UNIVERSIDADE FEDERAL DO PARANÁ
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ETIÉLI MARA WENDLER

O PAPEL DO ESTRIADO DORSOLATERAL, ESTRIADO DORSOMEDIAL E NÚCLEO ACCUMBENS CORE NO APRENDIZADO E EXTINÇÃO DOS COMPONENTES PAVLOVIANO E INSTRUMENTAL DE RESPOSTAS CONDICIONADAS DE ESQUIVA

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Orientador: Prof. Dr. Cláudio Da Cunha

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PARECER

A Comissão Examinadora da Dissertação de Mestrado "O PAPEL DO ESTRIADO DORSOLATERAL, ESTRIADO DORSOMEDIAL E NÚCLEO ACCUMBENS CORE NO APRENDIZADO E EXTINÇÃO DOS COMPONENTES PAVLOVIANO E INSTRUMENTAL DAS RESPOSTAS CONDICIONADAS DE ESQUIVA", de autoria da pós-graduanda **ETIÉLI MARA WENDLER,** sob orientação do Prof. Dr. Cláudio da Cunha e composta pelos professores: Prof. Dr. Cláudio da Cunha (Presidente - Farmacologia - UFPR); Prof. Dr. Roberto Andreatini (Farmacologia – UFPR) e Prof.ª Dr.ª Tatiana Lima Ferreira (UFABC), reuniu-se e, de acordo com o Regimento Interno do Programa de Pós-Graduação em Farmacologia, a pós-graduanda foi APROVADA. Para a devida publicação o trabalho deverá sofrer as modificações sugeridas, que serão conferidas pelo seu orientador. Em Curitiba, 13 de agosto de 2012.

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“A mente que se abre a uma nova idéia
jamais voltará ao seu tamanho original”.
- Albert Einsten -

RESUMO

Nesse estudo avaliamos os efeitos de lesões excitotóxicas bilaterais do núcleo accumbens core (NAc-co), estriado dorsomedial (DMS) ou estriado dorsolateral (DLS) de ratos no aprendizado e extinção do condicionamento de medo (tom-choque) e esquiva ativa de duas vias. Nenhum rato apresentou qualquer défict motor ou no tempo de reação ao choque. Lesões do NAc-co, mas não do DMS ou do DLS, diminuíram os escores de *freezing* condicionado. Ratos com lesão no NAc-co ou DLS aprenderam a tarefa de esquiva ativa de duas vias mais lentamente e ratos com lesões no NAc-co, DLS ou DMS apresentaram extinção mais rápida das respostas instrumentais de esquiva. Os escores de medo condicionado e de respostas instrumentais de esquiva apresentaram uma correlação negativa no grupo com lesão no NAc-co. Esses resultados sugerem que o NAc-co é necessário para o condicionamento Pavloviano de medo e para a aprendizagem e/ou desempenho de respostas instrumentais de esquiva. Entretanto, apenas o NAc-co parece mediar o impacto do medo condicionado sobre a aprendizagem e desempenho das respostas instrumentais de esquiva. Esses resultados sugerem ainda que o DLS contribui para uma extinção lenta das respostas de hábito instrumentais de esquiva. Porém, o DLS parece não afetar o aprendizado lento dessas respostas. Por outro lado, nossos resultados não suportam a hipótese de que o DMS é importante para um aprendizado rápido de respostas instrumentais de esquiva direcionadas a um objetivo, mas sugere que o DMS pode ser importante para manutenção dessas respostas durante a fase inicial da extinção.

Palavras-chave: Estriado dolsolateral; estriado dorsomedial; núcleo accumbens; condicionamento clássico; condicionamento instrumental; esquiva ativa de duas vias.

ABSTRACT

This study examined the effects of bilateral excitotoxic lesions of the nucleus accumbens core (NAc-co), dorsomedial striatum (DMS) or dorsolateral striatum (DLS) of rats on learning and extinction of tone-footshock fear conditioning and two-way active avoidance. Such lesions did not cause sensorimotor deficits that could affect locomotion or unconditioned responses to footshocks. Lesions of the NAc-co, but not DMS or DLS, caused decreased conditioned freezing scores. NAc-co and DLS lesioned rats learned the two-way avoidance task more slowly and NAc-co, DLS, and DMS lesioned rats presented faster extinction of the conditioned avoidance responses when the tones and footshocks were presented in a non-contingent manner. The scores of conditioned fear and instrumental avoidance were indirectly correlated in the NAc-co lesioned rats. These results suggest that the NAc-co is needed for Pavlovian fear conditioning and instrumental avoidance learning and/or performance. However, only the NAc-co seems to mediate the impact of the conditioned fear to the tone on learning and/or performance of instrumental avoidance responses. The present findings support a role of the DLS in slow-extinction of the putatively habitual aspect of instrumental avoidance responses, but not support a role of this structure in the slow-learning of habitual avoidance responses. On the other hand, the present findings do not support a role for the DMS in the fast-learning (putatively goal-directed) aspects of instrumental avoidance responses , but suggest that it might play a role in maintenance of instrumental avoidance responses during the early phase of extinction.

Keywords: Dorsolateral striatum; dorsomedial striatum; nucleus accumbens; classical conditioning; instrumental conditioning; two-way active avoindance.

LISTA DE ABREVIATURAS E SIGLAS

A-O – Ação-Consequência (do inglês *action-outcome*)

BG – Núcleos da base (do inglês *basal ganglia*)

CS – Estímulo condicionado (do inglês, *conditioned stimulus*)

DA - Dopamina

DLS – Estriado dorsolateral (do inglês, *dorsolateral striatum*)

DMS – Estriado dorsomedial (do inglês, *dorsomedial striatum*)

DV – Dorso-ventral (do inglês, *dorsoventral*)

GPe – Globo pálido externo

GPi – Globo pálido interno

i.p. – Intraperitoneal

ML – Linha média (do inglês, *midline*)

NAc – Núcleo *accumbens*

NAc-co – Núcleo *accumbens core*

SHAM - falsamente lesado.

SNc – Substância negra parte compacta (do latim, *substantia nigra pars compacta*)

SNr – Substância Negra parte reticulada (do latim, *substantia nigra pars reticulata*)

S-R – Estímulo-resposta (do inglês *stimulus-response*)

US – Estímulo incondicionado (do inglês, *unconditioned stimulus*)

VTA – Área tegmentar ventral (do inglês, *ventral tegmental area*)

SUMÁRIO

APRESENTAÇÃO

Esta dissertação apresenta os resultados desse estudo sob forma de artigo científico (Anexo I), no qual se encontram descritos os itens, Material e Métodos, Resultados e Discussão apresentados na íntegra.

Nos itens 1 e 6 são apresentadas uma introdução e as conclusões gerais da dissertação. As Referências Bibliográficas, item 7, referem-se apenas às citações da introdução geral da dissertação.

1. INTRODUÇÃO

A sobrevivência depende, entre outros fatores, do aprendizado de como obter recompensas e evitar condições aversivas. (CYBULSKA-KLOSOWICZ *et al*., 2009) Nas situações de perigo, como no confronto com um predador, os animais contam com uma série de comportamentos defensivos que vão da fuga à luta e que incluem comportamentos relacionados ao medo (BRANDÃO *et al*., 2003). Além da presença do predador, outros estímulos aversivos que põem em risco a integridade física, tais como os que causam dor (choque elétrico, objetos cortantes, altas temperaturas, etc.), também desencadeiam reações de medo (BRANDÃO & GRAEFF, 2006).

As respostas condicionadas de esquiva constituem um recurso precioso com o qual os animais conseguem prever e evitar situações de risco antecipando respostas de defesa tais como a fuga. Para tanto, o animal precisa aprender a antecipar as situações de perigo através de estímulos ambientais que sinalizam a sua iminência. Esse tipo de aprendizagem é chamado de condicionamento clássico ou condicionamento Pavloviano. Ele ocorre quando um estímulo neutro é repetidamente pareado com um estímulo incondicionado (US, do inglês *unconditioned stimulus*), aquele que causa uma resposta inata. Nesse caso, o estímulo que era neutro passa a sinalizar a iminência do US, permitindo que o animal possa antecipar a mesma resposta. O estímulo que passa a funcionar como um aviso de que o US irá aparecer em breve, é chamado de estímulo condicionado (CS, do inglês *conditioned stimulus*) (SCHULTZ, 2006; PAVLOV, 1927).

Reações defensivas frente a um estímulo que prediz uma consequência aversiva são geradas no condicionamento Pavloviano de medo. Em um experimento típico de condicionamento de medo para roedores, um CS, geralmente um estímulo sonoro, que é pareado com um US aversivo (choque nas patas). Em geral, antes do pareamento, os roedores exibem uma resposta defensiva tênue a um som. Após o pareamento, a simples apresentação do tom faz iniciar uma cascata de respostas incluindo o congelamento (*freezing)* (BLANCHARD & BLANCHARD, 1969; FANSELOW & BOLLES, 1979), reações

autonômicas (Le DOUX *et al*., 1980), respostas neuroendócrinas (KORTE *et al*., 1992a,b) e uma potenciação de reflexos somáticos, tais como o sobressalto (DAVIS, 1986) e o piscar de olhos (WEISZ & McINERNEY, 1990). Um esquema de pareamento de estímulos comumente usado no condicionamento Pavloviano e das respostas condicionadas de medo são ilustradas na Figura 1.

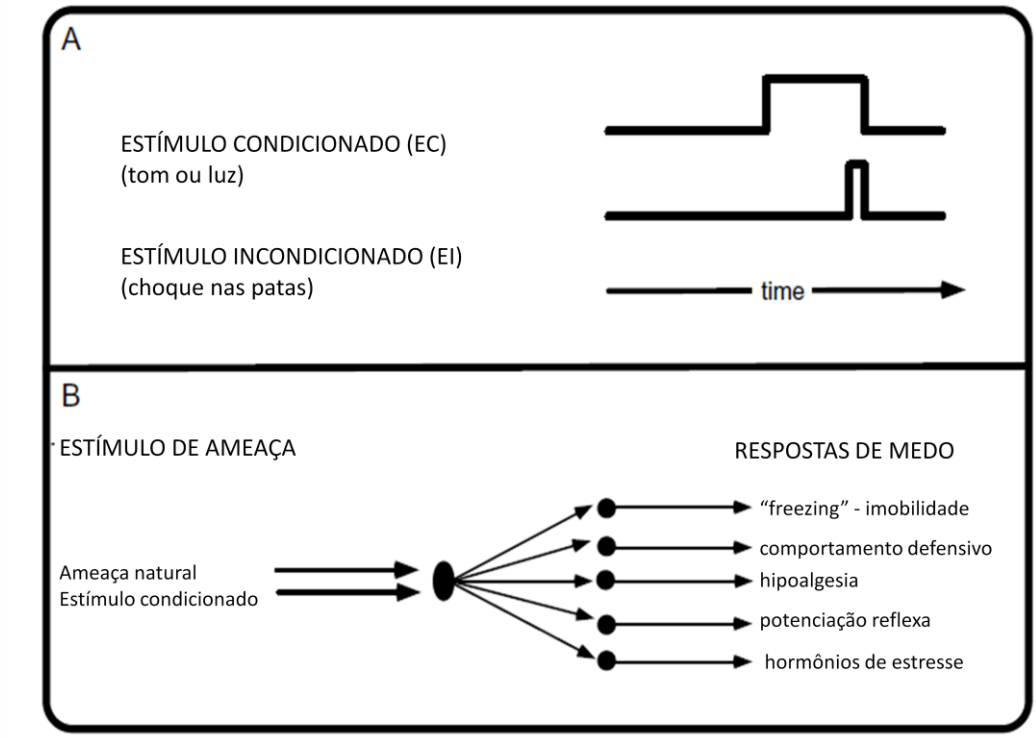

Figura 1. Esquema de condicionamento Pavloviano de retardo (A) e respostas de medo em mamíferos (B) (Le DOUX, 2000).

Na presença do CS, o sujeito antecipa a emoção que é produzida de forma inata pelo US. Entre os USs mais comuns para os animais estão o predador e outros eventos potencialmente perigos tais como o contato com objetos cortantes, altas temperaturas etc. Para seres humanos, os eventos aversivos envolvem, além de situações que colocam em risco a integridade física, situações sociais aversivas, cuja iminência pode ser antecipada por CSs. Tal aprendizado é fundamental para a nossa sobrevivência e convivência social, mas pode também pode contribuir para várias doenças afetivas tais como a ansiedade e a depressão (LOVIBOND *et. al.*, 2011).

Além do condicionamento clássico, outro tipo de aprendizagem, chamada de condicionamento instrumental ou operante, contribui para as respostas condicionadas de esquiva. No condicionamento clássico, o sujeito

aprende apenas a antecipar a iminência do estímulo incondicionado (US), mas não como evitá-lo. No condicionamento instrumental, o sujeito aprende a emitir um comportamento capaz de evitar um evento aversivo (esquiva) ou causar a liberação de um estímulo gratificante (THORNDIKE, 1911; BALLEINE *et al*., 2009). Portanto, o aprendizado de respostas condicionadas de esquiva depende tanto de condicionamento clássico como de condicionamento instrumental.

Existe um número bem maior de estudos de condicionamento instrumental, onde são usados estímulos incondicionados (US) gratificantes do que aqueles onde se empregam USs aversivos. Entre os USs gratificantes mais comumente usados estão pellets de alimentos palatáveis (aqueles que contêm maior conteúdo de açúcares e lipídeos são os com maior poder reforçador), líquidos doces e drogas de abuso. Nos modelos animais de condicionamento aversivo os USs mais comuns são choques nas patas, um ruído alto e sopros de ar (CYBULSKA-KLOSOWICZ *et al.*, 2009).

São apresentados pareamentos de som e choque para ratos, em um experimento típico de condicionamento instrumental de esquiva ativa de duas vias, enquanto eles estão posicionados de um lado de uma caixa de dois compartimentos. O movimento do animal para o lado oposto da caixa faz com que o tom seja cessado e previne a apresentação do choque. Essas respostas de esquiva servem como uma medida dependente do aprendizado de medo e os animais exibem respostas mais frequentemente e com latências menores à medida que o treinamento progride (CAIN & LeDOUX, 2008).

No caso do condicionamento instrumental com motivação apetitiva, estudos comportamentais mostram evidências consistentes de dois processos associativos diferentes. O primeiro, chamado de aprendizado ação-consequência (A-O, do inglês *action-outcome*), permite a formação de associações entre as ações e suas consequências. Ações sob o controle de memórias do tipo A-O são críticas para aquisição e desempenho de ações voluntárias (também chamadas de ação direcionadas a um objetivo) e para o controle executivo das tomadas de decisão. O segundo, chamado de aprendizado de hábitos estímulo-resposta (S-R, do inglês *stimulus-response*), envolve a formação de uma associação entre a resposta (ou ação) e o estímulo a antecedente. Os comportamentos controlados por hábitos S-R estão

relacionados a ações automáticas, impulsivas ou habituais, aquelas onde a tomada de decisão não leva em conta as consequências da ação. (BALLEINE & O'DOHERTHY, 2010).

Há duas características determinantes no aprendizado de hábito que diferem daquelas das ações direcionadas a um objetivo. A primeira delas é a falta de regulação pelas consequências de uma ação, ou seja, as ações de hábito são insensíveis à desvalorização da consequência. A segunda é o pareamento estímulo-resposta, ou seja, a resposta de hábito é deflagrada pelo estímulo que o antecede, independente de uma expectativa de consequência daquela resposta (BALLEINE *et al*., 2009; REDGRAVE *et. al*., 2010).

Outros fatores também determinam se o sujeito (animal ou humano) se comporta visando atingir um objetivo ou por hábito. (REDGRAVE *et. al*., 2010). Um é o tempo de treinamento. A aprendizagem de hábitos S-R depende de um treinamento longo: inicialmente, o controle do comportamento é do tipo A-O, mas após um treinamento longo ocorre uma mudança gradual do controle do comportamento para o modo de hábito S-R. (ADAMS & DICKINSON, 1981, BALLEINE *et al.*, 2009). Outro fator é a previsibilidade das consequências. Por exemplo, dirigir falando ao celular é tranquilo enquanto tudo está conforme o previsto. Nesse momento escolhemos as ações necessárias para dirigir por hábito S-R. No entanto, quando alguma coisa inesperada ocorre, como alguém atravessando a rodovia, imediatamente ocorre uma mudança de hábito para o controle do comportamento por memórias A-O. (HIKOSAKA & ISODA, 2010; SCHWABE & WOLF, 2011). O estresse é outro fator que tem uma influência poderosa sob o modo de controle de comportamento. O S-R assegura que o controle do hábito predomine quando as situações demandam reações rápidas – agimos de forma automática, sem pensar nas consequências da ação. O estresse crônico também favorece o controle do comportamento por hábito S-R. (HEUER *et. al*., 1998; DIAS-FERREIRA, 2009). Exemplo, ratos submetidos a um estresse crônico apresentam respostas comportamentais que são insensíveis a mudanças no valor da consequência e são resistentes a mudanças na contingência de A-O. (DIAS-FERREIRA, 2009).

O aprendizado instrumental, tanto as ações direcionadas a um objetivo (A-O) como dos hábitos estímulo-resposta (S-R), depende dos núcleos da base (BG, do inglês *basal ganglia*). Os BG são uma coleção de núcleos sub-corticais

que estão funcionalmente ligados ao córtex frontal (ASHBY *et al*., 2010). Na visão moderna dos grupos que pesquisam as funções cognitivas dos BG, eles constituem um sistema de seleção do comportamento adequado para as diversas demandas do ambiente, incluindo aquelas que envolvem o medo (REDGRAVE *et al*., 2008; NICOLA *et al*., 2007). Entre as estruturas que compõem os núcleos da base em roedores encontram-se: o caudado-putâmen (também chamado estriado dorsal), o núcleo accumbens e tubérculo olfatório (chamados de estriado ventral), o globo pálido (externo e interno), a substância negra (reticulada e compacta) e o núcleo subtalâmico.

Existem muitas evidências de que a associação entre um estímulo ambiental e a resposta comportamental (ou ação apropriada) se forma no estriado, a principal porta de entrada de informação para os BG (Da CUNHA *et al*. 2009). O estriado recebe projeções de todo o córtex e pode ser subdividido nas regiões límbica, associativa e sensoriomotora, dependendo de que região cerebral ele recebe essas projeções. O estriado límbico: núcleo *accumbens* (NAc) e tubérculo olfatório), recebe projeções do sistema límbico, tais como hipocampo e amígdala. Ele também recebe projeções de neurônios dopaminérgicos da área tegmentar ventral (VTA, do inglês *ventral tegmental area*). O estriado associativo, que inclui todos os núcleos caudados e o putâmen anterior, recebe projeções de regiões associativas do lobo temporal e do córtex pré-frontal. Por outro lado, o estriado sensoriomotor, que inclui a parte posterior do putâmen, recebe projeções dos lobos parietais e do córtex motor e pré-motor. Em roedores, o caudado e o putâmen são indiferenciados e o estriado associativo inclui o estriado dorsomedial (DMS, do inglês *dorsomedial striatum*), enquanto o estriado sensoriomotor inclui o estriado dorsolateral (DLS, do inglês *dorsolateral striatum*) (ASHBY et al., 2010). O estriado dorsal recebe projeções glutamatérgicas topograficamente organizadas do córtex cerebral, sendo que diferentes regiões corticais projetam para diferentes regiões do estriado (Mc GEORGE & FAULL, 1989). Os neurônios estriatais projetam para o globo pálido e substância negra parte reticulada (SNr, do latim *substantia nigra pars reticulata*), e são modulados por neurônios dopaminérgicos provenientes da substância negra pars compacta (SNc, do latim *substantia nigra pars compacta*). Do globo pálido e SNr saem

projeções para o núcleo subtalâmico e tálamo, sendo que este último projeta para o córtex, fechando o circuito córtico-basal (HEIMER *et al*., 1995).

Embora os estudos antigos considerassem o estriado como um tecido homogênio, trabalhos mais recentes mostraram a natureza heterogênea do estriado dorsal, especialmente quando se trata das divisões, medial e lateral. (FEATHESTONE & McDONALD, 2004a). Alguns estudos mostram que essas sub-regiões estriatais desempenham diferentes funções (PISA & SCHRANZ, 1988; PISA & CYR, 1990; DEVAN *et al*., 1999; KANTAK *et al*., 2001), porém ainda não está totalmente elucidado seu papel no aprendizado e memória.

Em estudos mais recentes de condicionamento instrumental, foram mostradas evidências, funcionais e anatômicas de circuitos distintos envolvendo o estriado dorsolateral (DLS) e o estriado dorsomedial (DMS) que contribuem para dois processos de aprendizagem instrumental independentes. Essas evidências sugerem que a formação da associação A-O que medeia a ação direcionada ao objetivo está localizada no DMS (YIN, KNOWLTON & BALLEINE, 2006), enquanto que o DLS, equivalente ao putâmen em primatas, recebe projeções dos córtices sensoriomotor primário e manda projeções que podem influenciar redes cerebrais de controle motor. Uma variedade de estudos usando diferentes metodologias tem mostrado que essa área é um componente importante da circuitaria neural mediando o comportamento de hábito (BROWN *et al*., 1998; DEVAN *et al*., 1999; GRILLNER *et al*, 2005). A inflexibilidade e a compulsão observadas em algumas doenças podem ser atribuídas a uma potenciação de memórias de hábito S-R (CARDINAL *et al*., 2002; EVERITT & WOLF, 2002).

O paradigma Pavloviano pode explicar apenas em parte os déficits de funções executivas observados em doenças neuropsiquiátricas. As funções executivas incluem aspectos de planejamento e ação (LEZAK, 1995), que saem desse domínio Pavloviano. Diversos pesquisadores propuseram que sintomas distintos podem refletir na desconexão de áreas corticais de regiões subcorticais como tálamo mediodorsal (na doença de Alzheimer (CHU *et al.*,1997), áreas do estriado (nas doenças de Parkinson, Huntington e desordens obsessivas compulsivas (BROWN & MARSDEN, 1998; HODGES *et al*., 2006) e amígdala (em vários transtornos de ansiedade , DAMASIO,1996).

As informações processadas pelos núcleos da base (BG) são comunicadas a estruturas efetoras através do tálamo e também através da SNr diretamente para o tronco encefálico, de forma a desinibir as respostas adequadas. Entre os efetores estão o córtex frontal, a substância cinzenta periaquedutal dorsal, o hipotálamo medial e também regiões autonômicas do tronco encefálico (NICOLA *et al*. 2007). Quando os BG estão processando estímulos aversivos, a desinibição destes núcleos determina as características motoras, endócrinas e vegetativas de reações de medo e ansiedade.

O processamento de informações corticais no estriado é modulado por projeções dopaminérgicas da SNc e da VTA (ZHANG et. al., 2010). A integridade da via nigroestriatal é necessária para o aprendizado de hábito (Da CUNHA et al., 2003) - a aprendizagem de associações A-O e S-R não ocorre quando há uma depleção de dopamina no estriado (ASHBY *et al*., 2010). Portanto, o estriado constitui o sistema neural que faz a aquisição e a retenção dos tipos de memórias não-declarativas que envolvem conexões entre estímulos, respostas e consequências durante o aprendizado ou condicionamento instrumental.

O funcionamento dos BG durante o condicionamento instrumental depende de forma crítica da liberação fásica de dopamina (DA). Neurônios dopaminérgicos mesencefálicos da SNc e VTA disparam de forma fásica na presença de estímulos desconhecidos e salientes (BERRIDGE, 2007). Sabe-se que estímulos aversivos também desencadeiam uma resposta fásica da DA. A liberação fásica de DA no estriado, é crítica para os processos de plasticidade sináptica que medeiam o condicionamento clássico, e as associações A-O e S-R (CALABRESI *et al*. 2007).

Os estudos do nosso grupo mostraram que ratos com uma lesão parcial da SNc apresentaram déficits no aprendizado destas associações, tal como na tarefa da esquiva ativa de duas vias (Da CUNHA *et al*. 2001, 2002; GEVAERD *et al*. 2001) e na versão S-R do labirinto aquático de Morris (MIYOSHI *et al*. 2002; Da CUNHA *et al*. 2003, 2006, 2007). Pacientes com doença de Parkinson também apresentam déficits equivalentes na formação destas memórias de procedimento (KNOWLTON *et al*. 1996). Além disso, a formação destas memórias associativas que envolvem reações de medo são um componente importante de doenças afetivas tais como o estresse pós-

traumático (Mc NALLY, 2006) e a síndrome do pânico (JACOBS & NADEL, 1999).

A DA está envolvida em outras situações, como por exemplo, procura pela droga, controle motor, aprendizado de recompensa, motivação e processos de atenção (SCHULTZ, 2002; WISE, 2004). O sistema dopaminérgico também tem sido associado com aprendizado relacionado ao medo (PEZZE & FELDON 2004; FADOK *et al*. 2010, DARVAS, 2012)

Estudos de microdiálise in vivo e de registro de células unitárias em animais acordados divergem sobre o que ocorre durante a aprendizagem de tarefas com motivação aversiva. Os primeiros sugerem que os neurônios dopaminégicos são excitados por estímulos aversivos, enquanto os outros sugerem que eles são inibidos (HORVITZ, 2000; NICOLA, 2007).

Em um trabalho do nosso grupo, com microdiálise in vivo, observou-se que a dopamina é liberada no estriado de ratos durante a aprendizagem de uma tarefa de esquiva ativa de duas vias. Os ratos SHAM (ou sem lesão) aprenderam a tarefa normalmente, já os ratos que sofreram lesão no estriado por 1-metil-4-fenil-1,2,3,6-tetraidropiridina (MPTP), uma neurotoxina que destróis os neurônios dopaminérgicos, não aprenderam a tarefa. O trabalho apresenta evidências de que, pelo menos na tarefa de esquiva ativa de duas vias, a DA liberada no estriado para promover o aprendizado. (DOMBROSWKI *et. al*., 2012)

É de grande importância estudar como a neurotransmissão dopaminérgica afeta a aprendizagem, pois isso pode ajudar a esclarecer os mecanismos sinápticos sobre como as motivações, apetitiva e aversiva, promovem a aprendizagem por condicionamento instrumental que leva à formação de memórias de procedimento. Esses mecanismos parecem estar alterados em várias doenças neurológicas e psiquiátricas, tais como na doença de Parkinson, transtorno de déficit de atenção/hiperatividade, abuso de drogas e esquizofrenia. Além disso, estudar os mecanismos neurais envolvidos no Condicionamento Pavloviano de Medo, pois este é fundamental para a nossa sobrevivência e convivência social e pode também pode contribuir para várias doenças afetivas tais como a ansiedade e a depressão.

2. OBJETIVOS

Estudar o papel do NAc-co, DMS e DLS na aprendizagem, expressão e extinção de respostas condicionadas de medo e respostas instrumentais de esquiva do tipo hábito S-R e A-O.

3. MATERIAIS E MÉTODOS

Vide anexo I página 32.

3. RESULTADOS

Vide anexo I página 37.

4. DISCUSSÃO

Vide anexo I página 44.

5. CONCLUSÕES

Nossos resultados sugerem que:

- O NAc-co é importante para o condicionamento Pavloviano de medo e seu impacto sobre a aquisição e manutenção de respostas instrumentais de esquiva.

- O DLS pode ser importante para a aprendizagem e manutenção de respostas instrumentais de esquiva.

- O papel do DLS e do DMS podem desempenhar um papel na seleção de respostas instrumentais de esquiva do tipo A-O e hábito S-R, mas esse papel pode não ser o mesmo evidenciado para respostas instrumentais com motivação apetitiva.

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ANEXO I

The roles of the nucleus accumbens core, dorsomedial and dorsolateral striatum in learning and extinction of the Pavlovian and instrumental components of conditioned avoidance responses

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Abstract

This study examined the effects of bilateral excitotoxic lesions of the nucleus accumbens core (NAc-co), dorsomedial striatum (DMS) or dorsolateral striatum (DLS) of rats on learning and extinction of tone-footshock fear conditioning and two-way active avoidance. Such lesions did not cause sensorimotor deficits that could affect locomotion or unconditioned responses to footshocks. Lesions of the NAc-co, but not DMS or DLS, decreased conditioned freezing. The NAc-co and DLS lesioned rats learned the 2-way active avoidance task more slowly. The NAc-co and the DLS lesioned rats presented lower avoidance scores in all extinction sessions while the DMS lesioned rats presented lower scores only in the last 3 extinction sessions. The scores of conditioned fear and instrumental avoidance were indirectly correlated in the NAc-co lesioned rats. These results suggest that: (i) The NAc-co mediates the impact of conditioned fear to the tone on learning and/or performance of instrumental avoidance responses. (ii) The DLS plays a role of in the slow-extinction of (putatively habitual) avoidance responses. (ii) The DMS plays a role late (putatively habitual), phase of extinction of avoidance responses. However, the present findings do not support the hypotheses that: (i) the DLS plays a role the slow-learning of (putatively habitual) avoidance responses; (ii) the DMS plays a role in the fast-learning and fast extinction (putatively goal-directed) of avoidance responses.

Keywords: Classic conditioning; Pavlovian fear conditioning; instrumental conditioning; conditioned avoidance responses, nucleus accumbens core, dorsolateral striatum, dorsomedial striatum; neostriatum; caudoputamen.

Introduction

During dangerous situations animals express species-specific fear, typically fleeing or fighting. Freezing and escape attempts are common fear and flee behaviors in rodents (Blanchard and Blanchard, 1989; Martinez et al., 2008). The chance of survival is highly increased if an animal can predict and anticipate the need for such responses based on environmental cues learnt through Pavlovian conditioning (Pavlov, 1927). Rodents also learn instrumental actions for avoid danger (Bolles, 1970). Pavlovian fear conditioning and instrumental avoidance responses are also critical for human beings to deal with situations involving physical risks and aversive social challenges. Deficits in such abilities contribute to a range of affective diseases, such as anxiety disorders and depression (Lovibond et al., 2012).

The 2-way active avoidance task is a rat model of avoidance learning that depends on both Pavlovian fear conditioning and conditioned instrumental avoidance (Maia, 2010; Mowrer, 1956). The task involves presenting a conditioned stimulus (CS, e.g. a tone) that is paired with an aversive unconditioned stimulus (US, e.g. footshock). Rats learn to predict an imminent aversive US and avoid it by crossing to the opposite side of the shuttle box. Pavlovian fear conditioning can be studied separately of 2-way active avoidance by measuring the conditioned response of freezing observed in rats re-exposed to the same tones in the shuttle box (Blanchard and Blanchard, 1969; Fanselow and Bolles, 1979)

Performance of unconditioned, conditioned, and instrumental responses under dangerous situations demands a system to select the response that results in the most beneficial outcome. A growing body of evidence suggests that the basal ganglia are the evolutionary solution for action selection (Da Cunha et al., 2009; Grillner et al., 2005; Nicola, 2007; Redgrave et al., 2010). In studies using appetitive stimuli, selection of unconditioned and conditioned responses seems to be dependent on the shell (NAc-sh) and core (NAc-co) parts of the nucleus accumbens and nucleus, respectively (Da

Cunha et al., 2012; Ikemoto, 2007). The dorsomedial striatum (DMS) and dorsolateral striatum (DLS) are seen to be needed for selection of goal-directed (action-outcome, A-O) and stimulus-response (S-R) habitual actions, respectively (Ikemoto, 2007; Yin et al., 2006). An action is considered to be goal-directed if it is sensitive to outcome devaluation - for example by pre-feeding the animal or by causing malaise with post-feeding injection of lithium chloride) (Dickinson and Balleine, 1994). On the other hand, habits are considered to be insensitive to outcome devaluation, being performed not with an intended goal but as an automatic response to a stimulus that signals the action's outcome (Yin et al., 2008). Habits are proposed to be learned slowly as an association of the predictive stimulus and the automatic response. During instrumental conditioning, early responding seems to be goal-directed and progress slowly to habitual responding. In addition, under extinction (when a response is no longer rewarded), goal-directed actions rapidly fade, while habitual responses persist for a longer time (Balleine and O'Doherty, 2010; Devan and White, 1999; Yin et al., 2006). The inflexible nature of S-R habits is proposed to contribute to compulsive behaviors observed in diseases such as drug abuse (Everitt and Robbins, 2005).

Separation of habitual and goal-directed instrumental avoidance responses is more difficult because there is no available protocol for devaluation of aversive outcomes. It can be assumed that goal-directed and habitual avoidance responses present the corresponding learning and extinction timing to appetitive ones but do they depend on the same brain regions? The present investigation tested whether learning of unconditioned, conditioned, goal-directed and habitual avoidance responses are differently affected by lesions of the rat NAc core, DMS, and DLS in the same way that we know appetitive responses are.

Methods and Materials

Subjects

Adult male Wistar rats from the colony of the Universidade Federal do Paraná, weighing 200-260 g at the beginning of the experiments were used. The rats were maintained in a temperature-controlled room (22±2ºC) on a 12-h light/dark cycle (lights on, 7:00 a.m.) with water and food available ad libitum. These procedures were approved by the Animal Care and Use Committee of the Universidade Federal do Parana (protocol number 545) and are consistent with the Brazilian (11.794/ 8 October 2008) and UK (EC Council Directive, 24 November 1986; 86/609/EEC) laws.

Sixty three rats were randomly assigned to 4 experimental groups given lesions in the: nucleus accumbens core (NAc-co, n = 21), DMS (n = 15), DLS (n = 15), and an additional group that was sham-operated (n=12). From these rats 17 died and 13 were discarded due to inappropriate lesion location. Only the remaining rats had their behavioral data analyzed: 5 NAc-co, 5 DMS, 6 DLS, and 11 sham rats.

Surgery

The rats received atropine sulfate (0.4 mg/kg, i.p.) and penicillin G-procaine (20,000U in 0.1 mL, i.m.) and were anesthetized with 3 mL/kg equithesin (1% sodium thiopental, 4.25% chloral hydrate, 2.13% magnesium sulfate, 42.8% propylene glycol, and 3.7% ethanol in water), placed in the stereotaxic frame with the nose bar adjusted to -3.3 mm. The skull were drilled and the neurotoxin quinolinic acid (20 µg/µL) infused with a Hamilton syringe fitted to a microinfusion pump (Stoelting, QSI-quintessential Stereotaxic Injector, Wood Dale, IL) into the NAc-co, DMS, and DLS according to the coordinates adapted from Castañé et. al. (2010), as shown in Table 1. Sham rats received vehicle (PBS solution composed of phosphate buffer 0.1 M, 0.9% NaCl, pH 7.4) in the NAc-co, DMS or DLS, instead of quinolinic acid. After surgery, rats were allowed to recover from anesthesia in a temperature controlled chamber and then placed back in their home cages.

GROUP	TOTAL VOLUME*	ANGLE	AP	LL	DV
NAc-co	0.22 µl	14°	+ 0.23 cm	± 0.36 cm	- 0.77cm
DLS	0.40 µl	0°	- 0.01 cm	± 0.45 cm	- 0.56; - 0.59 cm
		0°	+ 0.05 cm	± 0.40 cm	- 0.56; - 0.59 cm
DMS	0.40 µl	0°	+ 0.16 cm	± 0.17 cm	- 0.47; - 0.52 cm
		0°	+ 0.19 cm	± 0.14 cm	- 0.47; - 0.52 cm

Table 1. Coordinates adapted from the Paxinos and Watson Atlas (2005) for the neurotoxins microinfusions in the nucleus accumbens shell (NAc-sh), nucleus accumbens core (NAc-co), dorsomedium striatum (DMS), dorsolateral striatum (DLS), anteroposterior (AP), laterolateral (LL), dorsoventral (DV), *total volume per hemisphere.

Behavioural apparatus

Pavlovian fear conditioning and 2-way active avoidance were carried out in an automated shuttle box (Insight Instruments, Ribeirao Preto, Brazil). The box (23 x 50 x 70 cm) has walls made of Plexiglas and floor made of parallel 5 mm caliber stainless-steel bars, 15 mm apart. The floor was divided (unmarked) into 6 12.5 x 10 cm rectangles. The number of tones (CS), footshocks (US), and crossings between the 2 sides of the box were recorded automatically.

The open field apparatus was constructed according to Broadhurst (1960): a wooden round arena (97 cm diameter, 32.5 cm high) painted white with the floor divided into 3 concentric circles that subdivided into 19 approximately equal regions. All experiments were videotaped from above.

Pavlovian fear conditioning

Fifteen days after surgery, rats were submitted to a Pavlovian fear conditioning training session followed by 3 test sessions under extinction in the next 3 days. Rats were placed individually in the automated shuttle box. The training session was carried out immediately after the 10 min the rats habituated within the shuttle box. Ten tones

(1.5 KHz, 60 dB, 10 s) were delivered, each of them paired with 0.4 mA inescapable footshock delivered in the last second of the tone presentation. The interval between each pair of stimuli varied randomly between 30 and 120 s. The rats returned to their home cages just after the delivery of the last pair of stimuli. The test sessions were carried out in the same box; during 10 min and same 10 s tones were presented for 10 times, separated by the same random intervals, without presentation of US. The time the rats remained in freezing (no movements, except for respiratory and vibrices' movements) in the test sessions were recorded by a blind observer. This protocol was adapted from the study of Albrechet-Souza et. al. (2011).

The two-way active avoidance task

Three days after the Pavlovian fear conditioning, the animals were trained in the two-way active avoidance task for 3 days and tested under extinction for 2 additional days (2 training/extinction sessions per day). Trained were carried out according to Da Cunha et al. (2001). The 2-way active avoidance training sessions started just after the rat was placed in the shuttle-box and consisted of 40 pairings of the same tone (maximum duration of 20 s) with a 0.4 mA footshock (maximum of 10 s) that started 10 s after the beginning of the tone. The rat could interrupt the tone and avoid the shock by making the instrumental action of crossing to the opposite side of the shuttle box. In the extinction sessions the same mean number and duration of footshocks and tones were presented, but in an inescapable, unpredictable and unavoidable manner: stimuli of different durations (varying from 1-10 s for the shocks and from 1-20 s for the tones) were presented in a random order and intervals; the tones and shocks were presented in a temporally uncontiguity manner, except for 2 times in order to not allow the animal to learn that the tone was a safe signal. We opted for this protocol (adapted from Dombrowski et al., 2012) because, different of extinction of an appetitive instrumental response, after the rat learn the action instrumental to avoid the US, an US omission contingent to the instrumental response represents a reward, thus reinforcing it. Three

measures of behaviour were taken: (i) avoidance: during presentation of the CS, the rats could turn off the sound and actively avoid the shock by crossing to the opposite side of the box; (ii) response failure: a trial in which the rat did not cross the opposite side during either the CS or US presentation; (iii) inter-trial crossing (ITC): number of crossings between the two sides of the box made during the intervals between the CS-US pairings.

Locomotor activity

Two days after the end of the behavioral experiments, rats were placed individually in the open field apparatus The number of crossings through regions in the periphery (the outsider circle) and in central areas (within the two inner circles) was counted during 5 min. This protocol was modified from Broadhurst (1960).

The locomotor activity during 10 min of habituation to shuttle box (before Pavlovian fear conditioning training session) was also evaluated. The number of transitions among the 6 of 12.5 x 10 cm rectangles in which the floor was imaginarily divided was counted for 10 min.

Histology

At the end of the experimental procedures histological analysis was carried out on all rats. They were killed with an overdose of pentobarbital and brains were fixed *in situ* using transcardial perfusion at room temperature of saline solution (0.9%), followed by 4% paraformaldehyde in phosphate buffer, pH 7.4, and the brains were placed in the same fixative containing 20% sucrose for 72 h at 4^{o}C. Series of 40 µm thick sections were cut in the frontal plane with a vibrating blade microtome (Leica, VT1000 S, Bensheim, Germany). Some sections were immediately mounted on gelatin-coated slides, after 48 h stained with thionin, and examined in a light microscope (DM 2500, Leica, Heerbrugg, Switzerland) to evaluate the lesions of the NAc-co, DMS and DLS groups and non-lesion of sham rats. The next section were processed free floating to

demonstrate neuronal nuclear protein (NeuN) using immunohistochemical techniques. The sections were incubated for 45 min in goat serum based blocking solution (20% serum, 0.1% Triton, in PBS). Primary antibody was mouse anti-NeuN (1:20.000/ overnight) (Chemicon International Inc., Temecula, CA, USA), followed by IgG anti-mouse secondary antibody (1:10.000/ 90 min) and Elite Peroxidase ABC kits (Vector Labs, Peterborough,UK) and Sigma fast DAB substrate (Sigma Chemical Co., St Louis, MO, USA). After the NeuN stained sections were mounted, it was examined at the same light microscope to estimate the damage of NAc-co, DMS, and DLS and the photos of these sections were captured with a scanning microscope. (Zeiss/ Metafer Slide Scanning System – Metasystems, Altlusshein, Germany)

Statistical analysis

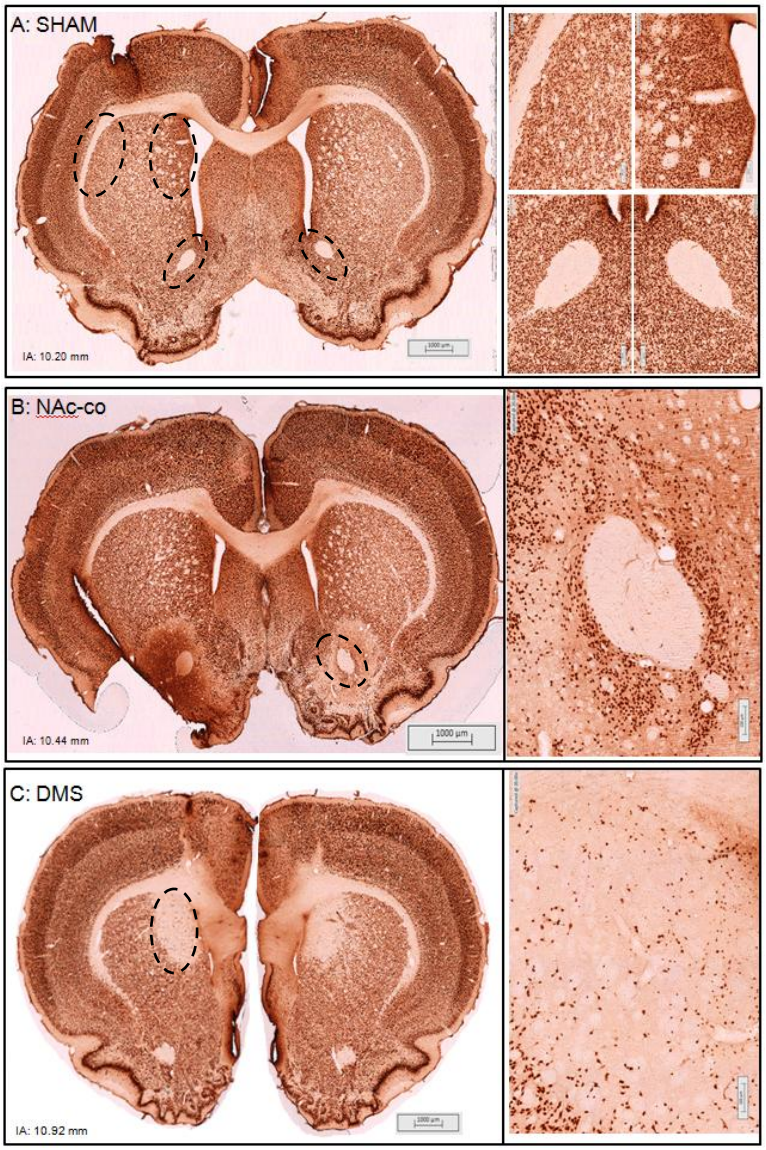
The results were analyzed by one-way ANOVA or two-way ANOVA with repeated measures (lesion as independent factor and session as repeated factor) followed by the post hoc Newman-Keuls test. The Pearson's Correlation Coefficient was used for correlation analysis. Statistical significant were considered when $p < 0.05$.

Results

Histology

Representative pictures of Neu-N immunostained brain slices of sham and NAc-co, DMS, and DLS are shown in Figure 1. Figure 2 displays the maximum and minimum damage resulting from the lesions for the animals included in the behavioral analyses. NAc rats showed substantial neuronal loss in the core region bilaterally and typically extended in the anteroposterior direction from 0.6 to 1.7 mm anterior to bregma. DMS lesions destroyed neurons in the part of the dorsal striatum that is closer

to the lateral ventricle bilaterally and typically extended in the anteroposterior direction from 2.4 mm anterior to bregma to 0.2 mm posterior to bregma. DMS lesions. DLS lesions were also substantial and typically confined to the part of the dorsal striatum that extends laterally from the medial part to the lateral vicinity of the corpus callosum; typically, in the anteroposterior direction it extended from 2.1 mm anterior to bregma to 0.4 mm posterior to bregma. Eight animals with damage outside the target nuclei and 12 rats with unilateral damage were excluded from the behavioral analysis.



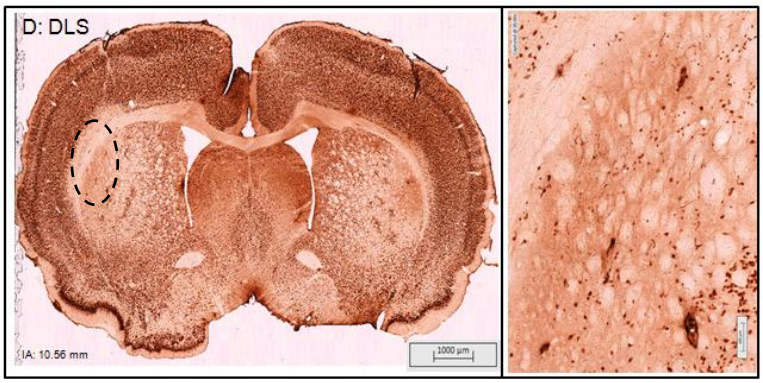


Figure 1. Photos showing NeuN immunohistochemistry coronal sections. Representative SHAM (A); representative core lesion: NAc-co (B); representative dorsomedial lesions: DMS (C); and representative dorsolateral lesions: DLS (D). Dotted areas represent regions showed in right side of the images in a higher magnification.

Post-surgery evaluation of health and motor parameters

No weight loss statistically different from the controls was observed in the in the NAc-co, DMS, and DLS lesioned rats during 21 days after surgery. A two-way ANOVA showed non-significant group effect [$F_{(3,23)} = 0.59$; $p = 0.70$], significant session effect [$F_{(3,69)} = 507.20$; $p < 0.001$], and non-significant group vs session interaction [$F_{(9,69)} = 1.91$, $p = 0.06$] (see supplemental illustrations – S1). As shown in supplemental illustrations S2, NAc-co, but not DMS or DLS lesioned rats, presented higher locomotor activity in the peripheral part of the open field [$F_{(3,23)} = 4.27$, $p < 0.05$, ANOVA; $p < 0.05$, Neuman-Keuls test]. No significant different among groups was observed in the number of crossings in the central part of the open field [$p > 0.05$, pos-hoc Neuman-Keuls test]. No significant difference was found between any of the lesioned groups and the sham group on habituation locomotor activity to the shuttle box [$p > 0.05$, ANOVA, Neuman-Keuls test, see supplemental illustrations - S3A]. In addition, no significant difference was found in the number of inter-trials crossings (ITC) during the two-way active avoidance sessions: a two-way ANOVA showed non-significant group effect [$F_{(3,23)} = 0.47$; $p = 0.70$], session effect [$F_{(5,115)} = 0.76$; $p = 0.58$], and group vs session interaction [$F_{(15,115)} = 0.70$, $p = 0.77$, see supplemental illustrations - S3B].

Therefore, the learning and memory deficits observed in the lesioned rats do not seem to be a result of healthy problems, motor impairment or to anxious-like behavior.

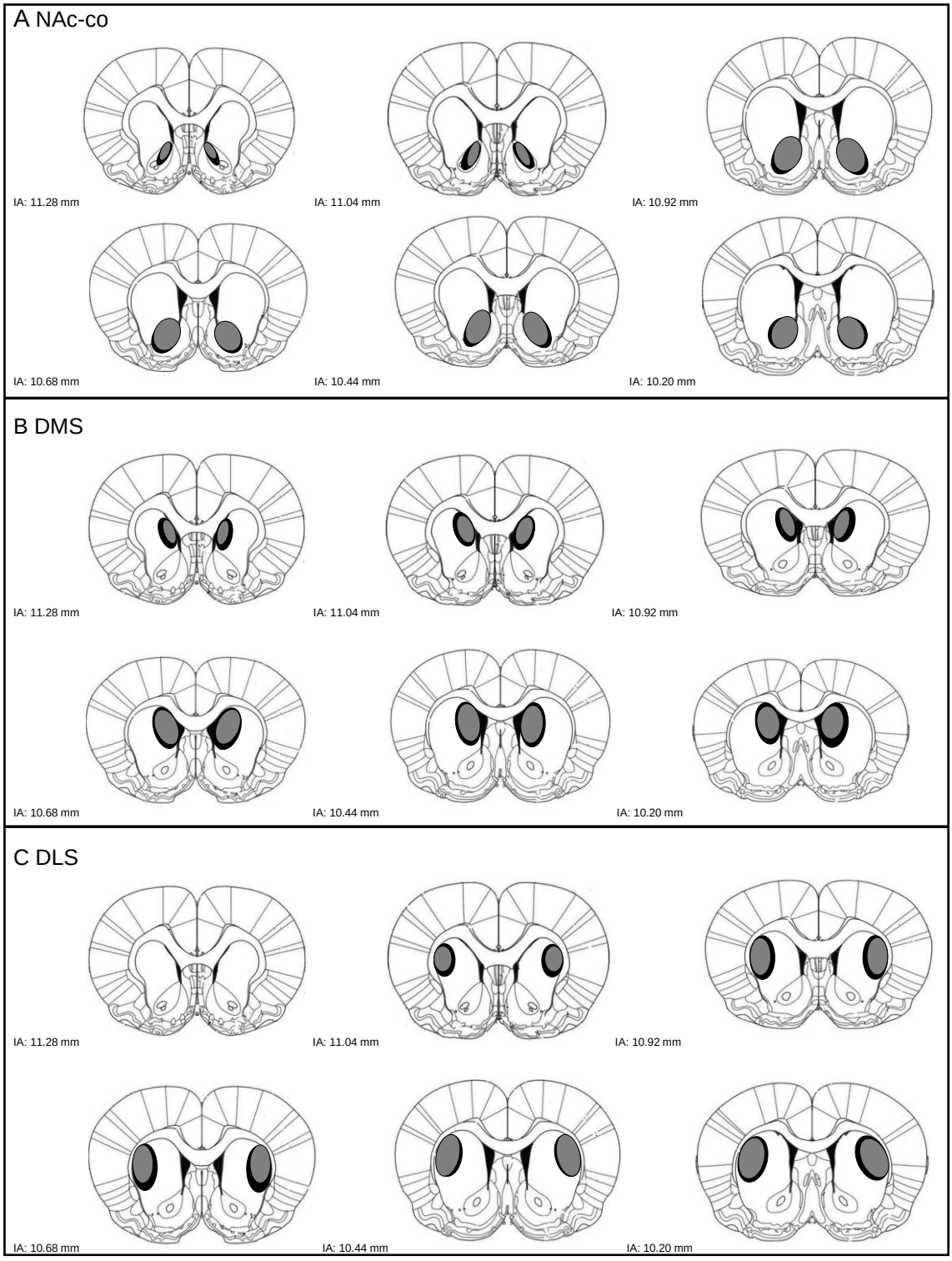


Figure 2. Schematic representation of excitotoxic lesions of the NAc-core (A), DMS (B), and DLS (C). Shaded areas represent the maximum (black) and minimum (gray) extent of the

lesions for the animals included in the behavioral analyses (adapted from Paxinos and Watson,2005).

Unconditioned responses to the footshock

Unconditioned response to footshock was evaluated by the first latency to escape to the opposite (safe) side of the shuttle box and by number of escape failures in the first session of the two-way active avoidance task. A one-way ANOVA showed no significant difference among groups for the escape latency [$F_{(3,23)} = 1.39$, $p = 0.27$] and a two-way ANOVA showed non-significant group effect [$F_{(3,23)} = 0.39$; $p= 0.75$] [session effect [$F_{(5,115)} = 1.34$; $p =0.25$], and group vs session interaction [$F_{(15,115)} = 0.96$, $p = 0.49$] in the number of escape failures during the two-way active avoidance sessions: (Figures 3A and 3B, respectively).

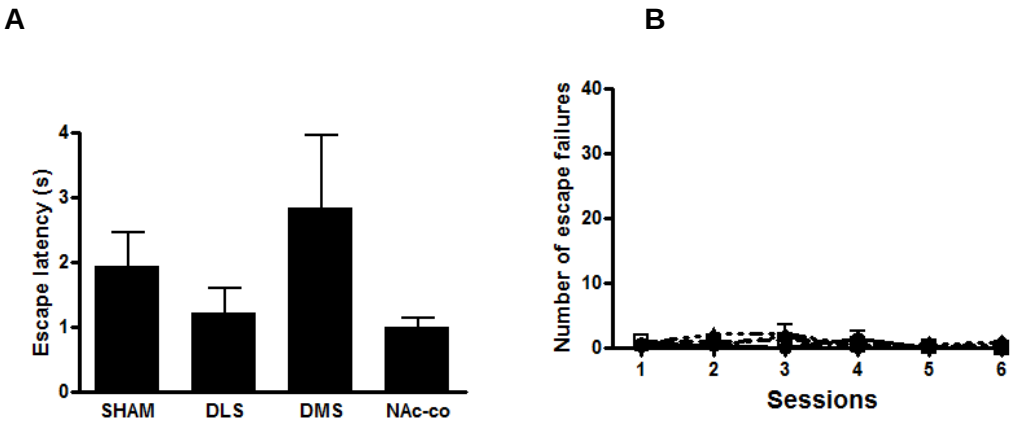


Figure 3. Effect of lesions in the rat nucleus accumbens core (NAc-co), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) for the escape latency [$F_{(3,23)} = 1.39$, $p = 0.27$] (A) and number of escape failures [$F_{(3,23)} = 1.16$; $p= 0.34$] (B).

Conditioned fear response

As shown in Figure 4, in the first test session the rats of NAc-co group, but not of the DMS and DLS groups, showed freezing times significantly lower compared to the sham group [two-way ANOVA, group factor: $F_{(3,23)} = 5.81$; $p < 0.01$, $p < 0.05$ Newman-Keuls test]. However, the lesions in the NAc-co did not completely prevent fear conditioning – rats of these group did not freeze during the 10-min habituation to the

shuttle box but spent between 20 and 225 s in freezing during the 10-min of the first test session. Furthermore, the same lesions did not prevent extinction of the tone-conditioned fear. In the next 2 sessions carried out under extinction, the freezing times decreased significantly in all lesioned groups [two-way ANOVA, session factor: $F_{(2,46)} = 110.40$; $p < 0.001$; $p < 0.05$ pos-hoc Newman-Keuls test], but no significant difference was observed in the freezing time between any of the lesioned groups and the sham group [$p > 0.05$ pos-hoc Newman-Keuls test].

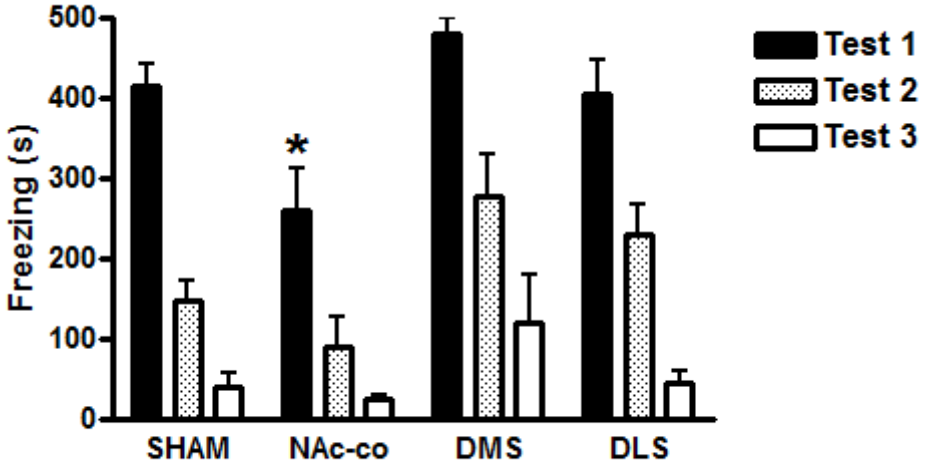


Figure 4. Effect of lesions in the rat nucleus accumbens core (NAc-co), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) on tone fear conditioning. The rats were submitted to 10 tone-footshock pairing and the time of freezing in test sessions carried out 1, 2 or 3 days after trainings were scored. * [$F_{(3,23)} = 5.81$; $p < 0.01$, $p < 0.05$ Newman-Keuls test] compared to scores of the sham group in the first day.

Instrumental avoidance response to the tone

Though lesions in the NAc-co, DMS, or DLS did not prevent learning of the 2-way active avoidance task, learning of the NAc-co and DLS lesioned rats were slower to learn, compared to the sham-lesioned group (Figure 5A). A two-way ANOVA showed the following effects: (i) Number of avoidances: significant group [$F_{(3,23)} = 5.51$; $p < 0.01$] and session [$F_{(5,115)} = 79.08$; $p < 0.001$] effects, and a significant group X

session interaction [$F_{(15,115)} = 3.70$; $p < 0.001$]. The sham and DMS rats seemed to have achieved asymptotic active avoidance performance after 3 training sessions and no significant difference between them was observed in any of the training sessions [$p > 0.20$; Newman-Keuls post-hoc test]. On the other hand, the DLS and NAc-co rats scored significantly lower than the sham rats in the 3 first sessions [$p < 0.05$, Newman-Keuls test].

The NAc-co, DMS, and DLS seem to be important for long-term memory retention and/or expression of instrumental conditioned avoidance responses, because rats with lesions in these structures showed faster extinction of the instrumental avoidance responses in the sessions carried out under extinction. As shown in Figure 5B, the NAc-co, and DLS rats had avoidance scores significantly lower than the sham rats all of these sessions and the DMS lesioned rats scored lower than the sham rats in the last 3 sessions of extinction [$p < 0.05$, post-hoc Newman-Keuls]. A two-way ANOVA showed the following effects: (i) Number of avoidances: significant group effect [$F_{(3,23)} = 3.49$; $p < 0.05$] and session effect [$F_{(3,69)} = 69.06$; $p < 0.001$], and non-significant group X session interaction [$F_{(9,69)} = 1.13$; $p = 0.35$].

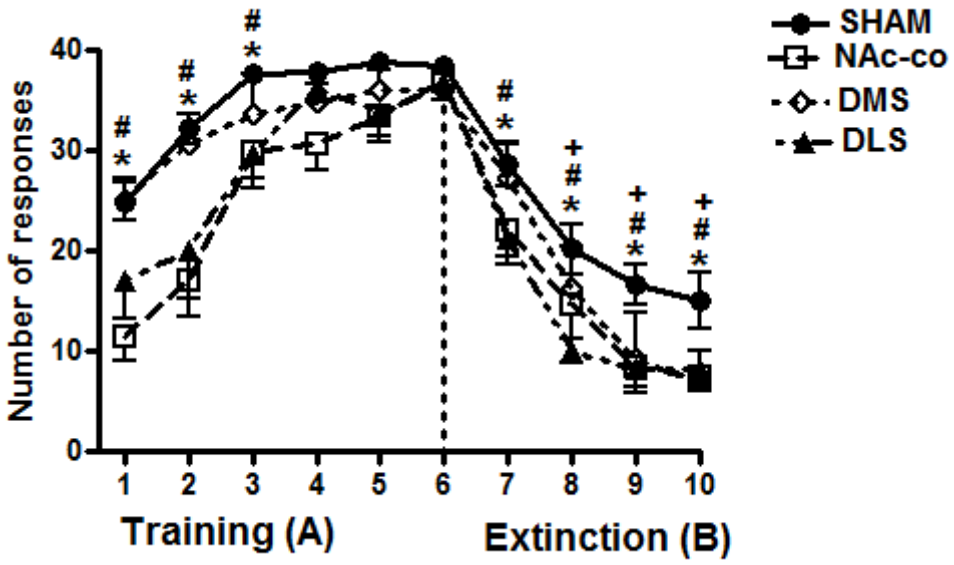


Figure 5. Effect of lesions in the rat nucleus accumbens core (NAc-co), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) on two-way active avoidance learning (A) and extinction (B). For training, the rats were submitted to 6 sessions of 40 tone-footshock pairing and the

avoidance responses were automatically computed. For extinction, the rats were submitted to 4 sessions of 40 non-pairing tone-footshock and the avoidance responses were automatically computed.* NAc-co [$p < 0.05$, ANOVA; $p < 0.05$, Neuman-Keuls test] compared to scores of the sham group in the testing sessions of training and extinction; + DMS [$p < 0.05$, ANOVA; $p < 0.05$, Neuman-Keuls test] compared to scores of the sham group in the extinction's testing sessions; # DLS [$p < 0.05$, ANOVA; $p < 0.05$, Neuman-Keuls test] compared to scores of the sham group testing sessions of training and extinction.

Interactions between locomotor arousal, conditioned fear and instrumental avoidance

As mentioned above, the NAc-co rats showed higher locomotor activity in the periphery of the open field. Had this potentially arousal effect influenced fear conditioning? It is unlikely because a Pearson test detected no significant correlation between these scores was found for the NAc-co [$r = 0.77$, $p= 0.12$] and sham [$r = -0.005$, $p = 0.98$] groups. However, the same test detected a significant positive correlation between these scores in the DLS lesioned group [$r = 0.84$, $p < 0.05$], what might mean that locomotor arousal might contribute to decreased conditioned fear, but in normal rats this effect is buffered by the DLS.

Does conditioned fear impact instrumental avoidance, as proposed by the two-factor learning theory (Mowrer, 1956)? A Pearson test did not found a positive correlation between the scores of conditioned fear (first test day) and conditioned avoidance responses (second training day). However, this test showed a negative correlation for the NAc-co group [$r = -0.92$, $p < 0.05$]. This suggests that NAc-co dependend conditioned fear might impact avoidance learning, but this effect is not evident when this structure is operating normally.

Discussion

The results of the present study shed light into specific contributions of the different regions of the striatum to learning, performance and extinction of conditioned fear and avoidance responses. A summary of the main lesion effects on Pavlovian fear conditioning and two-way active avoidance learning and extinction is shown in Table 2.

Rats submitted to cued, but unavoidable and inescapable footshocks, freeze when re-exposed to the same warning cue (Blanchard and Blanchard, 1969; Bolles, 1970). On the other hand, rats exposed to cued, but escapable and avoidable footshocks, learn to escape and perform conditioned instrumental responses to avoid footshocks. According to the two-factor theory (Mowrer, 1956; Maia, 2010), learning and performance of conditioned avoidance responses depend on both Pavlovian and instrumental conditioning.

	CF (test)	CF (extinction)	2-WAA (training)	2-WAA (extinction)
NAc-co	↓	---	↓	↑
DMS	---	---	---	↑
DLS	---	---	↓	↑

CF, Conditioned fear; 2-WAA two-way active avoidance; NAc-co, nucleus accumbens core; DMS, dorsomedial striatum; DLS , dorsolateral striatum; ↑ increase; ↓ decrease; --- no effect.

Instrumental actions to get positive reinforcers (e.g. appetitive stimuli) are believed to be learned both as goal-directed actions and S-R habits (Dickinson and Balleine, 1994). Recent studies suggest that goal-directed actions and habitual S-R responses learned under appetitive reinforcement are dependent on the dorsomedial and dorsolateral regions of the striatum, respectively (Yin et al., 2006; Ikemoto, 2007). However, it is not clear whether these regions of the striatum are also involved in habitual responses or goal-directed actions instrumental to avoid aversive outcomes. Here this hypothesis is tested in relation to speed of learning and extinction of the two-way active avoidance tasks. Correlations between scores of fear conditioning and two-way active avoidance learning were also used to test whether impairments in conditioned avoidance response (CAR) learning is affected by poor fear conditioning in lesioned rats.

The results of the present study showed that NAc-co, but not DMS and DLS lesioned rats presented reduced conditioned freezing when re-exposed to the tones previously paired with footshocks in the shuttle box. The reduced conditioned freezing of NAc-co lesioned rats may be attributed to their higher locomotor activity in periphery of the open field since we found a non-significant correlation between these parameters. Coherent with our findings, further evidence that the NAc-co plays a role in fear conditioning also includes decreased acquisition of conditioned freezing in rats after intra-NAc infusion of lidocaine (Haralambous and Westbrook, 1999), increased BOLD signal (Klucken et al., 2012), and increased dopamine release (Young, 2004; Martinez et al., 2008) in the NAc during the expression of conditioned fear.

Lesions of the NAc-co, DMS or DLS did not affect extinction of the conditioned freezing response in the present study. However, other studies showed both decreased and increased extinction of fear conditioning in rats submitted to deep brain stimulation of the portions of the NAc respectively dorsomedial and dorsolateral to the anterior commissure (Rodriguez-Romaguera et al., 2012), and decreased extinction of conditioned fear in rats that received intra-NAc infusions of a D2 receptor antagonist (Holtzman-Assif et al., 2010). It might have happened due the permanent inactivation (neuronal death) of the brain areas by lesions with quinolinic acid in the present work.

We also observed impaired learning of the two-way active avoidance task by the NAc-co lesioned rats, a finding in agreement with previous studies showing dopamine release during the early training sessions of this task (Dombrowski et al., 2012) and that infusion of D1 (Wietzikoski et al., 2012) or D2 (Boschen et al., 2011) dopamine receptor antagonists into the rat NAc impaired learning of this task. The present study suggests a role for the NAc-co in the mediation of Pavlovian influence of fear conditioned on instrumental avoidance response.

In the present study lesions in the NAc-co or DLS, but not in the DMS, delayed learning of instrumental avoidance responses in the two-way active avoidance task. This finding is in agreement with a recent study showing that deficits to learn the two-

way active avoidance task observed in dopamine-deficient mice can be reversed by restoration of DA signaling to whole striatum together with the amygdala, but not by restoration of DA signaling restricted to the ventral striatum and amygdala (Darvas et al., 2011).

Evidence from studies in which instrumental responding was reinforced by appetitive stimuli supports the view that learning and performance of instrumental goal-directed actions and S-R habits in rodents depends respectively on the dorsomedial and dorsolateral parts of the striatum (Yin et al., 2004, 2006; Redgrave et al., 2010; Dezfouli and Balleine, 2012). These studies showed that the behavior of bar-pressing for appetitive rewards is sensitive to outcome devaluation in rats after lesion or inactivation of the dorsolateral striatum (Yin et al., 2004, 2006), but not in rats with a lesion in the dorsomedial striatum or in controls trained under a schedule that favors S-R habit learning (Yin et al., 2004). However, the present findings do not support a role for the DMS in instrumental avoidance learning. If the DMS was important for goal-directed avoidance actions, it would be expected that the DMS lesioned rats had learned the two-way active avoidance more slowed and stop responding more slowly under non-contingent presentation of the tones and footshock. However, the DMS lesioned rats learned the two-way active avoidance as well as the controls and stop responding more quickly than the controls under this condition. This suggests that though the DMS may contribute to maintain this instrumental avoidance behavior under extinction, it is not needed for the learning of such behavior.

The present results are not consistent with the hypothesis that the DLS plays a role in the habitual avoidance responding. Because habits S-R are learned more slowly and are more resistant to extinction, DLS lesioned rats are expected to learn the two-way active avoidance more quickly and stop responding more quickly under extinction. The DLS lesioned rats of our study learned more slowly the two-way active avoidance and stop responding more quickly than the controls under this condition. Our data are

in agreement with the hypothesis that DLS plays a role only in extinction, but not in acquisition of habits S-R.

However, a role for the DLS in slowly learned habitual avoidance responding is supported by other two recent studies showing that the infusion of D1 (Wietzikoski et al., 2012) or D2 (Boschen et al., 2011) antagonists into the rat DLS did not affect avoidance responding during a learning session of two-way active avoidance, but decreased avoidance responses in a test session carried out 24 h later. On the other hand, administration of the same drugs in the rat NAc caused decreased avoidance scores in the early training and test session, what is coherent with the view that the NAc is needed for a fast learning (goal-directed?) or to the invigoration of avoidance responding, as suggested by other studies (Wadenberg et al., 1990).

Finally, it is important to stress that the present deficits of the lesioned rats in the fear conditioning and two-way active avoidance cannot be attributed to motor deficits. The NAc-co, DMS and DLS did not reduce locomotor activity in an open field, or in the shuttle box – either before habituation to the apparatus and in the inter-trial intervals of the two-way active avoidance sessions. Rats of all lesioned groups also presented the same locomotor activity scores observed in the control rats to explore the central part of the open field arena – what suggest that they did not present anxiety-related behaviors. On the contrary, the NAc-co lesioned rats presented increased locomotor activity (in the periphery) in the open field, a finding also observed in a previous study (Maldonado-Irizarry and Kelley, 1995).

In conclusion, the present study supports the view that learning and expression of conditioned avoidance responses depends on the NAc-co and DLS. It also supports that the NAc-co plays a critical role in learning and/or expression of conditioned avoidance responses, but suggests that this role is not directly related to conditioned fear. It is possible that it is related to learning of the predictive value of the conditioned stimulus (e.g. an auditory cue) or to another aspect of motivational maintenance of the instrumental avoidance response (e.g. action-arousal). However, a direct role of the

NAc-co in learning and expression of instrumental avoidance responses cannot be discarded. The present findings support only a role of the DLS in slow-extinction (habitual aspect) of instrumental avoidance responses, but not in slow-learning. On the other hand, the present data did not support a role for the DMS in the fast-learning (putatively goal-directed) aspects of instrumental avoidance responding, but suggest that it might play a role in the impact of Pavlovian fear conditioning in instrumental performance and might be important for maintenance of instrumental avoidance responding during an early phase of extinction.

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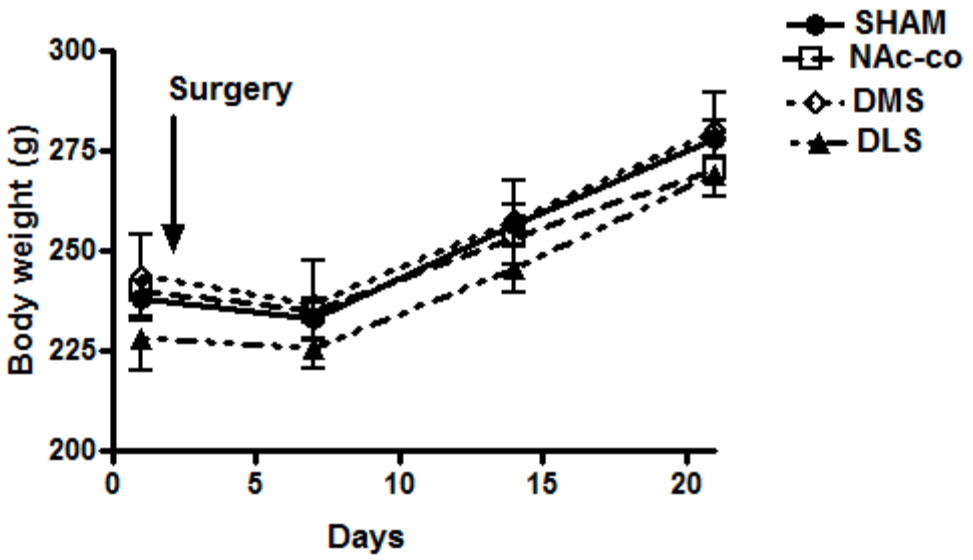
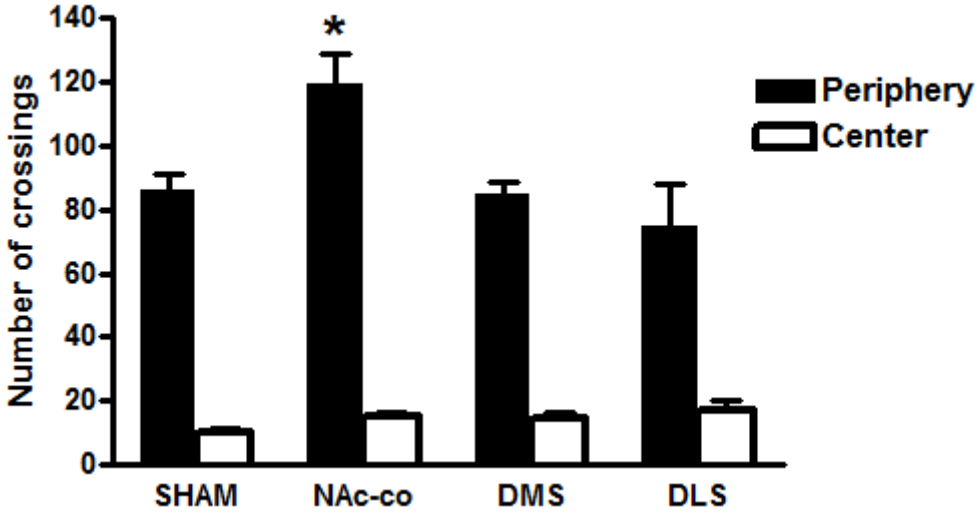
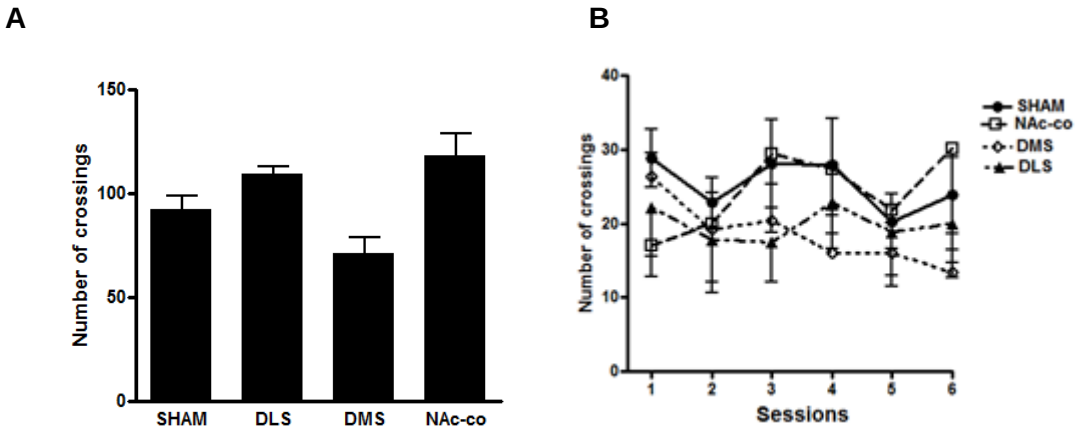
Supplemental illustrations



S1. Effect of lesions in the rat nucleus accumbens core (NAc-co), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) on weight loss during 21 days after surgery. A two-way ANOVA showed non-significant group effect [$F_{(3,23)} = 0.59$; $p = 0.70$], significant session effect [$F_{(3,69)} = 507.20$; $p < 0.001$], and non-significant group vs session interaction [$F_{(9,69)} = 1.91$, $p = 0.06$]



S2. Effect of lesions in the rat nucleus accumbens core (NAc-co), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) on periphery * [$F_{(3,23)} = 4.27$, $p < 0.05$, ANOVA; $p < 0.05$, Neuman-Keuls test] and central part of the open field compared to scores of the sham group.



S3. Effect of lesions in the rat nucleus accumbens core (NAc-co), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) in the locomotor activity during the habituation to the shuttle box in the 10 min before the conditioned fear training [$p > 0.05$, ANOVA, Neuman-Keuls test] (A) and in the number of inter-trials crossings (ITC) during the 2-way active avoidance sessions [$p > 0.05$, ANOVA, Neuman-Keuls test] (B)