UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO - UENF

JOAQUIM BARBOSA LEITE JUNIOR

EFEITO DA ADMINISTRAÇÃO DE MORFINA E MK-801 NA EXPRESSÃO DE UMA RESPOSTA LOCOMOTORA CONDICIONADA E SENSIBILIZADA E NA ATIVAÇÃO DA PROTEÍNA ERK 1/2 EM ESTRUTURAS ENCEFÁLICAS RELACIONADAS À DEPENDÊNCIA QUÍMICA

CAMPOS DOS GOYTACAZES - RJ

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Tese apresentada ao Centro de Ciências e Tecnologias Agropecuárias da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências do Doutorado no programa de Pós-Graduação em Ciência Animal.

Orientadora: Profª. Drª. Marinete Pinheiro Carrera

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Tese apresentada ao Curso de Doutorado do Programa de Pós-graduação em Ciência Animal, Centro de Ciências e Tecnologias Agropecuárias da Universidade Estadual do Norte Fluminense Darcy Ribeiro, Área de Concentração da Sanidade Animal e Psicofarmacologia, como requisito para obtenção do grau de Doutor em Ciência Animal.

Aprovada em 29 de março de 2023

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RESUMO

Joaquim Barbosa Leite Junior, Médico Veterinário, filho de Joaquim Barbosa Leite e Carla Aparecida Torres Leite e irmão de Jhean Torres Leite, nasceu na cidade de Bom Jesus do Itabapoana – RJ, em 15 de junho de 1990. Morou em Carabuçu (4º distrito de Bom Jesus do Itabapoana) até 2009. Ingressou na Universidade Estadual do Norte Fluminense - RJ em 2010. Desde 2010 mora em Campos dos Goytacazes.

RESUMO

A dependência química é considerada uma doença grave, que evolui de forma complexa, crônica e progressiva. Sendo um problema de saúde pública, que se caracteriza pela tendência constante à recaída, mesmo após um longo período de abstinência. A morfina é um analgésico opioide para dores intensas e possui expressiva taxa de abuso nos últimos anos. Em altas doses a morfina provoca ativação das vias dopaminérgica e glutamatérgica e atua causando hiperlocomoção. O maleato de dizocilpina (MK-801), é um antagonista glutamatérgico dos receptores do tipo NMDA (N-metil-D-aspartato), em baixas, doses produz diminuição da locomoção e, em altas doses, causa hiperlocomoção. A dopamina é o neurotransmissor associado como o prazer e a recompensa, enquanto o glutamato se relaciona à recaída, abstinência e à memória de longo prazo. A sensibilização e o condicionamento são processos importantes para manter da dependência. A sensibilização é o processo de aumento progressivo da resposta, com a mesma dose do fármaco. O condicionamento é o processo onde um estímulo inicialmente neutro se torna um estímulo incondicionado, após constantes pareamentos. A sensibilização é mediada pela ERK (proteína quinase ativada por mitógenos), na via mesocorticolímbica (área tegmental ventral, córtex pré-frontal, Amigdala, Hipotálamo e núcleo accumbens), e está relacionada à memória de longo prazo na dependência química. O objetivo do presente trabalho foi verificar o efeito das manipulações dopaminérgicas, por meio de menor atividade dopaminérgica com apomorfina (0,05 mg/kg) ou maior ativação dopaminérgica com o uso de morfina (10 mg/kg) e MK-801 (0,025, 0,1 e 1,0 mg/kg) em um protocolo de condicionamento pavloviano de atraso, avaliando a resposta locomotora condicionada e sensibilizada e ativação da ERK. Para tanto, foram desenvolvidos 2 conjuntos experimentais. O primeiro conjunto experimental testou o efeito de doses baixas de apomorfina (0,05 mg/kg) na atenuação/bloqueio da aquisição de sensibilização locomotora e condicionamento por morfina (10 mg/kg). O segundo conjunto experimental examinou os efeitos de diferentes doses de MK-801 (0,025, 0,1 e 1,0 mg/kg) na aquisição de sensibilização locomotora e condicionamento. Os resultados mostram aumento na locomoção após tratamentos repetidos, porém sem alteração locomotora nos tratamentos agudos tanto nos experimentos com MK-801, quanto Morfina. Os grupos tratados com morfina, apresentaram hiper locomoção, sinalizando sensibilização comportamental. No primeiro conjunto experimental, os resultados mostraram maior ativação de ERK, nas regiões da área tegmental ventral e núcleo accumbens, nos grupos tratados com Morfina. Entretanto os grupos Morfina tratados previamente com Apomorfina não tiveram maior ativação de ERK, se igualando aos grupos veículo. Apenas uma dose de apomorfina é capaz de interferir na aquisição de sensibilização locomotora, mostrada por meio de diminuição da locomoção e menor ativação de ERK. No segundo conjunto experimental, os resultados dos tratamentos com dose alta de MK-801 (1.0 mg/kg) mostraram sensibilização locomotora. Por outro lado, nos animais do grupo MK-801 (0,1 mg/kg), só foi possível observar sensibilização locomotora a partir do quinto dia de experimentação.

Nos tratamentos com MK-801 (0,025 mg/kg), não houve diferença na locomoção se comparados ao grupo veículo. Portanto os resultados obtidos no presente trabalho indicam que manipulações dopaminérgicas, com morfina ou MK-801, assim como a avaliação da ERK, se traduzem em um modelo mais completo para examinar o processo de condicionamento e sensibilização induzido por drogas, fornecendo informações importantes sobre os processos neurobiológicos para a compreensão das complexidades da dependência de opioides.

Palavras-chave: Morfina; MK-801; Dopamina; Glutamato; ERK.

ABSTRACT

Addiction is considered a serious disease, which evolves in a complex, chronic and progressive way. Being a public health problem, which is characterized by the constant tendency to relapse, even after a long period of abstinence. Morphine is an opioid analgesic for severe pain and has had a significant rate of abuse in recent years. In high doses, morphine activates the dopaminergic and glutamatergic pathways and acts by causing hyper locomotion. Dizocilpine maleate (MK-801) is a glutamatergic antagonist of NMDA (N-methyl-D-aspartate) receptors. In low doses, it reduces locomotion and in high doses it causes hyperlocomotion. Dopamine is the neurotransmitter associated with pleasure and reward, while glutamate is related to relapse, withdrawal and long-term memory. Sensitization and conditioning are important processes to maintain addiction. Sensitization is the process of progressively increasing response with the same drug dose. Conditioning is the process where an initially neutral stimulus becomes an unconditioned stimulus after constant pairing. Sensitization is mediated by ERK (mitogen-activated protein kinase), in the mesocorticolimbic pathway (ventral tegmental area, prefrontal cortex, amygdala, hypothalamus and nucleus accumbens), and is related to long-term memory in addiction. The objective of the present study was to verify the effect of dopaminergic manipulations, through lower dopaminergic activity with apomorphine (0.05 mg/kg) or greater dopaminergic activation with the use of morphine (10 mg/kg) and MK-801 (0.025 , 0.1 and 1.0 mg/kg) in a delayed Pavlovian conditioning protocol, evaluating the conditioned and sensitized locomotor response and ERK activation. For this purpose, 2 experimental sets were developed. The first experimental set tested the effect of low doses of apomorphine (0.05 mg/kg) on attenuating/blocking the acquisition of locomotor sensitization and conditioning by morphine (10 mg/kg). The second experimental set examined the effects of different doses of MK-801 (0.025, 0.1 and 1.0 mg/kg) on the acquisition of locomotor sensitization and conditioning. The results show an increase in locomotion after repeated treatments, but without locomotor alteration in the acute treatments both in the experiments with MK-801 and Morphine. The groups treated with morphine showed hyper locomotion, signaling behavioral sensitization. In the first experimental set, the results showed greater activation of ERK, in the regions of the ventral tegmental area and nucleus accumbens, in the groups treated with Morphine. However, the morphine groups previously treated with apomorphine did not have greater ERK activation, matching the vehicle groups. Only one dose of apomorphine is capable of interfering with the acquisition of locomotor sensitization, shown by decreased locomotion and lower ERK activation. In the second experimental set, the results of treatments with a high dose of MK-801 (1.0 mg/kg) showed locomotor sensitization. On the other hand, in the animals of the MK-801 group (0.1 mg/kg), it was

only possible to observe locomotor sensitization from the fifth day of experimentation. In treatments with MK-801 (0.025 mg/kg) there was no difference in locomotion compared to the vehicle group. Therefore, the results obtained in the present work indicate that dopaminergic manipulations, with morphine or MK-801, as well as the ERK evaluation, translate into a more complete model to examine the drug-induced conditioning and sensitization process, providing important information about the neurobiological processes for understanding the complexities of opioid Addiction.

Keywords: Morphine; MK-801; Dopamine; Glutamate; ERK.

1 **SUMÁRIO**

1 - INTRODUÇÃO

A dependência química ou vício, como é mais comumente determinada, é uma doença grave na qual se desenvolvem processos como a sensibilização, o condicionamento, a abstinência, a recaída e a tolerância. Trata-se de uma doença incurável induzida pelo uso de substâncias psicoativas, ademais se manifesta de forma crônica e progressiva (CUNHA e NOVAIS, 2004). Essa doença se caracteriza pela tendência constante à recaída, mesmo após um longo período de abstinência o que envolve alterações biológicas e comportamentais. A dependência e o alto risco à recaída acompanham o indivíduo por toda sua vida (WISE, 1996; O'BRIEN *et al*., 1998; WISE, 2000). O dependente tem prejuízos cognitivos na percepção, atenção, associação, memória, raciocínio, juízo, imaginação, pensamento e linguagem que podem variar em intensidade de indivíduo para indivíduo (MANN *et al*., 1999).

A ONU qualifica a dependência química como uma epidemia mortal, sendo o uso de opioides uma das maiores crises de saúde atuais, em todo o mundo cerca de 1068 pessoas morrem diariamente vítimas de overdose por opioides (KOLODNY *et al*., 2015; CHANG *et al*., 2018; UNODC, 2019). No cenário atual, os opioides já contabilizam 76% das mortes envolvendo distúrbios relacionados ao uso de drogas no mundo (UNODC, 2022). Só em 2021, ocorreram mais de 107 mil mortes por overdose de drogas nos EUA, 17% maior quando comparado ao ano de 2020 (UNODC, 2022). Nos últimos 5 anos, cerca de 80% dos usuários de heroína dos Estados Unidos buscaram a substância ilícita após fazerem uso de opioides comerciais (HUECKER E LEAMING, 2020; KIM *et al*., 2019). Segundo dados do UNODC 2022, a produção mundial de ópio aumentou 7% entre 2020 – 2021.

Dados do Relatório Mundial sobre Drogas em relação ao uso de opioides, tanto para uso médico quanto para uso recreativo como droga de abuso, indica o Brasil como o maior mercado consumidor da América do Sul, com cerca de 600 mil usuários (UNODC, 2016). No Brasil, houve 1,6 milhões de prescrições de opioides no ano de 2009, já em 2015 foram 9 milhões de prescrições, um aumento de 465% em um período de 6 anos (KRAWCZYK *et al*., 2018). Cabe ressaltar que os fármacos com maiores incrementos foram a codeína (alcaloide natural do ópio) e a oxicodona (alcaloide semi-sintética), ambas substâncias tem ação semelhante à da morfina (KRAWCZYK *et al*., 2018; ALEXANDER *et al*., 2020).

Nesse contexto, o desenvolvimento da dependência química ocorre devido a uma complexa interação entre fatores sociais, biológicos e genéticos (ROBBINS e EVERITT, 1999). O dependente, na ausência da droga, quando interrompe o uso, sente forte motivação para recobrar o consumo, mesmo depois de decorridos meses ou até anos do último uso. A recaída é uma situação em que o dependente volta ao consumo depois de algum tempo de abstinência (ZALESKI *et al*., 2017).

Atualmente se sabe que a recaída é mantida não apenas pela sensação de prazer ou ainda pela tentativa de evitar os possíveis efeitos da abstinência da droga, mas sim pelos mecanismos que envolvem aprendizagem de longa duração, ou seja, a memória associada às drogas (NESTLER, 2002). Para a dependência química, o desejo resultante da associação entre os estímulos ambientais e os efeitos subjetivos da droga (particular a cada indivíduo) estão amplamente ligados com os fatores de risco de longa duração para a recaída (HYMAN e MALENKA (2001).

As alterações comportamentais geradas pelo uso de drogas de forma crônica produzem adaptações neurobiológicas e moleculares permanentes, instituindo um importante modelo de neuroplasticidade (ROBINSON e KOLB, 1999; EISCH *et al*., 2000). Dentre os processos relacionados com a gênese e manutenção da dependência, dois processos são fundamentais: o primeiro consiste em uma forma de aprendizagem associativa estabelecida entre os efeitos da droga (estímulo farmacológico) e a interação com o ambiente (objetos, lugares e som), conhecido como processo de condicionamento (SHALEV *et al*., 2002); o segundo é uma forma de aprendizagem não associativa, denominada sensibilização comportamental, e se caracteriza pelo aumento progressivo de uma determinada resposta comportamental, quando se administra a mesma dose da droga repetidas vezes (ROBINSON e BERRIDGE, 1993). Dessa forma, o estudo do condicionamento e sensibilização comportamental é de grande importância para o entendimento dessa doença. Os dois processos explicam os comportamentos compulsivos de desejo, ingestão e recaída presentes na dependência química (WISE e ROMPRE, 1989; PIERCE e BARI, 2001; SEE, 2002).

Já se encontra bem aceito na literatura científica que a característica comum a todas as substâncias de abuso é a ativação do sistema dopaminérgico e glutamatérgico (SCHULTZ *et al*., 1993; TRUDEAU *et al*., 2014; SAKAE *et al*., 2015). Entretanto o fator de maior importância compartilhado por essas substâncias é a transformação dos estímulos contextuais contíguos (próximos) em estímulos condicionados e estímulos de incentivo, os

quais podem motivar e manter os comportamentos relacionados à dependência (ROBBINS e EVERITT, 2002).

As catecolaminas, como a dopamina, atuam no circuito de recompensa no sistema nervoso central (SNC), e são importantes para a aquisição de memórias de longo prazo, pois variações nos seus níveis circulantes estão envolvidas na modificação do aprendizado, tornando o aprendizado prazeroso ou ainda angustiante (ORDOÑEZ, 2012). Níveis altos de dopamina e de glutamato facilitam o aprendizado, contribuindo para a consolidação de memórias mal adaptadas como é o caso da dependência química. Por outro lado, níveis baixos de dopamina podem dificultar a consolidação de memórias e assim serem usados como estratégia para o tratamento do dependente químico (NODA *et al*., 1998; NODA e NABESHIMA, 2004).

Os sistemas, dopaminérgico bem como o glutamatérgico estão implicados na dependência por opioides e outras drogas psicoativas como o álcool e a cocaína. Nesse contexto, os estudos terapêuticos atuais se voltam para drogas, que se sejam capazes de equilibrar a transmissão do glutamato e da dopamina no contexto de dependência química (COLLINS *et al*., 1998; OLIVE *et al*., 2012; D'SOUZA, 2015).

Trabalhos de DOBI e colaboradores (2010) e WYLLIE e colaboradores (2013) mostraram diminuição da locomoção quando antagonistas glutamatérgicos NMDA como por exemplo o MK-801 (maleato de dizocilpina) foram administrados em baixas doses (0,01-0,025 mg/kg), os receptores do subtipo GLUN-2A são os mais impactados e deixam de inibir a glicina (FRANTZ e HARTESVELDT, 1999; TANG *et al*., 2006). Essa diminuição da locomoção aponta para a importância do glutamato no processo da dependência química (TANG *et al*., 2006).

A morfina é uma droga psicoativa capaz de gerar dependência química, pois é uma substância que apresenta uma característica comum com as drogas de abuso, a capacidade de aumentar os níveis circulantes de glutamato pós-sináptico (NARITA *et al*., 2008) e também de dopamina (KREEK, 2007). De acordo com os trabalhos do nosso grupo, que mostraram através de experimentos com administração de apomorfina como pré-tratamento antes da administração de morfina, houve uma menor ativação de ERK 1/2 em regiões encefálicas como a área tegmental ventral (VTA) e o NAc (DE MELLO BASTOS *et al*., 2019; LEITE JUNIOR *et al*., 2019; DIAS *et al*., 2021; CRESPO *et al*., 2022), essas estruturas que são responsáveis por reforçar o aprendizado e aumentar a probabilidade de

se usar a droga novamente, como o NAc e VTA, são capazes de gerar e manter a dependência química (SHOBLOCK *et al*., 2005).

A hipótese do presente é que o aumento da atividade da dopamina por meio dos tratamentos com MK-801 em alta dose (1.0 mg/kg) e com morfina (10 mg/kg), administrados imediatamente antes do teste experimental, provocam aumento da locomoção, como também um aumento de ERK, sugerindo aumento das respostas condicionada e sensibilizada. Bem como a diminuição da atividade dopaminérgica pelo uso de apomorfina (0.05 mg/kg), dificulta a consolidação de memórias associadas às drogas psicoativas, resultando em menor atividade locomotora e menor ativação de ERK.

Assim sendo, o objetivo do presente trabalho foi verificar o efeito das manipulações dopaminérgicas, por meio de menor atividade dopaminérgica com apomorfina (0,05 mg/kg) ou maior ativação dopaminérgica com o uso de morfina (10 mg/kg) e MK-801 (0,025, 0,1 e 1,0 mg/kg) em um protocolo de condicionamento pavloviano de atraso, avaliando a resposta locomotora condicionada e sensibilizada e a ativação da proteína ERK. Para tanto, foram desenvolvidos 2 conjuntos experimentais. O primeiro conjunto experimental testou o efeito de doses baixas de apomorfina (0,05 mg/kg) na atenuação/bloqueio da aquisição de sensibilização locomotora e condicionamento por morfina (10 mg/kg). O segundo conjunto experimental examinou os efeitos de diferentes doses de MK-801 (0,025, 0,1 e 1,0 mg/kg) na aquisição de sensibilização locomotora e condicionamento. O presente trabalho busca uma maior compreensão da interação do glutamato e da dopamina na dependência química, principalmente de drogas como as opioides.

1.1 HIPÓTESE

Trabalhos de colaboradores (1992) e Carey (1995), utilizando MK-801 nas doses (0,1 – 0,3 mg/kg), em ratos, descreveram alguns efeitos excitatórios como a hiperatividade locomotora. Nos trabalhos do nosso grupo CRESPO e colaboradores (2022), utilizando morfina na dose (10 mg/kg), observou-se aumento da atividade locomotora e maior ativação de ERK em regiões como o VTA, Nac. Outros resultados foram encontrados por De Mello e colaboradores (2020), com o uso de injeções prévias de apomorfina (0,05 mg/kg) antes de injeções de morfina (10 mg/kg), em que houve bloqueio da aquisição de sensibilização e condicionamento.

Assim sendo, a hipótese do presente estudo é que manipulações dopaminérgicas por meio de uma menor atividade dopaminérgica com o uso de apomorfina (0,05 mg/kg) dificultam ou bloqueiam as respostas locomotoras que indicam condicionamento e sensibilização, como a hiperlocomoção. Em outro ponto, uma manipulação onde ocorra maior ativação dopaminérgica com o uso de morfina (10 mg/kg) e MK-801 (1,0 mg/kg) em um protocolo de condicionamento pavloviano de atraso, provocam aumento na resposta locomotora condicionada e sensibilizada e também na ativação da ERK.

II - OBJETIVOS

Objetivo Geral:

O objetivo do presente trabalho foi verificar o efeito das manipulações dopaminérgicas, por meio de menor atividade dopaminérgica com apomorfina (0,05 mg/kg) ou maior ativação dopaminérgica com o uso de morfina (10 mg/kg) e MK-801 (0,025, 0,1 e 1,0 mg/kg) em um protocolo de condicionamento pavloviano de atraso, avaliando a resposta locomotora condicionada e sensibilizada e a ativação da proteína ERK

Objetivos Específicos:

a) Avaliar a administração de MK-801 1.0 mg/kg no desenvolvimento de uma resposta condicionada e sensibilizada, empregando-se um protocolo de condicionamento pavloviano de atraso;

b) Avaliar a administração de morfina 10 mg/kg no desenvolvimento de uma resposta condicionada e sensibilizada, juntamente com a ativação da ERK 1/2, empregando-se um protocolo de condicionamento pavloviano de atraso;

c) Avaliar a administração de Apomorfina na dose de 0,05 mg/kg, pré-arena experimental, na atenuação e/ou bloqueio de uma resposta locomotora previamente condicionada e sensibilizada por morfina 10 mg/kg;

d) Avaliar a administração de Apomorfina na dose de 0,05 mg/kg antes de uma administração de morfina 10 mg/kg pré-arena experimental na atenuação e/ou bloqueio da ativação da proteína ERK nas regiões do VTA, Nac, Hipo, AM e CPF.

III - REVISÃO DE LITERATURA

3.1 Dependência Química

A dependência química é uma doença crônica que tem como característica a tendência recorrente à recaída e pode ser entendida dessa forma como um problema complexo e contínuo, sendo classificada entre os transtornos psiquiátricos (CID10, F19).

De acordo com as últimas estimativas do Relatório Mundial sobre Drogas de 2022, cerca de 284 milhões de pessoas (3,55% da população mundial) entre 15 e 64 anos usaram drogas no ano de 2020, enquanto cerca de 40 milhões de pessoas (13% da população de usuários) sofrem de transtornos associados ao uso de drogas. As projeções estatísticas sugerem um aumento de 11% no número de usuários globalmente até 2030 e um aumento acentuado de 40% na África devido ao rápido crescimento da população jovem, juntamente com um acréscimo do número de usuárias mulheres (UNODC, 2022).

A dependência é uma forma de aprendizado, que ocorre devido ao consumo de substâncias psicoestimulantes que provocam alterações do SNC como o aumento de níveis circulantes de dopamina, o que ocasiona uma ação estimulatória na vigília e na atenção. Dessa forma, a dependência pode ser separada em dependência física, na qual o organismo do dependente apresenta distúrbios físicos quando se faz a interrupção do uso da droga (DIAZ, 1996). A dependência ainda pode ser entendida por dependência psicológica, em que o uso tem por objetivo o bem-estar e os efeitos iniciais da droga (RIBEIRO e MINAYO, 2015).

A dependência pode ser tratada e controlada objetivando a diminuição dos sintomas, sendo, por vezes, de difícil controle (PÉREZ-CAJARAVILLE *et al*., 2005). Os indivíduos toxicodependentes tendem a ter uma rejeição por atividades que não estejam ligadas ao consumo da droga, pois ocorre diminuição da sensação de prazer (NERY FILHO *et al*. 2009).

Por muito tempo, a dependência foi entendida como sendo desvio de caráter, falta de personalidade ou diminuição da força de vontade, pela qual o dependente era tratado por nomes pejorativos, o que levava a uma interpretação equivocada sobre o uso de drogas (DE MORAIS *et al*., 2012). Somente em 1964 foi que a Organização Mundial da Saúde

(OMS) introduziu o termo “dependência” em modificação ao termo “vício” e “habituação” que eram usados até então, assim o problema passou a ser entendido como uma doença (DUPONT, 2005).

O tratamento da dependência química é um desafio árduo, pois suas causas são diversas, ou seja, sendo determinada por vários fatores. Para o seu entendimento se faz necessária uma abordagem cuidadosa, feita por equipes multidisciplinares, pois se refere a um problema em que se relacionam fatores sociais, familiares, emocionais e da psiquê (FONTES *et al*., 2006). Na maior parte dos pacientes, cerca de 90%, ocorre recaída em até 1 ano depois de iniciado o tratamento, tornando o processo bastante penoso (MILTON e EVERITT, 2012).

O consumo de substâncias psicoestimulantes é bem antigo e de certa maneira contemporâneo ao próprio nascimento das civilizações (NUNES e JÓLLUSKIN, 2007). No princípio os homens consumiam drogas em cerimônias religiosas como parte da celebração, depois o consumo de drogas mudou de contexto e passou a ser feito com a finalidade de diminuir o sofrimento, diminuir a dor, o cansaço e também para se alcançar sensações prazerosas (MARTINS e CORRÊA, 2004).

Dois fatos históricos ocorridos no século XIX contribuíram bastante para que a dependência química se tornasse pela primeira vez um grave problema social, o primeiro foi a guerra travada entre China e Inglaterra pelo abastecimento mundial de ópio e o segundo foi durante a Guerra Civil Americana nos EUA (ARAÚJO e MOREIRA, 2006). Nesses dois conflitos os soldados feridos em combate fizeram o uso de morfina nas formas oral e subcutânea, e isso provocou um enorme número de casos de dependência química entre os sobreviventes que retornaram à Europa e aos EUA (BARAKA, 2000; DUARTE, 2005).

O abuso ou uso nocivo seria um momento intermediário entre o uso recreativo (de baixo risco, não sendo caracterizado como um problema médico) e a dependência. Já há prejuízo decorrente do consumo da substância, mas ainda há algum controle do indivíduo quanto à quantidade consumida e à duração dos efeitos (TAMELINI e MARTINS, 2007).

Alguns pontos são importantes para entender os limites entre o uso recreativo, o abuso e a dependência de substâncias psicoativas. Postula-se que sejam fenômenos que ocorrem em conjunto, com parâmetros orientando a transição de um estágio para o outro, como: o impacto funcional no trabalho e na família, restrições judiciais e morais, as

consequências decorrentes do consumo da substância, assim como o desenvolvimento de mecanismos fisiológicos de adaptação à presença da substância, como tolerância e abstinência. Tolerância é a diminuição dos efeitos esperados de uma droga por exposição excessiva do dependente ao seu princípio ativo, enquanto a abstinência é a privação do uso da droga e de suas sensações prazerosas, podendo causar perturbações fisiológicas no organismo como: irritabilidade, depressão, ansiedade, suores, enjoos, dores de cabeça (RAITH e HOCHHAUS, 2004).

As drogas ativam o circuito da recompensa na via mesocorticolímbica (MORGANE *et al*., 2005) liberando neurotransmissores responsáveis por uma sensação de bem-estar e prazer como a dopamina e o glutamato, como consequência, o desejo por repetir a experiência se mantém. Na dependência química, o comportamento de busca ativa da droga é no sentido de reequilibrar o sistema de recompensa (FERREIRA *et al*. 2001).

3.2 Morfina

A morfina é um opióide analgésico. A droga foi isolada pelo alemão Friedrich Serturner em 1806, mas teve sua difusão somente em 1853 após a invenção da agulha hipodérmica (PATHAN e WILLIAMS, 2012). Sendo o primeiro fármaco narcótico derivado do ópio (*Papaver somniferum*) e usado frequentemente na intervenção cirúrgica e clínica de doenças com presença de dor crônica. A morfina pode ser administrada por via oral, subcutânea, intramuscular, intravenosa, epidural e ainda pelas vias transdérmica e intranasal (NESTLER, 2004). Quando usada pelas vias intramuscular e subcutânea, o pico de ação se dá aos 15-20 minutos, por via intravenosa, o pico do efeito analgésico é obtido aos 20 minutos. Caso seja administrada em bolus epidural ou intratecal, o pico ocorre de 5 a 10 minutos após a administração e tem a duração da ação analgésica de 4 a 5 horas. Quando administrada por via oral, a morfina tem seu pico de ação em 30-90 minutos, com 6 horas de duração da ação analgésica (GLARE e WALSH, 1991; LUGO e KERN, 2002; STEIN, 2015). A droga consegue ultrapassar a placenta e a barreira hematoencefálica e sua excreção ocorre na maior parte pela via renal, apenas uma pequena parte é excretada por via biliar. A figura 1 mostra a fórmula estrutural da morfina (SJOGREN *et al*., 1994).

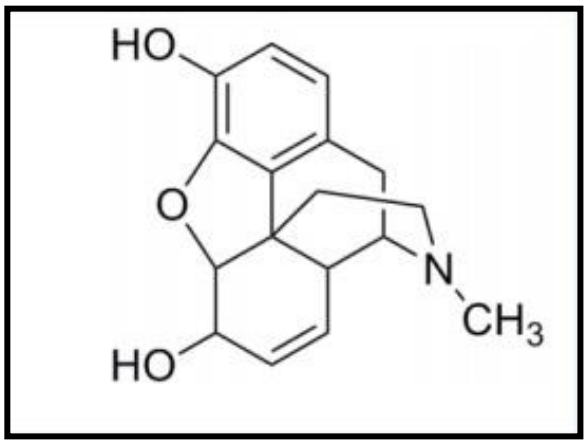

Figura 1: Fórmula estrutural da morfina (7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol). Adaptado de Trescot e colaboradores (2008).

Os receptores dos opioides são classificados em 3 tipos principais: μ (Mi), κ (Kappa), δ (Delta) e ainda mais 2 tipos que se chamam Nor (nociceptina), e, mais recentemente, o ζ (Zeta). Esses receptores fazem parte da família de receptores ligados à proteína G no mecanismo de analgesia. Os receptores do tipo μ (OP3) regulam funções como a nocicepção, o controle do ciclo respiratório, miose, bradicardia, indiferença aos estímulos do meio circulante e o trânsito intestinal. São receptores onde a maior parte dos fármacos opioides atua, eles estão localizados nas lâminas III e V do córtex cerebral, no tálamo, área tegmental ventral (VTA), substância cinzenta periaquedutal, substância gelatinosa e trato gastrintestinal (PERT e SNYDER, 1973). Já os receptores do tipo κ (OP2) tem relação com as funções de nocicepção, termorregulação, controle de diurese e secreção neuroendócrina, eles estão localizados no hipotálamo, substância cinzenta periaquedutal, substância gelatinosa na medula espinhal, além de neurônios sensitivos periféricos (DHAWAN *et al*., 1996). Quanto aos receptores do tipo δ (OP1), eles são mais fortemente expressos nos gânglios da base e nas regiões do neocortex. Esse tipo de receptor tem relação com o controle do ciclo respiratório, nocicepção e modulação de funções cognitivas (BROWNSTEIN, 1993). O quarto tipo de receptor o Nor (OP4) é encontrado no córtex, amigdala, hipocampo e hipotálamo, sendo relacionado com regulação do humor e do apetite (STEIN *et al*., 2003). Por fim, há o receptor tipo ζ (ZOR) que tem importância para o crescimento do tecido celular e embrionário, e, ainda na regulação da proliferação de células cancerosas, esse tipo de receptor se encontra em regiões como o coração, os rins, os músculos esqueléticos, o cérebro e o pâncreas (VARGA *et al*., 2004; MARTINS *et al*., 2012).

Os receptores opioides estão envolvidos em uma série de sinais intracelulares, incluindo a inibição da adenilato ciclase, a diminuição da abertura dos canais de cálcio, o aumento das correntes de potássio e a ativação da proteína quinase C (PKC). O principal efeito dos opioides é a redução da excitabilidade celular e da neurotransmissão. Em nível celular, a morfina age como agonista total nos receptores μ acoplados a proteína G do tipo inibitória (Gi), impedindo a ação da enzima adenilato ciclase, essa enzima é responsável pela transformação de ATP (adenosina trifosfato) em AMPc (Monofosfato cíclico de adenosina), dessa forma, não serão feitas novas sínteses de AMPc (importante mensageiro intracelular); como resultado, há a diminuição das respostas celulares (PETROFF, 2002).

Outro ponto de destaque é o aumento do efluxo de potássio, ou seja, ocorre a passagem dos íons potássio para o exterior da célula, devido à abertura dos canais de potássio e fechamento dos canais que levam sódio para a porção interior da célula (COHEN, 1979). A diminuição da concentração de potássio no meio intracelular faz com que a membrana da célula fique hiperpolarizada, dessa forma não é gerado o potencial de ação ocorrendo o impedimento do impulso nervoso (SCHROLL e HAMKER, 2013).

Em nível de SNC, a morfina tem sua ação nos receptores do tipo μ (Mi) dos neurônios gabaérgicos, onde ocorre uma diminuição da síntese do ácido gama-aminobutírico (GABA) e posterior aumento da dopamina e glutamato circulantes devido à excitação dos neurônios dopaminérgicos e desinibição dos neurônios glutamatérgicos, posteriormente ocorre a ativação do sistema de recompensa das vias mesolímbica e mesocortical, duas vias simpáticas e também ativação das vias glutamatérgicas descendentes (via córtico-troncar, córtico-estriatal, córtico-accumbens, córtico-talâmica). Essa excitação do sistema nervoso central pelo aumento de dopamina e glutamato é o mecanismo responsável por causar a dependência química (YAMAKAGE e NAMIKI, 2002).

Com as diminuições da fração de mensageiros celulares e da liberação de neurotransmissores inibitórios pelas células que compõem o SNC respectivamente, somado a um aumento da liberação de dois importantes neurotransmissores excitatórios glutamato e dopamina, ocorre uma excitação neuronal com ativação do sistema de recompensa cerebral (GOLDBERG *et al*., 2013). Com o aumento de glutamato e dopamina há uma redução da síntese de importantes neurotransmissores inibitórios, responsáveis pela regulação do humor, como a glicina e o GABA (WATANABE *et al*., 2002).

Os receptores opioides estão localizados em neurônios de diversas áreas do encéfalo (córtex pré-frontal, tálamo, hipocampo, bulbo olfatório, núcleos pontinhos, substância gelatinosa na medula espinhal e na substância cinzenta periaquedutal no mesencéfalo). Além disso, podem ser encontrados no sistema nervoso periférico. A ativação desses receptores é feita pelos opióides endógenos, como as endorfinas e as encefalinas que são neurotransmissores semelhantes à morfina (HEMMINGS e JEVTOVIC-TODOROVIC, 2013).

A morfina atuando nos receptores μ (Mi) tem as seguintes ações: analgesia, miose, bradicardia, depressão respiratória e indiferença aos estímulos do meio circulante. Quando sua atuação é nos receptores δ (Delta), ocorre primariamente a analgesia, mas também a modulação de funções cognitivas e de dependência física, enquanto que sua ação nos receptores κ (Kappa), vai alterar a nocicepção, a termorregulação, o controle de diurese, a secreção neuroendócrina e a reduzir a atividade motora e reflexos (MARTINS *et al*., 2012). Os receptores do tipo OP1 ou σ (sigma) estimulam as funções simpáticas provocando taquicardia, hipertensão, midríase, náuseas e vômitos, também podem causar alucinações. Os receptores σ (sigma) são parcialmente opioides, depois de reanalisados por alguns pesquisadores, foi visto que eles interagem com uma variedade de drogas psicoativas e seu ligante endógeno não é conhecido (embora eles possam reagir com certos esteroides endógenos), chegou-se à conclusão de que seriam, na verdade, decorrentes do bloqueio de receptores glutamatérgicos do tipo NMDA (PERT e SNYDER, 1973; BROWNSTEIN, 1993; MARTINS *et al*., 2012).

A morfina é metabolizada no fígado, essencialmente pela UDP-glucoronosiltansferase 2B7 (UGT2B7). A partir daí, são obtidos dois metabólitos principais: a morfina-6-glucuronideo (M6G) que é um metabólito ativo e responsável por efeitos clínicos da morfina como a analgesia (LÖTSCH e GEISSLINGER, 2001) e a morfina-3-glucoronideo (M3G), que em excesso pode levar a hiperalgesia (sensibilidade aumentada a dor), através da reação de fase II (glucoronidação) do grupo alcoólico 6-OH e do grupo fenólico 3-OH (figura 2); (DAHAN *et al*., 2008; SMITH, 2000).

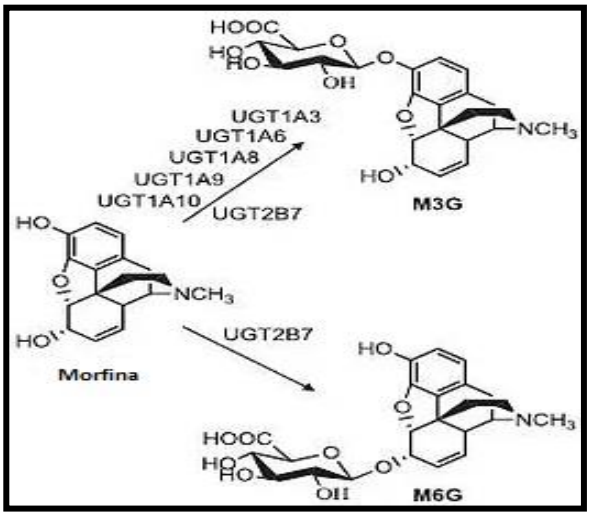

Figura 2: Metabolismo da morfina e os seus metabólitos M6G e M3G. Adaptado de Wahlström *et al*. (1988).

3.3 Condicionamento Pavloviano e Morfina

Rehman e Rehman (2018) descrevem que o condicionamento clássico é um método de aprendizado inconsciente, uma forma direta através da qual os animais podem aprender. O condicionamento clássico é o processo em que uma resposta automática e condicionada é emparelhada com estímulos específicos.

O condicionamento pode ser segmentado em dois tipos, o primeiro tipo é o condicionamento operante ou instrumental, em que se manifesta um aumento na probabilidade de ocorrência de um determinado comportamento, por exemplo, se a consequência for reforçadora aumenta a probabilidade dela se repetir, mas se a consequência for punitiva, pode diminuir ou extinguir a probabilidade de sua ocorrência futura (STADDON e CERUTTI, 2003). O segundo tipo é o condicionamento pavloviano clássico, que é o processo pelo qual um estímulo ambiental neutro adquire funções similares às de um estímulo incondicionado através de emparelhamentos, com drogas de abuso ou outro estímulo, de forma prévia e repetida (SCHULTZ e SCHULTZ, 1992; BARDO e BEVINS, 2000).

O condicionamento clássico foi postulado por Ivan Pavlov, através de trabalhos com cães. Em seu experimento mais conhecido, ele tocava um sino, Estímulo Neutro (EN), que ocasionava uma Resposta Inespecífica e Neutra (RN), antes de apresentar comida aos cães, a comida sendo um Estímulo Incondicionado (EI) e que gerava uma Resposta

Incondicionada (RI), que era a sialorreia. Após consecutivos pareamentos entre Estímulo Incondicionado e Estímulo Neutro, os cães passaram a salivar, Resposta Incondicionada (RI), ao som do sino sozinho, nesse caso, tendo uma Resposta Condicionada (RC). O Estímulo que originalmente era um Estímulo Neutro, se torna um Estímulo Condicionado (EC) (PAVLOV, 1927).

Segundo Catania (1999), o condicionamento clássico pode ser diferido em relação a sua programação temporal, em dois tipos: atraso e traço. No condicionamento de atraso, o tratamento farmacológico é administrado antes da colocação do animal no ambiente onde ele será testado. Desse modo, a resposta ao fármaco vai ocorrer concomitantemente com o estímulo do ambiente de testes. O outro tipo, o condicionamento de traço, tem-se o Estímulo Condicionado terminando antes do início da administração do fármaco, ou seja, o animal recebe a administração do fármaco após a saída da arena experimental e, para tanto, o intervalo entre os dois estímulos precisa ser breve. Em se tratando do condicionamento de atraso, que é um modelo menos utilizado, o indivíduo experimenta os estímulos da arena e do fármaco em consonância, ou seja, não existe intervalo temporal entre cada um dos estímulos (DE MELLO BASTOS *et al*., 2014; SANTOS *et al*., 2015). No condicionamento de traço o animal vivencia os estímulos de forma separada, ou seja, primeiro ele experimenta os efeitos causados pela permanência na arena experimental, imediatamente depois, ele vai experimentar os efeitos provocados pelo fármaco (CAREY *et al*., 2014).

Sabe-se que a morfina, assim como outras drogas psicoativas, por exemplo, anfetamina e cocaína, causa mudanças permanentes no SNC, e que essa neuroplasticidade, após uso repetido, está envolvida com a dependência química (JACOBS *et al*., 2003). Para tanto, os seus estímulos incondicionados podem ser facilmente condicionados a estímulos presentes em ambientes experimentais (SIEGEL, 2001).

Trabalhos de Walter e Kuschinsky (1989), usando três grupos de ratos: o primeiro grupo (associado) foi condicionado 8 vezes com morfina 15 mg/kg, na presença de vários estímulos condicionados definidos (auditivo, olfativo e tátil). O segundo grupo (não-associado), foi exposto ao mesmo esquema de tratamento de morfina e estímulos, mas sem associação positiva entre droga e os estímulos. Um terceiro grupo (veículo) foi tratado com solução salina NaCl 0,9%, e exposto aos mesmos estímulos que os outros dois grupos. Todos os grupos foram testados para respostas condicionadas na presença dos

estímulos condicionados. Uma série de experimentos foi realizada com solução salina, depois de um intervalo de dois dias dos testes de condicionamento, uma segunda série foi testada com soro fisiológico após um intervalo de 7 dias, uma terceira série com morfina 15 mg/kg após um intervalo de 2 dias, uma quarta série com a mesma dose de morfina após um intervalo de 7 dias. Os resultados mostraram que, quando a morfina foi usada após um intervalo de 2 dias, a tolerância farmacodinâmica "clássica" – mas não os fenômenos condicionantes – poderia explicar a mudança de comportamento, enquanto que, nos outros três protocolos descritos, alguns efeitos comportamentais condicionados puderam ser observados na presença ou na ausência de morfina, para os parâmetros monitorados (ativação locomotora, melhora das funções motoras, cheirar, roer). Consequentemente, o condicionamento contribui para a mudança de comportamento após a administração repetida de morfina.

3.4 Sensibilização e Morfina

A sensibilização é um processo onde se tem o aumento progressivo da atividade locomotora, compulsão, comportamento de fuga, sendo que esse aumento ocorre após a administração repetitiva de psicoestimulantes, usando-se a mesma dose da substância química (WELLS e WELLS, 1971).

Também conhecida como tolerância reversa, a sensibilização comportamental é essencial para o processo de dependência química. A administração de drogas causa mudanças em nível cerebral, na via dopaminérgica mesolímbica associada ao núcleo accumbens (Nac), provocando sensibilização neural. Como consequência, esses sistemas se tornam hipersensíveis aos efeitos da droga e também a estímulos associados a eles, mesmo após cessada a administração da substância química. Mesmos depois de algum tempo sem o uso, a atividade locomotora ainda estará aumentada, devido à hiperpersensibilidade comportamental que é duradoura (CADOR *et al*., 1995). Dessa forma, a sensibilização neural aumenta a busca pela droga (GARCIA *et al*., 2011). Um único tratamento com anfetamina ou morfina induz a hiperlocomoção associada à hiper-responsividade da transmissão de dopamina mesocorticolímbica (VANDERSCHUREN *et al*., 1999).

A sensibilização depende do padrão temporal da exposição ao medicamento, regimes de tratamento intermitente, com intervalos de 24 horas (dose diária) entre as administrações, são geralmente mais eficazes na indução da sensibilização do que os esquemas que empregam exposição a altas doses crescentes de medicamentos (ROBINSON e BECKER, 1986; STEWART e BADIANI, 1993; VANDERSCHUREN *et al*., 1997). É possível notar ainda, que até mesmo uma única exposição a drogas psicoestimulantes, como morfina, pode ser suficiente para provocar uma sensibilização duradoura (NASCIMENTO, 2011).

No trabalho de Vanderschuren e colaboradores (1997), foi observado efeito de hiperlocomoção em um protocolo de sensibilização comportamental por morfina, mesmo após transcorrer três semanas da última aplicação, o experimento consistia em uma aplicação diária de morfina 10 mg/kg, por via subcutânea (SC), em ratos Wistar, por um período de 14 dias consecutivos.

Viganò e colaboradores (2003), após administrarem doses crescentes de morfina (10 mg/kg - 20 mg/kg - 40 mg/kg), em ratos, duas vezes ao dia, durante três dias consecutivos, puderam constatar indução de sensibilização comportamental. O teste de sensibilização foi feito usando-se de uma dose baixa de morfina (5 mg/kg) por via SC, depois de passados 15 dias da última aplicação. Os resultados mostraram que houve aumento da locomoção, quando comparado ao grupo controle.

Em outro trabalho de Vanderschuren e colaboradores (2001), animais pré-expostos à dose única de morfina 10 mg/kg, exibiram um significativo aumento psicomotor. O mesmo tipo de exposição à morfina com dose diferente, 2 mg/kg não afetou o efeito psicomotor. Os testes de locomoção foram feitos três semanas após o tratamento o último farmacológico. Portanto, uma única exposição à morfina, em dose alta, induz a sensibilização comportamental duradoura e também a neuroadaptação associada.

3.5 MK-801, Sistema Dopaminérgico e o Sistema Glutamatérgico

A presença da monoamina dopamina no SNC sugere um papel de neuromodulação celular, que está ligada ao glutamato no processo da dependência química. A liberação glutamatérgica reflete em excitação neuronal e, por outro lado, a presença do

neurotransmissor GABA causa inibição celular (JONAS *et al*., 1998; NABEKURA *et al*., 2004)

O maleato de dizocilpina-MK-801 (figura 3) é um fármaco antagonista glutamatérgico dos receptores ionotrópicos do tipo NMDA (N-metil-D-aspartato), tendo os seus efeitos dependentes da dose, pois se trata de um antagonista não competitivo (FORD *et al*., 1989; ÖGREN e GOLDSTEIN, 1994).

O uso de doses baixas (0,025 mg/kg) de MK-801 provoca diminuição da locomoção em ratos em campo aberto e diminuição da ansiedade/medo ligada as reações defensivas antipredadoras (BLANCHARD *et al*., 1992). No caso do uso em doses elevadas (0,1 – 0,3 mg/kg), observaram-se os seus efeitos excitatórios como hiperatividade locomotora em ratos, esses são os resultados encontrados por Carey (1995) e Hargreaves e colaboradores (1992). As alterações locomotoras como a hipoatividade e hiperatividade, nos mostram que há uma estreita relação dos níveis de glutamato com algumas mudanças comportamentais, pois o glutamato é um neurotransmissor que desempenha um papel fundamental no comportamento locomotor e na memória a longo prazo no processo dependência química (SPENCER *et al*., 2016).

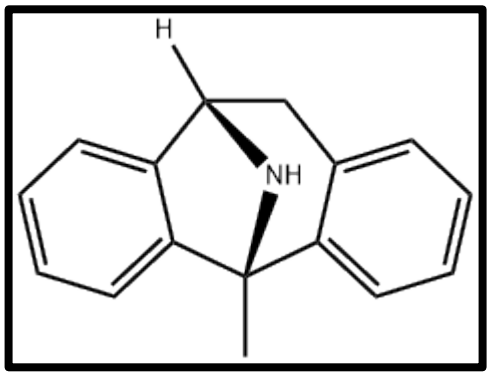

Figura 3: Fórmula estrutural do maleato de dizocilpina-MK-801 (5S,10R) -(+) -5 Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-iminemaleate). Adaptado de Yi e colaboradores (2020).

Há algum tempo, a importância dos gânglios da base no comportamento locomotor já é bem esclarecida. Estruturas como o núcleo accumbens, córtex pré-frontal, amígdala, hipotálamo e área tegmental ventral, medeiam a interação entre as vias glutamatérgicas e

dopaminérgicas, e dessa forma a atividade inibitória glutamatérgica é equilibrada pela atividade excitatória dopaminérgica (DAI e CAREY 1995).

Diversos estudos mostraram que fármacos agonistas dopaminérgicos diretos ou indiretos dopaminérgicos podem induzir hiperatividade e promover condicionamento e sensibilização (MATTINGLY *et al*., 1997, 1988; ANAGNOSTARAS e ROBINSON, 1996; ROWLETT *et al*., 1997; KELLER e DELIUS, 2001; BLOISE *et al*., 2007; BRAGA *et al*., 2009; DE MATOS *et al*., 2010). Da mesma forma, fármacos antagonistas glutamatérgicos do tipo NMDA, como o MK-801, podem induzir hiperatividade em ratos, e ainda, que essa hiperatividade se torna progressiva com o uso de tratamentos repetitivos, indicando sensibilização (CAREY *et al*. 1995).

Os sistemas dopaminérgicos bem como o glutamatérgico estão implicados na adição por opioides e outras drogas psicoativas como os opioides, o álcool e a cocaína. Nesse contexto os estudos terapêuticos atuais se voltam para drogas, que sejam capazes de equilibrar a transmissão do glutamato e da dopamina no contexto de dependência química (OLIVE *et al*., 2012; COLLINS *et al*., 1998; D'SOUZA, 2015).

A dopamina é um neurotransmissor endógeno da família das catecolaminas, sua produção ocorre na substância nigra e na área tegmental ventral (VTA) no cérebro. Existem, pelo menos, 5 receptores dopaminérgicos, D1, D2, D3, D4 e D5. Sendo D2, D3 e D4 com grande afinidade entre si, enquanto D1 e D5 compartilham de características semelhantes. A dopamina está envolvida no controle da cognição, humor, aprendizado, memória, emoções e na manutenção das funções renal, gastrointestinal e cardiovascular (MATSUMOTO *et al*., 2005).

Kenna e colaboradores (2007) mostraram que propriedades reforçadoras de drogas psicoativas com potencial de abuso têm relação direta e indireta com o sistema dopaminérgico. Os receptores dopaminérgicos se distribuem de forma ampla no hipotálamo, na substância nigra e na área tegmental ventral (VTA), dando origem às quatro principais vias dopaminérgicas (figura 4) no sistema nervoso central, a via mesocortical, a via mesolímbica, a via nigroestriatal e a via túbero-infundibular (BERKE, 2018).

A compreensão e o estudo das quatro vias dopaminérgicas, do sistema nervoso central e dos aspectos envolvidos na neurotransmissão são fundamentais para entender a vasta gama de funções desempenhadas por estas vias. Falhas nas estruturas de neurotransmissão das vias dopaminérgicas mesolímbica e mesocortical, localizadas no

sistema nervoso central estão relacionadas com doenças graves, como a dependência química (SALAMONE e CORREA, 2012).

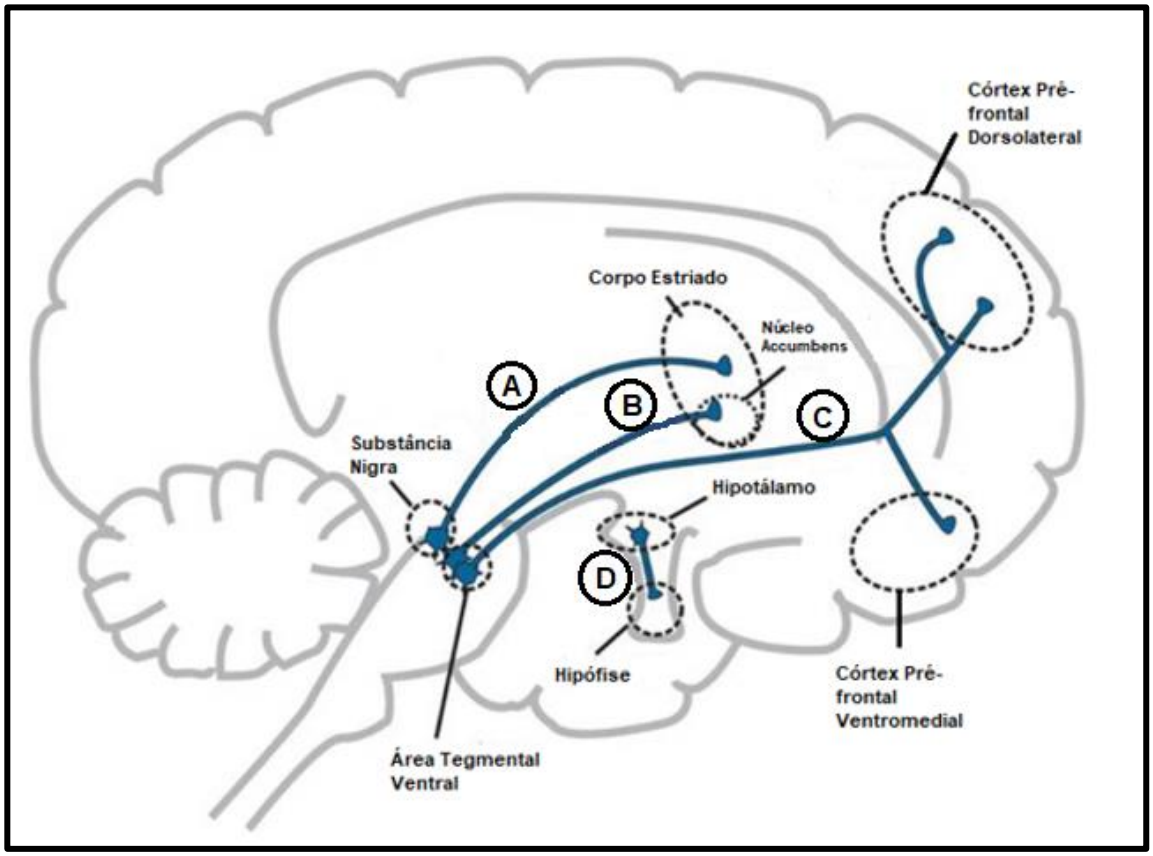

Figura 4: Vias Dopaminérgicas. (A): Via Nigroestriatal; (B): Via Mesolímbica; (C): Via Mesocortical; (D): Via Tubero-infundibular.

A via mesolímbica é uma das vias dopaminérgicas mais importantes, por suas funções destacadas com relação à dopamina. Está ligada diretamente ao sistema de recompensa cerebral. A via mesolímbica se origina na área tegmental ventral, uma região rica em neurônios dopaminérgicos e que cobre parte do mesencéfalo, e projeta os seus feixes nervosos para outras áreas importantes do sistema nervoso central, como o córtex pré-frontal, o hipocampo, o complexo amigdaloide e, também, para o *núcleo accumbens*. É no *núcleo accumbens* que a dopamina exerce, em um primeiro instante, sua ação como mediadora dos sentimentos de prazer e recompensa (ADINOFF, 2004).

Portanto, no instante em que um determinado indivíduo tem contato com um estímulo de caráter recompensador ou prazeroso, como comida, sexo ou drogas, ocorre liberação de dopamina cujos sinais percorrem da área tegmental ventral ao núcleo accumbens. O caminho feito pela dopamina possibilita a criação e o reforço de sensações

positivas que acabam por acentuar e modular o comportamento do indivíduo (SOMALWAR *et al*., 2018).

Outra via dopaminérgica importante com projeções originadas na área tegmental ventral é a via mesocortical, suas projeções se encaminham para o córtex pré-frontal. A via mesocortical é uma das maiores vias dopaminérgicas junto com a via mesolímbica e tem grande importância para o desenvolvimento normal das funções cognitivas, recompensa, aprendizagem, atenção, tomadas de decisão, memória de trabalho e resposta emocional. Está presente na fisiopatogênese da esquizofrenia, patologia em que ocorre a diminuição de dopamina nessa via cerebral (DIAZ, 1996; MORGANE *et al.,* 2005).

A via nigroestriatal tem projeções dopaminérgicas, com origem na substância nigra, indo até o corpo estriado e integra os gânglios basais. A ativação da via nigroestriatal, produz um aumento na liberação de dopamina no corpo estriado dorsal, e também produz estereotipias. Essa via está relacionada com a locomoção e promove aprendizado dos comportamentos do vício (RIVERA *et al*., 2013). Os neurônios dopaminérgicos presentes nessa via são responsáveis pela estimulação motora voluntária e contém em torno de 80% da reserva de toda a dopamina do sistema nervoso central (YADAV *et al*., 2014).

O Glutamato é um aminoácido proteinogênico presente em diversos tipos de alimentos, tem grande importância para processos metabólicos como a gliconeogênese, a glicólise e o ciclo dos ácidos tricarboxílicos (REEDS *et al*., 2000). O glutamato é um neurotransmissor com grande importância para o SNC, principalmente por seus efeitos excitatórios (CONTI, 1998). Ele está presente em cerca de 80% de todas as sinapses nervosas, regula muitas emoções e está envolvido na formação de lembranças, aprendizado e na atenção, assim como nas situações patológicas responsáveis por diversas desordens neuropsiquiátricas agudas e crônicas como na dependência química, ansiedade generalizada, dor neuropática, esquizofrenia e depressão, (LI *et al*., 2019; MELDRUM, 2000; FEATHERSTONE, 2010).

Existem dois grandes grupos de receptores glutamatérgicos, os ionotrópicos (iGluRs) e os metabotrópicos (mGluRs). Os receptores metabotrópicos se dividem em 3 grupos: grupo 1 (mGluR1 e mGluR5), grupo 2 (mGluR2 e mGluR3) e grupo 3 (mGluR4, mGluR6, mGluR7 e mGluR8), (REINER e LEVITZ, 2018; BONSI *et al*., 2005). Os receptores ionotrópicos podem ser divididos em três tipos: N-metil-D-aspartato (NMDA), ácido-amino-3-hidroxi-5-metil-isoxazol-4-propiônico (AMPA) e cainato (KA) (RUGGIERO *et al*., 2011). Os receptores ionotrópicos NMDA, AMPA e KA possuem alguns subtipos e

esses são classificados conforme as diferentes combinações das subunidades proteicas que os formam.

A neurotransmissão quando mediada pelos receptores ionotrópicos é rápida, pois o fluxo de íons é afetado de maneira imediata (principalmente Na+ e Ca2+) e da mesma forma o estado eletroquímico da membrana pós-sináptica (BOWIE, 2008). O receptor do tipo AMPA é responsável pela maioria das transmissões sinápticas excitadoras rápidas, o tipo KA participa nas respostas pós-sinápticas em sinapses excitadoras, podendo ainda modular a liberação pré-sináptica do transmissor glutamato em determinadas sinapses, enquanto o receptor do tipo NMDA é fundamental na indução de formas específicas de plasticidade sináptica (ENGELHARDT, 2003).

Existem pelo menos sete vias glutamatérgicas (figura 5). A primeira via se chama córtico-troncar, essa via tem projeções indo do cortéx até o tronco cerebral. A via córtico-troncar descende dos neurônios piramidais corticais no córtex pré-frontal e seguem para o tronco cerebral. É uma via glutamatérgica muito importante pois é a chave na regulação da liberação de neurotransmissores, envolve também a substância nigra e VTA para a dopamina, os núcleos da rafe para a serotonina e o locus coeruleus para a norepinefrina.

A segunda, via também descendente se projeta do córtex pré-frontal ao corpo estriado dorsal (via glutamatérgica córtico-estriatal) ou pode ainda se projetar do córtex pré-frontal ao núcleo accumbens no corpo estriado ventral (via glutamatérgica cortico-accumbens), e constituem juntas a porção das alças cortico-estriatal-talâmica. Essas vias descendentes de glutamato terminam nos neurônios GABA que têm como destino uma estação de retransmissão chamada globo pálido, essa estação tem grande importância na coordenação motora, na manutenção e aquisição de informações e processamento de emoções (FOX *et al*., 1974; CAMARGO *et al*., 1981).

A terceira via glutamatérgica tem grande importância, pois algumas teorias vinculam essa via específica à esquizofrenia. Esta via se projeta do hipocampo para o núcleo accumbens e é conhecida como via glutamatérgica hipocampal-accumbens. Da mesma forma que a segunda via glutamatérgica (vias córtico-estriatal e córtico-accumbens), a terceira via também tem neurônios que saem do hipocampo e fazem sinapses com neurônios gabaérgicos presentes no núcleo accumbens, que por sua vez se projetam para uma estação de retransmissão no globo pálido.

A quarta via é uma via ascendente, os neurônios saem do tálamo e fazem sinapses com neurônios piramidais no córtex (via glutamatérgica tálamo-cortical). Normalmente, essa via está envolvida no processamento de informações sensoriais.

A quinta via é descendente, e segue do córtex pré-frontal indo até o tálamo (via glutamatérgica córtico-talâmica). Essa via pode direcionar a forma pela qual os neurônios reagem às informações sensoriais.

A sexta via é a córtico-cortical direta e é formada pelos neurônios piramidais intracorticais que liberam o neurotransmissor glutamato, que é excitatório.

A sétima via é a via córtico-cortical indireta, nessa via os neurônios piramidais fazem sinapses com interneurônios (que liberam o GABA) e estes fazem sinapses com neurônios presentes no córtex.

Quatro das sete vias glutamatérgicas se projetam a partir do córtex frontal e penetram em áreas mais profundas do cérebro, onde exercem controle sobre as estruturas neuroanatômicas como o corpo estriado ventral e dorsal, o núcleo accumbens, o tálamo e o tronco cerebral (STAHL, 2008; SCHWARTZ *et al*., 2012).

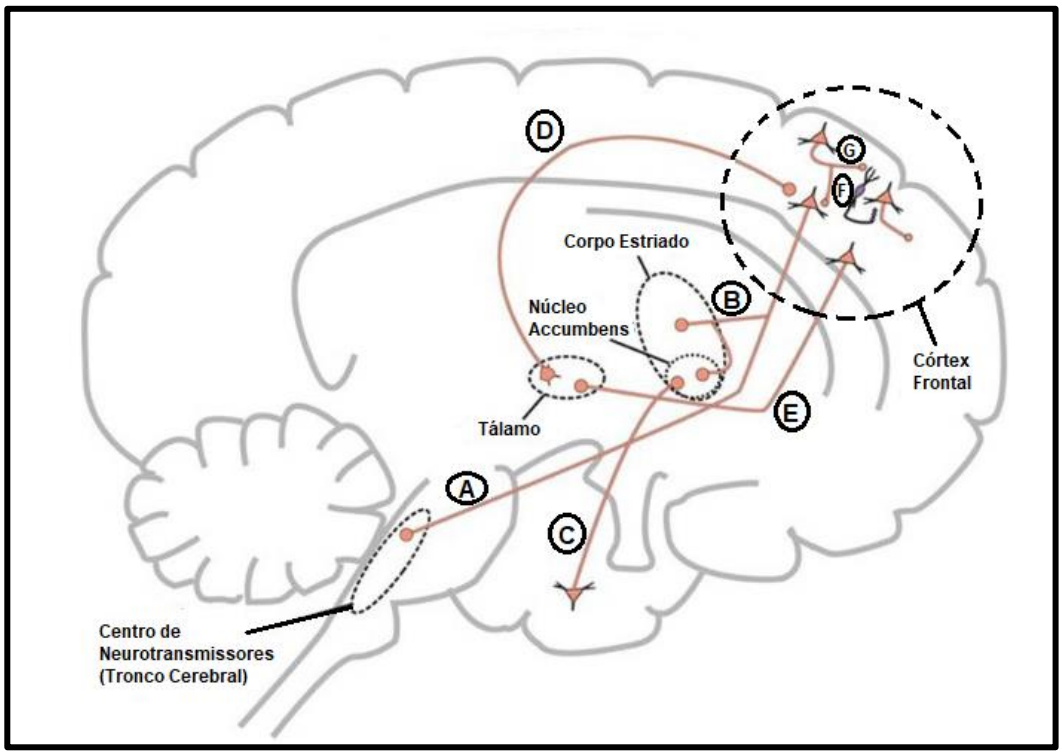

Figura 5: Vias glutamatérgicas. (A): via córtico-troncar; (B): via córtico-estriatal e córtico-accumbens; (C): Via hipocampal-accumbens; (D): via tálamo-cortical; (E): via córtico-talâmica; (F): via córtico-cortical direta; (G): via córtico-cortical indireta.

Spencer e colaboradores (2016) esclarecem que o envolvimento da dopamina na dependência química não é completo, ou seja, ela contribui como o neurotransmissor responsável pelas associações relacionadas à droga como, por exemplo, o prazer, e, dessa forma, induzem ao desejo por voltar ao consumo da substância, enquanto que o glutamato é o maior causador da recaída após um tempo em abstinência, portanto a memória a longo prazo está mais ligada aos efeitos excitatórios do glutamato pelas vias descendentes do córtex (córtico-troncar, córtico-estriatal, córtico-accumbens, córtico-talâmica) no SNC.

Os opioides, como a morfina, provocam um aumento da atividade glutamatérgica e dopaminérgica na via mesolímbica, que acontece por meio da inibição dos neurônios gabaérgicos; dessa forma, ocorre diminuição do GABA e aumento da dopamina liberada pelos neurônios dopaminérgicos, por outro lado ocorre liberação pré-sináptica de glutamato por meio da desinibição por neurônios gabaérgicos no VTA (CHEN *et al*., 2015; CHARTOFF e CONNERY, 2014). A morfina, quando administrada repetidamente em um ambiente determinado, produz uma série de neuroadaptações que levam ao desenvolvimento de tolerância, dependência e sensibilização. Assim como outras drogas de abuso, ela afeta as vias dopaminérgicas mesolímbica e mesocortical do SNC. A ativação do sistema dopaminérgico mesolímbico (circuito de recompensas) provoca um aumento na liberação de dopamina no *núcleo accumbens* e é responsável pelas propriedades de reforço dos fármacos (WISE, 1996; BARDO, 1998).

Existe uma diferença no curso temporal em que se tem um pico de atividade motora e níveis de concentração extracelulares no corpo estriado cerebral, para o glutamato, esse tempo é de 30 – 90 minutos, diferindo da dopamina, que ocorre entre 15 e 30 minutos após estímulo com psicoestimulantes como exemplo a morfina (GRAY *et al*., 2001; RAWLS e MCGINTY, 2000).

A ativação das vias dopaminérgicas normalmente está ligada a respostas ocasionadas por riscos e recompensas imprevisíveis, enquanto que a ativação das vias glutamatérgicas é informativa sobre o contexto da situação (SCHULTZ, 2010).

3.6 Proteína Quinase Ativada por Mitógenos – ERK

A ERK é uma proteína quinase regulada por sinais extracelulares (mitógenos); (JOHNSON e LAPADAT, 2002). A proteína é ativada de forma a coordenar a proliferação, diferenciação e sobrevivência de muitos tipos de células, principalmente os neurônios no SNC (LEWIS *et al*., 1998; DAVIE e SPENCER, 2001; MORRISON, 2012).

Algumas doenças como o câncer, as síndromes imunológicas, inflamatórias e degenerativas podem decorrer de defeitos na via relacionados à ERK (HINDLEY e KOLCH, 2002). Na dependência química, a sinalização celular está desregulada, o que ocasiona um aumento dos níveis de produção de ERK, quando comparado ao aumento por estímulos naturais, dessa forma contribuindo para a neuroplasticidade sináptica após doses repetidas de drogas (GIRAULT *et al*, 2007; SHIFLETT e BALLEINE, 2011).

A dopamina modula a transmissão de glutamato e controla a plasticidade estriatal induzida por drogas de abuso por meio do receptor do tipo D1. Um dos principais alvos dos receptores D1 presentes no estriado cerebral é a proteína quinase ativada por mitógenos (ERK). A ativação da ERK por drogas de abuso se comporta como um fator integrador chave de sinalização dos receptores dopaminérgicos tipo D1 e glutamatérgicos tipo NMDA, formando um núcleo central de neuroplasticidade (WICKENS, 2009; CAHILL *et al*., 2014).

Sanguedo e colaboradores (2015 e 2017), mostraram aumento da locomoção e ativação da ERK1/2 no córtex pré-frontal, amígdala, núcleo accumbens, hipotálamo lateral em grupos de ratos que receberam apomorfina (2,00 mg/kg), em dose alta, a substância provoca aumento dos níveis circulantes de dopamina. Essas estruturas cerebrais são as principais relacionadas ao circuito de recompensa e motivação e são também responsáveis pela aquisição e manutenção da dependência química.

Mitra e Sinatra (2004) mostraram um aumento na densidade e alteração dos receptores opioides acoplados à proteína G, após o uso crônico de morfina, juntamente com a alteração dos receptores foi visto ainda uma maior ativação de ERK 1/2 em indivíduos que se tornaram dependentes de opioides após passarem por procedimentos cirúrgicos.

A ativação de ERK1/2 *in vivo* após uso de morfina sinaliza a expressão gênica em regiões como o núcleo accumbens e amigdala, e dessa forma contribui para a sua

permanência a longo prazo (ASENSIO *et al*., 2006). Ainda pode ocorrer sinalização no início da abstinência com aumento da ERK 1/2 em regiões como amígdala, locus coeruleus, hipotálamo, córtex cerebral, septo lateral (CICCARELLI *et al*., 2013; HOFFORD *et al*., 2009).

MATERIAL E MÉTODOS

4.1 Sujeitos

Foram utilizados ratos machos, albinos, Wistar, pesando entre 200 - 250g, oriundos do Biotério Central da UENF, Campos Dos Goytacazes, RJ. Os animais foram mantidos em gaiolas individuais de plástico (BEIRA MAR, São Paulo) medindo 25x18x17 cm, com acesso livre à ração padronizada de laboratório e à água. As gaiolas foram mantidas em uma sala no setor de Farmacologia do Laboratório de Morfologia e Patologia Animal (LMPA), com umidade e temperatura controladas (22 ± 2.0ºC), e com ciclo de luz claro e escuro de 12 em 12 horas (luz das 07:00 às 19:00 horas). O experimento foi conduzido na fase clara, horário entre 09:00 e 14:00 horas. Os animais foram manipulados individualmente, por um único indivíduo, pelo tempo de 5 minutos durante 7 dias antes do início do procedimento experimental e foram habituados ao procedimento de injeção com solução de veículo durante 3 dias antes do início do experimento. O presente projeto foi aprovado pela comissão de ética de uso de animais (CEUA – UENF), sob o nº 029/2022, Protocolo 473.

4.2 Ambiente Experimental

O presente experimento foi desenvolvido em quatro salas experimentais padronizadas medindo 1,40 x 1,40 metros. Cada sala constituída de iluminação vermelha, temperatura controlada (22 ± 2,0 ºC) e o som de um ventilador (30 cm de hélice) ligado em cada uma das salas como ruído de fundo. Cada sala contendo uma arena quadrada medindo 60x60x45cm, com assoalho e paredes pintados na cor preta. Para o registro do

comportamento locomotor, foram utilizadas câmeras (IIKEGAMI, modelo ICD – 49 e Panasonic, modelo WV – BP334), posicionadas 60 cm acima das arenas experimentais. As câmeras ficaram acopladas a um computador PC compatível, contendo um programa de análise de imagens, EthoVision (NOLDUS, Holanda), o qual estava localizado fora das salas de experimento, o sistema quantificou a atividade locomotora em distância percorrida (metros).

4.3 Fármacos

O sulfato de morfina (Dimorf 10mg/ml, Cristália®, São Paulo, SP, Brasil) foi utilizado na dose de 10 mg/kg (VANDERSCHUREN *et al*., 1997, 2001) e administrado por via subcutânea (SC). O volume de administração foi de 1ml/kg.

A apomorfina-Hcl (Sigma-Aldrich®, Saint Louis, Mo, USA) foi utilizada na dose de 0,05 mg/kg, dissolvida em uma solução salina (NaCl 0,9%) e administrada por via subcutânea (volume de administração: 1ml/kg).

O maleato de dizocilpina (MK-801, Sigma Aldrich®, Saint Louis, Mo, USA) foi utilizado nas doses de 0,025 mg/kg, 0,1 mg/kg e 1.0 mg/kg por via subcutânea (SC). O volume de administração foi de 1ml/kg.

A solução salina (NaCl 0,9%) foi utilizada como veículo na concentração de 1 ml/kg e administrada por via subcutânea.

A solução Carbamato de etila (Urethane, Sigma Aldrich®, Saint Louis, Mo, USA) foi utilizado na dose de 3000 mg/kg (CFMV, 2013; CEUA-UNIFESP, 2017; HENKE *et al*., 2016) e administrado por via intraperitoneal. O volume de administração foi de 15 ml/kg.

As soluções foram preparadas antes de cada dia experimental.

4.4 Procedimento Experimental

Os experimentos foram conduzidos segundo o protocolo experimental de Carrera e colaboradores (2013), Carrera e colaboradores (1995), Miczek e colaboradores (2011), Crespo e colaboradores (2022) com modificações.

5. MANUSCRITOS

Os dois manuscritos com os dados desta tese serão apresentados em dois capítulos:

Primeiro capítulo, o manuscrito “Morphine and dopamine: Low dose apomorphine can prevent both the induction and expression of morphine locomotor sensitization and conditioning”, submetido à revista “Behavioural Brain Research” foi apresentado.

Segundo capítulo, o manuscrito MK-801 “Induces dose dependent stimulant sensitization effects but dose independent conditioned stimulant effects: MK-801 effects on sensory information processing versus learning and memory, submetido à revista “Psychopharmacology” foi apresentado.

5.1 - Capítulo I

Morphine and dopamine: Low dose apomorphine can prevent both the induction

and expression of morphine locomotor sensitization and conditioning.

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Abstract

The disinhibition of dopamine neurons in the VTA by morphine is considered an important contributor to the reward potency of morphine. In this report, three separate experiments were conducted in which a low dose of apomorphine (0.05 mg/kg) was used as a pretreatment to reducedopamine activity in the brain. Locomotor hyperactivity was used as the behavioral response to morphine (10.0 mg/kg). In the first experiment, five treatments with morphine induced the development of locomotor and conditioned hyperactivity. Apomorphine, given 10 min prior to morphine, prevented the development of morphine-induced hyperactivity and conditioning. Apomorphine before either vehicle or morphine injections induced equivalent reductions inspontaneous activity in the vehicle- and morphine-treated groups. In the second experiment, a pretreatment with apomorphine was performed after the morphine-induced conditioned hyperactivity and apomorphine eliminated the expression of the morphine-conditioned response. To assess the effects of the apomorphine on VTA and the nucleus accumbens, ERK measurementswere carried out. Morphine increased ERK activation in these encephalic areas and the increase inERK activity was associated with enhanced locomotion. A third experiment was conducted to assess the effects of acute morphine on ERK before locomotor stimulation was induced by morphine. Acute morphine did not increase locomotion but a robust ERK response was produced.The increases in ERK activation were impaired by the apomorphine. The results provide substantial support for the importance of ongoing activity in dopamine neurons for the efficacy ofmorphine disinhibition of dopamine reward systems in the mediation of morphine behavioral sensitization and conditioning.

Key words: Morphine; Dopamine; VTA; ERK; Behavioral sensitization; Drug conditioning.

1. Introduction

It has been well established that dopamine neurons in the ventral tegmental area (VTA) are of substantial importance in the mediation of behavioral reward effects [12, 18, 41]. Not surprisingly, dopaminergic drugs that can mediate reward effects also activate VTA dopaminergicneurons [17, 36, 40]. Morphine can also activate VTA dopaminergic neurons but, unlike psychostimulant dopaminergic drugs, morphine produces this pharmacological action indirectly by a disinhibitory and inhibitory effects. Morphine binds to μ-opioid receptors located on □- aminobutyric acid (GABA)ergic neurons that inhibit VTA dopaminergic neurons. This inhibitionof GABAergic neurons by morphine recruiting μ-opioid receptor-signaling can reduce the GABAergic inhibition on dopaminergic neurons and thereby enhance the ongoing activity in the dopaminergic neurons [6, 16, 21, 23, 34, 38]. Somewhat analogous to the indirect effects of morphine on VTA dopaminergic neurons, it has been suggested [8, 11] that morphine-induced behavioral increases in spontaneous activity [20, 26, 28, 35, 39] are not a result of a direct morphine activation of the motor system but rather a consequence of morphine activation of VTA-induced reward effects that adventitiously occur in association with ongoing spontaneous activity and reinforce and potentiate the spontaneous behavior.

The magnitude of the impact of the disinhibitory effect of morphine on dopaminergic neurons to increase dopamine activity is presumably related to the level of the activity of the dopaminergic neurons. Seemingly, the lower the ongoing activation state of VTA dopaminergic neurons, the less the magnitude of the increase in VTA dopamine activity induced by a reductionof GABAergic inhibition. Clearly, if the dopaminergic system is inactive then the morphine disinhibitory effects would not increase VTA dopaminergic system reward activation and, consequently, there would be no reinforcement and potentiation of spontaneous behavioral

activity. One pharmacologically effective treatment for reducing activity in dopaminergic neuronsis the use of a low dose of apomorphine. At low dose range in rats (<0.1 mg/kg), apomorphine caninduce inhibition of dopaminergic neurons by preferential stimulation of dopamine autoreceptors,thereby inhibiting dopaminergic activity in the brain [2, 7, 10, 24].

This work was undertaken to use the pretreatment with apomorphine (0.05 mg/kg) followed by the treatment with morphine to reduce ongoing activity in dopaminergic neurons. We measured the effects of apomorphine pretreatment on morphine-induced effects on spontaneous activity and on extracellular signal regulated kinase activity (ERK) in subcortical brain areas implicated in dopamine-related reward system nuclei namely the VTA and nucleus accumbens (NAc). The rationale for measuring ERK in these encephalic structures was based on findings that have reported increases in ERK activity in dopaminergic projection areas [1, 14, 22, 29] includingthe NAc [5, 13, 19, 37] following treatment with psychostimulant drugs such as cocaine. Additionally, in previous studies [30, 31, 32, 33] focused on apomorphine behavioral sensitization, we showed that this sensitization was associated with a strong ERK signal in brain dopaminergic systems. In the present study, we used ERK as an indicator of activation in VTA and NAc subcortical areas associated with reward systems. This report details the effects of a low dose apomorphine pretreatment on morphine-induced behavioral sensitization and conditioning, and ERK activity in the VTA and NAc of laboratory animals submitted to three independent experiments.

2. Method

2.1. Subjects

Male Wistar rats provided by the Universidade Estadual do Norte Fluminense Darcy Ribeiro, initially weighing 200-300 g, were housed in individual plastic cages (25 X 18 X 17 cm)until the end of the experiment. Food and water were freely available at all times. The vivarium was maintained at a constant temperature (22 ± 2 ºC), and a 12h/12h light/dark cycle (lights on at7 a.m. and off at 7 p.m.). All experiments were performed between 8 a.m. and 12 p.m. For 7 daysprior to all experimental procedures, each animal was weighed and handled daily for 5 min. This process included being placed in a transport cage and taken to the injection administration bench located in an anteroom adjacent to the experimental testing room. The animals were given vehicle(VEH) injections. In this way, the animals were familiarized with the handling and injection aspects of the testing protocol. All the experiments were performed in compliance with Brazilian Council for Animal Experimentation (CONCEA), which are based on the US National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Drugs

Morphine sulfate (Cristália, São Paulo, Brazil) was used from 10 mg ampoules (1 ml) and was injected subcutaneously in the nape of the neck at a dose of 10 mg/kg. Apomorphine-HCl (Sigma, St. Louis, MO, USA) was dissolved in 0.9% NaCl solution and was injected subcutaneously in the nape of the neck in a dose of 0.05 mg/kg. Physiological saline was used as

vehicle (VEH). All doses were administered in a volume of 1.0 ml/kg body weight. Drug solutionswere freshly prepared before each experiment.

2.3. Apparatus and behavioral measurements

The behavioral measurements were conducted in a black open field test enclosure (60 x 60x 45 cm). A closed-circuit camera (IKEGAMI, model ICD-49; Ikegami Electronics Inc., Torrance,California, USA) mounted 60 cm above the arena was used to record behavioral data. Locomotion,measured as distance traveled (m), was automatically analyzed using EthoVision (Noldus, the Netherlands). The complete test procedure was conducted automatically without the presence of the experimenter in the test room. All behavioral testing was conducted under dim red light that favors exploratory behavior and avoids the possible aversive quality of white light [25] as well asto enhance the contrast between the white subject and the dark background of the test chamber andreduce the animal's shadow.

2.4. General experimental procedure

Initially, all rats were acclimated to the test arena by being injected with VEH and placed in the arena for 30 min on 3 successive days (habituation phase). The habituation protocol was conducted so that a stable baseline of the locomotor behavior could be established prior to the startof the drug treatments. On the basis of the locomotion distance scores from the third habituation session, groups were formed that equated for the distance scores. Next, the groups received their pharmacological treatment immediately prior to the 5 min placement in the open-field arena and the locomotor activity was measured. These treatments were administered for 5 consecutive days

(experiments 1 and 2), one trial per day, and were designed as the induction phase. On the following day, the final test was performed, in which the animals received their treatmentimmediately before being placed into the open-field arena for a 5-min session. Immediately uponcompletion of the final test, the animals were euthanized, intracardially perfused with physiological saline and fixative and immunochemistry was performed. Figure 1A shows the experimental timeline.

2.5. Experiment 1: Effect of a low dose of apomorphine pretreatment on the acquisition of morphine-induced locomotor sensitization, conditioning, and ERK activity in VTA and NAc.

The protocol described in the general experimental procedure was followed. In the induction phase, the rats received the first injection and were then put back into home-cage and after 10 min they received the second injection and were immediately placed into the open-field test arena (Fig. 1C). There were 2 VEH+VEH groups, when rats received 2 injections of VEH; 2 VEH+MOR, in which the first injection was VEH and the second was morphine at a dose of 10 mg/kg (MOR); 2 APO+VEH groups in which the first injection was apomorphine at 0.05 mg/kg (APO) and the second was VEH and; 2 APO+MOR groups in which the first injection was apomorphine at 0.05 mg/kg (APO) and the second was MOR at 10 mg/kg. For the final test, all groups received VEH as the first injection, and for the second injection, received either VEH or MOR. The experimental groups (n=7 for each group) were labeled in terms of (a) their induction treatments /(b) their final test treatments. The treatment groups are summarized and presented in Table 1.

2.6. Experiment 2: The effect of APO on the expression of morphine-induced conditioned locomotor stimulation.

The protocol described in the general experimental procedure was followed. In the induction phase, the rats received the first injection and were put back into home-cage. After 10 min they received the second injection and were immediately placed into the open-field test arena. There were 2 VEH+VEH groups, in which rats received 2 injections of VEH, and 2 VEH+MOR groups, in which the first injection was VEH and the second was morphine at a dose of 10 mg/kg (MOR). After completion of the 5 sessions induction phase, the VEH/MOR groups developed a strong locomotor stimulant response and the next day a conditioning test was conducted to assess the effects of APO on the expression of the conditioned MOR response. For the conditioning test, the two VEH/MOR groups were divided into 2 groups (n=7) with comparable locomotor stimulant responses, and one group received two injections of VEH, while the other group received APO as the first injection and VEH as the second injection. The experimental groups (n=7 for each group) were labeled in terms of (a) their induction treatments /(b) their final test treatments. The treatment groups are summarized and presented in Table 2A.

2.7. Experiment 3: Acute 1-day experiment.

After the 3 days of the habituation phase, that was used in the previous two experiments, the rats received the first treatment injection and were put back into home-cage and after 10 min. they received the second treatment injection and immediately were placed into the open-field test arena. All groups received a one day 5 min test and immediately upon completion of the test the animals were euthanized, perfused and immunochemistry was performed. The objective of this experiment was to assess the effects of morphine on ERK activity in the VTA and NAc before

locomotor stimulant effects were induced. There were 4 groups (n=6 for each group): VEH+VEHgroup in which rats received 2 injections of VEH; VEH+MOR group in which the first injection was VEH and the second was morphine at 10 mg/kg (MOR); APO+VEH in which the first injection was apomorphine at 0.05 mg/kg (APO) and the second injection was VEH; andAPO+MOR group where the first injection was APO and the second injection was MOR. The treatment groups are summarized and presented in Table 2B.

2.8. Immunohistochemistry

The immunohistochemistry protocol was conducted as previously described by Crespo and co-workers [8] and Dias and co-workers [11]. In brief, rats were rapidly anaesthetized by intraperitoneal injection of urethane (15 ml//kg; Sigma Aldrich, Saint Louis, MO, USA) prior to intracardiac perfusion with 4% paraformaldehyde (500 ml) in 0.1 M sodium phosphate buffer (pH7.4) for 30 min. The encephalon of each rat was removed and post-fixed for 2 h in 4% paraformaldehyde solution before transferring to 20% sucrose in 0.1 M sodium phosphate buffer (pH 7.4) for 48 h at 4 ºC. Each brain was placed on an aluminum paper base and cryoprotected using a solution of water-soluble glycols and resins (Tissue Tek® O. C. T. Sakura Finetek®, USA).The brain tissue was then frozen and maintained in liquid nitrogen until being processed for immunohistochemistry.

The encephalic areas sampled for the immunohistochemical analysis were ventral tegmental area (approximately -4.80 mm to -5.04 mm from bregma) and nucleus accumbens (core and shell approximately +2.52 mm to +2.76 mm from bregma). The coordinates adopted as the reference were obtained from Paxinos and Watson [27]. Four slices were collected sequentially from each brain structure of each animal and sectioned in a cryostat (Zeiss, Germany) at a thickness

of 30 µm. The sections were placed onto previously gelatinized glass microscope slides to allow fixation. For immunohistochemistry, sections were rinsed three times for 10 min in phosphate- buffered saline (PBS) and placed in blocking buffer (3% normal goat serum and 0.25% Triton X-100 in PBS) for 1 h at 22 ºC. The sections were then treated with a freshly prepared solution of hydrogen peroxide (3% vol/vol) in chilled absolute methanol for 10 min at 22 °C. Sections were then incubated for 24 h at 4 ºC in 1:500 dilution of anti-phosphorylated-ERK protein antibody diluted in blocking buffer (Cat # 9101, Cell Signaling Technology®, Boston, MA, USA) as previously described. After the end of the incubation time, sections were washed 3 times for 10 min each in PBS and incubated at 22 ºC with a 1:100 dilution of biotinylated goat anti-rabbit IgG secondary antibody (BA-1000, Vector Laboratories®, CA, USA) in 1% normal goat serum and 0.25% Triton X-100 in PBS. Sections were then washed three times for 10 min in PBS and processed using an ABC Elite kit (Vector Laboratories ®, Burlingame, CA, USA). In the next step, sections were washed again in PBS and processed with a DAB substrate kit for peroxidase (SK-4100, Vector Laboratories®, Burlingame, CA, USA) and incubated in DAB substrate simultaneously and timed precisely at 22 °C for 3 min for color development of signal intensity. Sections were lightly counterstained with hematoxylin. After drying, the slides were mounted with DPX (Sigma ®, USA).

Photomicrographs of the brain sections were obtained using a CCD camera (Nikon Photometrics Cool Snap) attached to a light microscope (Nikon 80i, USA). Digital images were obtained at low magnification (10X) from regions of the brain identified from the Paxinos and Watson [27] rat brain atlas and the labeled nuclei from each brain structure of each animal were quantified using the Image J® software "multi-point" tool. The number of labeled neurons was counted bilaterally from four sections and the average count of labeled neurons for these sections

was used as the score for each animal. The number of p-ERK-labeled cells was normalized to the area of the quadrant counted. In order to demonstrate details of the ERK-labeled cells, specific sections were photographed at higher magnification (40X).

Negative control slices were incubated with normal serum instead of primary antibody (data not shown). In order to minimize any potential bias in the scoring, two experimenters unaware of the treatment groups independently performed the labeled nuclei counts. The counts obtained by each experimenter for each encephalic area and for each group were compared using Student's t-tests and no statistically significant differences ($p>0.05$) were found between groups.

2.9. Statistics

After the determination of normal distributions of data using the Shapiro-Wilk normality test, considering the induction treatment phase, a repeated two-way analysis of variance (ANOVA) was used in order to determine the group effect, day effect, as well as the interactions between both factors. When a significant effect of interaction was recorded, data were further statistically evaluated by one-way ANOVA followed by Tukey's test with $p<0.05$ as the criterion for statistical significance. For the final test, a one-way ANOVA was performed followed by Tukey's post-hoc tests using $p<0.05$ as the criterion for statistical significance. For the morphine induced ERK activation analysis, a one-way ANOVA was performed followed by Tukey's post-hoc tests using $p<0.05$ as the criterion for statistical significance.

3. Results

3.1 Experiment 1: Effect of a low dose of apomorphine pretreatment on the acquisition of morphine-induced locomotor sensitization, conditioning, and ERK activity in the VTA and NAc.

Figure 2 shows the locomotor activity over the course of days 1 to 5 of the induction phase. According to a repeated two-way ANOVA, there were significant effects of interaction of groupsX days [$F(28, 192) = 12.75$; $p<0.01$], of groups [$F(7, 48) = 45.30$; $p<0.01$], and of days of treatment [$F(4, 192) = 10.10$; $p<0.01$]. According to a one-way ANOVA followed by Tukey's post hoc test, to further analyze the interaction of group X days, on day 1 there was a significant difference among the groups [$F(7, 48) = 11.11$; $p<0.01$], the groups that received apomorphine ata dose of 0.05 mg/kg before being placed in the arena had lower locomotion than all other groups(Tukey's post hoc test; $p<0.05$). There was no significant difference between the groups that received vehicle only and the groups that received morphine at a dose of 10 mg/kg (Tukey's posthoc test; $p>0.05$). On day 2 [$F(7, 48) = 9.0$; $p<0.01$], the results showed that the VEH+MOR groups had higher locomotion than the APO groups ($p<0.05$). On day 3 [$F(7, 48) = 32.50$; $p<0.01$],day 4 [$F(7, 48) = 51.70$; $p<0.01$], and day 5 [$F(7, 48) = 84.0$; $p<0.01$], the VEH+MOR groups had higher locomotion than all other groups ($p<0.05$). The APO, APO+MOR and APO+VEH groups had significantly lower locomotion than all other groups ($p<0.01$). For both VEH+MOR groups, there was a significant increase in locomotion across the days of the induction phase ($p<0.01$).

Figure 3 shows the locomotor activity during the final test. According to a one-way ANOVA, there was a significant difference among the groups [$F(7, 48) = 106.43$; $p<0.01$], and the VEH+MOR/VEH+MOR and VEH+MOR/VEH+VEH groups had higher locomotion than the other groups (Tukey's post hoc test; $p<0.01$). These findings suggest that the conditioned

morphine locomotor stimulant response was equivalent to the morphine induced locomotor stimulantresponse. The APO+MOR/VEH+MOR group had the highest locomotion of all other groups ($p<0.05$), except the APO+MOR/VEH+VEH group, which was not significantly different ($p>0.05$). There was no significant difference between the APO+MOR/VEH+MOR group and theAPO+MOR/VEH+VEH group ($p>0.05$). In addition, rats from the APO+MOR/VEH+VEH group had higher locomotion than the VEH+VEH/VEH+VEH group ($p<0.05$). There was neither a significant difference among the APO+MOR/VEH+VEH-, APO+VEH/VEH+VEH-, APO+VEH/VEH+MOR- and VEH+VEH/VEH+MOR-treated groups ($p>0.05$), nor among the VEH+/VEH+VEH-, APO+VEH/VEH+VEH-, APO+VEH/VEH+MOR- and VEH+VEH/VEH+MOR-treated groups ($p>0.05$).

Figure 4 shows the values for ERK immunoreactive nuclei for the ventral tegmental area. Regarding the number of Phosphor ERK protein immunoreactive nuclei (Fig. 4A), according to aone-way ANOVA, there was a significant effect of the treatment [$F(7, 48) = 89.01$; $p<0.01$]. VEH+MOR/VEH+MOR and VEH+MOR/VEH+VEH groups had a significantly higher number of phosphors ERK immunoreactive nuclei than all other groups (Tukey's post hoc test; $p<0.01$). Figures 4B-D show examples of the sections used for counting phosphorylated-ERK- immunoreactive cells in the VTA following VEH, MOR, and APO administration.

Figure 5 shows the values for ERK immunoreactive nuclei in the nucleus accumbens. Regarding the number of Phosphor ERK immunoreactive nuclei (Fig. 5A), according to a one- way ANOVA, there were significant differences among the treatments [$F(7, 48) = 94.91$; $p<0.01$]. VEH+MOR/VEH+MOR and VEH+MOR/VEH+VEH groups had a significantly higher number of phosphor ERK immunoreactive nuclei than all the other groups (Tukey's post hoc test; $p <$

0.01). Figures 4B-D show examples of the sections used for counting phosphorylated-ERK- immunoreactive cells in the NAc following VEH, MOR, and APO administration.

3.2. Experiment 2: Effect of a low dose of apomorphine on the expression of conditioned morphinelocomotor stimulant effects, and ERK activity in VTA and NAc.

Figure 6 shows the locomotor activity over the course of days 1-5 induction phase. According to a repeated two-way ANOVA, there was a significant effect of interaction between groups X days [$F(12, 96) = 15.34$; $p<0.01$], of groups [$F(3, 24) = 33.32$; $p<0.01$], and of days oftreatment [$F(4, 96) = 9.52$; $p<0.01$]. According to one-way ANOVAs followed by Tukey's post hoc test on day 1, there was no significant difference among the groups [$F(3, 24) = 0.30$; $p>0.05$].On day 2 [$F(3, 24) = 30.0$; $p>0.05$], day 3 [$F(3, 24) = 33.0$; $p<0.01$], day 4 [$F(3, 24) = 23.45$; $p<0.01$], and day 5 [$F(3, 24) = 37.61$; $p<0.01$], the VEH+MOR-treated groups had significantly higher locomotion than all of the VEH-treated groups (Tukey's post hoc test; $p<0.01$).

Figure 7 shows the locomotor activity during the final test. According to a one-way ANOVA, showed that there was a significant effect of treatment [$F(3, 24) = 120.0$; $p<0.01$]. The VEH+MOR/VEH+VEH-treated group had the highest locomotion score (Tukey's post hoc test; $p<0.01$), indicative of a strong conditioned morphine response. There was no significant difference between the VEH+VEH/VEH+VEH- and VEH+MOR/APO+VEH-treated groups ($p>0.05$), indicating a complete blockade of the expression of the morphine-induced conditioned response.

Figure 8 shows the ERK values for the ventral tegmental area and for the nucleus accumbens. Regarding the number of Phosphor ERK protein immunoreactive nuclei for the VTA(Fig. 8A), according to a one-way ANOVA, there was a significant effect of

treatment [F (3, 24) /= 8.0; p<0.01]. The VEH+MOR/VEH+VEH-treated group had a higher number of phosphors ERKimmunoreactive nuclei than all other groups (Tukey's post hoc test; p<0.01) and the APO pretreatment blocked the ERK response. Considering the number of Phosphor ERK protein immunoreactive nuclei for the NAc (Fig. 8C), according to a one-way ANOVA, there was a significant effect of treatment [F (3, 24) = 11.50; p<0.01]. The VEH+MOR/VEH+VEH-treated group had a higher number of phosphors ERK immunoreactive nuclei than all other groups (Tukey's post hoc test; p<0.01), and the APO pretreatment blocked the ERK response. Figures 8Band 8D show examples of the sections used for counting phosphorylated-ERK-immunoreactive cells in the VTA and NAc following VEH, MOR, and APO administration.

3.3. Experiment 3: Acute 1-day experiment.

Figure 9 shows the locomotor activity during the one-day test session. According to a one-way ANOVA, there was a significant effect of treatment [F (3, 20) = 69.30; p<0.01]. The VEH+VEH- and VEH+MOR-treated groups had higher locomotion than the APO+VEH- and APO+MOR-treated groups (Tukey's post hoc test; p<0.01). Importantly, the VEH+VEH and VEH+MOR groups did not significantly differ in activity levels (p>0.05).

Figure 10 shows the ERK values for the ventral tegmental area and for the nucleus accumbens. Regarding the number of Phosphor ERK protein immunoreactive nuclei for the VTA (Fig. 10A), according to a one-way ANOVA, there was a significant effect of treatment [F (3, 20)
= 48.0; p<0.01]. The VEH+MOR-treated group had a higher number of phosphors ERK immunoreactive nuclei than all the other groups (p<0.01). Regarding the number of Phosphor ERKprotein immunoreactive nuclei for the NAc (Fig. 10C), according to a one-way ANOVA, there was a significant effect of treatment [F (3, 20) = 55.31; p<0.01]. The

VEH+MOR-treated group had a higher number of phosphors ERK immunoreactive nuclei than all the other groups (Tukey's post hoc test; $p<0.01$). Figures 10B and 10D show examples of the sections used for counting phosphorylated-ERK-immunoreactive cells in the VTA and NAc following VEH, MOR, and APO administration.

4. Discussion

In two separate experiments, morphine (10.0 mg/kg) administered immediately prior to 5 min placements in an open field test arena generated a marked hyperactivity. In both experiments, the first morphine treatment had no significant effect on activity level as compared to vehicle controls. Hyperactivity began to emerge by the second treatment with morphine and steadily increased with repeated treatments. In the first experiment, morphine groups that were given a pretreatment with apomorphine (0.05 mg/kg) 10 min before the morphine injection had a substantial reduction in spontaneous activity during the first test session and that reduction persisted unchanged over the course of the five test sessions. Vehicle-treated groups that received the apomorphine pretreatment 10 min before placement in the test arena also had a reduction in spontaneous activity that was indistinguishable from the combined apomorphine/morphine treatment. After completion of these five test sessions, the rats from different groups were given an additional test to assess if the drug induced changes in spontaneous activity were conditioned to the test environment contextual cues. In the conditioning test the groups that received morphine without the apomorphine pretreatment remained hyperactive regardless of whether they received either morphine or vehicle immediately pretest or not, indicating that the morphine-induced hyperactivity response was conditioned to the test arena cues. In contrast, the groups that had received the apomorphine pretreatment with or without morphine were hypoactive throughout the

five-day acquisition phase and when tested for a conditioned drug response exhibited either vehiclelevel activity or were slightly more active than the vehicle-treated groups. Whereas morphine stimulant effects were conditioned to test arena cues the apomorphine response inhibitory effectswere not conditioned.

While the initial acquisition experiment established that apomorphine prevented the development of sensitization and conditioning of morphine-induced hyperactivity, the second experiment was conducted to determine if the apomorphine would also block the expression of the morphine conditioned stimulant effect. As in the first acquisition experiment, morphine administered immediately before testing did not elicit a hyperactivity response on the first test session, but hyperactivity developed progressively over the five test sessions. These morphine- treated groups were then tested for a conditioned stimulant response. One morphine-treated groupreceived the apomorphine pretreatment 10 min pretest and the other morphine group received vehicle 10 min pretest. The morphine-vehicle pretreatment group exhibited a strong conditioned stimulant response whereas the morphine-treated group given the apomorphine pretreatment did not have a conditioned stimulant response, and its behavioral response was equivalent to the vehicle-treated control group. Thus, apomorphine prevented both the acquisition and expression of the morphine conditioned stimulant response.

The rationale for using a test protocol, in which the behavioral testing is done immediately post-injection and is brief (5 min), was developed in our recent study [8]. The behavioral and ERKeffects of the morphine treatments in the vehicle morphine groups in the present report essentiallyreplicate this previous report. While no unpaired morphine groups were used in our experiments, we have shown previously [11] that the same morphine treatments (10.0 mg/kg) given unpaired 15 min after a 5 min test session are not significantly different from vehicle injections given

immediately pretest and do not generate hyperactivity effects to test either cues or morphine. Thus,the immediate morphine pretest injection stimulant effects are not context independent sensitization effects.

Ostensibly, the present conditioning findings do not readily fit into a Pavlovian drug conditioning framework. That is, morphine did induce a strong behavioral stimulant response butinitially did not elicit an unconditioned behavioral stimulant drug response so that there was no initial unconditioned behavioral response to be associated with the test arena cues. The ERK findings, however, appear to have relevance to the morphine conditioning effects. That is, while there was no behavioral response elicited by morphine in the first arena test session in the first twoexperiments, the ERK findings obtained in experiment three, the acute treatment-related experiment, showed that there was a strong ERK response evoked during the first test session, in which no behavioral response was elicited. From a conditioning perspective, the ERK response could be considered an unconditioned response. This unconditioned response could be viewed in terms of either instrumental or Pavlovian conditioning. When considered in terms of instrumental conditioning, the ERK activation in the VTA could be seen as the elicitation of a reward response that served to reinforce and enhance the preceding behavioral activity and possibly even exertingthis effect on the behavioral trace during the post-trial consolidation process. In line with this latterconsideration, we have demonstrated in several previous reports [8, 9, 11] that morphine (10 mg/kg) administered immediately after a brief 5 min exposure to a test arena induced a marked behavioral stimulant response, whereas the same morphine treatment given 15 min after removal from the test arena was without effect [11]. This analysis seems to align with an operantconditioning process wherein morphine administered after a lever press response increases the frequency of the response as the morphine reward effect is associated with the lever press response

trace. The response trace to a lever press response is brief [15]. In that the lever press response trace is occurring in the context of ongoing behavior responses, the lever press trace would quickly be replaced by subsequent occurring response traces, so the reinforcement necessarily needs to beprecisely timed to immediately follow the emission of the lever press response. In the present open-field testing arrangement, the initial 5 min sensory/motor exploration of the open-field test arena occurs concurrently with the activation of VTA and NAc as manifested in the ERK activation. Thus, it can be argued that this apparent activation of reward systems during this initial locomotoractivation serves to reinforce and strengthen this behavior analogous to a conventional instrumental reinforcement [3, 4].

In contrast, to conventional instrumental conditioning in which the experimenter delivers the reward following a specified response in this open-field test drug treatment arrangement, the drug treatment is administered so the drug induced activation of reward systems occurs during a prepotent locomotor exploration response. This arrangement seemingly creates the opportunity fora Pavlovian/Instrumental conditioning fusion. The drug induced Pavlovian unconditioned response of activation of reward brain areas becomes associated with the test arena cues and consequently can reinforce and strengthen the associated occurring behavior. Consequently, the conditioning effects occur as both locomotor stimulation and ERK activation of selected brain reward areas. This creates an interesting situation in which the non-drug conditioning tests wouldappear to be able to function as additional drug treatment sessions. Indeed, in the conditioning tests we performed either with or without morphine, both the locomotor and ERK responses evoked were equivalent. When considered in this way, it would seem possible that the initial drug induced behavior could, following conditioning, be maintained independent from further drug use. This is

an issue of both theoretical importance as well as pragmatic importance from the perspective of drug addiction.

In the groups treated with apomorphine, a strong inhibitory response was elicited with the first drug treatment so this inhibitory response seemingly could become associated with the arenatest cues. This inhibitory response, however, was not conditioned. In that low dose apomorphine decreases dopamine activity broadly [7], including sensory/motor and reward systems, the absenceof inhibitory conditioning is not surprising as attention to arena cues would be severely suppressedand essentially would be equivalent to not being placed in the test arena. Indeed, the performance of the apomorphine-treated groups in the conditioning tests did not differ from the performance ofthe vehicle groups during their first test session.

The ERK results were consistent with the behavioral results in that the morphine stimulant effects were associated with a marked ERK response in the VTA and NAc, and these ERK responses were impaired by the apomorphine pretreatments that eliminated the morphine stimulantresponses. Thus, the apomorphine pretreatments eliminated both the behavioral effects of the morphine treatment, as well as the morphine activation of the ERK response in the VTA and NAc.In all three experiments, pretreatments with apomorphine at low dose prevented morphine-inducedincreases in ERK activity in the VTA and NAc. Together, these findings are consistent with the dependence of morphine GABAergic disinhibitory effects on the level of ongoing activity in the dopaminergic system.

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Figure captions

Fig. 1. Timeline and scheme of injections.

Fig. 2. Distance scores (M) during the induction phase of experiment 1. Data were represented asmean and S.E.M.; ** p<0.01 (higher locomotion) in comparison to all other groups; * p<0.05 in comparison to APO-treated groups; + p<0.05 (lower locomotion) in comparison to all other groups;# denotes a significant difference (p<0.05) between the first and the last day of the induction phasefor the same experimental group, according to the repeated measure two-way ANOVA followed by Tukey's post hoc test.

Fig. 3. Distance scores (M) during the final test of experiment 1. Data were represented as mean and S.E.M.; ** p<0.01 (higher locomotion) in comparison with all other groups; & denotes the second higher locomotion score (p<0.05) except for the APO+VEH/VEH+VEH-treated group; § p<0.05 in comparison to VEH+VEH/VEH+VEH-treated group, according to a one-way ANOVAfollowed by Tukey's post hoc test.

Fig. 4. ERK activation in the ventral tegmental area (VTA) from experiment 1. **A:** Quantificationof immunohistochemical labeling for ERK phosphorylation in the VTA. Data represent the mean

± S.E.M.; ** p<0.01 in comparison to the other groups, according to one-way ANOVA followed by Tukey's post hoc test. **B-C:** Modified drawing of a brain parasagittal section displaying, at thevertical black bar, the location of the coronal midbrain section, obtained from the Paxinos and Watson atlas (2005). **D:** Representative photomicrographs of low (10X) and high (40X inserts)

magnification images of ERK-P-immunoreactive cells. The scale bar indicates 100 µm in the lowmagnification images.

Fig. 5. ERK activation in the nucleus accumbens (NAc) from experiment 1. **A:** Quantification of immunohistochemical labelling for ERK phosphorylation in the nucleus accumbens (NAc). Data represent the mean ± S.E.M.; ** p<0.01 in comparison to the other groups, according to a one-wayANOVA followed by Tukey's post hoc test. **B-C:** Modified drawing of sagittal section of the braindisplaying, at the vertical black bar, the location of the coronal section of forebrain, obtained fromthe Paxinos and Watson atlas (2005). **D:** Representative photomicrographs of low (10X) and high(40X inserts) magnification images of ERK-P-immunoreactive cells. The scale bar indicates 100 µm in the low magnification images.

Fig. 6. Distance scores (M) during the induction phase from experiment 2. Data were representedas mean and S.E.M.; ** denotes higher locomotion ($p < 0.01$) than all the other groups; # denotes asignificant difference ($p < 0.05$) between the first and the last day of the induction phase for the same experimental group, according to the repeated measure two-way ANOVA followed by Tukey's post hoc test.

Fig. 7. Distance scores (M) during the final test of experiment 2. Data were represented as mean and S.E.M.; ** denotes higher locomotion ($p < 0.01$) than all the other groups; + denotes lower locomotion ($p < 0.05$) than all the other groups, according to a one-way ANOVA followed by Tukey's post hoc test.

Fig. 8. ERK activation in the ventral tegmental area (VTA) and in nucleus accumbens (NAc) from experiment 2. **A:** Quantification of immunohistochemical labelling for ERK phosphorylation in the VTA. **C:** Quantification of immunohistochemical labeling for ERK phosphorylation in the NAc. Data represent the mean ± S.E.M.; ** denotes higher numbers ($p<0.01$) of immunoreactive nuclei than the other groups, according to a one-way ANOVA followed by Tukey's post hoc test. **B-D:** Representative photomicrographs of low (10X) and high (40X inserts) magnification images of ERK-P-immunoreactive cells. The scale bar indicates 100 µm in the low magnification images.

Fig. 9. Distance scores (M) during the acute one-day experiment (experiment 3). Data represent the mean ± S.E.M.; ** denotes higher locomotion than all the other groups ($p<0.05$; one-way ANOVA followed by the Tukey test).

Fig. 10. ERK activation in the ventral tegmental area (VTA) and in nucleus accumbens (NAc) from experiment 3. **A:** Quantification of immunohistochemical results for ERK phosphorylation in the VTA. **C:** Quantification of immunohistochemical labeling for ERK phosphorylation in the NAc. Data represent the mean ± S.E.M.; ** denotes higher numbers ($p<0.01$) of immunoreactive nuclei than in the other groups, according to a one-way ANOVA followed by Tukey's post hoc test. **B-D:** Representative photomicrographs of low (10X) and high (40X inserts) magnification images of ERK-P-immunoreactive cells. The scale bar indicates 100 µm in the low magnificationimages.

Highlights

- Acute MOR did not increase locomotion but repeated MOR induced hyperactivity.
- Conditioned MOR hyperactivity was equivalent to MOR induced hyperactivity.
- APO pretreatment prevented the development and expression of MOR conditioning.
- Acute MOR and conditioned MOR increased ERK activation in the VTA and NAc.
- All MOR induced increases in ERK activity were eliminated by the APO pretreatment.

Figure 1: Timeline and scheme of injections.

A: Timeline – Experiment 1 and 2.

Days	1	2	3	4	5	6	7	8	9
	Habituation			Induction Phase					Final Test

B: Timeline – Experiment 3.

Days	1	2	3	4
	Habituation			One Test Session

C: Scheme of injections.

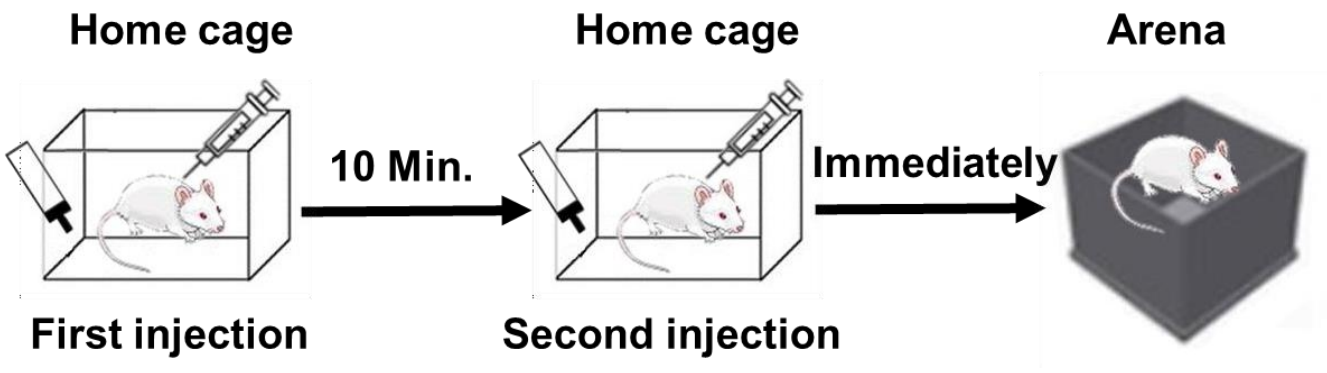


Table 1

Table 1: Groups of experiment 1.

Induction Phase 5 Days	Final Test 1 Day	Groups Induction Phase / Final Test
VEH + VEH	VEH + VEH	VEH+VEH / VEH+VEH (n=7)
VEH + VEH	VEH + MOR	VEH+VEH / VEH+MOR (n=7)
VEH + MOR	VEH + VEH	VEH+MOR / VEH+VEH (n=7)
VEH + MOR	VEH + MOR	VEH+MOR / VEH+MOR (n=7)
APO + VEH	VEH + VEH	APO+VEH / VEH+VEH (n=7)
APO + VEH	VEH + MOR	APO+VEH / VEH+MOR (n=7)
APO + MOR	VEH + VEH	APO+MOR / VEH+VEH (n=7)
APO + MOR	VEH + MOR	APO+MOR / VEH+MOR (n=7)

VEH=Vehicle; MOR=Morphine 10 mg/kg; APO=Apomorphine 0.05 mg/kg.

Table 1

Table 2: Groups of experiment 2 (A) and 3 (B).

A

Induction Phase 5 Days	Final Test 1 Day	Groups Induction Phase / Final Test
VEH + VEH	VEH + VEH	VEH+VEH / VEH+VEH (n=7)
VEH + VEH	APO + VEH	VEH+VEH / APO+VEH (n=7)
VEH + MOR	VEH + VEH	VEH+MOR / VEH+VEH (n=7)
VEH + MOR	APO + VEH	VEH+MOR / APO+VEH (n=7)

B

One Test Session	Groups
VEH + VEH	VEH+VEH (n=6)
VEH + MOR	VEH+MOR (n=6)
APO + VEH	APO+VEH (n=6)
APO + MOR	APO+MOR (n=6)

VEH=Vehicle; MOR=Morphine 10 mg/kg; APO=Apomorphine 0.05 mg/kg.

FIG. 2

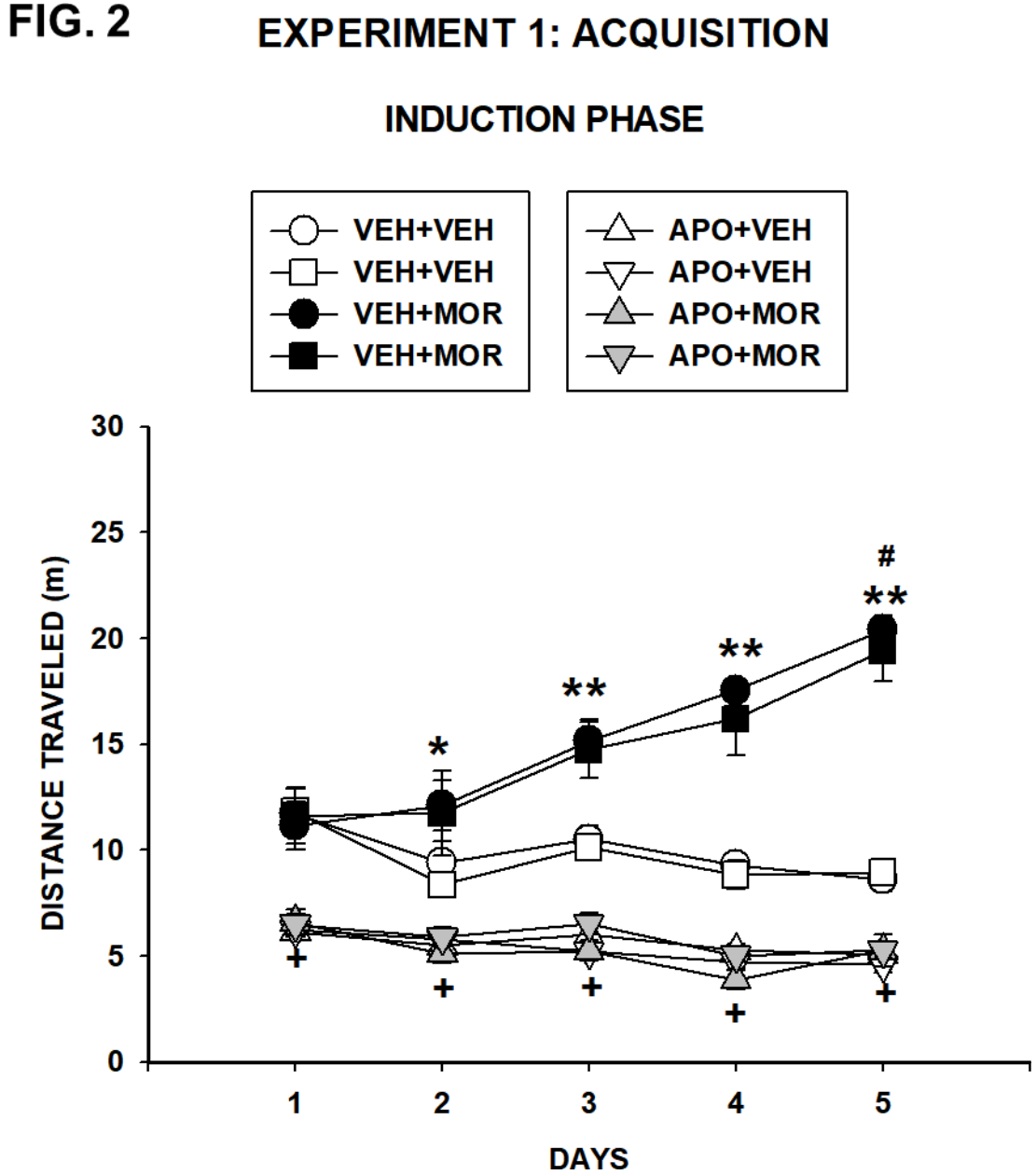

EXPERIMENT 1: ACQUISITION
INDUCTION PHASE
VEH+VEH
VEH+VEH
VEH+MOR
VEH+MOR
APO+VEH
APO+VEH
APO+MOR
APO+MOR
DISTANCE TRAVELED (m)
30
25
20
15
10
5
0
1
2
3
4
5
DAYS
*
**
**
**
#
+
+
+
+
+

Fig. 3

EXPERIMENT 1: ACQUISITION

FINAL TEST

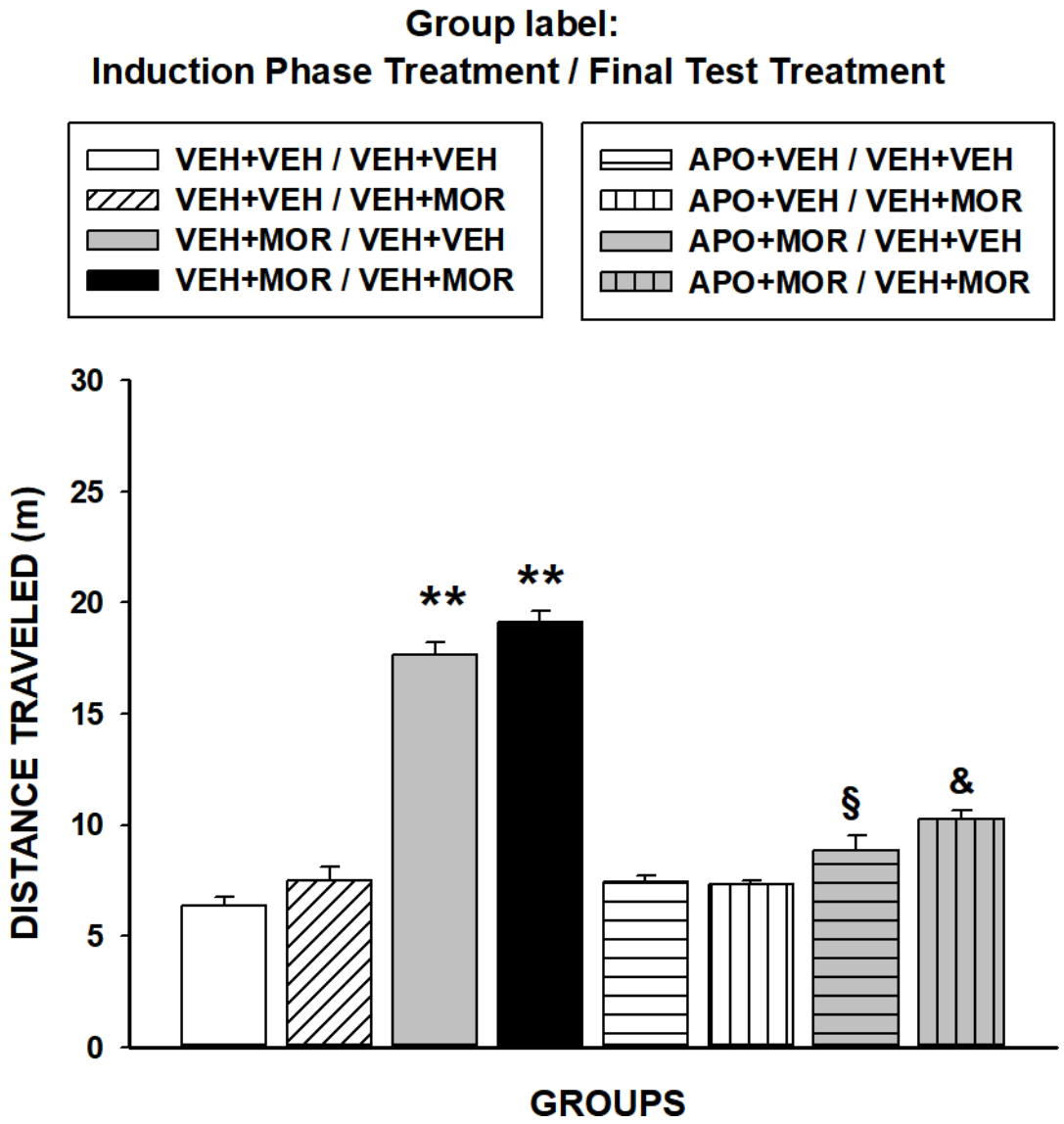


FIG. 4

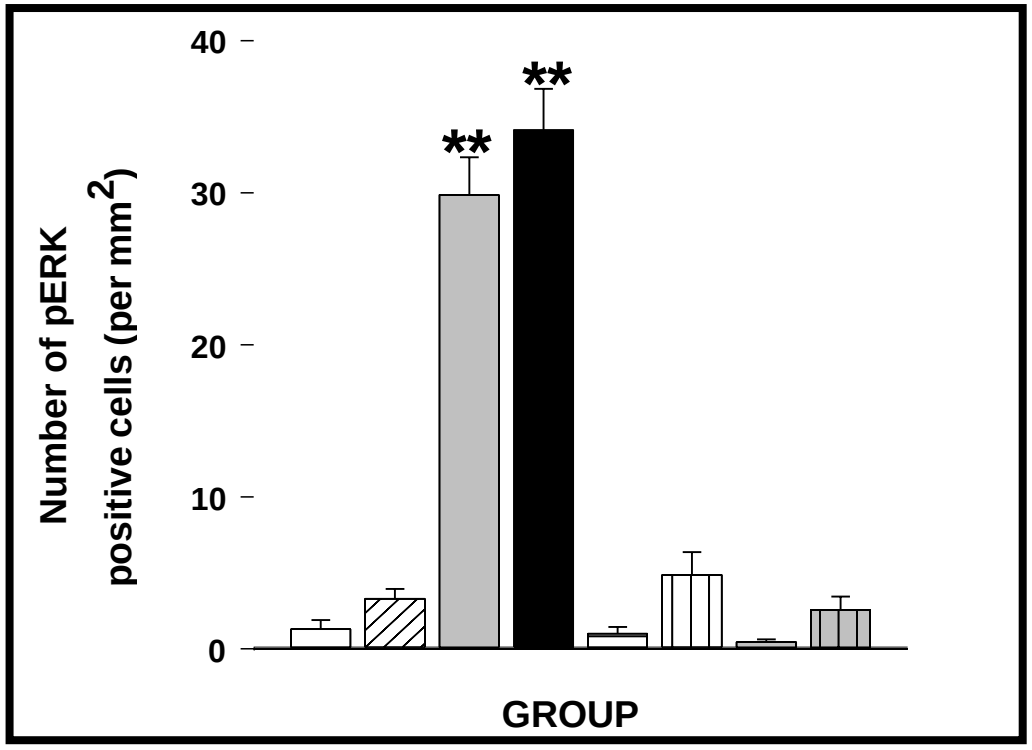
EXPERIMENT 1: ACQUISITION

ERK ACTIVATION - VTA

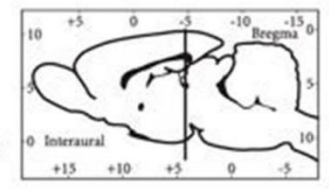
Group label:
Induction Phase / Final Test

- VEH+VEH / VEH+VEH
- VEH+VEH / VEH+MOR
- VEH+MOR / VEH+VEH
- VEH+MOR / VEH+MOR
- APO+VEH / VEH+VEH
- APO+VEH / VEH+MOR
- APO+MOR / VEH+VEH
- APO+MOR / VEH+MOR

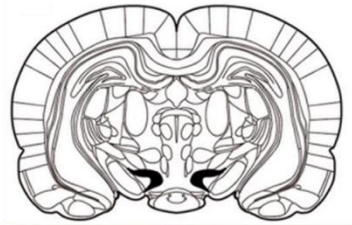
A



B



C



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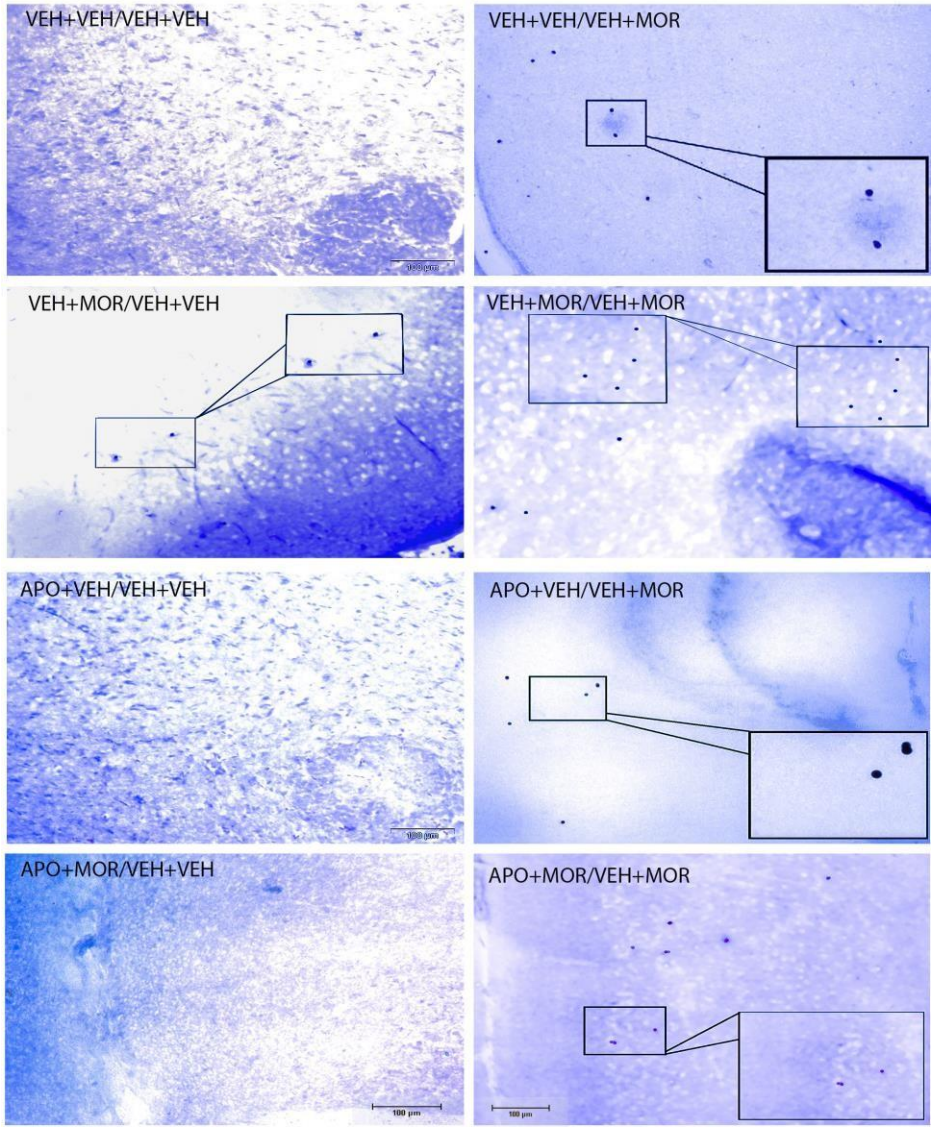


FIG. 5

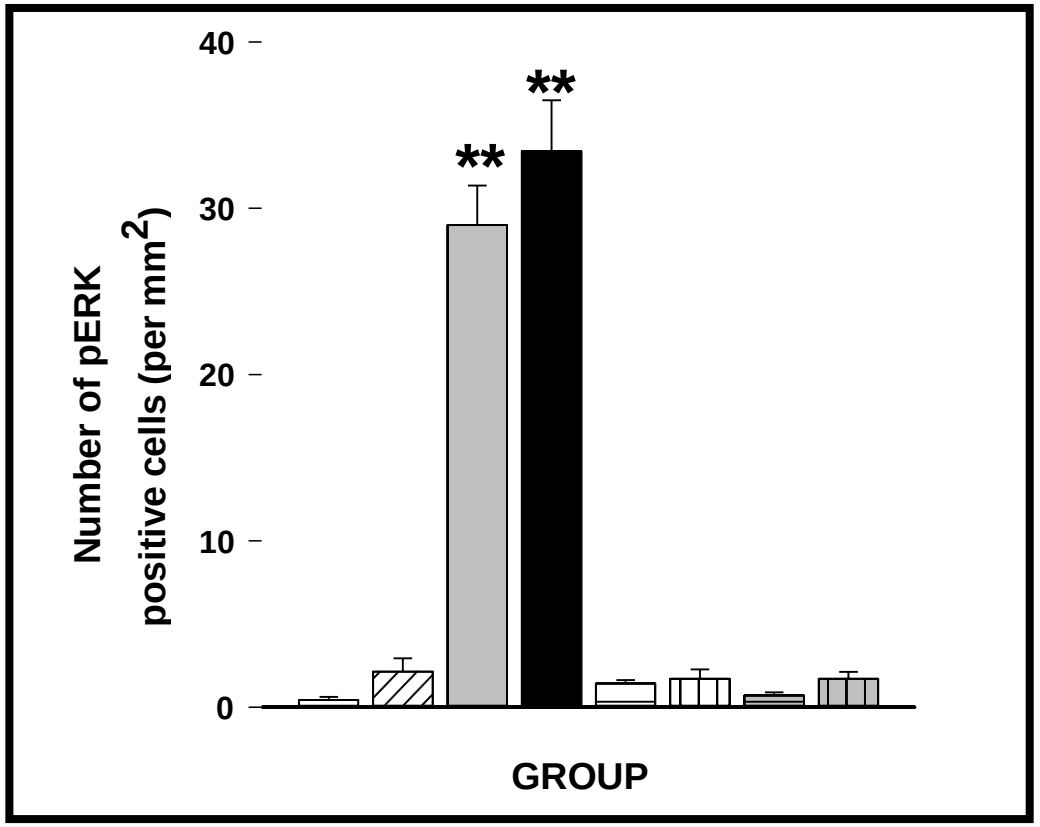
EXPERIMENT 1: ACQUISITION

ERK ACTIVATION - NAc

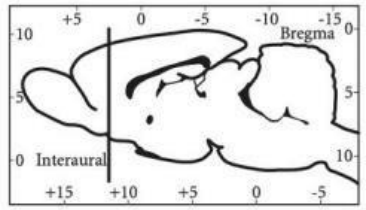
Group label:
Induction Phase / Final Test

- VEH+VEH / VEH+VEH
- VEH+VEH / VEH+MOR
- VEH+MOR / VEH+VEH
- VEH+MOR / VEH+MOR
- APO+VEH / VEH+VEH
- APO+VEH / VEH+MOR
- APO+MOR / VEH+VEH
- APO+MOR / VEH+MOR

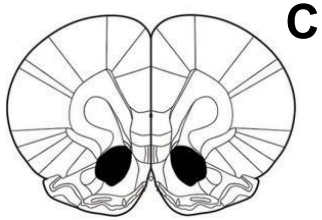
A



B



C



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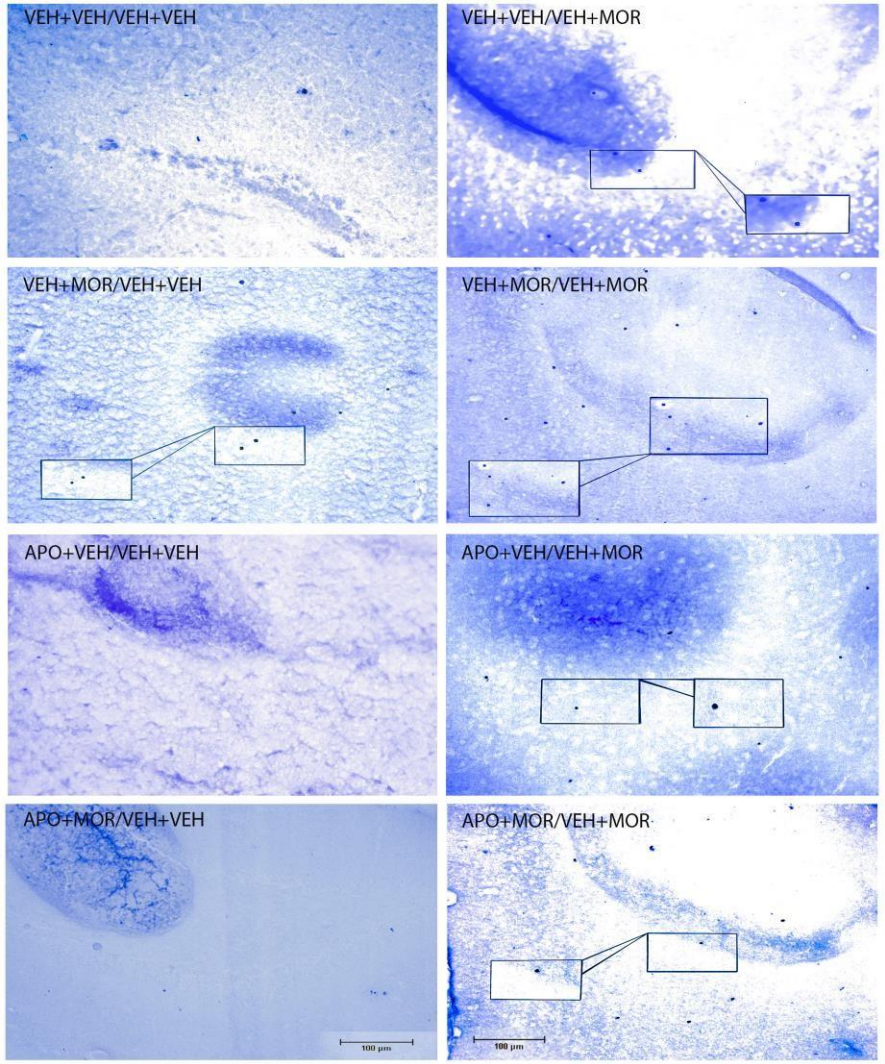


FIG. 6

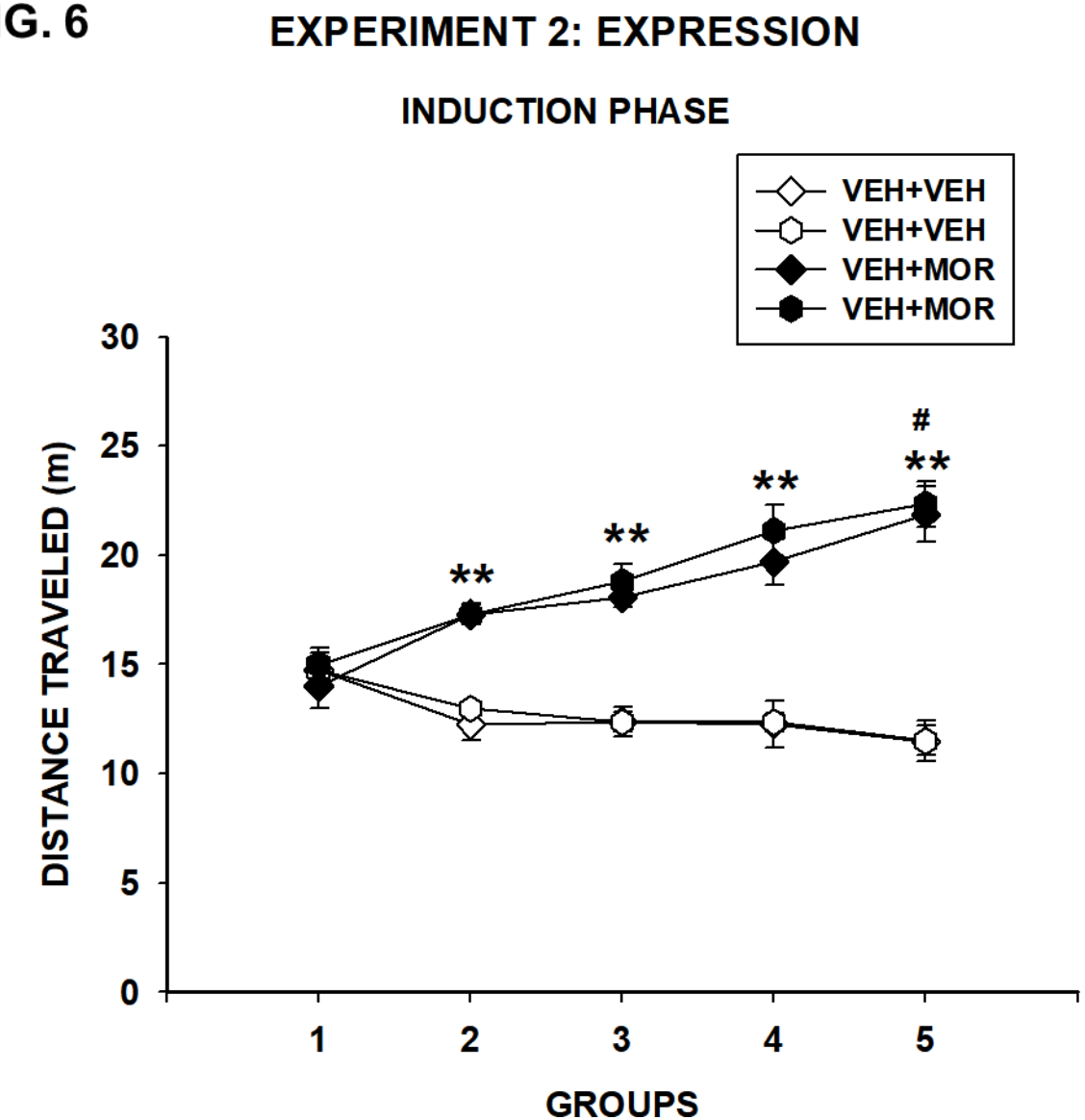

EXPERIMENT 2: EXPRESSION
INDUCTION PHASE
VEH+VEH
VEH+VEH
VEH+MOR
VEH+MOR
DISTANCE TRAVELED (m)
30
25
20
15
10
5
0
**
**
**
#
**
1
2
3
4
5
GROUPS

FIG.7

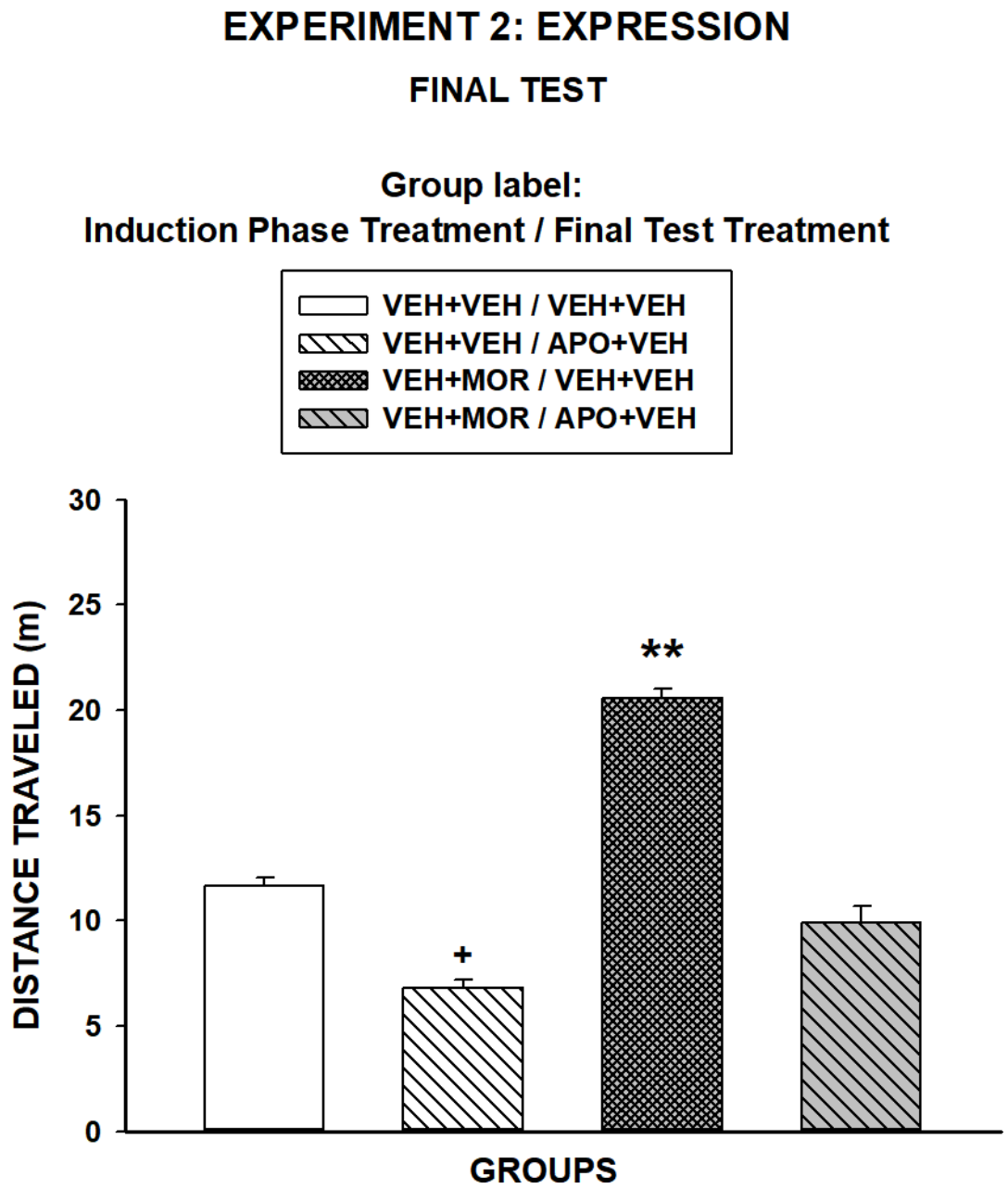
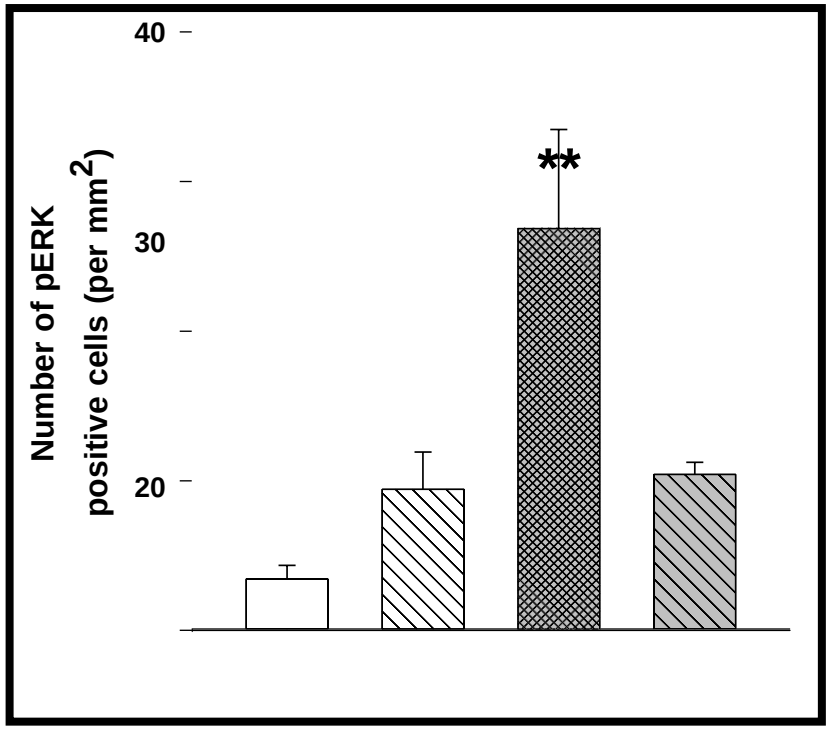

EXPERIMENT 2: EXPRESSION
FINAL TEST
Group label:
Induction Phase Treatment / Final Test Treatment
VEH+VEH / VEH+VEH
VEH+VEH / APO+VEH
VEH+MOR / VEH+VEH
VEH+MOR / APO+VEH
DISTANCE TRAVELED (m)
30
25
20
15
10
5
0
+
**
GROUPS

FIG. 8

EXPERIMENT 2: EXPRESSION - ERK ACTIVATION

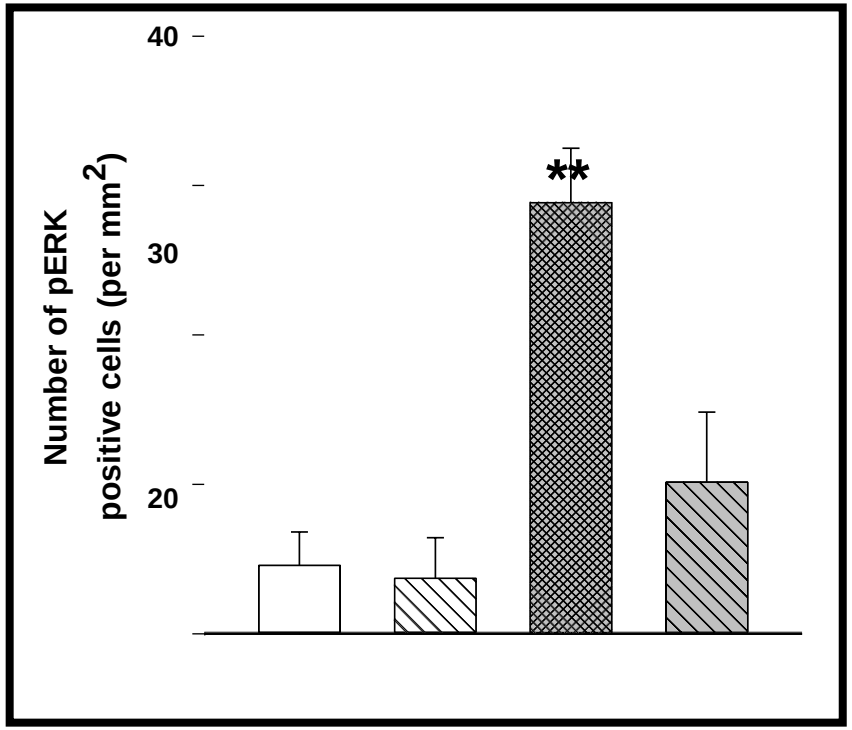
A

VTA

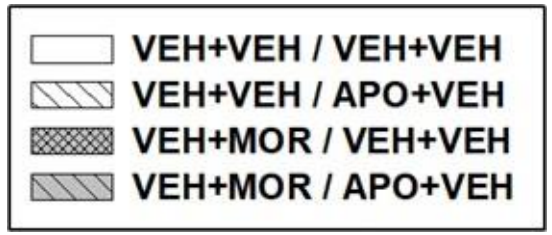


C

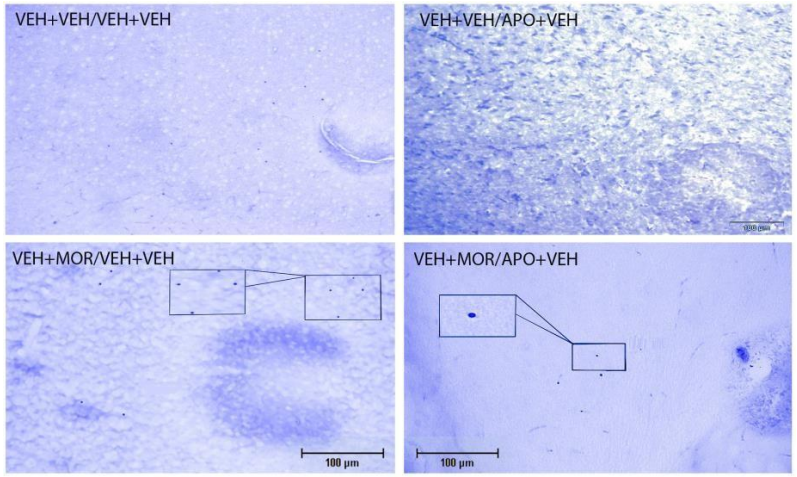
NAc



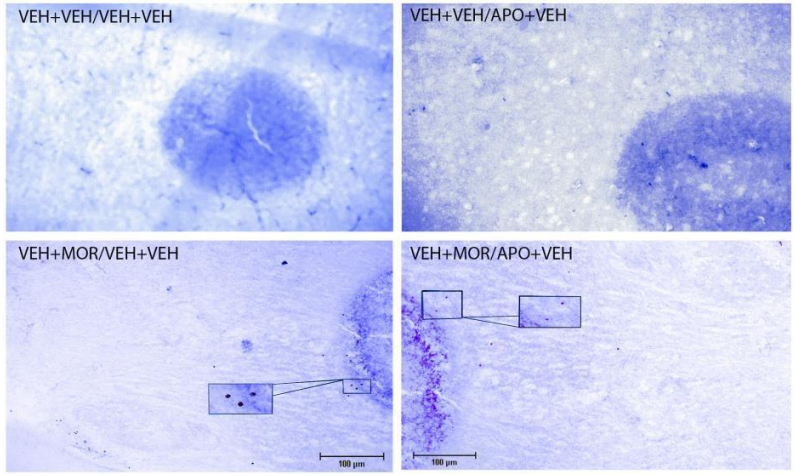
Group Legends:
Induction Phase / Final Test



B



D



1

FIG. 9

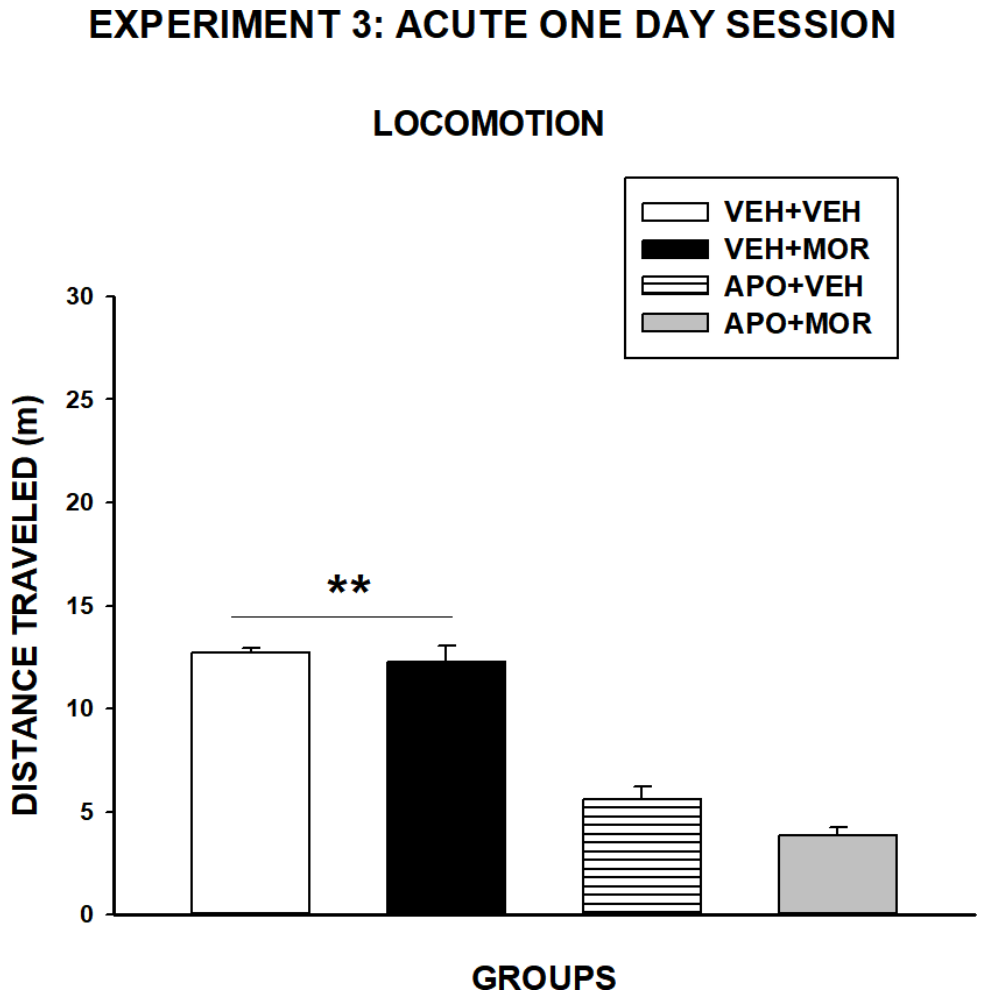
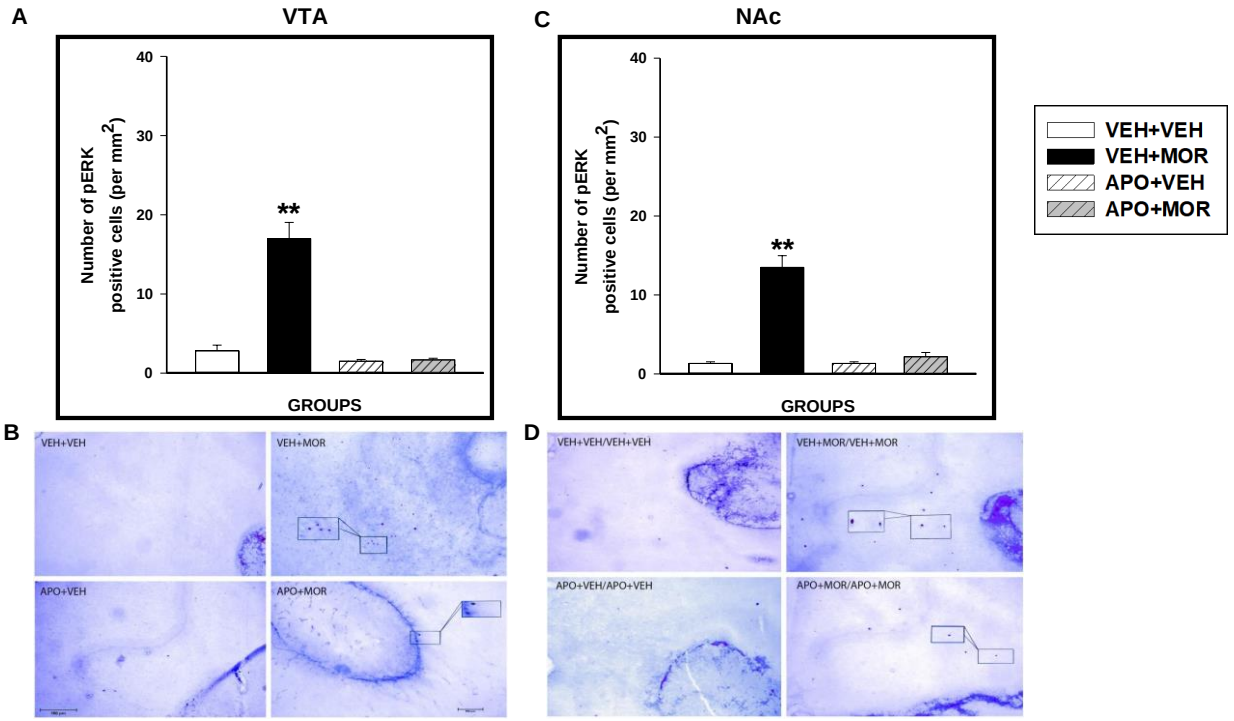

EXPERIMENT 3: ACUTE ONE DAY SESSION
LOCOMOTION
VEH+VEH
VEH+MOR
APO+VEH
APO+MOR
DISTANCE TRAVELED (m)
30
25
20
15
10
5
0
**
GROUPS

FIG. 10 EXPERIMENT 3: ACUTE ONE DAY SESSION - ERK ACTIVATION



5.2 – Capítulo II

MK-801 induces dose dependent stimulant sensitization effects but dose independent conditioned stimulant effects: MK-801 effects on sensory information processing versus learning and memory.

Joaquim Barbosa Leite Junior1, João Marcos de Mello Bastos1, Flávia Regina Cruz Dias1, Richard Ian Samuels2, Robert J Carey3 and Marinete Pinheiro Carrera1

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Abstract

Rationale: The NMDA receptor antagonist MK-801 can prevent sensitization induced by dopaminergic stimulant drugs and also induces sensitization.

Objectives: To determine whether repeated MK-801 treatments can induce dose dependent locomotor sensitization and conditioned effects.

Methods: Initially, rats received vehicle (VEH) prior to the 30 minutes open-field sessions for 3 days (habituation) and locomotion was measured. Afterwards, groups were formed with comparable locomotion and the pharmacological phase was initiated with the treatments administered immediately prior to the five daily 30 min. test sessions. Three groups received one of the three MK-801 doses (0.025, 0.1 and 1.0 mg/kg) and one group received VEH. Two days later there was a conditioning test where rats received VEH immediately prior to a single 5 min. (experiment 1) test session or a 30 min. test session (experiment 2).

Results: A habituation response occurred in all groups. In experiment 1, sensitization effects were induced in the 1.0 mg/kg MK-801 group but not in the 0.025 mg/kg group. Surprisingly, equivalent conditioned effects were observed in all MK-801 groups. Experiment 2 confirmed the responses obtained in experiment 1. Additionally, the conditioned and habituation day 3 locomotor responses were equivalent in all MK-801 groups, whereas for the VEH group, the conditioned response was lower than on habituation day 3.

Conclusions: We relate the conditioned response to sensory neglect effects induced by MK-801. The impact of MK-801 on the sensory processes is independent of the motor stimulant responses and can mimic effects on memory.

Key-words: MK-801, NMDA receptor, motor stimulation, learning and memory, sensory neglect, drug stimuli.

Introduction

The importance of the basal ganglia in movement processes has long been established. Indeed, it is the interplay between glutamate and dopamine (DA) systems in the basal ganglia that mediates normative movement wherein glutamatergic inhibitory activity is balanced by dopaminergic excitatory activity (Dai and Carey 1995). Thus, excessive movement can occur either by decreasing glutamate activity or increasing dopamine activity. Numerous studies have shown that in rats direct as well as indirect acting dopamine agonists can induce locomotor hyperactivity and that these effects undergo conditioning and sensitization (Anagnostaras and Robinson 1996; Bloise *et al*. 2007; Braga *et al*. 2009; de Matos *et al*. 2010; Keller and Delius 2001; Mattingly *et al*. 1997, 1988; Rowlett *et al*. 1997). Similarly, antagonism at the glutamate N-methyl-D-aspartate (NMDA) receptor by drugs such as dizocilpine (MK-801) can induce hyperactivity in rats and that with repeated treatments this hyperactivity becomes exaggerated, indicative of sensitization (Carey *et al*. 1995).

Interestingly, the sensitization and conditioned effects induced by dopamine agonists can be prevented if the glutamate NMDA receptor non-competitive antagonist MK-801 is given in combination with DA agonists such as amphetamine and cocaine (Stewart and Druhan 1993; Vezina and Queen 2000; Zweifel *et al*. 2008), including the hyper motoric stimulation induced by L-DOPA in an animal model of Parkinson's (Pinheiro-Carrera *et al*. 1995). In that behavioral drug sensitization and conditioning are persistent and lasting effects, and that NMDA receptors are important for neuronal changes such as long-term potentiation (LTP), drug induced activation of the NMDA receptor site has provided a possible explanation for drug sensitization and conditioning. While a substantial body of evidence supports the efficacy of NMDA antagonists in preventing the development of sensitization to other drugs, it is also the case that repeated MK-801 treatments can induce MK-801 behavioral sensitization effects (Carey *et al*. 1995). Thus, while MK-801 can prevent the sensitization induced by dopaminergic stimulant drugs it also induces behavioral stimulant effects that also undergo sensitization with repeated treatments. Thus, MK -801 has the seemingly puzzling capability to both prevent and induce stimulant sensitization

effects. In that MK-801 can prevent the Pavlovian conditioning of dopaminergic drug induced hyperactivity (Damianopoulos and Carey 1995), the question arises as to whether MK-801 induced hyperactivity can undergo Pavlovian conditioning as occurs with dopamine agonist induced hyperactivity. Specifically, the present study was undertaken to determine whether repeated MK-801 treatments that can induce dose dependent behavioral stimulant sensitization effects and also induce conditioned stimulant effects. In this study, a replication experimental design was used in which the experiment was conducted twice in two separate independent experiments to enhance the reliability of the findings.

Methods

Subjects

Male Wistar albino rats provided by the State University of North Fluminense Darcy Ribeiro, initially weighing 200-300 g, were housed in individual plastic cages (25 X 18 X 17 cm) until the end of the experiment. Food and water were always available. The vivarium was maintained at a constant temperature (22 ± 2 ºC) and a 12h/12h light/dark cycle (lights on at 07:00h and off at 19:00h). All experiments occurred between 8:00 and 12:00h. For 7 days prior to all experimental procedures, each animal was weighed and handled daily for 5 min. This process included being placed in a transport cage and taken to the injection administration bench located in an anteroom adjacent to the experimental testing room. The animals were given vehicle (VEH) injections. In this way the animals were familiarized with the handling and injection aspects of the testing protocol. The experimental procedures were in accordance with the recommendations of the Commission of Ethics in Animal Experimentation (CEUA) of the University of North Fluminense Darcy Ribeiro-UENF (process 395/2018). These recommendations are in agreement with the ethical principles of animal research adopted by the Brazilian College of Animal Experimentation (COBEA) and by the National Council for Animal Experimentation Control (CONCEA).

Drugs

Dizocilpine [(+)-MK-801;+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate]—further referred to as MK-801—was purchased from Sigma–Aldrich (Sigma, St Louis, USA) was dissolved in saline (0.9% NaCl solution) at doses of 0.025 mg/kg, 0.1 mg/kg and 1.0 mg/kg (Carey *et al*. 1998; Frantz and Van Hartesveldt 1999; Qi *et al*. 2008). Saline alone was administered to vehicle controls. MK-801 or vehicle was subcutaneously (s.c.) injected in a volume of 1.0 ml/kg body weight.

Apparatus and behavioral measurements

The behavioral measurements were conducted in a black open field chamber (60 X 60 X 45 cm). A closed-circuit camera (IKEGAMI, model ICD-49) mounted 60 cm above the arena was used to record behavioral data. Locomotion, measured as distance traveled (m), was automatically analyzed using software EthoVision (Noldus, the Netherlands). The complete test procedure was conducted automatically without the presence of the experimenter in the test room. All behavioral testing was conducted under dim red light that favors exploratory behavior and avoids the possible aversive quality of white light (Nasello *et al*. 1998) as well as to enhance the contrast between the white subject and the dark background of the test chamber and reduce the animal's shadow.

Experimental procedure

Two similar experiments were conducted. In experiment 1 rats were initially given three 30 min tests in the open field arena on 3 successive days (habituation period). Immediately prior to each of the habituation test days, the subjects

received vehicle (VEH) injections. The locomotor distance scores were used to form four groups (n=5 for each group) with equivalent distance scores. Subsequently the groups were injected with VEH, 0.025, 0.1 or 1.0 mg/kg MK-801 immediately before 30 min test sessions in the open field arena on 5 successive days (pharmacological treatment phase). Following 2 days of non-testing (withdrawal period), all groups were given VEH injections and tested for 5 min to assess possible conditioned drug effects (conditioning test; CT).

Experiment 2 was undertaken because of the finding in experiment 1 that all MK-groups expressed similar conditioned stimulant responses in the conditioning test despite having very different locomotor stimulant responses in the five days of drug treatment phase. In that the number of animals per group in experiment 1 was modest and to have a more substantial conditioning test the protocol used in experiment 1 was repeated and the conditioning test session was expanded to 30 minutes (n=6-7 per group). Table 1 present, respectively, the experimental timeline (A) and the treatment protocols (B).

Statistics

For the drug treatment phase, a repeated two-way analysis of variance (ANOVA) was used to determine the group effect, day effect, as well as the interactions between both factors. When a significant effect of interaction was recorded, data were further statistically evaluated using a one-way ANOVA followed by Tukey's test with $p<0.05$ as the criterion for statistical significance. For the 5 and 30 min conditioning test a one-way ANOVA followed by Tukey's post-hoc test were used with $p<0.05$ as the criterion for statistical significance. For the within-treatment assessment of the behavioral activity during conditioning test of the experiment 2, the total activity score was divided into 6 intervals of 5 min each and a repeated measure two-way ANOVA was used to determine treatments, intervals, and interactions between factors. When a significant effect of treatment group and

intervals was obtained, data were further evaluated by one-way ANOVA followed by Tukey's post-hoc tests using $p<0.05$ as the criterion for statistical significance.

Results

Pre-treatment habituation effects

Prior to the initiation of each experiment, a three-day habituation procedure was conducted and locomotion was measured. In experiment 1, a one-way ANOVA showed there was an effect of days of treatment [$F(2, 63) = 24.0$; $p<0.01$] and the Tukey's test showed that day 1 had higher locomotion than the day 2 and day 3 ($p<0.05$) (data not shown). In experiment 2, a one-way ANOVA showed that there was an effect of days of treatment [$F(2, 69) = 18.0$; $p<0.01$] and the Tukey's test showed that day 1 had higher locomotion than day 2 and day 3 ($p<0.05$) (data not shown).

Experiment 1: 5 minute conditioning test

Figure 1 shows the locomotor activity over the course of days 1-5 of the induction phase of the first experiment. For the total locomotor activity over the course of days 1-5 (induction phase), a repeated two-way ANOVA showed a group X days interaction [$F(12, 72) = 7.64$; $p<0.01$], an effect of groups [$F(3, 18) = 22.25$; $p<0.01$] and an effect of days of treatment [$F(4, 72) = 7.33$; $p<0.01$]. A one-way ANOVA followed by Tukey test to further analyze the interaction of group X days showed that on day 1, the MK-1.0 group had higher locomotion than the VEH group ($p<0.05$). There was no difference among the MK-1.0, MK-0.1 and MK-0.025 groups ($p>0.05$). On days 2-5, the MK-1.0 group had higher locomotion than all other groups ($p<0.05$). However, on day 4 and day 5, the MK-0.1 had

higher locomotion than the VEH group ($p<0.05$). There was no difference between the MK-0.1 and MK-0.025 groups ($p>0.05$).

Figure 2 shows the locomotor activity during the conditioning test for the first experiment. A one-way ANOVA showed that there were differences among the groups [$F(3, 18) = 8.0$; $p<0.01$] and the Tukey test showed that the MK groups, (MK-0.025, MK-0.1 and MK-1.0 groups), had higher locomotion than all VEH groups ($p<0.05$) and were not significantly different from each other ($p> 0.05$).

Experiment 2: 30 minute conditioning test

Figure 3 shows the locomotor activity over the course of days 1-5 of the induction phase of the second experiment. A repeated two-way ANOVA showed an interaction group x day [$F(12, 80) = 7.54$; $p<0.01$], an effect of groups [$F(3, 20) = 50.80$; $p<0.01$] and an effect of days of treatment [$F(4, 80) = 11.25$; $p<0.01$]. A one-way ANOVA followed by the Tukey test to further analyze the interaction of group X days, showed on day 1, that the MK-1.0 group had higher locomotion than the VEH group ($p<0.05$). There was no significant difference among the VEH, MK-0.025 and MK-0.1 groups ($p>0.05$). Also, there was no significant difference among the MK-0.025, MK-0.1 and MK-1.0 groups ($p>0.05$). From day 2 until day 5, the MK-1.0 groups had higher locomotion than all groups ($p<0.05$). However, from day 2 until day 4, the MK-0.1 group had higher locomotion than the VEH group ($p<0.05$). There was no difference between the MK-0.1 and MK-0.25 groups ($p>0.05$) and no difference between the VEH and MK-0.025 groups ($p>0.05$). On day 5, the MK-0.1group had higher locomotion than the VEH and MK-0.025 groups ($p<0.05$). There was no difference between the VEH and MK-0.025 groups ($p>0.05$).

Figure 4 shows the within session locomotor activity during the 5 induction days of the second experiment. For induction day 1 (Fig. 4A), a repeated two-way ANOVA showed an interaction group X interval [$F(15, 100) = 7.32$; $p<0.01$], an effect of groups [$F(3, 20) = 3.35$; $p<0.05$] and an effect of intervals [$F(5, 100) =$

31.53; p<0.01]. A one-way ANOVA followed by the Tukey test to further analyze the interaction showed that on interval 1 [F (3, 20) = 14.63; p<0.01], showed that there was difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than all other groups (p<0.05). On interval 2 [F (3, 20) = 5.0; p<0.01], there was difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than the VEH group (p<0.05). There was no difference among the MK-1.0, MK-0.1 and MK-0.25 groups (p>0.05) and no differences among the VEH, MK-0.1 and MK-0.25 groups (p>0.05). On interval 3 [F (3, 20) = 0.25; p>0.05], interval 4 [F (3, 20) = 1.80; p>0.05], interval 5 [F (3, 20) = 1.90; p>0.05] and interval 6 [F (3, 20) = 1.45; p<0.05], there were no differences among the groups.

For induction day 2 (Fig. 4B), a repeated two-way ANOVA showed an interaction group X interval [F (15, 100) = 5.91; p<0.01], an effect of groups [F (3, 20) = 34.13; p<0.01] and an effect of intervals [F (5, 100) = 22.02; p<0.01]. A one-way ANOVA followed by the Tukey test to further analyze the interaction, showed that on interval 1 [F (3, 20) = 2.22; p>0.05], there was no difference among the groups. On interval 2 [F (3, 20) = 23.53; p<0.01], there was a difference among the groups and Tukey's test showed that the MK-1.0 groups had higher locomotion than all other groups (p<0.05). On interval 3 [F (3, 20) = 16.0; p<0.01], there was a difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than all other groups (p<0.05). The results also showed that the MK-0.1 group had higher locomotion than the VEH, and MK-0.25 groups (p<0.05). There was no difference between the MK-0.1 and MK-0.25 groups (p>0.05) and no difference between VEH and MK-0.25 groups (p>0.05). On interval 4 [F (3, 20) = 1.50; p>0.05], there was no difference among the groups. On interval 5 [F (3, 20) = 8.54; p<0.01], there was difference among the groups and Tukey's test showed that the MK-1.0 groups had higher locomotion than all the other groups (p<0.05), except the MK-0.1 group in which there was no difference between these groups (p>0.05). However, the MK-0.1 group had higher locomotion than the VEH group (p<0.05), but there was no difference between MK-0.1 and MK-0.025 groups (p>0.05) and no difference between the

MK-0.025 and VEH groups ($p>0.05$). On interval 6 [$F(3, 20) = 0.70$; $p>0.05$], there was no significant difference among the groups.

For induction day 3 (Fig. 4C), a repeated two-way ANOVA showed an interaction group X interval [$F(15, 100) = 5.10$; $p<0.01$], an effect of groups [$F(3, 20) = 28.0$; $p<0.01$] and an effect of intervals [$F(5, 100) = 18.30$; $p<0.01$]. A one-way ANOVA followed by the Tukey test to further analyze the interaction, showed that on interval 1 [$F(3, 20) = 20.70$; $p<0.01$], there was a difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than all other groups ($p<0.05$). The results also showed that the MK-0.1 and MK-0.025 groups had higher locomotion than the VEH group ($p<0.05$), there was no difference between the MK-0.1 and MK-0.025 groups ($p>0.05$). On interval 2 [$F(3, 20) = 23.00$; $p<0.01$] and interval 3 [$F(3, 20) = 15.50$; $p<0.01$], the MK-1.0 group had higher locomotion than all other groups ($p<0.05$). On interval 4 [$F(3, 20) = 5.0$; $p<0.01$] and interval 5 [$F(3, 20) = 5.50$; $p<0.01$], there was a difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than all the other groups ($p<0.05$), except the MK-0.1 group. There was no difference between the MK-1.0 and MK-0.1 groups ($p>0.05$) and no difference among VEH, MK-0.1 and MK-0.25 groups ($p>0.05$). On interval 6 [$F(3, 20) = 4.40$; $p<0.05$], the MK-1.0 and MK-0.1 groups had higher locomotion than the VEH group ($p<0.05$). There was no difference between the MK-1.0, MK-0.1 and MK-0.025 groups ($p>0.05$) and no differences between the VEH and MK-0.025 groups ($p>0.05$).

For induction day 4 (Fig. 4D), a repeated two-way ANOVA showed an interaction group X interval [$F(15, 100) = 5.23$; $p<0.01$], an effect of groups [$F(3, 20) = 27.0$; $p<0.01$] and an effect of intervals [$F(5, 100) = 14.21$; $p<0.01$]. A one-way ANOVA followed by the Tukey test to further analyze the interaction, showed on interval 1 [$F(3, 20) = 14.21$; $p<0.01$], that there was a difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than all the other groups ($p<0.05$). On interval 2 [$F(3, 20) = 45.10$; $p<0.01$], there was a difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than all the other groups ($p<0.05$). The results also showed

that the MK-0.1 and MK-0.025 groups had higher locomotion than the VEH group ($p<0.05$), and there were no differences between the MK-0.1 and MK-0.025 group ($p>0.05$). On interval 3 [$F(3, 20) = 12.20$; $p<0.01$] and interval 4 [$F(3, 20) = 8.53$; $p<0.01$], the MK-1.0 group had higher locomotion than all the other groups ($p<0.05$). On interval 5 [$F(3, 20) = 6.0$; $p<0.01$] and interval 6 [$F(3, 20) = 5.10$; $p<0.01$], there was a difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than the VEH and MK-0.025 groups ($p<0.05$). There was no difference between the MK-1.0 and MK-0.1 groups ($p>0.05$) and no difference among VEH, MK-0.1 and MK-0.25 groups ($p>0.05$).

For induction day 5 (Fig. 4E), a repeated two-way ANOVA showed an interaction group X interval [$F(15, 100) = 3.32$; $p<0.01$], an effect of groups [$F(3, 20) = 27.10$; $p<0.01$] and an effect of intervals [$F(5, 100) = 13.20$; $p<0.01$]. A one-way ANOVA followed by the Tukey test showed that on interval 1 [$F(3, 20) = 8.70$; $p<0.01$], there was difference among the groups and Tukey's test showed that the MK-1.0, MK-0.1 and MK-0.025 groups had higher locomotion than the VEH group ($p<0.05$). There was no difference among the MK-1.0, MK-0.1 and MK-0.025 groups ($p>0.05$). On interval 2 [$F(3, 20) = 15.73$; $p<0.01$], there was a difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than all the other groups ($p<0.05$). The results also showed that MK-0.1 had higher locomotion than the VEH group ($p<0.05$). There was no difference between MK-0.1 and MK-0.025 groups ($p>0.05$) and no difference between MK-0.025 and VEH groups ($p>0.05$). On interval 3 [$F(3, 20) = 12.60$; $p<0.01$], the MK-1.0 groups had higher locomotion than all the other groups ($p<0.05$). On interval 4 [$F(3, 20) = 12.30$; $p<0.01$], the MK-1.0 group had higher locomotion than the VEH and MK-0.025 groups ($p<0.05$). There was no difference between the MK-1.0 and MK-0.1 groups ($p>0.05$). The results also showed that the MK-0.1 group had higher locomotion than the VEH group ($p<0.05$). There was no difference between the MK-0.1 and MK-0.025 groups ($p>0.05$) and no difference between the VEH and MK-0.025 groups ($p>0.05$). On interval 5 [$F(3, 20) = 4.83$; $p<0.01$] there was difference among the groups and Tukey's test showed that the MK-1.0 group had higher locomotion than the VEH

group ($p<0.05$). There was no difference among the MK-1.0, MK-0.1 and MK-0.025 groups ($p>0.05$) and no difference among VEH, MK-0.1 and MK-0.25 groups ($p>0.05$). On interval 6 [$F(3, 20) = 4.71$; $p<0.01$], the MK-1.0 group had higher locomotion than the VEH and MK-0.025 groups ($p<0.05$). There was no difference between the MK-1.0 and MK-0.1 groups ($p>0.05$) and no difference among VEH, MK-0.1 and MK-0.025 groups ($p>0.05$).

Figures 5 A, B and C show the locomotor activity during the 30 min. conditioning test in experiment 2. For the first 5 min. interval (Fig. 5A), a one-way ANOVA showed that there was a difference among the groups [$F(3, 200) = 7.0$; $p<0.01$] and the Tukey test showed that the MK groups, i. e., MK-0.025, MK-0.1 and MK-1.0, had higher locomotion than the VEH group ($p<0.05$). For the 30 min. total locomotion measurements (Fig. 5B), a one-way ANOVA showed that there was a difference among the groups [$F(3, 20) = 6.0$; $p<0.01$] and the Tukey test showed that the MK-1.0, MK-0.1 and MK-0.025 groups had higher locomotion than the VEH group ($p<0.05$). There were no differences among the MK-801 groups ($p>0.05$). For the within session analysis, the 30 min total locomotion evaluation was divided into 6 intervals of 5 min. each (Fig. 5C). A repeated two-way ANOVA showed an interaction group X interval [$F(15, 100) = 3.0$; $p<0.01$], an effect of groups [$F(3, 20) = 5.70$; $p<0.01$] and an effect of intervals [$F(5, 100) = 60.0$; $p<0.01$]. A one-way ANOVA followed by Tukey test to further analyze the interaction of groups X intervals showed that on interval 1 [$F(3, 20) = 7.0$; $p<0.01$] and interval 2 [$F(3, 20) = 10.40$; $p<0.01$], the MK-1.0, MK-0.1 and MK-0.025 groups had higher locomotion than the VEH group ($p<0.05$). There was no difference among the MK-1.0, MK-0.1 and MK-0.025 groups ($p>0.05$). On interval 3 [$F(3, 20) = 1.12$; $p>0.05$], interval 4 [$F(3, 20) = 0.24$; $p>0.05$] and interval 5 [$F(3, 20) = 0.40$; $p>0.05$], there was no difference among the groups ($p>0.05$). On interval 6 [$F(3, 20) = 3.44$; $p<0.01$], there was only a difference between the MK-1.0 and MK-0.025 groups, i. e., the MK-0.025 group had higher locomotion than the MK-1.0 group ($p<0.05$).

To assess the possibility of possible differential habituation changes between the MK and VEH groups in experiment 2, Figures 6A and 6B present the changes in

locomotor activity during the habituation and the day 9 conditioning test. Figure 6A compares activity levels on habituation day 1 versus habituation day 3. A repeated two-way ANOVA showed that there was only an effect of days of treatment [$F(1, 20) = 65.0$; $p<0.01$]. There was no effect of groups [$F(3, 20) = 0.74$; $p>0.05$] and no interaction groups X days [$F(3, 20) = 0.52$; $p>0.05$]. The paired t-test showed that habituation day 1 had higher locomotion than habituation day 3 [$t(23) = 8.32$; $p<0.01$]. Thus, all groups showed comparable habituation. Figure 6B compares habituation day 3 versus conditioning test session 9. A repeated two-way ANOVA showed that there was a groups X days interaction [$F(3, 20) = 3.14$; $p<0.05$] and an effect of days of treatment [$F(1, 20) = 8.0$; $p<0.01$] but no effect of groups [$F(3, 20) = 2.40$; $p>0.05$]. The one-way ANOVA showed on habituation day 3 [$F(3, 20) = 1.30$; $p>0.05$], that there was no difference among the groups. However, for the conditioning test on session 9, there was a difference among the groups [$F(3, 20) = 5.70$; $p<0.01$] and the Tukey's test showed that all MK-801 groups had higher locomotion than the VEH group ($p<0.05$). The paired t-tests showed that for the VEH group, the activity level on during the conditioning test day was lower than for habituation day 3 [$t(5) = 3.04$; $p<0.05$], whereas for all MK-801 groups, there were no differences ($p>0.05$) between habituation day 3 and the ninth test session.

In that Fig. 6A and Fig. 6B suggest that the MK treatments prevented the progression of habituation that occurred in the VEH group, Figure 7 presents the locomotor activity for the 30 minute conditioning test including the 30 min day 3 habituation test for the VEH group. For the within analysis, the 30 min total locomotion was divided into 6 intervals of 5 min. each. A repeated two-way ANOVA showed an interaction group X interval [$F(20, 125) = 2.23$; $p<0.01$], an effect of groups [$F(4, 25) = 3.10$; $p<0.05$] and an effect of intervals [$F(5, 125) = 70.74$; $p<0.01$]. A one-way ANOVA followed by the Tukey test to further analyze the interaction of groups X intervals, showed that on interval 1 [$F(4, 25) = 5.45$; $p<0.01$], the MK-1.0, MK-0.1 and MK-0.025 groups had higher locomotion than the VEH group ($p<0.05$). There was no difference among the VEH-HAB-DAY3, MK-1.0, MK-0.1 and MK-0.025 groups ($p>0.05$) On interval 2 [$F(4, 25) = 4.13$;

p<0.01], the MK-1.0, MK-0.1 and VEH-HAB-DAY3 groups had higher locomotion than the VEH group (p<0.05). There was no difference among the VEH-HAB-DAY3, MK-1.0, MK-0.1 and MK-0.025 groups (p>0.05). On interval 3 [F (4, 25) = 1.43; p>0.05], interval 4 [F (4, 25) = 0.20; p>0.05], interval 5 [F (4, 25) = 0.35; p>0.05] and interval 6 [F (4, 25) = 2,50; p>0.05], there was no difference among the groups (p>0.05).

Discussion

In line with previous reports (Carey *et al*. 1995; Lefevre *et al*. 2016; Wolf and Khansa 1991), the present study found that MK-801 can induce locomotor sensitization with repeated treatments. During the pharmacological treatment phase, sensitization effects were dose related and largely manifested in the MK-801 1.0 mg/kg group. While the 30 min session totals indicated a rather smooth increase in locomotion with repeated treatments, the analysis of the locomotor stimulant effects in successive five-minute intervals during the 30 min test session indicated a more complicated progression. In fact, the initial treatment (day 1 of the pharmacological treatment phase) only induced a modest stimulant effect and only at the highest dose level (1.0 mg/kg) that was limited to the initial five minutes of the post-injection period. By the second treatment however, potent locomotor stimulant effects emerged and after only five minutes post-injection. These stimulant effects expanded to later intervals with additional MK-801 treatments but the peak remained during the 5-10 min interval. Essentially, the same pattern of hyperlocomotion was observed in the second experiment. In addition to the potent stimulant effects induces by the 1.0 MK-801 dose after 4 treatments, a modest locomotor stimulation developed following the 0.1 mg/kg MK-801 treatments, but no locomotor stimulation occurred in the 0.025 MK-801 treatment group. The behavioral responses to the different MK-801 doses, which ranged from no difference from the vehicle injections (0.025 mg/kg MK-801), to the major hyperlocomotion pattern with the 1.0 mg/kg MK-801treatment dose, provided the

opportunity to investigate the capacity of MK-801 to induce a conditioned drug response. In Pavlovian conditioning the conditioned response is an attenuated replica of the unconditioned response. In that the 0.025 dose did not induce a locomotor response, no conditioned stimulant response was expected while the profound behavioral effect of the 1.0 mg/kg MK-801 dose would be expected to induce some type of locomotor stimulant response. On the other hand, if the NMDA antagonism interfered with the formation of an association, then no conditioning would be expected in any of the MK-801 groups. Surprisingly in both experiments, all MK groups expressed equivalent conditioned stimulant responses.

In that the result obtained in the conditioning test does not make sense in terms of a conditioned drug response, we looked for other possible explanations. While all groups were given three habituation test sessions prior to the start of drug treatments, there were an additional five days of testing for the vehicle groups. This additional testing would have permitted an enhanced degree of habituation for the vehicle groups. When we examined the three-pre-drug habituation sessions we found that a strong habituation response occurred in all experimental groups. Interestingly, when we compared activity levels in the day three habituation session with the conditioning session, we found that the activity levels in all MK-801 groups in the conditioning test were the same as their third habituation session level whereas, the conditioning test activity level of the vehicle group in the conditioning test was significantly less than the activity level in the third habituation session. These findings indicated that the MK-801 treatments had interfered with the acquisition of additional habituation over the course of the 5 MK-801 treatment sessions. It is important to note that the MK-801 treatment did not reverse the habituation acquired between habituation day 1 and day 3, indicating that MK-801 did not interfere with previously acquired habituation but rather that MK-801 interfered with the acquisition of new additional habituation. One possible explanation for the efficacy of MK-801 in preventing the acquisition of additional habituation is that the exposures to the test environment with MK-801 were drug state dependent, so that the possible habituation acquired

under the MK-801 drug state did not transfer to the non-drug vehicle state. This explanation suggests that the drug state cues in the 0.025 MK-801 group were sufficient to induce a complete drug state dependent effect. Another possibility is suggested by the report (Dai and Carey 1994) that low doses of MK-801 (e.g. 0.03 mg/kg) block attention to external stimuli. In this study the low dose MK-801 treatments that did not affect activity levels prevented the spontaneous responding of rats to an object placed in an open-field. Whereas non-drug animals would stop and briefly interact with the object, the MK-801 treated rats which had similar contacts with the object, did not stop and interact with the object but rather seemingly ignored the object. In that the MK-801 treated rats' behavior at the location of the object was the same with or without the object being present suggested an inattention to the external environment. This could also be viewed as a general behavioral neglect of external stimuli. This characterization would fit with other reported effects of MK-801 (Baker and Azorlosa 1996), in which MK-801 blocked the effects of extinction exposures to conditioned stimuli. Also, consistent with an MK-801 induced neglect of external stimuli, it has been reported that MK-801 behavioral sensitization is context independent (Lefevre *et al*. 2016). In that MK-801 generates interoceptive drug stimuli (Grant *et al*. 1996; Koek *et al*. 1990), then the sensitization would be exclusively associated with the MK-801 drug stimuli. When MK-801 sensitization is viewed in this way, the external contextual stimuli paired with the MK-801 sensitization would be ignored and irrelevant. Consequently, the drug induced response would therefore only be paired with the drug stimuli. An implication of this analysis is that other drugs that generate drug stimuli that can substitute for MK-801 in drug discrimination experiments (Grant *et al*. 1996; Koek *et al*. 1990) seemingly could substitute for the MK-801 drug stimuli and evoke the behavioral sensitization response (Carey and Damianopoulos 1993). In addition, the conditioning results obtained in the present study, wherein the non-motoric 0.025 mg/kg MK-801 dose was as effective as the hyper-motoric 1.0 mg/kg dose in terms of the conditioned response, suggests that rats sensitized to the 1.0 MK-801 dose would also express this sensitization if tested with the 0.025 dose. This would suggest that

MK-801 sensitization is associated with MK-801 drug stimuli is a testable possibility.

In conclusion, the behavioral properties of MK-801, such as drug stimuli and MK-801 induced inattention to external stimuli, provide potential explanations for important effects of MK-801 that do not require invoking the NMDA receptor in the mediation of memory processes (Chan *et al*. 2019; Jackson *et al*. 1992). The reports showing that combined MK-801 dopaminergic drug treatments prevent the expression of context sensitization to the dopaminergic drugs is readily explicable by the removal of MK-801 drug stimuli in tests with the dopaminergic drug. In fact, there have been several reports in which drugs with stimuli different from dopaminergic drugs that are paired with dopaminergic drug treatments can acquire control over the behavioral expression of the dopaminergic drug sensitization effects so that the dopaminergic drug sensitization effects only occur when the non-dopaminergic drug stimuli are present (Carey *et al*. 1999; Carey *et al*. 2005). While the possible linkage of the NMDA receptor with memory processes has had heuristic value in initiating behavioral pharmacology investigations with drugs such as MK-801, the behavioral properties of drugs such as interoceptive drug stimuli, remain of fundamental importance in the elucidation of the behavioral drug effects.

Figure Captions

Fig. 1: The Means and SEMs for distance scores (M) during the five 30 minute pharmacological treatment sessions in which VEH or MK-801 (0.025, 0.1 and 1.0 mg/kg) were administered immediately before testing sessions. ** denotes difference for all groups, *denotes difference from the VEH group. + denotes difference from VEH group ($p<0.05$; repeated two-way ANOVA followed by the Tukey test).

Fig. 2: The Means and SEMs for distance scores during the 5 minute conditioning test sessions in which all groups received VEH before testing. ** denotes difference from the VEH group ($p<0.05$; one-way ANOVA followed by the Tukey test).

Fig. 3: The Means and SEMs for distance scores (M) during the five 30 minute pharmacological treatment sessions in which VEH or MK-801 (0.025, 0.1 and 1.0 mg/kg) were administered immediately before testing sessions. ** denotes difference for all groups, *denotes difference from the VEH group. ++ denotes difference for all groups, except the MK-1.0 group. + denotes difference from the VEH group ($p<0.05$; repeated two-way ANOVA followed by the Tukey test).

Fig. 4: The Means and SEMs for distance scores (M) during the within session locomotor activity during the 5 pharmacological treatment days (A-E) in experiment 2. The 30 min. total activity score was divided into 6 intervals of 5 min each. ** denotes difference for all groups, *denotes difference from the VEH group. ++ denotes difference for all groups, except the MK-1.0 group. + denotes difference from the VEH group ($p<0.05$; repeated two-way ANOVA followed by the Tukey test).

Fig. 5: The Means and SEMs for distance scores during the 30 minute conditioning test session. A. Distance scores during the first 5 min. interval.

B. Distance scores during the 30 min. total session.

C. Within group comparisons in which the 30 min. total activity score was divided into 6 intervals of 5 min. each.

*denotes difference from the VEH group. + denotes the MK-0.025 group had higher locomotion than the MK-1.0 group ($p<0.05$; repeated two-way ANOVA followed by the Tukey test).

Fig. 6: The Means and SEMs for distance scores for changes in locomotor activity during habituation and the conditioning test (day 9).

A. Comparison between habituation day 1 versus habituation day 3.

B. Comparison between habituation day 3 versus the conditioning test session 9.

*denotes difference from the VEH group. # denotes difference between the days ($p<0.05$; repeated two-way ANOVA followed by the Tukey test or the paired t-test).

Fig. 7: The Means and SEMs for distance scores for locomotor activity in the 30 minute conditioning test including the 30 minute habituation day 3 of the VEH group. For the within analysis, the 30 min. total locomotion was divided into 6 intervals of the 5 min. each. ** denotes difference for all groups. *denotes difference from VEH group ($p<0.05$; repeated two-way ANOVA followed by the Tukey test).

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TABLE. 1

Table 1: Experimental procedure

A: Timeline.

Days	1	2	3	4	5	6	7	8	9	10	11
	Habituation			Pharmacological Treatments					Withdrawal Period		CT

B: Treatment protocols.

Groups	Pharmacological Treatment	Conditioning Test (CT)
Experiment 1		
	30 min. arena	5 min. arena
VEH (n=5)	VEH	VEH
MK-0.025 (n=5)	MK-0.025	VEH
MK-0.1 (n=5)	MK-0.1	VEH
MK-1.0 (n=5)	MK-1.0	VEH
Experiment 2		
	30 min. arena	30 min. arena
VEH (n=7)	VEH	VEH
MK-0.025 (n=6)	MK-0.025	VEH
MK-0.1 (n=6)	MK-0.1	VEH
MK-1.0 (n=6)	MK-1.0	VEH

CT=Conditioning Test; VEH=vehicle; MK=MK-801.

FIG. 1

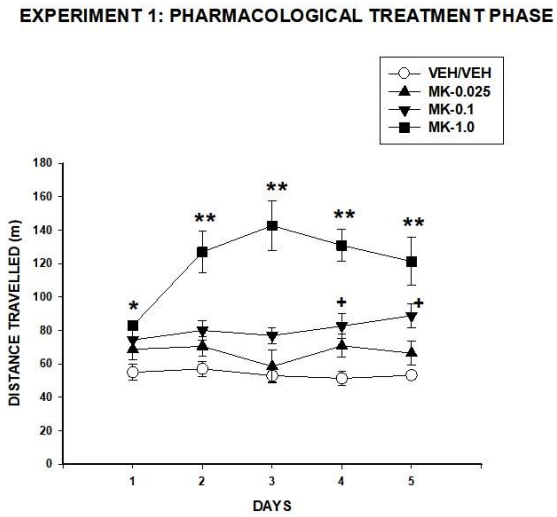

EXPERIMENT 1: PHARMACOLOGICAL TREATMENT PHASE
VEH/VEH
MK-0.025
MK-0.1
MK-1.0
DISTANCE TRAVELLED (m)
DAYS
0
20
40
60
80
100
120
140
160
180
1
2
3
4
5
*
**
**
**
**
+
+

FIG. 2

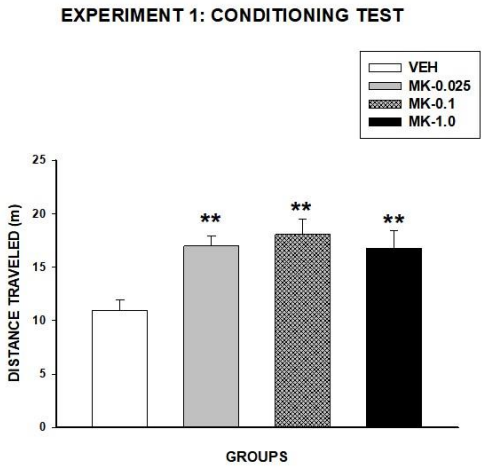

EXPERIMENT 1: CONDITIONING TEST
VEH
MK-0.025
MK-0.1
MK-1.0
DISTANCE TRAVELED (m)
GROUPS
0
5
10
15
20
25
**
**
**

FIG. 3

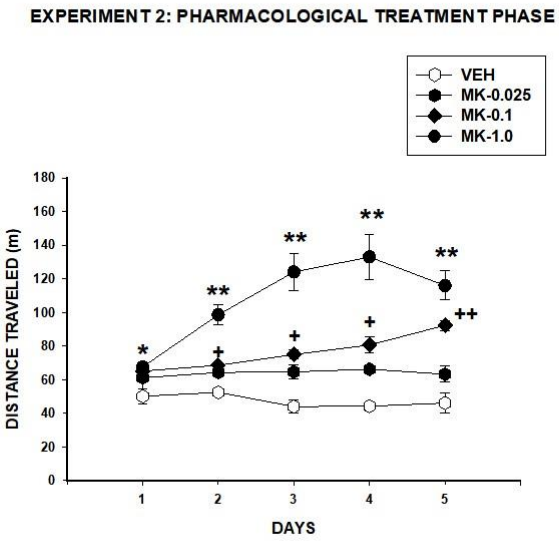

EXPERIMENT 2: PHARMACOLOGICAL TREATMENT PHASE
VEH
MK-0.025
MK-0.1
MK-1.0
DISTANCE TRAVELED (m)
DAYS
0
20
40
60
80
100
120
140
160
180
1
2
3
4
5
*
**
**
**
+
+
+
++

FIG. 4

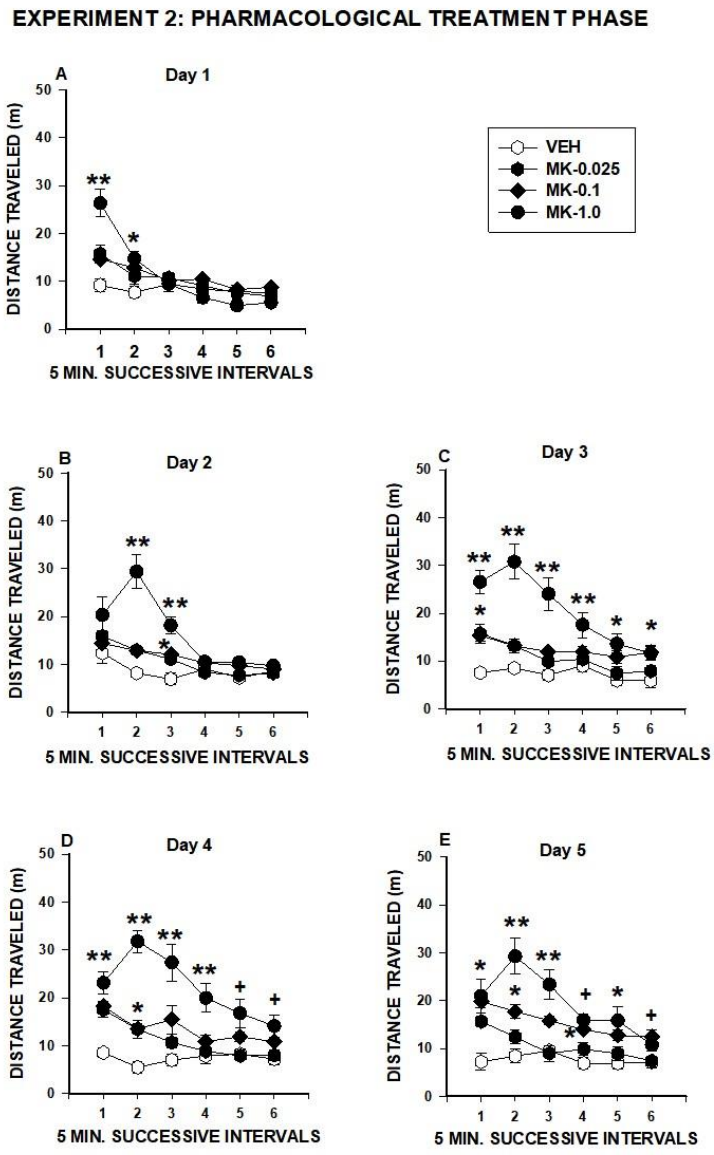

EXPERIMENT 2: PHARMACOLOGICAL TREATMENT PHASE
A
Day 1
B
Day 2
C
Day 3
D
Day 4
E
Day 5
VEH
MK-0.025
MK-0.1
MK-1.0
DISTANCE TRAVELED (m)
5 MIN. SUCCESSIVE INTERVALS

FIG. 5

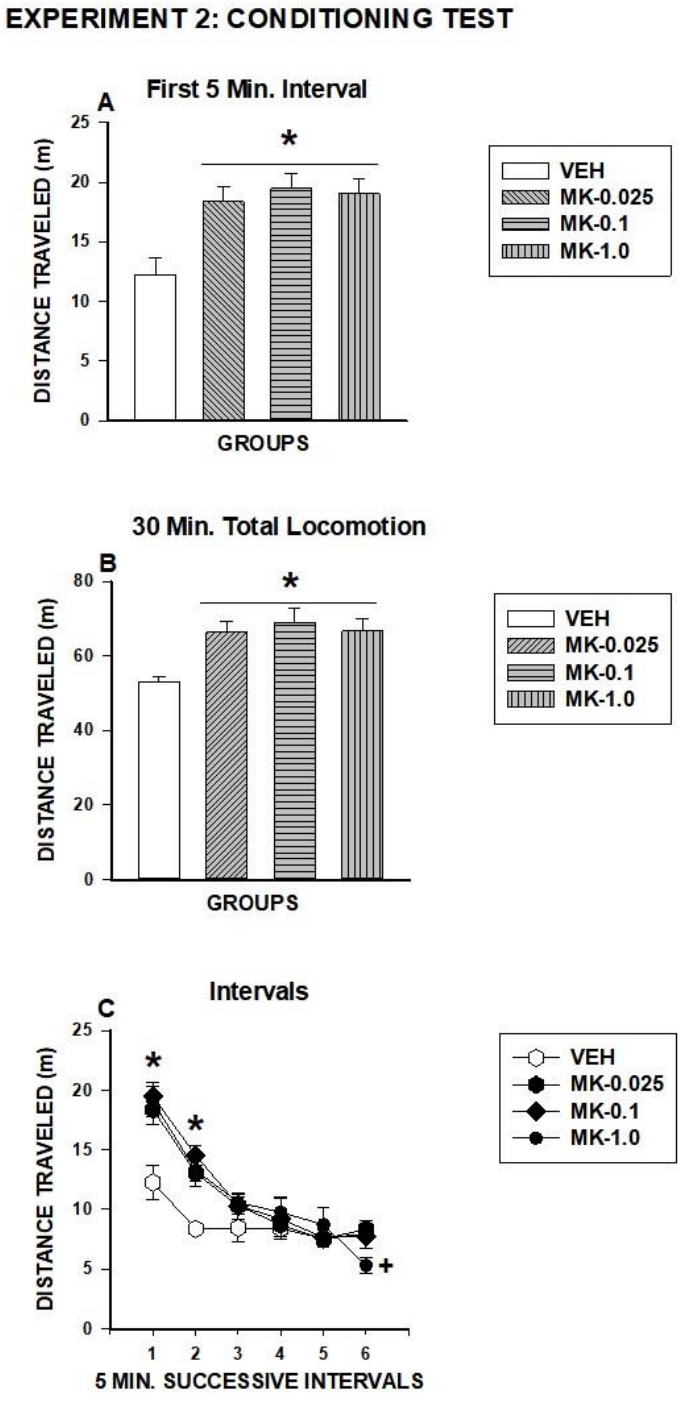

EXPERIMENT 2: CONDITIONING TEST
A
First 5 Min. Interval
DISTANCE TRAVELED (m)
GROUPS
VEH
MK-0.025
MK-0.1
MK-1.0
B
30 Min. Total Locomotion
DISTANCE TRAVELED (m)
GROUPS
VEH
MK-0.025
MK-0.1
MK-1.0
C
Intervals
DISTANCE TRAVELED (m)
5 MIN. SUCCESSIVE INTERVALS
VEH
MK-0.025
MK-0.1
MK-1.0

FIG. 6

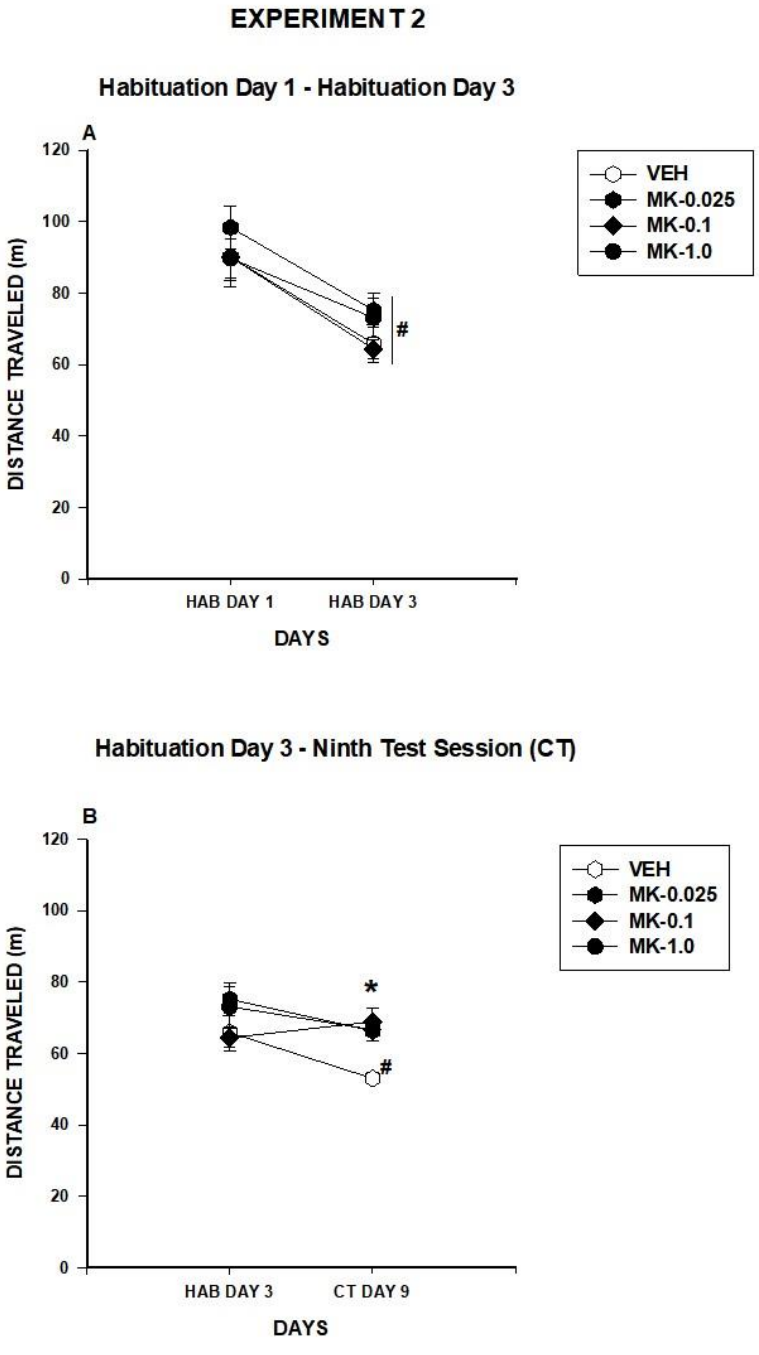
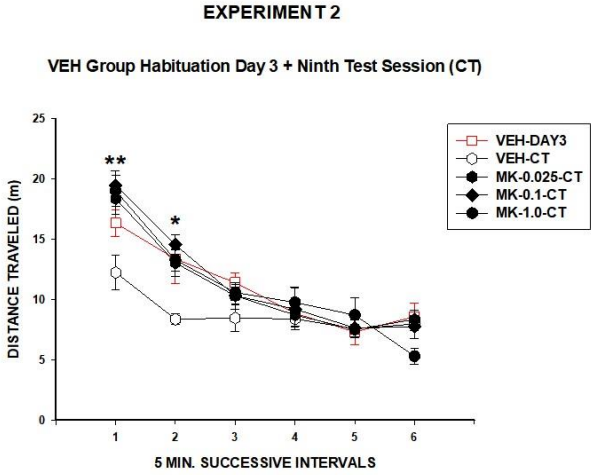

EXPERIMENT 2
Habituation Day 1 - Habituation Day 3
A
DISTANCE TRAVELED (m)
120
100
80
60
40
20
0
HAB DAY 1
HAB DAY 3
DAYS
#
VEH
MK-0.025
MK-0.1
MK-1.0
Habituation Day 3 - Ninth Test Session (CT)
B
DISTANCE TRAVELED (m)
120
100
80
60
40
20
0
HAB DAY 3
CT DAY 9
DAYS
*
#
VEH
MK-0.025
MK-0.1
MK-1.0

FIG. 7


EXPERIMENT 2
VEH Group Habituation Day 3 + Ninth Test Session (CT)
DISTANCE TRAVELED (m)
5 MIN. SUCCESSIVE INTERVALS
VEH-DAY3
VEH-CT
MK-0.025-CT
MK-0.1-CT
MK-1.0-CT
**
*

6 - DISCUSSÃO GERAL

Curiosamente, com apenas uma exposição ao ambiente experimental, a resposta comportamental de todos os animais do atual trabalho, incluindo os grupos dos dois conjuntos experimentais foi semelhante. Em estudos anteriores (SANTOS e colaboradores 2015) e (LEITE JUNIOR e colaboradores 2019) foram mostrados os mesmos padrões de resposta.

A primeira etapa de cada um dos conjuntos experimentais, a habituação, foi eficaz em reduzir a locomoção de todos os animais, deixando-os habituados à arena experimental, pois o efeito da novidade causa um acréscimo nos níveis circulantes de dopamina, gerando hiperatividade e aumento da locomoção (COSTA *et al.,* 2014). A morfina e apomorfina são duas drogas com capacidade de alterar os níveis dopaminérgicos, nesse caso, a influência de uma novidade pode confundir os resultados (CERBONE e SADILE, 1994).

A morfina se mostrou uma droga boa para o estudo da dependência química, os grupos tratados por 5 dias com morfina 10 mg/kg, tiveram um aumento da locomoção e sensibilização comportamental, como mostrado nas figuras 2, 3, 6 e 7 do conjunto experimental 1. Nos testes com apenas uma dose de morfina (figura 9), não houve aumento da locomoção quando comparado aos resultados do grupo veículo, mas houve hipolocomoção tanto nos animais do grupo tratados com Apomorfina e veículo, quanto nos grupos tratados com apomorfina e morfina, os mesmos resultados foram descritos por Leite Junior e colaboradores 2019 e Crespo e colaboradores 2022.

Após a avaliação do condicionamento e da sensibilização, figura 2 e 3 do primeiro conjunto, os grupos pré-tratados com apomorfina tiveram o bloqueio dos efeitos comportamentais comuns no tratamento com morfina, quando o tratamento foi feito antes da injeção de morfina, da mesma forma essa supressão foi descrita por Santos e colaboradores 2015; Sanguedo e colaboradores 2015 e 2017 e De Mello e colaboradores 2020. No teste final (figura 7), os tratamentos

com apenas uma dose de apomorfina foram eficazes em evitar o aparecimento dos efeitos do condicionamento previamente registrados com morfina.

Algumas estruturas encefálicas foram usadas nesse trabalho para avaliar posteriormente o condicionamento e sensibilização, sendo que as regiões do NAc e do VTA mostraram esses detalhes, descrito por Kobrin e colaboradores 2017. A morfina foi capaz de promover uma maior ativação da resposta ERK 1/2 no VTA e NAc. Conforte os dados expostos nas figuras 4, 5, 8 e 10 do conjunto experimental 1 nos mostram que, houve maior ativação de ERK nos grupos experimentais que receberam morfina na fase de indução (figura 4 e 5), mesmo naqueles grupos que receberam veículo no teste final, essa observação nos mostra que o condicionamento gerado pela morfina é duradouro e pode ocorrer na ausência da droga, na forma de hiperlocomoção que reflete numa maior ativação da ERK (DIAS *et al.,* 2021). A ativação da ERK foi bloqueada nos animais que receberam pré-tratamentos com apomorfina, sendo essa quantificação similar à encontrada no grupo veículo. No processo do uso de drogas e dependência ocorre uma maior ativação da via mesocorticolímbica, que se traduz em maior plasticidade em regiões responsáveis pelo prazer como o NAc (VOLLSTÄDT-KLEIN *et al.,* 2011; KANTAK e c DHONNCHADHA *et al.,* 2011). Dessa forma o paciente tem dificuldade em ter sucesso no tratamento pra inibir os estímulos de saliência deixados pelo uso de psicoativos, como por exemplo os opioides (THOMPSON *et al.,* 2020). Quando foi usado um protocolo experimental com apenas uma injeção de morfina (figuras 9 e 10 - experimento 3), não foi possível observar aumento da locomoção, mas houve maior ativação de ERK. Curiosamente pode ser que ocorra um aumento da plasticidade neuronal, que antecede o aumento da locomoção, ou seja, o reflexo ao uso de drogas não ocorre de forma aguda no comportamento, mas ocorre mudanças em regiões encefálicas como o VTA e NAc, similiar ao observado por Bender e Torregrossa, 2021 e Baimel e colaboradores 2019.

Aqui, os dados mostram que o MK-801 induz a sensibilização locomotora após tratamentos repetidos, o que corrobora com os resultados encontrados (WOLF E KHANSA 1991; CAREY *et al*., 1995 e LEFEVRE *et al.,* 2016). Durante

o tratamento farmacológico, fase de indução mostrada nos gráficos das figuras 1 e 4, os efeitos de sensibilização foram relacionados à dose alta e amplamente manifestados no grupo MK-801 1,0 mg/kg, como vistos em (TANG *et al*., 2006). Antagonistas NMDA como exemplo o MK-801, podem emular os sintomas psicóticos da Esquizofrenia, como a hiper locomoção, que também são comuns ao dependente químico (SNYDER e GAO, 2013). Quanto aos diferentes tratamentos com MK-801, houve diferença na locomoção dos animais que receberam as doses altas (1.0 mg/kg) e os animais tratados com as doses médias (0,1 mg/kg). A sensibilização locomotora nos animais tratados com 1.0 mg/kg ocorreu logo ao 3º dia, de tratamento de indução, e continuou sendo observada até o 5º dia, por outro lado os animais do grupo 0,1 mg/kg mostraram sensibilização locomotora mais demorada, essa só ocorreu no quinto dia de experimentação.

O fenômeno da sensibilização é muito importante para que um indivíduo se torne um dependente químico, essa sensibilização foi encontrada também por Cui e colaboradores 2014, com o uso de altas doses de MK-801 (0,5 mg/kg). Entretanto os animais que receberam MK-801 em baixa dose (0,025 mg/kg), não houve diferença na locomoção se comparados ao grupo veículo, essa ausência de efeito locomotor também foi observada recentemente por Chen e colaboradores 2022.

Um estudo Imunoistoquímico das estruturas encefálicas como o HIPO, VTA, NAc, CPF e AM, relacionadas à dependência assim como foi feito com o uso de apomorfina e morfina, se faz necessário para que os dados quanto ao uso do MK-801 sejam melhor compreendidos.

7 - CONCLUSÃO

Os tratamentos com morfina 10 mg/kg, de forma associada ao contexto ambiental, com uma duração de 5 dias, produziram tanto o condicionamento quanto a sensibilização comportamental;

A morfina 10 mg/kg produz uma memória associativa, desenvolvida pela associação do contexto ambiental como os efeitos da droga e também maior ativação da ERK no VTA e Nac;

Os pré-tratamentos com apomorfina eliminaram tanto os efeitos comportamentais da morfina no condicionamento e sensibilização, quanto a ativação da morfina da resposta ERK no VTA e NAc;

Os tratamentos pré-arena com MK-801 na dose de 1.0 mg/kg, produziram tanto sensibilização quanto condicionamento ambiental;

Para os efeitos da morfina, uma maior atividade do sistema dopaminérgico é dependente dos efeitos desinibidores do GABA;

Os resultados dos tratamentos com apomorfina fornecem suporte substancial para a importância o nível de atividade dos neurônios dopaminérgicos para a eficácia da desinibição da morfina nos sistemas de recompensa da dopamina na mediação da sensibilização comportamental e dos efeitos condicionados;

Portanto, para uma melhor compreensão dos dados relacionados ao MK-801, se faz necessário um estudo Imunoistoquímico da ERK nas estruturas encefálicas como o HIPO, VTA, NAc, CPF e AM, que estão relacionadas à dependência, assim como foi feito com o uso de apomorfina e morfina. Este questionamento é de grande interesse para investigações em experimentos futuros.

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