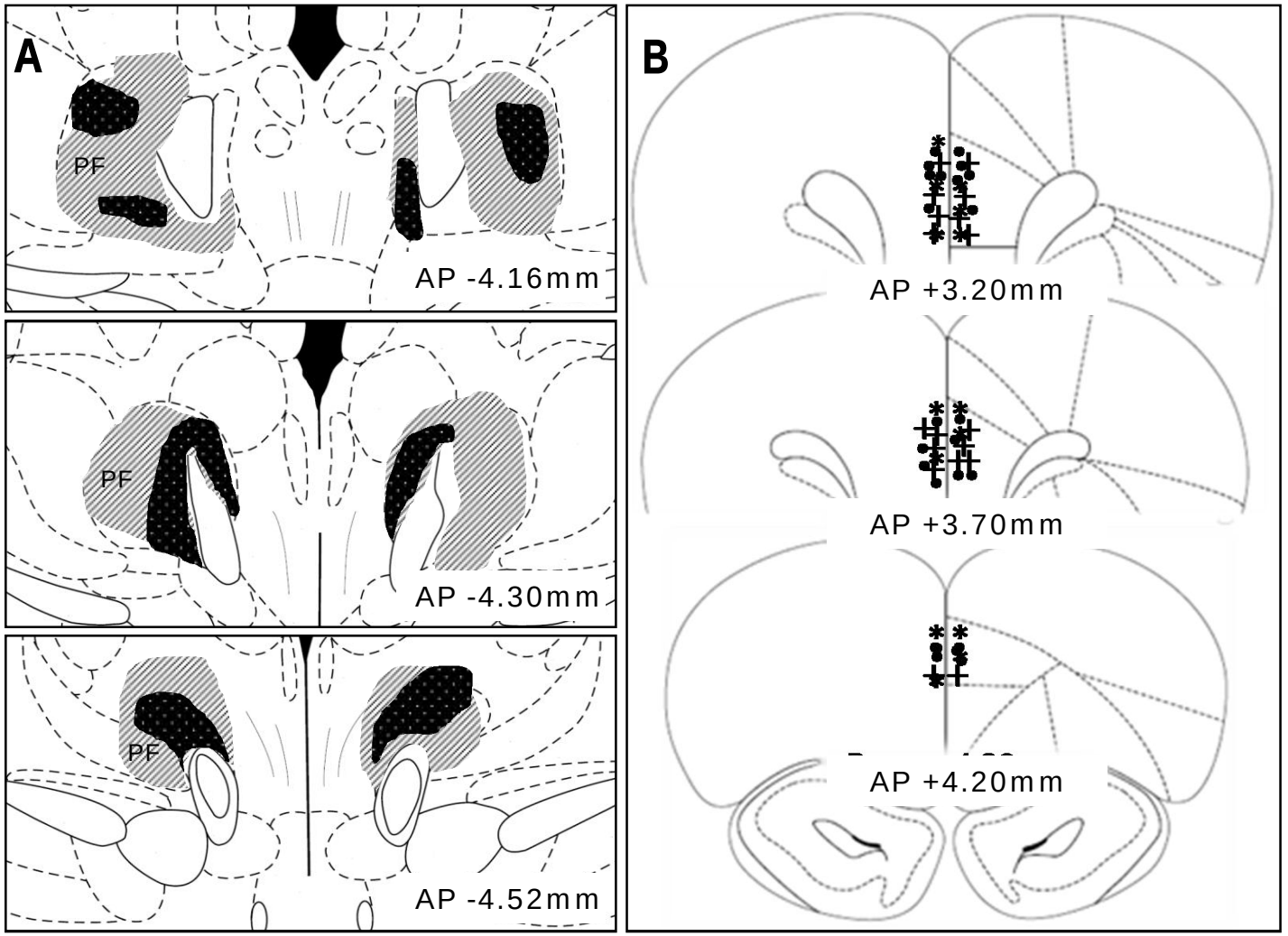
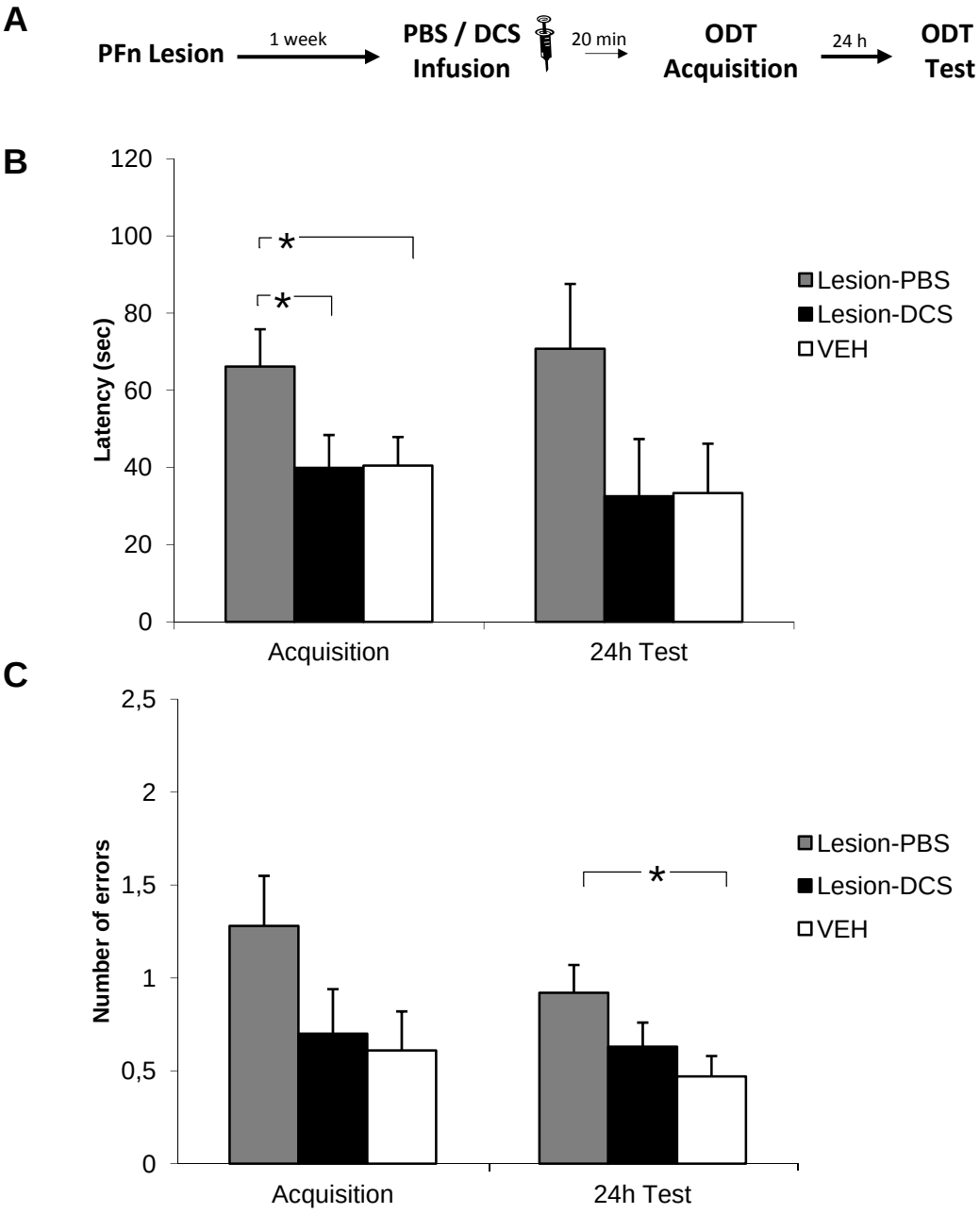

A
PF
AP -4.16mm
PF
AP -4.30mm
PF
AP -4.52mm
B
AP +3.20mm
AP +3.70mm
AP +4.20mm

Figure 2


A
PFn Lesion
1 week
PBS / DCS Infusion
20 min
ODT Acquisition
24 h
ODT Test
B
Latency (sec)
120
100
80
60
40
20
0
*
*
Acquisition
24h Test
Lesion-PBS
Lesion-DCS
VEH
C
Number of errors
2,5
2
1,5
1
0,5
0
*
Acquisition
24h Test
Lesion-PBS
Lesion-DCS
VEH