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ARCAÍNA REVERTE A PREFERÊNCIA CONDICIONADA POR LUGAR INDUZIDA POR MORFINA EM CAMUNDONGOS

DISSERTAÇÃO DE MESTRADO

Lediane Tomazi

Santa Maria, RS, Brasil
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ARCAÍNA REVERTE A PREFERÊNCIA CONDICIONADA POR LUGAR INDUZIDA POR MORFINA EM CAMUNDONGOS

Por

Lediane Tomazi

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Farmacologia, Centro de Ciências da Saúde, da Universidade Federal de Santa Maria (UFSM, RS), Área de concentração de Neuropsicofarmacologia e Imunofarmacologia como requisito parcial para obtenção do grau de Mestre em Farmacologia.

Orientadora: Drª Maribel Antonello Rubin

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ARCAÍNA REVERTE A PREFERÊNCIA CONDICIONADA POR LUGAR INDUZIDA POR MORFINA EM CAMUNDONGOS

elaborada por
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Como requisito parcial para obtenção do grau de
Mestre em Farmacologia

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RESUMO

Dissertação de Mestrado
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ARCAÍNA REVERTE A PREFERÊNCIA CONDICIONADA POR LUGAR INDUZIDA POR MORFINA EM CAMUNDONGOS

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A adicção a morfina consiste em uma doença crônica que envolve alterações biológicas, cognitivas e comportamentais desenvolvida após o uso repetido e compulsivo da droga. Mesmo após longos períodos de abstinência ocorrem recaídas aos usuários, principalmente quando se deparam com situações que lembram o uso da mesma. O protocolo de preferência condicionada por lugar (PCL) tem sido um dos modelos experimentais mais utilizados para mensurar os efeitos reforçadores positivos (preferência condicionada por lugar) e os negativos (aversão condicionada por lugar) de diversas drogas, incluindo a morfina. Estudos mostram que antagonistas do receptor N-Metil-D-Aspartato (NMDA) bloqueiam a PCL induzida por morfina, sugerindo que este receptor está envolvido nos efeitos da morfina. Uma vez que as poliaminas atuam no receptor NMDA, a espermidina (SPD) modulando de forma alostérica positiva e a arcaína agindo como antagonista do sítio das poliaminas neste receptor, o objetivo deste estudo foi avaliar o efeito das poliaminas sobre a preferência condicionada por lugar induzida por morfina. Camundongos Swiss machos foram pré-condicionados uma vez por dia, durante 15 minutos por dois dias consecutivos no aparelho de PCL, no dia seguinte, foram submetidos, duas vezes por dia às sessões de condicionamento, com diferentes drogas e protocolos durante quatro dias consecutivos. Vinte e quatro horas após a última sessão de condicionamento os animais foram submetidos ao teste. O escore de PCL foi calculado pelo tempo gasto no compartimento pareado com a droga no dia do teste, menos o tempo gasto no mesmo compartimento no segundo dia do pré-condicionamento. Os resultados deste estudo mostraram que a morfina (2,5-10 mg/kg, i.p.) induziu PCL, mas não aversão condicionada por lugar, a SPD (3-30 mg/kg, i.p.) e arcaína (0,3-3 mg/kg, i.p.) não induziram preferência e nem aversão condicionada por lugar. No entanto, a arcaína (3 mg/kg) administrada 15 minutos antes da morfina (5 mg/kg) no pré-treino atenuou a aquisição da PCL induzida por morfina. A arcaína (3 mg/kg) administrada imediatamente após o condicionamento com morfina (5 mg/kg) bloqueou PCL induzida por morfina. Ainda, arcaína (3 mg/kg) administrada 30 min pré-teste bloqueou a expressão PCL induzida por morfina. Além disso, o efeito da arcaína em atenuar o efeito da morfina foi prevenido pela administração de SPD antes do condicionamento, mas não foi revertido pela administração pós-condicionamento e pré-teste de SPD. Estes dados indicam que arcaína bloqueia o efeito de recompensa da morfina e sugere que a arcaína poderia ser um alvo terapêutico no desenvolvimento de drogas para tratar a adicção por morfina.

Palavras-chave: Arcaína. Espermidina. Morfina. Preferência condicionada por lugar. Receptor NMDA

ABSTRACT

Dissertation of Master's degree
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ARCAINE REVERSE THE MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE IN MICE

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The morphine addiction is a chronic disease that involves biological, cognitive and behavioral changes developed after repeated and compulsive drug use. Even after long periods of abstinence relapses occur to users, especially when faced with situations that resemble the use thereof. The protocol of conditioned place preference (CPP) has been one of the most widely used experimental models to measure the positive reinforcing effects (conditioned place preference) and negative (conditioned place aversion) of several drugs, including morphine. Studies show that antagonists of the N-methyl-D-aspartate (NMDA) block morphine-induced CPP, suggesting that this receptor is involved in the effects of morphine. Since polyamines act at the NMDA receptor, spermidine (SPD) positive allosteric modulating shape and arcaine acting as an antagonist of the polyamine site on this receiver, the purpose of this study the effect of polyamines on the preference induced conditioned place was to evaluate morphine. Adult male Swiss mice were pre-conditioned once a day for 15 minutes for two consecutive device in the CPP, the next day days were subjected twice daily for conditioning sessions with different drugs and protocols for four consecutive days. Twenty-four hours after the last conditioning session the animals were subjected to the test. The CPP score was calculated for the time spent in the compartment paired with the drug on test day, minus the time spent in the same compartment on the second day of the preconditioning. The results of this study showed that morphine (2.5-10 mg/kg, ip) induced CPP, but not aversion induced conditioned place aversion, the SPD (3-30 mg/kg, ip) and arcaine (0.3-3 mg/kg, ip) did not preferably nor induced conditioned place aversion. However, arcaine (3 mg/kg) administered 15 min before morphine (5 mg/kg) attenuated the pre-training acquisition of morphine-induced PCL. The arcaine (3 mg/kg) administered immediately after conditioning with morphine (5 mg/kg) blocked morphine induced PCL. Also, arcaine (3 mg/kg) administered 30 min pretest blocked morphine induced expression CPP. Furthermore, the effect of arcaine on attenuate the effect of morphine was prevented by the administration of SPD before conditioning, but was not reversed by postconditioning pre-test administration and SPD. These data indicate that arcaine blocks the rewarding effect of morphine and arcaine suggests that it could be a therapeutic target in the development of drugs to treat addiction to morphine.

Keywords: Arcaine. Spermidine. Morphine. Conditioned place preference. NMDA receptor

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

AC	Adenilil ciclase
AMPc	Adenilil monofosfato cíclico
AMPA	Ácido α-amino-3-hidroxi-5-metil-4-isoxazol propiônico
ACL	Aversão condicionada por lugar
AP5	Ácido-D-2-amino-5-fosfonopentanóico
ATV	Área tegumentar ventral
CPF	Córtex pré frontal
CREB	Proteína ligante do elemento responsivo ao AMPc
GABA	Ácido gama-aminobutírico
MK-801	(+)5-metil-10,11-dihidro-5H-dibenzo[a,b]-ciclohepteno-5-10-amino
NAc	*Nucleus accumbens*
NMDA	N-Metil-D-Aspartato
NTD	Domínio N-terminal
PCL	Preferência condicionada por lugar
PKA	Proteína cinase dependente de AMPc
SNC	Sistema nervoso central
SPD	Espermidina

SUMÁRIO

1 INTRODUÇÃO

1.1 EPIDEMIOLOGIA E ASPECTOS SOCIAIS DA ADICÇÃO

Atualmente a adicção é um problema amplamente divulgado e discutido na sociedade devido ao uso e abuso de substâncias psicoativas terem se tornado um problema social e de saúde pública, levando à mobilização de recursos e de ações que intervenham na atenção a usuários e adictos. A adicção apresenta um impacto considerável na sociedade, resultando em um dos maiores problemas de saúde, uma vez que assola todos os grupos étnicos e classes sociais em todo o mundo (CAMI e FARRE, 2003). Em linhas gerais, a adicção é uma doença crônica caracterizada pela compulsão a procura da droga, perda do controle sobre o consumo, problemas sociais e ocupacionais e, surgimento de um estado emocional negativo envolvendo sinais de disforia, ansiedade e irritabilidade quando o acesso à droga é impedido (KOOB et al., 2004; RODRIGUEZ AGUILAR e PILLON, 2005).

O estresse pode ser uma característica comum a vários fatores que aumentam a suscetibilidade ao consumo de drogas (VOLKOW e LI, 2004). A literatura confirma que a exposição a estressores ambientais tem impacto significativo sobre a adicção por acentuar a ação hedônica das drogas (GOEDERS, 2003).

Segundo o relatório da Organização das Nações Unidas (ONU), no ano de 2007 foi registrado que de 172 a 250 milhões de pessoas fizeram uso de alguma droga ilícita no mundo. Entre as drogas, a maconha é a de maior prevalência anual de uso (entre 143 a 190 milhões de pessoas), seguida por anfetamina, cocaína, opioides e ecstasy.

Nas últimas duas décadas, os custos com os cuidados à saúde, devido ao uso de opioides para tratar a dor crônica, têm aumentado. Um relatório do instituto de medicina sobre a dor na América divulgou que 116 milhões de americanos com dor que persistem por semanas ou anos, levou a custos financeiros variando de 560 a 635 bilhões de dólares por ano. Os americanos são os maiores usuários mundiais de opioides e o uso dessas substâncias se tornou uma epidemia, podendo causar efeitos adversos aos usuários (MANCHIKANTI et al., 2012).

As estratégias terapêuticas para o tratamento da adicção visam reduzir três aspectos importantes: a síndrome da abstinência, a fissura ou *craving* e a recaída pela droga

(VEILLEUX et al., 2010). Por isso torna-se importante uma melhor abordagem e estudo sobre a atuação das drogas de abuso, em especial sobre a morfina, de modo a buscar alternativas para o tratamento da adicção.

1.2 ASPECTOS NEUROBIOLÓGICOS DO USO DE DROGAS ADITIVAS

O conhecimento da neurobiologia das drogas e das alterações que ocorrem durante a adicção tem orientado novas estratégias para a prevenção e tratamento dessa doença, contribuindo também para identificar as áreas nas quais pesquisas adicionais são necessárias (VOLKOW e LI, 2004). As drogas de abuso agem sobre sistemas neuroquímicos que compõem o circuito de recompensa cerebral. Esse circuito anatômico foi inicialmente descrito por Olds e Milner em 1954, quando observaram um aumento nas respostas de auto-estimulação em regiões cerebrais específicas estimuladas eletricamente em ratos.

Estudos neurofarmacológicos têm relacionado propriedades reforçadoras das diferentes drogas com potencial de abuso com uma ação direta ou indireta no sistema dopaminérgico mesolímbico (KENNA et al., 2007). Três áreas principais estão relacionadas a este sistema e são consideradas determinantes neste processo: a área tegumentar ventral (ATV), o *nucleus accumbens* (NAc) e o córtex pré-frontal (CPF). Juntas, formam o que se convencionou chamar de circuito de recompensa cerebral e vem sendo amplamente estudadas no campo da adicção (Figura 1) (DI CHIARA, 1995; KOSTEN e GEORGE, 2002). Esse sistema é constituído por neurônios, principalmente dopaminérgicos, com corpos celulares localizados na área ventral do tegmento mesencefálico, projetando seus axônios para o NAc, tubérculo olfatório, CPF e amígdala (KOOB, 2000; SESACK e GRACE, 2010). Nesse sentido, tem sido demonstrado um aumento da disponibilidade de dopamina no NAc após a administração de praticamente todos os tipos de drogas com potencial de abuso. A liberação de dopamina leva à sensação de prazer e, através de conexões entre este circuito e estruturas relacionadas à formação de memórias, desenvolvem-se as respostas reforçadoras (DI CHIARA, 1995). Também, vale considerar que o sistema glutamatérgico atua nesse sistema sendo muito importante nos processos moleculares de aprendizado e memória (MCLELLAN et al., 2000; DI CHIARA et al., 2004).

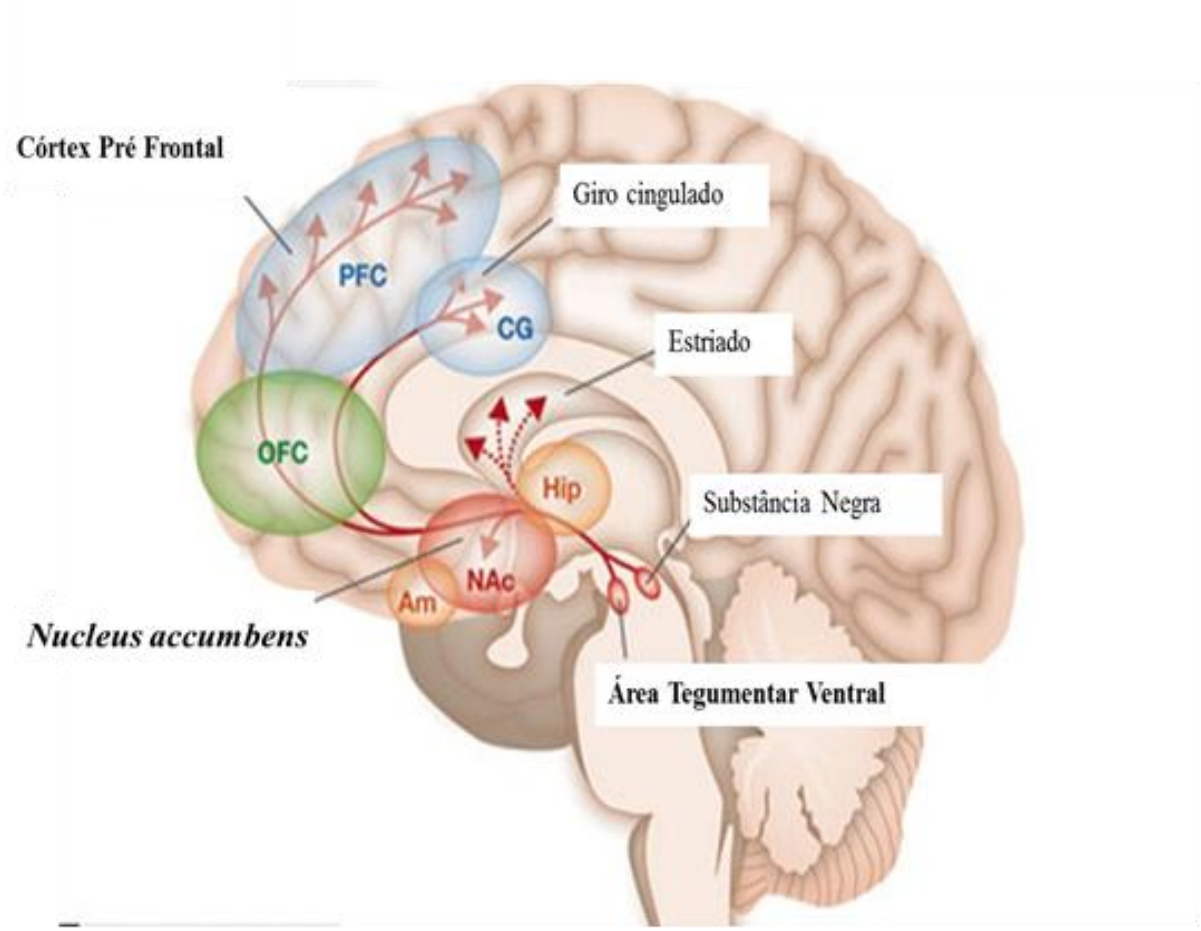

Figura 1: Sistema de recompensa cerebral. A ilustração mostra as estruturas cerebrais envolvidas no sistema de recompensa. Drogas estimulam esse sistema e promovem um aumento na liberação de dopamina, principalmete no NAc, provocando sensações de prazer. Am: Amígdala, CG: Giro cingulado, Hip: Hipocampo, NAc: *Nucleus accumbens*, OFC: Córtex orbitofrontal, PFC: Córtex Pré Frontal. Adaptado de Lee et al., 2012.

O uso abusivo de drogas é bem mais compreendido quando se identifica as fontes de reforço. Nesse contexto, as seguintes hipóteses de reforço foram sugeridas: reforço positivo (WISE, 1988), reforço negativo (WISE, 1988) aprendizado associativo (DI CHIARA, 1999), perda do controle inibitório sobre o uso e a busca pela droga (GOLDSTEIN e VOLKOW, 2002) e saliência de incentivo (ROBINSON e BERRIDGE, 2003 2008). No contexto das drogas de abuso, muitos fatores de reforço contribuem para seu uso compulsivo durante o curso da adicção, dessa forma, todas as hipóteses citadas contribuem em maior ou menor grau para o desenvolvimento e manutenção da adicção.

A hipótese do reforço positivo consiste em inserir um estímulo reforçador no ambiente, ou seja, usar drogas com intuito de promover estados afetivos positivos como euforia e prazer. A droga produz efeitos agradáveis e sensações prazerosas, esses efeitos de

reforço, promovidos pelas drogas de abuso, seriam responsáveis pelo uso e busca da droga (WISE, 1988).

Segundo a hipótese do reforço negativo, o adicto mantém o uso da droga para aliviar os sintomas aversivos (físicos ou psicológicos) com objetivo de diminuir sensações desagradáveis associados à abstinência desta (WISE, 1988). Portanto, o uso da droga mantém um determinado comportamento devido ao estado que alivia. Assim, o comportamento de um drogadicto, geralmente, é seguido por alguma forma de gratificação imediata como um estado de prazer máximo (reforço positivo) ou redução de um estado de tensão como meio de escapar de situações aversivas (reforço negativo).

A hipótese do aprendizado associativo postula que através da estimulação repetida promovida pela droga de abuso na transmissão dopaminérgica no sistema de recompensa, fortaleceria de forma anormal a associação estímulo-droga, levando a um aprendizado associativo anormal (DI CHIARA, 1999). Em contraste, o aprendizado associativo conecta os estímulos que podem estar associados a pistas presentes no ambiente.

Já a hipótese da perda do controle inibitório sobre o uso e a busca pela droga, descreve que durante a adicção ocorre uma disfunção do CPF, levando à perda do controle inibitório e ao desenvolvimento de comportamentos impulsivos, o que explica porque os usuários mantêm o uso da substância apesar dos prejuízos, riscos e consequências danosas provocadas pelo abuso de drogas (GOLDSTEIN e VOLKOW, 2002; DOM et al., 2005).

Por fim, a hipótese da saliência de incentivo postula que após o uso repetido de uma droga de abuso ocorre uma sensibilização de sistemas neurais dopaminérgicos, fazendo com que um intenso e compulsivo desejo pela droga se instale, independentemente do prazer promovido pela droga, de sintomas da abstinência e da formação de hábitos ou memórias (ROBINSON e BERRIDGE, 2003; 2008).

1.3 ÓPIO

O ópio, líquido extraído da papoula *Papaver somniferum*, contém substâncias naturais como a morfina, a codeína e a tebaína. O ópio é conhecido desde a antiguidade e tem sido usado pelo homem muito provavelmente antes da história escrita. Existem imagens arqueológicas que sugerem o seu emprego nas culturas sumérias. Além disso, diversos estudos demonstram que a maioria dos povos antigos já conhecia e utilizava esta substância,

incluindo os assírios, árabes, egípcios, gregos, romanos, chineses e persas**.** Em 4000 a.C. a papoula era descrita em ideogramas de povos Sumérios como "planta da alegria", provavelmente devido aos seus efeitos euforizantes (DUARTE, 2005).

A *Papaver somniferum* provavelmente evoluiu de uma espécie silvestre nativa da Ásia Menor, em torno do Mediterrâneo. No Papiro de Ebers (1552 a.C.) está descrito uma mistura de substâncias, entre as quais o ópio era empregado com eficiência para a sedação de crianças (Cohen, 1969). Na Grécia antiga, Hipócrates prescrevia um suco de papoula como purgativo, narcótico e para a cura da leucorréia (DUARTE, 2005).

Os Árabes dominaram o comércio no Oceano Índico e introduziram o ópio na Índia e posteriormente na China. Entre os séculos X e XII, o ópio se espalhou da Ásia para todas as regiões da Europa. Desde sua introdução, a papoula era utilizada apenas com finalidades medicinais na China, não havendo qualquer indício de uso recreativo até por volta do século XVII, no entanto, os chineses aderiram a uma nova forma de consumir o ópio, através da introdução de cachimbos de tabaco, e então, passaram a fumar também os extratos da papoula que antes eram ingeridos (BROWNSTEIN, 1993). Outro uso do ópio foi descrito pelo médico Inglês Thomas Sydenham, através de um láudano, uma preparação líquida feita com ópio, vinho de cereja, açafrão, cravo e canela (BARAKA, 2000). Ademais, em 1721, foi publicada na Farmacopeia de Londres, o Elixir Paregórico composto por ópio, mel, cânfora, anis e vinho (DUARTE, 2005).

A crença de que o ópio não acarretava prejuízo individual ou coletivo começou a ruir em 1830 e em 1860, essa droga se tornou problema médico e social em função dos dados estatísticos de mortalidade. Segundo esses dados, um terço de todos os envenenamentos fatais foi devido a casos de sobredose de ópio, quer tomado como fonte de prazer, quer com intenções suicidas (DUARTE, 2005).

Em 1803, Sertürner iniciou os seus trabalhos no isolamento de princípios ativos do ópio e publicou, em 1806, os primeiros resultados de uma série de experimentos realizados, em que conseguiu isolar um alcaloide, substância cristalina com propriedades alcalinas, a partir da resina da papoula. Ele descobriu o princípio ativo do ópio, dissolvendo-o em ácido, em seguida, neutralizando-o com amônia (BARAKA, 1982). Sertürner verificou, posteriormente, que o ópio, sem o alcaloide, não apresentava efeitos em animais, mas que o alcaloide sozinho era dez vezes mais poderoso que o ópio processado. Pela característica da droga em provocar sonolência, inicialmente foi denominada de *Principium somniferum* em homenagem a Morfeu, o Deus grego do sono, atualmente, morfina é denominação consagrada (HAMILTON e BASKETT, 2000). Alguns anos mais tarde, em 1827, a companhia Merck &

Co, na Alemanha, começa a produção comercial de morfina. Em 1843, Alexander Wood, de Edimburgo, na Escócia, descobre nova forma de administrar a morfina, através da seringa e agulha hipodérmica. Seus efeitos são instantâneos e três vezes mais potentes (BROWNSTEIN, 1993; DORRINGTON e POOLE, 2013).

A grande época do ópio foi o século XIX, quando esta droga se tornou um centro de conflito internacional que opôs a Inglaterra à China, desencadeando-se a denominada Guerra do Ópio. Além disso, a Guerra Civil Americana também criou grande oportunidade para o uso exacerbado tanto do ópio oral como da morfina subcutânea, em soldados feridos no combate. Como consequência, houve registros de vários casos de dependência física gerando um problema social para os EUA (BARAKA, 2000). Soldados britânicos que lutaram na guerra da Criméia também utilizaram morfina injetável, o mesmo aconteceu com os soldados prussianos durante a guerra de 1870 na França e Alemanha (DUARTE, 2005).

1.4 SISTEMA OPIOIDE

Na década de 1970, por meio de pesquisas com preparados de íleo de roedores, foi possível identificar três receptores da classe dos opioides, os quais foram nomeados com letras gregas de acordo com a correspondente inicial de cada substância específica utilizada para estimulá-lo. Os receptores opioides diferem entre si: nas propriedades farmacológicas; na sua distribuição no sistema nervoso central, periférico e em outros tecidos e na afinidade com os peptídeos opioides. Existem três classes de receptores opioides que são amplamente aceitas: o receptor mu (μ ou MOR), delta (δ ou DOR) e kappa (κ ou KOR) (PERT e SNYDER, 1973; GRANIER, 2012). Estes receptores apresentam sete domínios transmembrana, com três alças extracelulares e três intracelulares. A porção C-terminal se localiza no meio intracelular e a porção N-terminal no meio extracelular (TRESCOT et al., 2008; PASTERNAK, 2010). As três classes de receptores estão acopladas à proteína G inibitória (Gi) (DIETIS et al., 2011; FENG et al., 2012; ZHAO et al., 2012).

A presença de receptores de opioides levou a caracterização de vários ligantes endógenos chamados de peptídeos opioides endógenos, caracterizados quanto a natureza em: encefalinas (SIMANTOV e SNYDER, 1976), beta-endorfina (LI et al., 1976), dinorfina (LOWNEY et al., 1979), e nociceptina (REINSCHEID et al., 1995). Nos mecanismos neuroquímicos da recompensa à opioides, tem sido atribuída grande importância à ativação de

peptídeos opioides endógenos em especial aqueles que agem através de receptores mμ e kappa (FENG et al., 2012).

Os receptores mu são o principal alvo da morfina e dos peptídeos opioides endógenos beta-endorfinas (KIEFFER, 1999). Há dois tipos de receptores mu, o subtipo 1 medeia os efeitos reforçadores ou eufóricos, analgesia e a depressão respiratória, e o subtipo 2 é responsável pelos efeitos gastrintestinais (p. ex., constipação intestinal). A estimulação crônica desses receptores está associada a adicção de opioides (síndrome de abstinência e tolerância). No sistema nervoso, os receptores estão localizados no córtex cerebral, tálamo, NAc, amígdala, hipocampo, no corno dorsal da medula espinhal e nos nervos periféricos (DHAWAN et al., 1996; PASTERNAK, 2010).

Os receptores kappa exercem funções de nocicepção, termorregulação, controle de diurese e secreção neuroendócrina. Estão localizados no hipotálamo, pituitária, amígdala, ATV, núcleo caudado, além de neurônios sensitivos periféricos (DHAWAN et al., 1996). Essa classe apresenta três subtipos K1a, K1b, K2 (ZUKIN et al., 1988).

Os receptores delta são responsáveis primariamente pela analgesia, mas também por modular funções cognitivas e de dependência física, estão localizados na amígdala, bulbo olfatório, córtex cerebral profundo e nos neurônios sensitivos periféricos (DHAWAN et al., 1996).

Segundo Feng e colaboradores (2012), os receptores opioides, além de modulação da dor e da adicção, são amplamente envolvidos em várias atividades fisiológicas e fisiopatológicas, incluindo proliferação celular, resposta emocional, ataques epiléticos, função imune, alimentação, obesidade, doenças respiratórias e controle cardiovascular, bem como alguns distúrbios neurodegenerativos. Em algumas espécies desempenham um papel essencial no modo de hibernação.

1.5 MORFINA

O receptor μ, do qual a morfina (Figura 2) é o agonista protótipo, parece ser o mais intimamente relacionado aos efeitos de adicção. A morfina é introduzida no organismo por via oral ou injetada, onde rapidamente se espalha pela corrente sanguínea, chegando ao SNC (KAY et al., 2002).

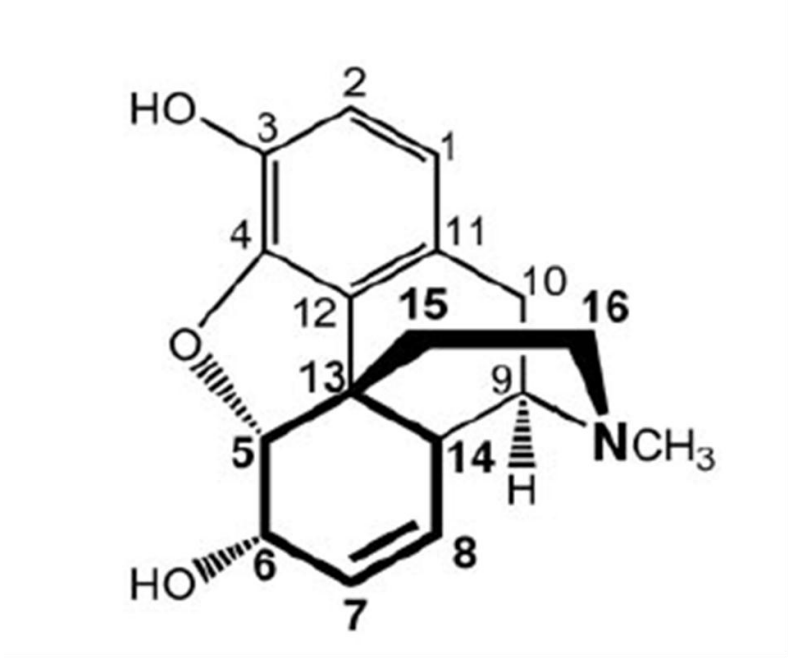

Figura 2: Estrutura química da morfina. Adaptado de Trescot et al., 2008).

Quando a morfina, heroína, oxicodona, ou qualquer outro opioide ativa receptores μ opioides, a ligação destes desencadeia os processos de recompensa cerebral (KOSTEN e GEORGE, 2002). Parece haver duas vias de interação dos opioides com o sistema de recompensa encefálico. Um local de ação situa-se na ATV, onde interneurônios GABAérgicos causam a inibição tônica dos neurônios dopaminérgicos responsáveis pela ativação da via de recompensa encefálica no NAc. Esses interneurônios GABAérgicos podem ser inibidos por encefalinas endógenas, que se ligam a receptores μ opioides nas terminações GABAérgicas. Como a morfina também se liga aos receptores μ opioides, a via de recompensa encefálica é ativada mediante desinibição dos neurônios dopaminérgicos na ATV. A outra via, que não está bem elucidada, está localizada no NAc. Os opioides que agem nessa região podem inibir neurônios GABAérgicos que se projetam de volta para a ATV, talvez como parte de uma alça de *feedback* inibitório. A importância relativa dessas duas vias ainda está sendo discutida (HYMAN et al., 2006; DAVID et al., 2009).

A administração aguda de opioides ativa receptores μ que estão acoplados à proteína Gi (Figura 3). Quando os receptores são estimulados por um fármaco opióde (morfina), ocorre a inibição da enzima adenilil ciclase (AC), reduzindo o nível intracelular de adenilil monofosfato cíclico (AMPc) (DUMAN et al., 1988). Com isso, também há efeito em canais catiônicos, pois promovem a abertura dos canais de potássio e inibem a abertura dos canais de cálcio voltagem dependente, assim, reduzem tanto a excitabilidade neuronal como a liberação de neurotransmisores (UEDA, 1989; TORRECILLA et al., 2002). Esse processo causa

hiperpolarização neuronal, bloqueando a transmição do estímulo doloroso (Jordan, 1999). Dessa maneira, a diminuição dos níveis da via sinalizadora AMPc diminui a fosforilação de outras proteínas, como a proteína cinase dependente de AMPc (PKA) e de fatores de transcrição gênica como a proteína ligante do elemento responsivo ao AMPc (CREB), proteínas essas importantes em outros processos neurais (CAMI e FARRE, 2003). Todavia, a administração crônica de opioides, está associada com a ativação da enzima AC, especialmente os tipos ACI e ACVIII (DUMAN et al., 1988; ROBISON e NESTLER, 2011; MAZEI-ROBISON e NESTLER, 2012), aumentando assim a fosforilação induzida por AMPc, PKA e tirosina hidroxilase, originando efeitos de tolerância (GUITART e NESTLER, 1989). Em vista disso, a ativação crônica desses receptores aumenta a fosforilação de fatores de transcrição, tais como CREB e FosB (CAMI e FARRE, 2003).

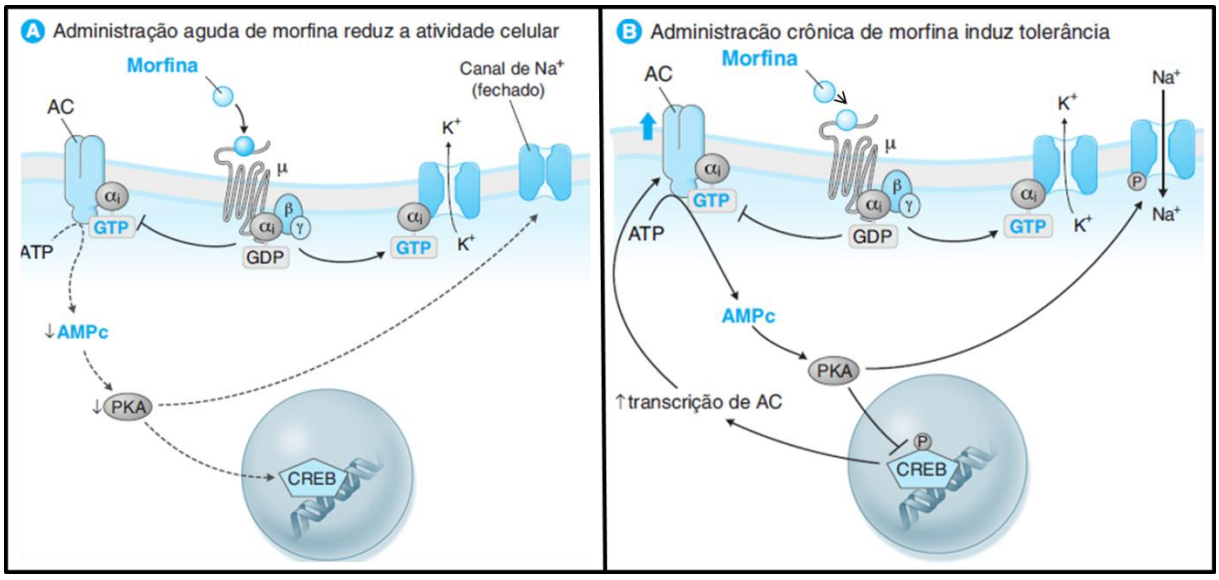

Figura 3: Mecanismo de ação da morfina aguda (a) e crônica (b). Adaptado de Golan et al., 2009.

Embora o uso agudo de opioides tem potencial para promover mudanças na transmissão do sistema opioide, é no uso crônico que se desenvolvem as alterações mais significativas e duradouras, interferindo também com outros sistemas de neurotransmissão. Múltiplas adaptações celulares são desencadeadas pelo uso crônico, relacionadas a três fenômenos maiores: o desenvolvimento de tolerância, a síndrome de abstinência e dependência química/adicção. As mais marcantes alterações são a diminuições do número e da sensibilidade dos receptores opioides no SNC (KOSTEN e GEORGE, 2002).

Assim, com o uso crônico, uma quantidade maior de agonistas opioides será necessária para se alcançar os efeitos desejados. Quando se interrompe o consumo da droga,

surgem os sintomas de abstinência relacionados ao desequilíbrio gerado pela estimulação insuficiente do sistema opioide (GINTZLER e CHAKRABARTI, 2000).

O Brasil é o maior consumidor de analgésicos opioides da América do Sul (BALTIERI et al., 2004; UNODC, 2009). Conforme o segundo levantamento realizado em 2005, pelo Centro Brasileiro de Informações sobre Drogas, envolvendo 108 cidades do país, 1,3% da população fez uso de analgésicos opioides na vida e em todas as faixas etárias, havendo predomínio de uso em mulheres em relação aos homens.

Dentre os medicamentos opioides prescritos a morfina, oxicodona e meperidina, juntamente com a ilícita heroína, configuram um dos grupos de drogas mais consumidas abusivamente e assim, levam a adicção (POULETTY, 2002; YARGEAU et al., 2014). Do ponto de vista clínico, a prescrição de opioides é problemática, pois a administração contínua pode levar a tolerância e dependência (ZHU et al., 1999). Estima-se que 18 a 45% de indivíduos que utilizam opioides no tratamento de dor crônica abusam da droga (WOLLER et al., 2012). A adicção por opioides é muito difundida e também está associado com alta mortalidade (BRADVIK et al., 2009).

O uso de opioides ocorre tanto por pacientes com prescrição médica, quanto em profissionais da saúde. Conforme o estudo de Alves e colaboradores (2005), uma pesquisa feita com 198 médicos em tratamento por dependência química, residentes em São Paulo (90 casos), Rio Grande do Sul (25 casos), Rio de Janeiro (20 casos) e Minas Gerais (17 casos), 26,7% fizeram o uso de opioides.

Os profissionais da saúde, principalmente médicos, passam por situações facilitadoras para o uso e abuso de drogas. Alguns fatores de risco para uso de substâncias psicotrópicas entre médicos são frequentemente relatados na literatura como: acesso facilitado aos medicamentos, perda do tabu em relação a injeções, problemas emocionais, estresse no trabalho e em casa, autoadministração no tratamento para dor e para o humor, fadiga crônica, e os de especialidade de alto risco: anestesiologia, emergência e psiquiatria (MCAULIFFE et al., 1987; WRIGHT, 1990; ALVES et al., 2005).

Atualmente, o tratamento da adicção pode ser dividido em duas categorias amplas: o tratamento farmacológico e o psicossocial. O tratamento farmacológico clássico da adicção concentra-se na desintoxicação aguda para aliviar os sintomas de abstinência que acompanham a interrupção do uso da droga. Já no tratamento psicossocial são empregadas técnicas de aconselhamento, que geralmente se concentram nas necessidades psicológicas individuais do paciente, as quais impedem a recuperação a longo prazo, como desemprego, transtornos familiares e falta de acesso e atenção a saúde (KOSTEN e GEORGE, 2002).

Sabe-se que os medicamentos existentes quando combinado com as terapias psicossociais, têm eficácia comprovada na redução dos aspectos da dependência de opioides, no entanto, infelizmente, esses medicamentos têm limitações críticas associadas a essas terapias. Muitos desses medicamentos utilizados (metadona, buprenorfina e naltrexona) causam dependência e outros efeitos indesejáveis. Além disso, e por outras circunstâncias, os medicamentos atuais não conseguem melhorar os aspectos chave da adicção, como por exemplo, as pistas de uso, o estresse e outras associações relacionadas ao comportamento de abuso. Assim, há uma necessidade de desenvolver novos tratamentos para adicção (CHARTOFF e CONNERY, 2014).

1.6 SISTEMA OPIOIDE E MEMÓRIA

Um dos enfoques mais atuais e interessantes da adicção é considerar este processo como uma forma de aprendizado. O uso repetido de morfina induz uma série de modificações neurobiológicas que fazem o indivíduo "aprender" a usar e buscar essa substância. De fato, esse fenômeno pode ser baseado em processos de aprendizagem e memória. Comportamentos crônicos e compulsivos pela busca de drogas constituem um fator de adicção. Ambientes contextuais que exprimem pistas do uso de drogas potencializam as propriedades de incentivo na manutenção de comportamentos de recaída (DIAS et al., 2012). Segundo Liddie e colaboradores (2012), a administração de uma droga que altera o estado afetivo do organismo em um contexto específico desencadeia um processo de aprendizagem associativo e formação de uma memória a longo prazo. Um grande número de estudos mostra que a adicção de opioides envolve mecanismos celulares envolvidos com a formação da memória (HYMAN e MALENKA, 2001).

Alguns autores afirmam que a memória da adicção jamais será esquecida (NESTLER, 2002; 2004), assim como a memória da ansiedade e a memória da dor, isso porque quando animais são colocados no ambiente em que uma droga foi apresentada no passado, ocorre um aumento da liberação de dopamina na amígdala e lembranças de comportamentos relacionados à busca pela droga fazem com que retornem ao uso (ROBINSON e BERRIDGE, 2000).

1.7 RECEPTOR N-METIL-D-ASPARTATO

O receptor N-metil-D-aspartato (NMDA) é um receptor de membrana ativado por glutamato, pertencente à família de receptores ionotrópicos glutamatérgicos responsável por mediar transmissões excitatórias tanto na medula espinhal como no encéfalo. Dentre esses sistemas de receptores, a neurotransmissão glutamatérgica mediada pelo receptor NMDA, tem merecido destaque em mecanismos de plasticidade sináptica, memória e aprendizagem. Atualmente são conhecidas sete subunidades, divididas em três subfamílias: uma única subunidade GluN1, quatro subunidades GluN2 (GluN2A, GluN2B, GluN2C e GluN2D), e duas subunidades GluN3 (GluN3A, GluN3B). Estas subunidades podem organizar-se em heterodímeros contendo subunidades GluN1 e GluN2 ou uma mistura de subunidades GluN2 e GluN3 (PAOLETTI et al., 2013; SANZ-CLEMENTE et al., 2013).

O receptor NMDA possui duas características peculiares: é o único ativado por ligante e dependente de voltagem e que necessita de dois ligantes, o glutamato e um coagonista (D-serina ou glicina) para ser ativado (KLECKNER e DINGLEDINE, 1988). Em condições de repouso, o poro do receptor NMDA está bloqueado por níveis fisiológicos de Mg^{2+}. A ativação do receptor NMDA resulta no influxo de sódio e cálcio e efluxo de potássio, através do seu poro. A ativação deste receptor e o subsequente influxo de cálcio dispara uma cascata de eventos que modifica a eficiência sináptica e a morfologia neuronal, cruciais para plasticidade sináptica (SANZ-CLEMENTE et al., 2013). O receptor NMDA é regulado por diferentes sítios de ligação tanto para ligantes endógenos quanto para ligantes exógenos, incluindo o glutamato, glicina, magnésio, zinco e também um sítio para as poliaminas (ZIGMOND et al., 1999).

1.8 POLIAMINAS

As poliaminas foram descritas pela primeira vez em 1678, por e Antoni van Leeuwenhoek, seus nomes comuns, putrescina, espermidina e espermina provem da fonte de onde foram primariamente isoladas: carne em putrefação e líquido seminal (COFFINO, 2001).

O caráter fortemente básico das poliaminas implica na protonação de todos os grupos amino em condições fisiológicas. As poliaminas podem modular a excitabilidade neuronal agindo em diferentes canais iônicos e receptores de membrana, como canais de cálcio e o receptor NMDA (MONY et al., 2009). Essas interações das poliaminas com sítios aniônicos de macromoléculas (ácidos nucléicos, proteínas, lipídios de membrana) é a base do mecanismo para a maioria das suas funções biológicas. Dentre estas se destacam: modulação do crescimento e diferenciação celular (TABOR e TABOR, 1984), apoptose (THOMAS e THOMAS, 2001), estabilização do DNA e RNA (IGARASHI e KASHIWAGI, 2000), regulação da expressão gênica (CELANO et al., 1989), síntese de proteínas (YOSHIDA et al., 1999), sinalização celular (JOHNSON e MCCORMACK, 1999; BACHRACH et al., 2001), diminuição da lipoperoxidação (BELLE et al., 2004) e ainda, possuem interações específicas com canais iônicos (Williams, 1997a; b) tais como canais de potássio (K^+), receptores AMPA permeáveis ao cálcio (Ca^{2+}), receptores cainato e o receptor NMDA (MOTT et al., 2003).

As poliaminas, putrescina, espermidina e espermina, são aminas alifáticas simples compostas por uma, duas ou três cadeias carbonadas flexíveis, respectivamente, as quais são conectadas por átomos de nitrogênio. Como mostra a figura 4**,** a putrescina (1,4-dianobutano) é uma diamina, a espermidina (mono-N-3-aminopropil-1,4-dianobutano) é uma triamina e a espermina (bis-N-3-aminopropil-1,4-dianobutano) é uma tetraamina (TETI et al., 2002).

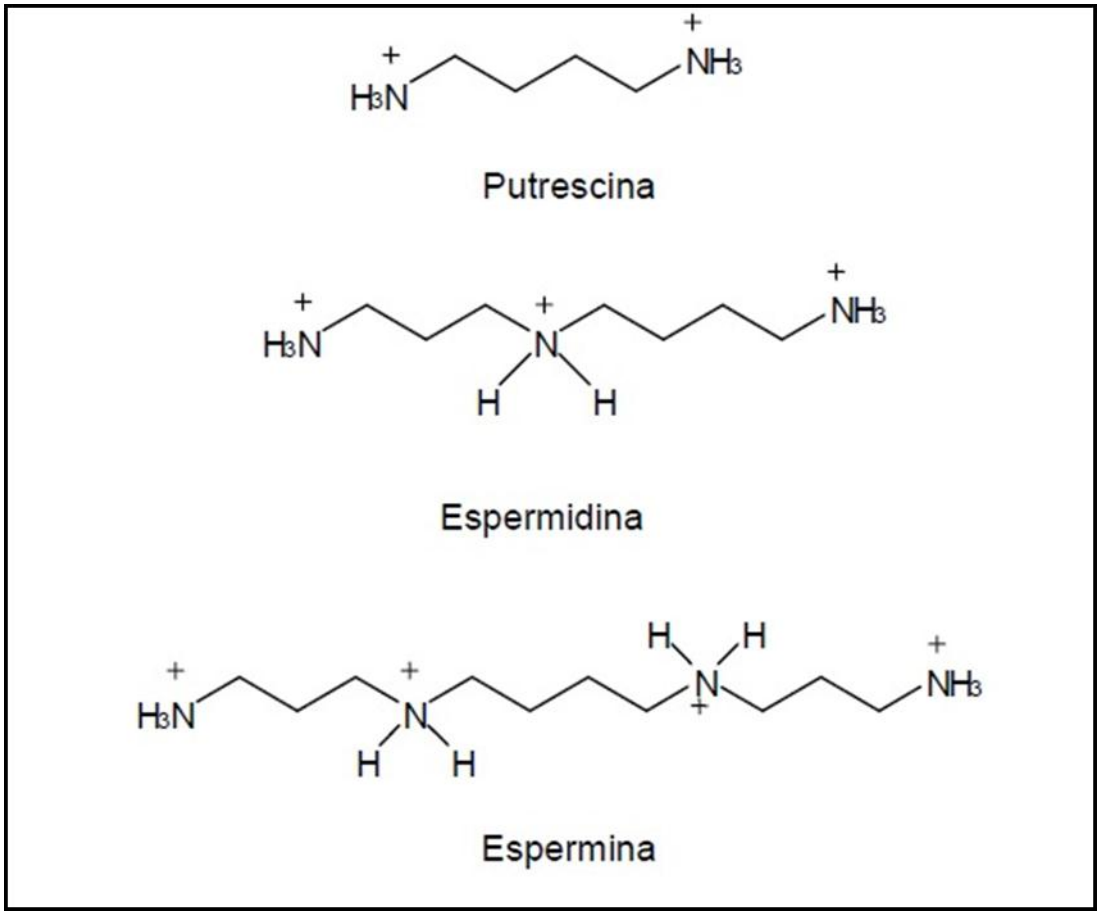

Figura 4- Estrutura química das poliaminas. Adaptado de Teti et al., 2002.

As poliaminas estão amplamente distribuídas no SNC de seres humanos, sendo as maiores concentrações de espermidina, seguida pela espermina e putrescina, cada qual estando distribuída diferentemente em cada região cerebral. São encontradas principalmente em regiões como hipocampo, hipotálamo, NAc, bulbo, hipocampo e córtex cerebelar (MORRISON e KISH, 1995; THOMAS e THOMAS, 2001; KRAUSS et al., 2007).

Recentemente, através de técnicas moleculares e eletrofisiológicas, foi identificado que as poliaminas se ligam na interface das subunidades GluN1/GluN2B do receptor NMDA (Figura 5) (MONY et al., 2011). No modelo proposto por Mony e colaboradores (2011), a espermina liga-se na interface formada pelos lóbulos inferiores do domínio N-terminal (NTD) das subunidades GluN1/GluN2B do receptor NMDA. O dímero formado pelo NTD das subunidades GluN1/GluN2B que possuem formato de concha, pode alternar entre dois estados conformacionais: um estado ativo e outro estado "tipo-dessensibilizado". No estado ativado, a região NTD das duas subunidades está aberta, o que mantém os lóbulos inferiores próximos e aumenta a probabilidade de ligação do agonista. Já no estado dessensibilizado, cargas eletrostáticas mantém os lóbulos inferiores de GluN1 e GluN2B separados, o que provoca o fechamento da região NTD. A espermina e a espermidina agem estabilizando o

receptor em um estado ativado, aliviando a repulsão eletrostática que separa os lóbulos inferiores das subunidades GluN1 e GluN2B.

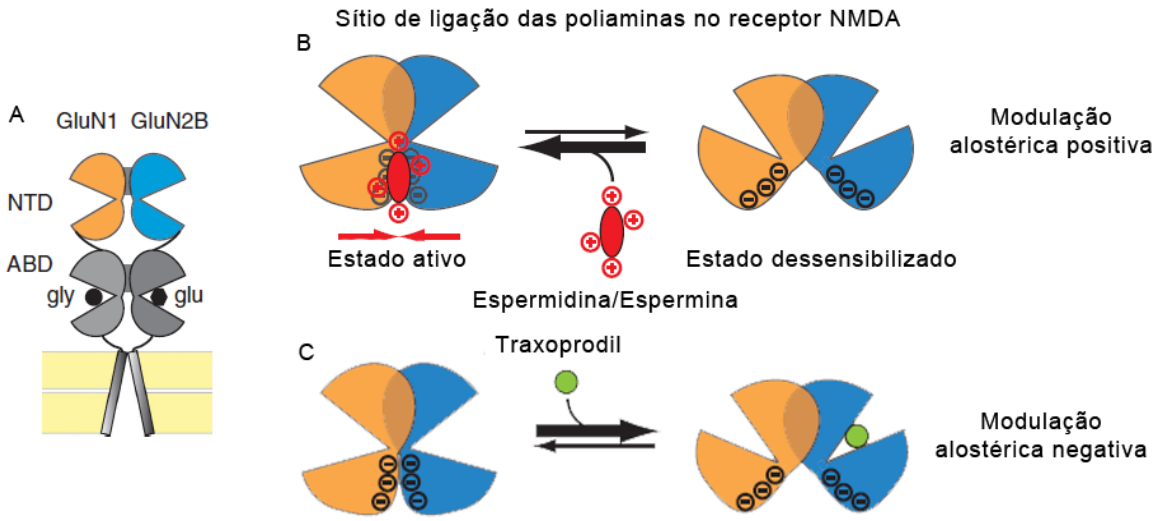

Figura 5: Sítios de modulação alostérica no receptor NMDA. A) Visão esquemática do heterodímero formado pelas subunidades GluN1 e GluN2B. B) Sítio de ligação das poliaminas no receptor NMDA. C) Sítio de ligação de antagonistas da subunidade GluN2B do receptor NMDA. Adaptado de Mony et al., 2011.

A arcaína é um antagonista competitivo do sítio de ligação das poliaminas do receptor NMDA (LYNCH et al., 1995) e um análogo das poliaminas, possuindo estrutura semelhante a estas. É composta por uma cadeia carbonada, a qual está conectada por átomos de nitrogênio. Ela também apresenta grupamentos amino em suas extremidades, além de conter uma molécula de ácido sulfúrico, a qual confere o nome de 1, 4-diguanidinobutano (REYNOLDS, 1990).

1.9 PREFERÊNCIA CONDICIONADA POR LUGAR (PCL)

A preferência condicionada por lugar (PCL) é um comportamento aprendido, testado em várias espécies e um dos modelos experimentais mais utilizados para mensurar os efeitos reforçadores positivos (preferência condicionada por lugar) e os negativos (aversão condicionada por lugar) de diversas drogas (BARDO e BEVINS, 2000). A preferência ocorre quando o animal prefere um determinado lugar, porque o local preferido tem sido associado

previamente com eventos gratificantes, enquanto na aversão, o compartimento pareado com a droga elicia um comportamento aversivo (HUSTON et al., 2013). A PCL é baseada no condicionamento Pavloviano clássico no qual a droga (estímulo incondicionado - EI) é repetidamente pareada com um ambiente específico. Esse ambiente reflete a capacidade de estímulos ambientais originalmente neutros passarem a atuar como estímulo condicionado (EC), quando a administração da droga (EI) adquire propriedades motivacionais positivas após pareamentos repetidos, essa associação do EC com EI provoca comportamentos de aproximação, resultando em uma resposta condicionada (TZSCHENTKE, 2007). Ratos e camundongos experienciam repetidamente dois ambientes neutros distintos que são pareados espacial e temporalmente com estados distintos de droga ou não-droga (Figura 6). Em uma sessão de pós-condicionamento dá-se, então, a oportunidade de o animal escolher e permanecer nos dois ambientes e o tempo de permanência no compartimento previamente associado à droga de abuso é considerado um índice do valor reforçador dessa droga. Com efeito, ratos e camundongos apresentam uma clara preferência condicionada pelo ambiente previamente associado a diferentes drogas de abuso, como a anfetamina, a metanfetamina, a cocaína, a nicotina, a morfina e o etanol, entre outras (BARDO e BEVINS, 2000). O efeito de condicionamento contextual observado na PCL varia de acordo com o número de sessões de condicionamento, o intervalo entre as sessões, a dose administrada, a via de administração, o intervalo entre a injeção e a sessão, o local onde a droga foi injetada e a natureza do estímulo ambiental (TZSCHENTKE, 2007).

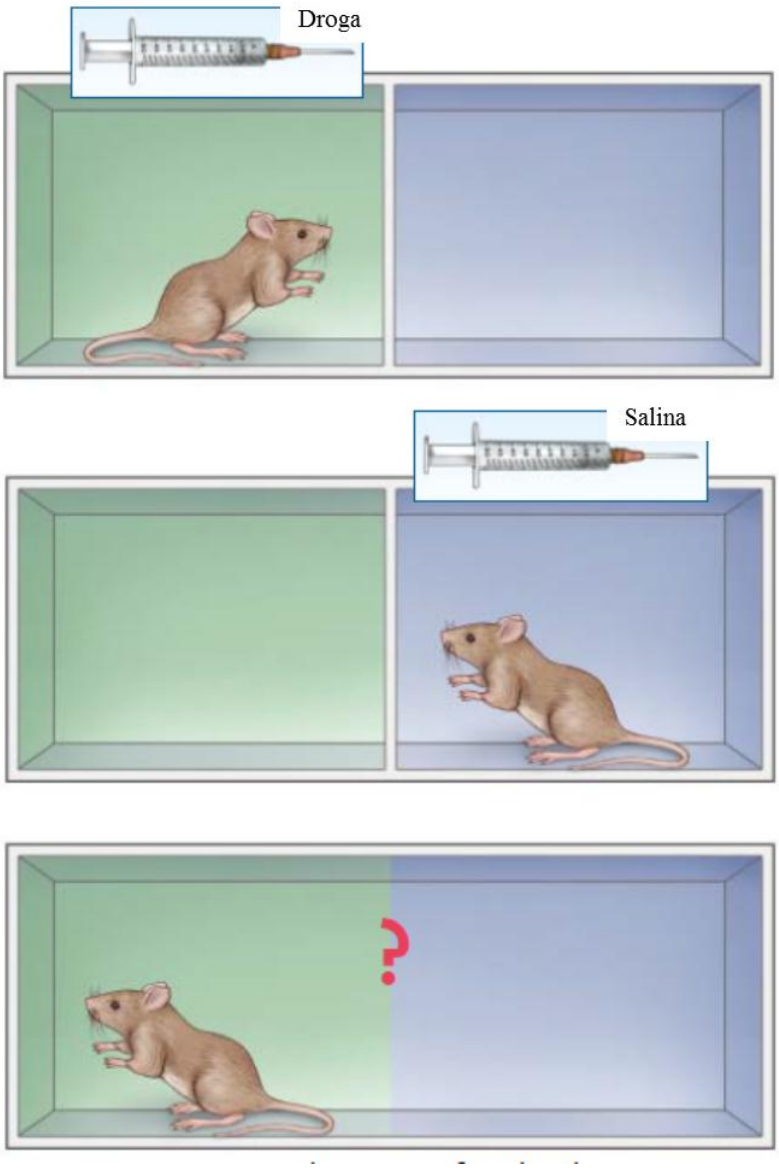

Figura 6: Preferência condicionada por lugar: Inicialmente, o animal é colocado na caixa de condicionamento com livre acesso aos compartimentos e o tempo de permanência em cada compartimento é registrado. Após vários pareamentos alternados de droga ou salina, no dia do teste, é evidenciado se a droga testada atuou como reforçador, devido à associação do ambiente com o efeito da droga. Adaptado de Camí e Farré, 2003.

1.10 INTERAÇÃO ENTRE OS SISTEMAS OPIOIDE E GLUTAMATÉRGICO

Tem sido proposto que existe uma interação entre os sistemas opioide e glutamatérgico, uma vez que o antagonista de receptores NMDA, MK-801((+)5-metil-10,11-dihidro-5H-dibenzo[a,b]-ciclohepteno-5-10-amino), potencializa a amnésia induzida por morfina (CESTARI e CASTELLANO, 1997) e previne a dependência de estado induzida por

opioides (ZARRINDAST et al., 2006), sugerindo que a dependência de estado à morfina envolve receptores opioides e NMDA. Também foi mostrado que a dependência de estado induzida pela arcaína envolve o sistema opioide uma vez que a administração de naloxona reverte a amnésia e a dependência de estado induzidas pela arcaína e morfina (MARIANI et al., 2011).

Muitos estudos mostram que a morfina induz PCL (SUZUKI et al., 1994; LIANG et al., 2006; TAHSILI-FAHADAN et al., 2006; MA et al., 2007; BYRNES et al., 2012; FAN et al., 2012; ZHANG et al., 2012) e que antagonistas do receptor NMDA reduzem a PCL induzida por morfina durante as fases (aquisição, consolidação e expressão) da memória de recompensa (LU et al., 2011; FAN et al., 2012). A ativação dos receptores NMDA é necessária para a expressão de efeitos comportamentais e fisiológicos, visto que a inativação genética desses receptores prejudica a aquisição da PCL induzida por comida e cocaína (ZWEIFEL et al., 2008; ZWEIFEL et al., 2009). Na mesma linha de evidências é interessante ressaltar que antagonistas do receptor NMDA, quando injetados pré-treino, inibem a aquisição, quando injetados pós-treino, inibem a consolidação e quando injetados pré-teste inibem a expressão da PCL induzida por morfina em ratos e camundongos (NODA e NABESHIMA, 2004), como por exemplo: MK-801 (TZSCHENTKE e SCHMIDT, 1995; DEL POZO et al., 1996; SUZUKI et al., 2000; RIBEIRO DO COUTO et al., 2004; YONGHUI et al., 2006; REZAYOF et al., 2007; LI et al., 2011; FAN et al., 2012), memantina (POPIK et al., 2003; RIBEIRO DO COUTO et al., 2004; CHEN et al., 2012), cetamina (SUZUKI et al., 2000; GAO et al., 2003), AP5 (Ácido D-2-amino-5-fosfonopentanóico) (HARRIS et al., 2004; WU et al., 2012), NPC 17742 (POPIK e KOLASIEWICZ, 1999), MRZ 2/579 (POPIK et al., 1998), CGP 37849 (TZSCHENTKE e SCHMIDT, 1995), dextrometorfano (LUE et al., 2007; CHEN et al., 2011) e ifenprodil (SUZUKI et al., 1999; MA et al., 2006; MA et al., 2011; XU et al., 2012). Ainda, Liu e colaboradores (2012) mostraram que gentiopicrosideo, um inibidor da expressão GluN2B do receptor NMDA, bloqueia a PCL induzida por morfina e Kao et al, (2011), mostram que usando silenciador genético (siRNA) da subunidade GluN2B também bloqueou a PCL induzida por morfina. Dessa forma, é possível considerar que antagonistas do receptor NMDA poderiam ser considerados como candidatos na busca pelo tratamento da adicção (MA et al., 2006; ALAGHBAND e MARSHALL, 2013).

No entanto, a maioria dos antagonistas do receptor de NMDA que bloqueiam a PCL induzida por morfina produzem efeitos psicoestimulantes e psicotomiméticos em modelos animais (BALSTER, 1987; KOEK et al., 1988; WILLETTS et al., 1990) e efeitos colaterais

adversos em estudos clínicos (SVEINBJORNSDOTTIR et al., 1993; GROTTA et al., 1995). Além disso, os antagonistas de NMDA possuem propriedades gratificantes e induzem PCL (TZSCHENTKE e SCHMIDT, 1995; PAPP et al., 1996; TZSCHENTKE, 1998). Tal efeito de recompensa tem sido demonstrado pela cetamina (SUZUKI et al., 2000), MK-801 (DEL POZO et al., 1996; SUZUKI et al., 2000), fenciclidina (MARGLIN et al., 1989; NODA et al., 1998; NODA e NABESHIMA, 2004; SHIN et al., 2005), dextrorfano e dextrometorfano (SHIN et al., 2005), o que provavelmente implica em potencial de abuso. Por estas razões, o uso destes antagonistas NMDA parece acarretar efeitos indesejáveis para o tratamento da adicção.

Considerando-se que: 1) agonistas do receptor NMDA facilitam a PCL induzida por morfina (TZSCHENTKE e SCHMIDT, 1995; PANOS et al., 1999; ZARRINDAST et al., 2007; HU et al., 2012); 2) antagonistas do receptor NMDA bloqueiam o estabelecimento da PCL induzida por morfina (TZSCHENTKE e SCHMIDT, 1997); 3) e que mecanismos opioides desempenham um papel na memória de dependência de estado induzida pelo arcaína (MARIANI et al., 2011); no presente estudo investigamos se a arcaína e a espermidina alteram a aquisição, consolidação e expressão de PCL induzida por morfina.

2. OBJETIVOS

2.1 Objetivo Geral

Avaliar se a administração sistêmica de poliaminas induz PCL e se as poliaminas revertem a PCL induzida por morfina em camundongos.

2.2 Objetivos específicos

2.2.1. Avaliar o efeito da administração de morfina, espermidina e arcaína sobre a PCL e sobre a aversão condicionada por lugar;

2.2.2. Avaliar o efeito da administração de arcaína sobre a aquisição, consolidação e expressão da PCL induzida por morfina;

2.2.3. Avaliar se a combinação de espermidina e morfina em dose sem efeito na PCL, induz PCL;

2.2.4. Avaliar o efeito da administração combinada de espermidina e arcaína sobre a PCL induzida por morfina;

2.2.5. Avaliar se a administração de morfina, arcaína e espermidina alteram a atividade locomotora dos animais;

2.2.6. Avaliar o efeito das administrações de morfina, arcaína e espermidina sobre as enzimas marcadoras de toxicidade renal e hepática.

3 MANUSCRITO

Arcaine, a GluN2B Antagonist, Blocks the Establishment of Morphine-Induced Conditioned Place Preference in Mice

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Running title: Arcaine blocks morphine-induced conditioned place preference

ABSTRACT

Opioid addiction is a chronic, recurrent brain disease that is characterized by compulsive drug seeking and a high rate of relapse, even after long periods of abstinence. Several studies have shown that the systemic administration of a variety of N-methyl-D-aspartate (NMDA) receptor antagonists blocks the development of conditioned place preference (CPP) induced by rewarding drugs, such as morphine. However, no study has investigated whether polyamines alter abuse-related effects of morphine. In this study we examined whether polyamines induced CPP and/or modified morphine-induced CPP. Adult male Swiss mice received morphine, spermidine (an endogenous polyamine that physiologically modulates the NMDA receptor) arcaine (an antagonist of the polyamine-binding site at the NMDA receptor), or saline and were subjected to the CPP paradigm. Morphine (2.5–10 mg/kg, i.p.) significantly increased the time spent in the drug-paired compartment. Arcaine (0.3 – 3 mg/kg, i.p.) and spermidine (3–30 mg/kg, i.p.) did not induce CPP or aversion. Arcaine (3

mg/kg) 15 minutes before of morphine (5 mg/kg) attenuated the acquisition of morphine-induced CPP. Arcaine immediately after conditioning with morphine and 30 min before testing blocked morphine-induced CPP. Spermidine (30 mg/kg) 15 minutes before arcaine and morphine prevented the attenuating effect of arcaine on the acquisition of morphine-induced CPP. However, spermidine did not reverse arcaine-induced blockade of CPP consolidation and expression. These data indicate that arcaine blocks the rewarding effect of morphine and suggests that the polyamine binding site may be a target to treat morphine abuse.

Keywords: arcaine; spermidine; morphine; conditioned place preference; addiction; NMDA receptor

INTRODUCTION

Opiate addiction is a complex relapsing brain disease process that is characterized by the compulsive seeking and taking an opiate, and the emergence of a negative emotional state when access to the opiate is denied (Koob, 1998). Morphine is a potent opioid analgesic that is widely used for the relief of pain, but it produces both psychological and physical dependence (Suzuki *et al*, 1994; Suzuki *et al*, 1999). The reinforcing effects of opiates have long been known, and demonstrated in both humans and experimental animals (Leshner and Koob, 1999). The conditioned place preference (CPP) paradigm has been widely used to assess the rewarding effects of a variety of drugs, including opiates (Tzschentke, 2007). The CPP paradigm is based upon the idea that contextual stimuli can acquire conditioned rewarding properties when paired with addictive drugs, reflecting their liability to be abused (Ribeiro Do Couto *et al*, 2005). In fact, morphine induces CPP in both rats and mice (Liu *et al*, 2012; Lin *et al*, 2014), and this experimental paradigm has been used to unveil some of the neurochemical mechanisms involved in its rewarding effects and addiction development.

Accumulating evidence suggests a role for the mesolimbic dopaminergic system in the rewarding effects of morphine (Wise, 1988; Koob and Le Moal, 2001; Narita *et al*, 2010), although there is also evidence indicating that morphine may induce reward by mechanisms that do not involve dopamine (Hnasko *et al*, 2005). Morphine increases dopamine (DA) and glutamate extracellular concentrations in the nucleus accumbens (Kebabian and Calne, 1979) by activating dopaminergic neurons that project from the ventral tegmental area to the nucleus accumbens. The mechanism by which morphine increases dopaminergic activity probably involves the inhibition of GABAergic inhibitory interneurons in the ventral tegmental area (Leone *et al*, 1991; Johnson and North, 1992). However, neurotransmitters other than

dopamine have been implicated in the development and maintenance of addiction to opiates (Zarrindast *et al*, 2003; Zarrindast *et al*, 2006; Rezayof *et al*, 2007) and a role for the glutamatergic system has been proposed (Nestler, 1996; Tzschentke and Schmidt, 2003; Mazei-Robison and Nestler, 2012) .

Both competitive and uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonists have been found to inhibit the CPP induced by morphine, amphetamine and cocaine (Del Pozo *et al*, 1996; Xi and Stein, 2002; Huang *et al*, 2003; Danysz *et al*, 2005; Makarska-Bialek *et al*, 2005; Backstrom and Hyytia, 2006; Lue *et al*, 2007), suggesting a possible role for NMDA receptor antagonists in the treatment of drug addiction (Tzschentke and Schmidt, 1995; Narita *et al*, 2001; Ma *et al*, 2006; Ma *et al*, 2007; Chen *et al*, 2011; Hu *et al*, 2012). However, most of NMDA receptor antagonists produce severe psychostimulant and psychotomimetic-like effects in animal models (Balster, 1987; Koek *et al*, 1988; Willetts *et al*, 1990) and severe adverse side effects were observed in clinical trials (Sveinbjornsdottir *et al*, 1993; Grotta *et al*, 1995). In addition, NMDA antagonists have rewarding properties, and induce CPP (Tzschentke and Schmidt, 1995; Papp *et al*, 1996; Tzschentke, 1998). Such a putative rewarding effect has been shown for ketamine (Suzuki *et al*, 2000), dizocilpine (Del Pozo *et al*, 1996; Suzuki *et al*, 2000), phencyclidine (Marglin *et al*, 1989; Noda and Nabeshima, 2004; Shin *et al*, 2005), dextrorphan and dextromethorphan (Shin *et al*, 2005), implying that they would probably have abuse potential. For these reasons, the use of orthostheric NMDA antagonists for treating drug addiction sounds unlikely.

Polyamines, naturally occurring polycations that selectively enhance NMDA receptors containing the GluN2B subunit, bind at a dimer interface between GluN1 and GluN2B subunit (Mony *et al*, 2011). It has been shown that arcaine, a competitive antagonist of the NMDA receptor polyamine-binding site, and morphine induce cross state-dependency in rats (Mariani *et al*, 2011), suggesting that polyamine antagonists may interfere with behavioral responses previously associated to morphine. Therefore, one might suppose that polyamine binding site antagonists, as other NMDA receptor antagonists, could also modify the rewarding effects of opioids. As such, these drugs could be potentially useful to decrease morphine-driven behavior, particularly if they did not cause rewarding effects *per se*. Considering that: 1) NMDA receptor agonists facilitate morphine-induced CPP (Tzschentke and Schmidt, 1995; Panos *et al*, 1999; Zarrindast *et al*, 2007; Hu *et al*, 2012) ; 2) glutamate receptor antagonists block the development of CPP induced by rewarding drugs (Tzschentke and Schmidt, 1997); 3) opioid mechanisms play a role in the state-dependent memory induced

by arcaine (Mariani *et al*, 2011); in the current study we investigated whether arcaine and spermidine alter the acquisition, consolidation and expression of morphine-induced CPP.

MATERIALS AND METHODS

Animals

Adult male Swiss mice (25-30 g), bred in the animal house of the Federal University of Santa Maria, housed 6 to a cage and maintained in a day/night cycle at temperature 21º C with access to water and food *ad libitum* were used. All experiments were carried out in the light phase and are in accordance with Brazilian law nº. 11.794/2008, which is in agreement with the Policies on the Use of Animals and Humans in Neuroscience Research and with the Institutional and National Regulations for Animal Research (process 068/2011).

Drugs

1, 4-diguanidinobutane sulfate (arcaine) was obtained from Pfaltz & Bauer (Waterbury, CT, USA), N-(3- aminopropyl)-1, 4-butanediamine trihydrochloride (spermidine) was obtained from Sigma-Aldrich Co (St. Louis, MO, USA) and morphine sulfate was obtained from Cristália (Itapira, São Paulo, Brazil). All drugs solutions were prepared in saline (0.9% NaCl) and the injections were performed intraperitoneally (i.p.) in a 10 ml/kg injection volume.

Apparatus

The conditioned place preference apparatus consisted of three wooden compartments separated by guillotine doors. Two of the compartments (A and B) were identical in size (18 cm length ×16 cm width ×40 cm height), but had different walls and floor colors and floor texture. Compartment A had black walls and a white floor covered by a wire mesh grid. Compartment B had white walls and a smooth black floor. The small center compartment (10 x 10 x 40 cm) was gray. During the conditioning phases, the compartments were separated by guillotine door.

Conditioned Place Preference

Conditioned place preference (CPP) consisted of a 7-day schedule with three distinct phases: preconditioning, conditioning and postconditioning (test), and was carried out according to Fukushiro and colleagues (2007), with minor modifications.

Preconditioning. In these sessions each mouse was injected with saline (0.9% NaCl, 10 ml/kg, i.p.) and placed in the apparatus for 15 min. During this time the animal was allowed to freely explore the three compartments. In the second day of preconditioning, the time spent in each compartment was recorded. Placement in each compartment was considered as placement of the front paws and the head. Since mice preferred the black compartment (A) during the preconditioning session, the white compartment was chosen as the drug-paired compartment [mean permanence time in the black (A): 567 ± 101 s and white (B): 204 ± 65 s compartments in the preconditioning session, $t(27) = 11.99$; $p<0.001$. Moreover, animals were distributed among groups according to place preference, in such a way that all groups had similar baseline place preference.

Conditioning. Place conditioning was established in four consecutive days. In each day, two 30 min conditioning sessions were carried out, spaced 5:30 h apart. On the first and the third days of conditioning, between 8:00 and 12:00, each animal was injected with saline and immediately confined to the black compartment (A, non drug-paired compartment) of the apparatus for 30 min. Immediately after the session, the animal was returned to its home cage. Between 14:00 and 18:00 each animal was injected with morphine, arcaine or spermidine, and confined to the white compartment (B, drug-paired compartment). On the second and the fourth days of conditioning, the procedure was performed in the reverse order, that is, from 8:00 to 12:00 the animals were injected with morphine, arcaine or spermidine, and confined to the white compartment; between 14:00 to 18:00 mice were injected with saline and confined to the black compartment.

Postconditioning or testing. This phase was carried out 24 h after the last conditioning session. Animals were allowed to freely explore the compartments of the apparatus for 15 min. The time spent in each compartment during the 15-min session was recorded. Drug-associated place preference was calculated as the difference (in seconds) of time spent in the drug-paired compartment during the post- and preconditioning phases.

Measurement of locomotor activity. Locomotor activity in the drug-paired compartment during the test session was measured according to Tahsili-Fahadan (2006). The ground area of the compartment was divided into two equal segments by a transverse line, and locomotion was measured as the number of crossings from one-half to the other over 15 min of testing, corrected for the total time spent in the respective compartment.

CPP experimental design

Dose-response curves of morphine, arcaine and spermidine on CPP. In this experiment, we established dose-response curves for morphine, arcaine and spermidine. Mice received different doses of morphine sulfate (0, 1.25, 2.5, 5 or 10 mg/kg, i.p.), arcaine (0, 0.3, 1 or 3 mg/kg, i.p.) or spermidine (0, 3, 10 or 30 mg/kg, i.p.) immediately before the confinement to the drug-paired compartment in the conditioning sessions. The mice were subjected to the postconditioning session in a drug-free state.

Effects of arcaine on acquisition, consolidation and expression of morphine-induced CPP. In order to investigate the effect of arcaine on the acquisition of morphine-induced CPP, mice were injected with saline or arcaine (3 mg/kg, i.p.) and, fifteen minutes thereafter, they were injected with saline or morphine (5 mg/kg, i.p.), immediately before the confinement to the drug-paired compartment in the conditioning sessions. The test was carried out 24 hours after the last conditioning session, in a drug-free state.

To investigate the effect of arcaine on the consolidation of morphine-induced CPP, mice were injected with saline or morphine (5 mg/kg, i.p.) immediately before the confinement to the drug-paired compartment in the conditioning sessions, and saline or arcaine (3 mg/kg, i.p.) immediately after each conditioning session. The test was carried out 24 hours after the last conditioning session, in a drug-free state.

To investigate the effect of arcaine on the expression of morphine-induced CPP, mice were injected with saline or morphine (5 mg/kg, i.p.) immediately before the confinement to the drug-paired compartment in the conditioning sessions, and saline or arcaine (3 mg/kg, i.p.) 30 minutes prior to testing session.

Effects of spermidine and arcaine on the acquisition, consolidation and expression of morphine-induced CPP. In order to investigate the involvement of NMDA receptor polyamine-binding sites in the deleterious effect of arcaine on CPP acquisition, we administered the polyaminergic agonist spermidine before arcaine, and subjected the animals to morphine-induced CPP. Animals were initially injected with saline or spermidine (30 mg/kg, i.p.) and placed in a clean waiting cage (with the same dimensions of the home cage). Fifteen minutes thereafter they were injected with saline or arcaine (3 mg/kg, i.p.). Fifteen minutes after saline or arcaine injection the animals were injected with saline or morphine (5

mg/kg, i.p.) and subjected to the conditioning sessions. Animals were tested on the day of testing in a drug-free state.

The effect of spermidine on the deleterious effect of arcaine on the consolidation of morphine-induced CPP was also investigated. Animals received saline or morphine (5 mg/kg, i.p.) immediately before the confinement to the drug-paired compartment in the conditioning sessions. Saline or arcaine (3 mg/kg, i.p.) were injected immediately after the confinement to the drug-paired compartment in the conditioning sessions. The animals were transferred to a clean waiting cage and, fifteen minutes thereafter, the animals were injected with saline or spermidine (30 mg/kg, i.p.) and returned to their home cages. Animals were tested on the day of testing in a drug-free state.

The effect of spermidine on the deleterious effect of arcaine on the expression of morphine-induced CPP was also investigated. Animals received saline or morphine (5 mg/kg, i.p.) immediately before the confinement to the drug-paired compartment in the conditioning sessions and returned to their home cages. Saline or arcaine (3 mg/kg, i.p.) were injected 30 min before testing and saline or spermidine (30 mg/kg, i.p.) 15 min before testing.

Effect of spermidine and a noneffective dose of morphine CPP. The previous experiment (fig 3) showed that spermidine does not alter CPP induced by a fully effective dose of morphine. However, one might argue that this has occurred because of a ceiling effect of morphine on CPP. Therefore, we investigated whether combining a noneffective dose of morphine and spermidine induced CPP. The spermidine dose used (30 mg/kg, i.p.) was selected on the basis of its ability to prevent the deleterious effect of arcaine in the experiment shown in figure 3. Mice were injected with saline or spermidine and, fifteen minutes thereafter, they were injected with saline or morphine (1.25 mg/kg, i.p.) immediately before the confinement to the drug-paired compartment in the conditioning sessions. The subeffective dose of morphine was chosen from the dose-response curve shown in fig 1c. Animals were tested on the test day in a drug-free state.

Statistical analysis

Data analysis was performed using one, two or three-way analysis of variance (ANOVA), depending on the experimental design. *Post hoc* analyses were carried out by the Student–Newman–Keuls test, when indicated. A $p<0.05$ was considered significant.

RESULTS

Figure 1a shows the effect of morphine (1.25-10 mg/kg, i.p.) on the time spent in the white (drug-paired) compartment in mice. Statistical analysis (one-way ANOVA) revealed that morphine induced place preference ($F_{4,25}$=6.30; p<0.05, figure 1a). *Post hoc* analysis revealed that doses of 2.5-10 mg/kg of morphine increased the time spent in the white (drug-paired) compartment, compared with saline control group.

Figure 1b and c show the effect of arcaine (0.3-3 mg/kg, i.p.) and spermidine (3-30 mg/kg, i.p.) on the time spent in the white (drug-paired) compartment respectively. Statistical analyses (one-way ANOVA) showed that neither arcaine ($F_{3,24}$=1.78; p>0.05, Figure 1b) nor spermidine ($F_{3,19}$=0.11; p>0.05, Fig. 1c) induced CPP or aversion.

Insert fig. 1 here.

Figure 2a shows the effect of arcaine on the acquisition of morphine-induced CPP. Statistical analyses (two-way ANOVA) revealed a significant pretreatment (saline or arcaine) by treatment (saline or morphine) interaction ($F_{1,32}$=6.40; p< 0.05), suggesting that arcaine blocks morphine-induced CPP.

Figure 2b shows the effect of arcaine on the consolidation of morphine-induced CPP. Statistical analyses (two-way ANOVA) revealed a significant treatment (saline or morphine) by post-treatment (saline or arcaine) interaction ($F_{1,24}$= 10.48; p<0.05), indicating that arcaine administration after conditioning completely blocks morphine-induced CPP.

Figure 2c shows the effect of arcaine on the expression of morphine-induced CPP. Statistical analyses (two-way ANOVA) revealed a significant treatment (saline or morphine) by post-treatment (saline or arcaine) interaction ($F_{1,24}$= 21.99; p<0.05), indicating that arcaine administration before testing completely blocks morphine-induced CPP.

Insert fig. 2 here.

Figure 3 shows the effect of spermidine on arcaine-induced blockade of morphine-induced CPP. Statistical analysis (three-way ANOVA) revealed a significant pretreatment 1 (saline or spermidine) by pretreatment 2 (saline or arcaine) by treatment (saline or morphine) interaction ($F_{1,69}$ =20.92; p<0.05), indicating that spermidine prevents the deleterious effect of arcaine on morphine-induced CPP acquisition.

Insert fig. 3 here.

Figure 4 shows the lack of effect of the combination of a noneffective dose of morphine (1.25 mg/kg, i.p.) and spermidine (30 mg/kg, i.p.) on the time spent in the white (drug-paired) compartment. Statistical analyses (two-way ANOVA) showed no significant effects ($F_{1,24}$=0,69; p>0.05), suggesting that the combination of a noneffective dose of morphine and spermidine did not induce CPP or aversion.

Insert fig. 4 here.

Figure 5 shows the lack of effect of spermidine on arcaine-induced blockade of morphine-induced CPP during the consolidation phase. Statistical analyses (three-way ANOVA) revealed only a significant treatment (saline or morphine) by post-treatment (saline or arcaine) interaction ($F_{1,56}$ =51.51; p<0.001), indicating that, also in this experiment, arcaine blocks morphine-induced CPP during the consolidation phase.

Insert fig. 5 here.

Figure 6 shows the lack of effect of spermidine on arcaine-induced blockade of the expression of morphine-induced CPP. Statistical analyses (three-way ANOVA) showed only a significant treatment (saline or morphine) by post-treatment (saline or arcaine) interaction ($F_{1,56}$=30.24; p<0.001), indicating that, also in this experiment, arcaine blockade the expression of morphine-induced CPP.

Insert fig. 6 here.

DISCUSSION

In the current study we showed that the pre-training, post-training and pre-test administration of arcaine blocks morphine-induced CPP. The deleterious effect of pre-training arcaine on morphine-induced CPP was prevented by spermidine. Notwithstanding, post-training and pre-test arcaine-induced blockade of CPP in the consolidation and expression

was not reversed by spermidine. Moreover, spermidine did not alter morphine-induced CPP. Moreover, any treatment alters the locomotor activity of animals (data not shown).

Our findings are in agreement with previous studies that have shown that morphine induces CPP (Tahsili-Fahadan *et al*, 2006; Kao *et al*, 2011; Liu *et al*, 2012; Tabaeizadeh *et al*, 2013), an animal model to assess the rewarding effect of different drugs, including opioids (Bardo *et al*, 1984). It has been proposed that opioids induce CPP by activating the reward circuit (Hyman *et al*, 2006). According to this view, morphine binds to μ-opioid receptors present in GABAergic neurons located in the ventral tegmental area and decreases GABAergic release. By these means it would indirectly stimulate the ascending mesocorticolimbic dopamine system (Kalivas, 1993; van Ree *et al*, 1999). Such a disinhibition of DA neurons in the ventral tegmental area increases DA release in nucleus accumbens (Johnson and North, 1992; Xi and Stein, 2002), which has been regarded to play a central role in reward. In addition, there are μ opioid receptors expressed by nucleus accumbens and dorsal striatal neurons. Opiates can stimulate these receptors directly and produce reward in a dopamine-independent manner (Hyman *et al*, 2006).

Interestingly, the acquisition, consolidation and expression of morphine reward in the CPP paradigm have been attenuated or blocked by NMDA glutamate receptor antagonists in both rats and mice (Bisaga and Popik, 2000; Papp *et al*, 2002). Accordingly, MK-801 (Tzschentke and Schmidt, 1995; Del Pozo *et al*, 1996; Kim *et al*, 1996; Tzschentke and Schmidt, 1997; Suzuki *et al*, 2000; Yonghui *et al*, 2006; Rezayof *et al*, 2007; Li *et al*, 2011; Fan *et al*, 2012), memantine (Popik and Danysz, 1997; Popik *et al*, 2003; Ribeiro Do Couto *et al*, 2004), ketamine (Suzuki *et al*, 2000; Gao *et al*, 2003), ifenprodil (Suzuki *et al*, 1999; Ma *et al*, 2006; Ma *et al*, 2011), NPC 17742 (Popik and Kolasiewicz, 1999), AP5 (Harris *et al*, 2004; Hu *et al*, 2012), dextromethorphan (Lue *et al*, 2007; Chen *et al*, 2011), agmatine (Wei *et al*, 2005) and CGP37849 (Tzschentke and Schmidt, 1995) have been reported to decrease morphine-induced CPP.

Zhu et al, (1999) have reported that the expression of the GluN1 subunit mRNA is increased in the locus coeruleus and in the hypothalamic paraventricular nucleus following 3 days of intracerebroventricular morphine infusion, suggesting that it is involved in the development of morphine dependence. Accordingly, the pretreatment with a NMDA receptor GluN1 subunit antisense oligonucleotide attenuates morphine withdrawal syndrome (Zhu and Ho, 1998). However, since the GluN1 subunit is an obligatory component of functional NMDA receptors (Paoletti, 2011), GluN1 supression itself does not tell much about the role of NMDA receptor subtypes in morphine dependence or reward-related effects. In this regard,

it is important that NMDA receptor subunits GluN1, GluN2A and GluN2B, but not GluN2C and GluN2D, are consistently expressed in the reward circuitry (Mori and Mishina, 1995). In fact, GluN2A receptors have been suggested to play a role in morphine dependence. Although both wild-type and GluN2A knockout mice repeatedly treated with morphine show withdrawal signs after treatment with naloxone (Miyamoto *et al*, 2004), the signs of naloxone-precipitated morphine-withdrawal symptoms are significantly attenuated in GluN2A knockout, compared with wild-type mice. These findings have suggested that adaptive changes mediated by GluN2A subunit-containing NMDA receptors play a role in the development of morphine physical dependence (Miyamoto *et al*, 2004). Interestingly, enhancement of GluN2A protein expression is observed in the nucleus accumbens of wild-type mice after development of dependence by chronic morphine treatment (Inoue *et al*, 2003), and the rescue of GluN2A protein, by electroporation into the nucleus accumbens of GluN2A knockout mice, significantly reverses the loss of abstinence behaviors. These findings have suggested a locus-specific role for GluN2A in the development of morphine physical dependence (Inoue *et al*, 2003).

Pharmacological evidence supports that GluN2B-containing NMDA receptors may be more importantly involved in morphine addiction than GluN2A-containing NMDA receptors. Accordingly, the administration of ifenprodil, a selective antagonist of GluN2B-containing NMDA receptor, suppresses morphine-induced rewarding effects in mice (Suzuki *et al*, 1999) and rats (Ma *et al*, 2006; Ma *et al*, 2011). Moreover, anti-NR2B antibody administration abolishes morphine-induced rewarding effects in mice, whereas antibodies against GluN1 and GluN2A subunits do not (Narita *et al*, 2000). At last, gentiopicroside- (Liu *et al*, 2012) and small interference RNA-induced (Kao *et al*, 2011) GluN2B downregulation decreases CPP, further supporting a role for GluN2B in the rewarding effects of morphine. Since arcaine may displace polyamines from the polyamine binding site at a dimer interface between GluN1 and GluN2B subunit (Mony *et al*, 2011), decreasing its function, the currently reported attenuation of morphine-induced CPP by arcaine administration before conditioning session could be interpreted as additional evidence that GluN2B receptors are involved in the acquisition of morphine-induced CPP. Moreover, one could also suggest that arcaine may impair context/drug association (acquisition) or reduce the rewarding effect of morphine. The possibility that arcaine causes place aversion, however, was refuted in the experiments shown in Figure 1, that revealed that arcaine (Figure 1b) and spermidine (Figure 1c) do not cause place preference or aversion *per se*. As expected,

from the pharmacological point of view, this effect of arcaine was fully prevented by the injection of spermidine (Figure 3).

Interestingly, we verified that the injection of arcaine immediately after the conditioning sessions abolished morphine-induced place preference at testing. Since arcaine was injected immediately after each conditioning session, one might reasonably conclude that this effect of arcaine is not due to an alteration in the motivational state of the animal during conditioning, i.e., arcaine neither altered the reward properties of morphine nor impaired the association between context and drug state, because it was injected after conditioning. Although arcaine did not induce manifested preference or aversion, due to the fact that arcaine injection was contingent upon morphine injection it is possible that a second, maybe less rewarding association (a morphine+arcaine interoceptive state) has emerged immediately after (but behaviorally associated to) the conditioning sessions, resulting in decreased preference scores at testing. It is also possible that arcaine injection impaired the consolidation of the memory of the conditioning sessions, since arcaine impairs memory consolidation (Rubin *et al*, 2001; Rubin *et al*, 2004; Camera *et al*, 2007; Ceretta *et al*, 2008) and reconsolidation (Ribeiro *et al*, 2013). Interestingly, Zarrindast and colleagues (2007), have shown that NMDA potentiates the rewarding effect of morphine, a finding that is in agreement with the view that NMDA receptor activation is involved in this effect of morphine.

The injection of arcaine before testing blockade the expression of morphine-induced CPP. It has been repeatedly shown that arcaine injection before testing does not alter the performance in the inhibitory avoidance task (Ceretta *et al*, 2008; Mariani *et al*, 2011). Interestingly, it has been previously reported that the pre-test effect of arcaine depends on the activation of opioid receptors, since morphine-induced state dependency transfers to arcaine, and naloxone blocks pre-test arcaine-induced facilitatory effects in animals injected post-training with morphine or arcaine (da Rosa *et al*, 2012). Given that morphine-induced state dependency transfers to arcaine (Mariani *et al*, 2011), we expected that arcaine would analogously facilitate the expression of morphine-induced CPP. However, we found the opposite, which, in turn, is in agreement with the various studies that have shown that NMDA receptor antagonists block the expression of CPP (Popik *et al*, 1998; Popik and Kolasiewicz, 1999; Popik *et al*, 2003; Harris *et al*, 2004; Wei *et al*, 2005; Ma *et al*, 2006; Yonghui *et al*, 2006; Li *et al*, 2011). This discrepancy may have occurred because of adaptive changes due to daily injections of morphine (5 mg/kg) for 4 days. In fact, this protocol is known to double the ED_{50} for morphine in the tail flick paradigm (Kolesnikov *et al*, 1993). Moreover, a 4-day

10 mg/kg morphine CPP protocol has been associated to an adaptive increase in the immunoreactivity of phosphoCREB in the hippocampus, nucleus accumbens, ventral tegmental area, striatum, prefrontal cortex and cortex, an adaptive change in the opposite direction of that caused by acutely administered morphine (Moron *et al*, 2010). Therefore, it is reasonable that these adaptive changes in the opioid system may have modified the behavioral response to arcaine.

In conclusion, the present study reports that a putative antagonist of the polyamine binding site at the NMDA receptor blocks morphine-induced CPP. Whether the currently described effects of arcaine on morphine-induced CPP are due to an anti-reward effect of the compound or a disruption of a cognitive component of the addiction process remains to be determined.

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DISCLOSURE

The authors declare no conflict of interest. None of the authors have received compensation for professional services in any of the previous three years, or anticipate receiving such compensation in the near future.

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FIGURES AND LEGENDS

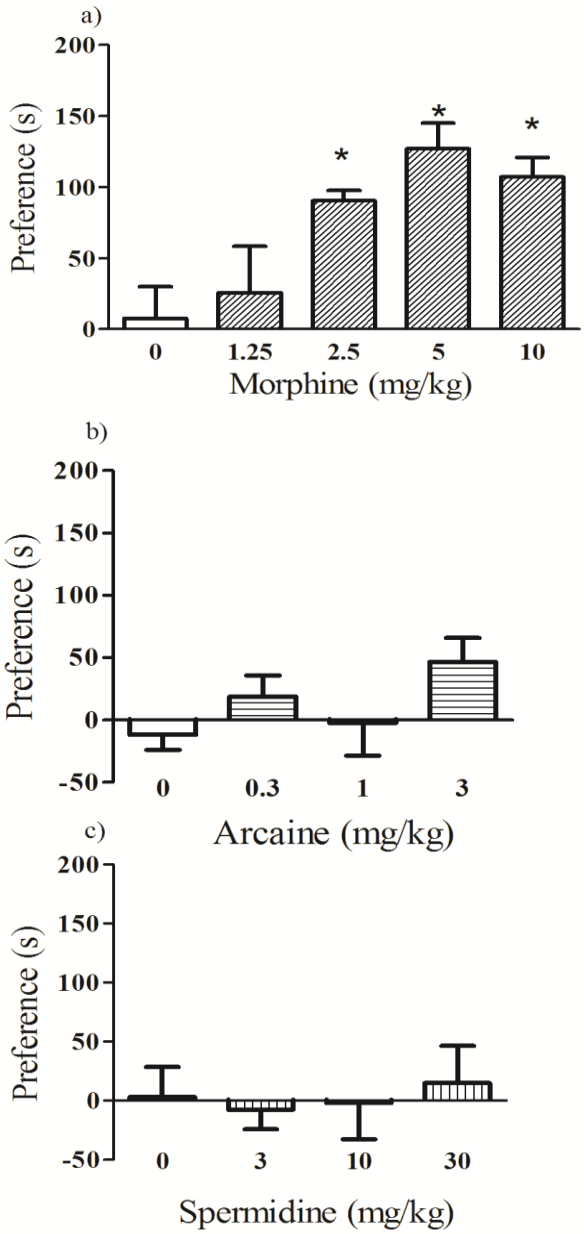

Figure 1 Effect of intraperitoneal administration of morphine (a), arcaine (b) and spermidine (c) on CPP. Animals received saline, morphine, arcaine or spermidine immediately before the confinement to the nonpreferred compartment in the 1st, 2 nd, 3rd and 4th days of conditioning. The change of preference was assessed as the difference between the time spent in the drug-paired compartment on the day of testing and the time spent in the drug-paired compartment on the second preconditioning session. Data are expressed as mean + SEM of 5-7 animals per group. *$p<0.05$ compared with control group.

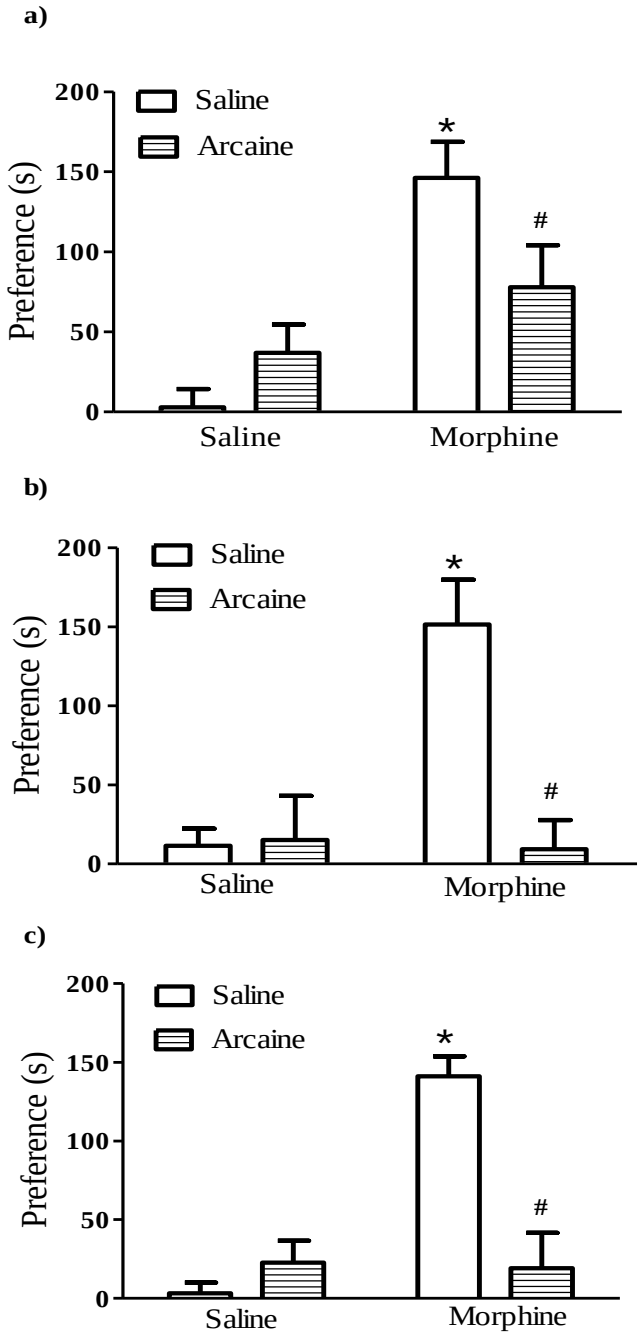

Figure 2 Effects of intraperitoneal administration of arcaine on acquisition (a), consolidation (b) and expression (c) of morphine-induced CPP in mice. Animals received saline or arcaine (3 mg/kg) 15 min before (a), immediately after each conditioning session with morphine (b) or 30 min before testing (c). The change of preference was assessed as the difference between the time spent in the drug-paired compartment on the day of testing and the time spent in the drug-paired compartment on the day of the second pre-conditioning session. Data are expressed as mean+SEM of 7 – 9 animals per group. *p <0.05 compared with the other groups, #p <0.05 compared with to saline/morphine group.

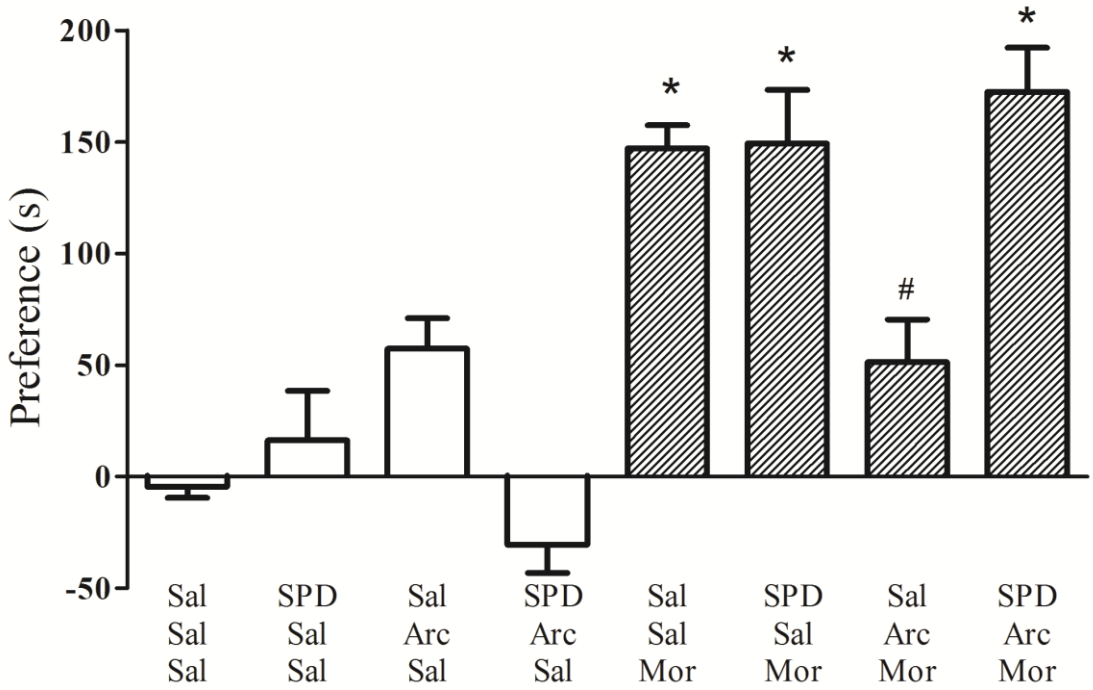

Figure 3 Effect of intraperitoneal administration of spermidine and arcaine on the establishment of morphine-induced CPP. Animals received saline or spermidine (SPD, 30 mg/kg) 30 min before the conditioning sessions, saline or arcaine (Arc; 3 mg/kg) 15 min before the conditioning sessions, and saline or morphine (Mor, 5 mg/kg) immediately before the conditioning sessions. The change of preference was assessed as the difference between the time spent in the drug-paired compartment on the day of testing and the time spent in the drug-paired compartment on the second pre-conditioning session. Data are expressed as mean+SEM of 9-11 animals per group. *$p<0.05$ compared to control (Sal/Sal/Sal) group, # $p<0.05$ compared to Sal/Sal/Mor group.

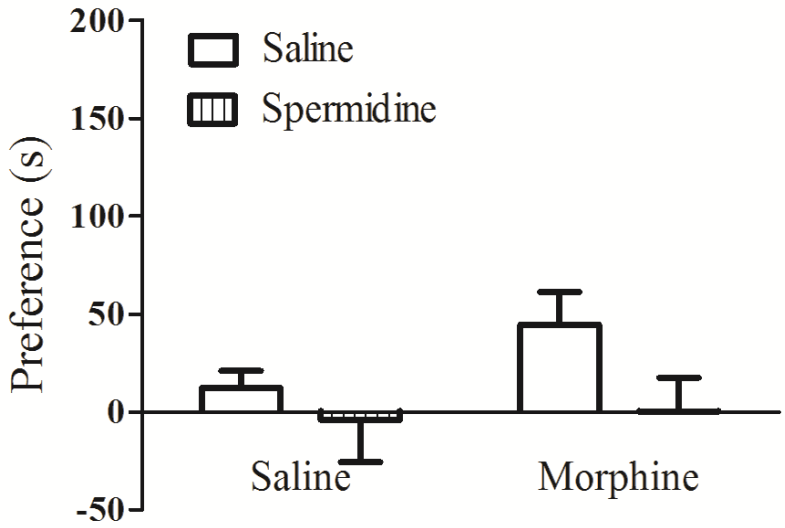

Figure 4 Effect of intraperitoneal administration of noneffective doses of spermidine and morphine on CPP. Animals received saline or spermidine (30 mg/kg) 15 min before saline or morphine (1.25 mg/kg) and were placed in the nonpreferred compartment in the conditioning sessions. The change of preference was assessed as the difference between the time spent in the drug-paired compartment on the day of testing and the time spent in the drug-paired compartment on the second pre-conditioning session. Data are expressed as mean+SEM of 7 animals per group.

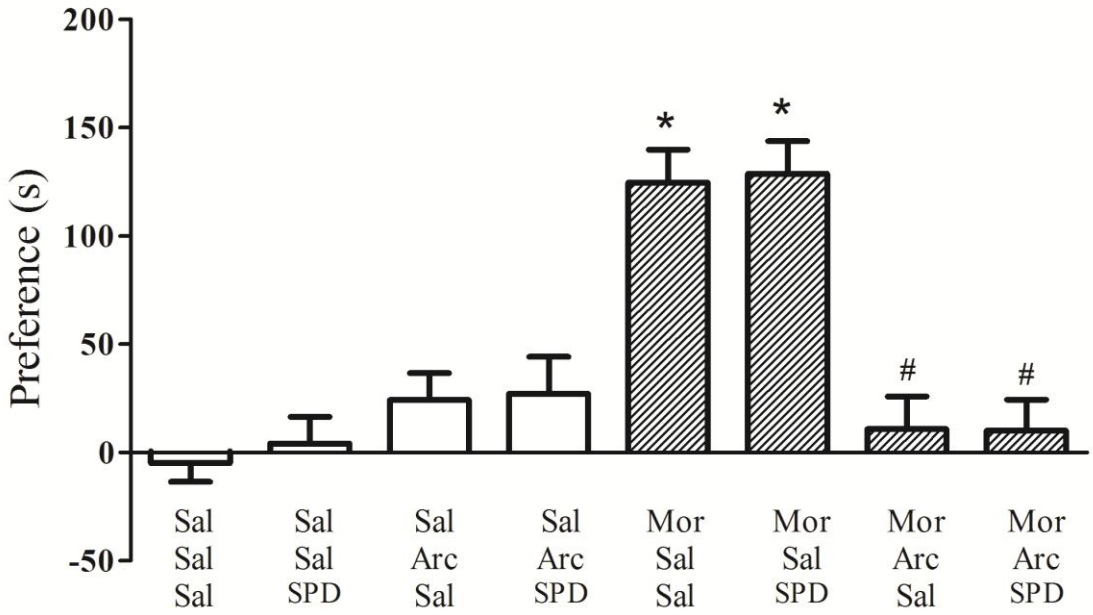

Figure 5 Effect of intraperitoneal administration of arcaine and spermidine on the consolidation of morphine-induced CPP. Animals received saline or morphine (Mor, 5 mg/kg) immediately before the conditioning sessions, saline or arcaine (Arc, 3 mg/kg) immediately after the conditioning sessions, and saline or spermidine (SPD, 30 mg/kg) 15 minutes after the conditioning sessions. Preference was assessed as the difference between the time spent in the drug-paired compartment on the day of testing and the time spent in the drug-paired compartment on the second pre-conditioning session. Data are expressed as mean+SEM of 8 animals per group. *$p<0.05$ compared to control (Sal/Sal/Sal) group, # $p<0.05$ compared to Mor/Sal/Sal group.

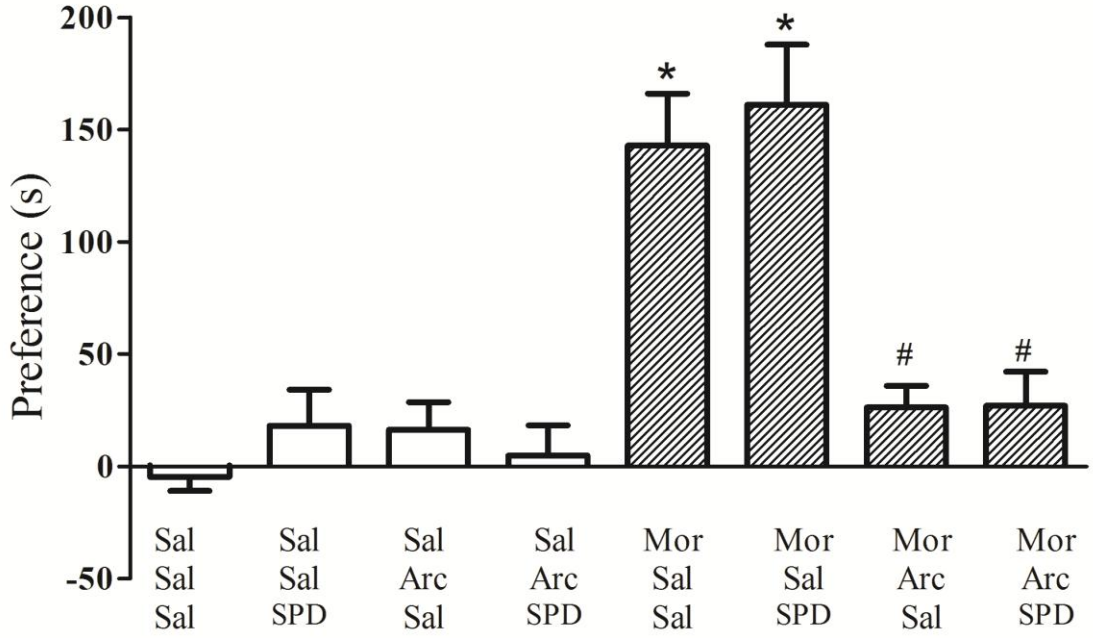

Figure 6 Effect of intraperitoneal administration of arcaine and spermidine on the expression of morphine-induced CPP. Animals received saline or morphine (Mor, 5 mg/kg) before the conditioning sessions, saline or arcaine (Arc, 3 mg/kg) was administered 30 minutes before the test, saline or spermidine (SPD, 30 mg/kg) 15 minutes before testing. Preference was assessed as the difference between the time spent in the drug-paired compartment on the day of testing and the time spent in the drug-paired compartment on the second pre-conditioning session. Data are expressed as mean+SEM of 8 animals per group. *$p<0.05$ compared to control (Sal/Sal/Sal) group, # $p<0.05$ compared to Mor/Sal/Sal group.

RESULTADO ADICIONAL I

Atividade locomotora

A tabela 1 mostra que os tratamentos não alteraram a atividade locomotora dos camundongos (resultado de ANOVA de uma via na tabela). A área do compartimento branco foi dividido em dois segmentos iguais e a locomoção foi medida pela contagem do número de cruzamentos de um segmento para outro, durante 15 min, ou seja, em todo o período do teste.

Tabela 1: Atividade locomotora

Tratamento (mg/kg)	**Média±SEM**	**n**
Salina	34,50±3,86	6
Morfina 1,25	41,33±6,31	6
Morfina 2,5	44,50±6,23	6
Morfina 5	49,67±5,74	6
Morfina 10	43,33±3,95	6
Análise estatística	F(4, 25)= 1,065 p > 0,05	
Saline	33±4,49	7
Arcaína 0,3	33,14±1,58	7
Arcaína 1	37,57±5,26	7
Arcaína 3	35,14±3,20	7
Análise estatística	F(3, 24)= 0,302 p > 0,05	
Salina	37,17±3,50	6
Espermidina 3	29,20±3,35	5
Espermidina 10	30,17±3,75	6
Espermidina 30	33,50±3,14	6
Análise estatística	F(3, 19)= 1,076 p > 0,05	
Salina+ saline	35,67±2,68	9
Salina+ morfina 5	39,22±4,96	9
Arcaína 3 + Saline	37,78±3,58	9
Arcaína 3 + morfina 5	39,78±3,60	9
Análise estatística	F(3, 32)= 0,233 p > 0,05	
Salina + salina	37,29±3,40	7
Salina + arcaína 3	30±3,41	7
Morfina 5 + salina	34,29±4,09	7
Morfina 5 + arcaína 3	28,57±3,64	7
Análise estatística	F(3, 24)= 1,195 p > 0,05	
Salina + salina	30,57±5,75	7

Salina + arcaína 3	31,57±3,40	7
Morfina + salina	36,86±5,03	7
Morfina + arcaína	32±6,09	7
Análise estatística	F(3, 24)= 0,293 p > 0,05	
Salina+ salina+ salina	37,44±3,62	9
Salina+ salina+ morfina 5	41,89±5,26	9
Salina+ arcaína 3 + salina	31,56±2,62	9
Salina+ arcaína 3 + morfina 5	34,09±3,05	11
Espermidina 30 + salina+ salina	31,11±2,75	9
Espermidina 30 + salina+ morfina 5	42,10±3,48	10
Espermidina 30 + arcaína 3 + salina	28,56±2,56	9
Espermidina 30 + arcaína 3 + morfina 5	35,82±3,50	11
Análise estatística	F(7, 69)= 0,0675 p > 0,05	
Salina + salina	36,71±2,72	7
Salina + morfina 1,25	34,43±5,34	7
Espermidina 30 + salina	37±3,28	7
Espermidina 30 + morfina 1,25	35,14±6,43	7
Análise estatística	F(3, 24)= 0,955 p > 0,05	
Salina + salina + salina	31,88±4,51	8
Salina + salina + espermidina 30	34,88±3,02	8
Salina + arcaína 3 + salina	30,88±1,87	8
Salina + arcaína 3 + espermidina 30	34,13±3,79	8
Morfina 5 + salina + salina	44,25±5,34	8
Morfina 5 + salina + espermidina 30	36,50±3,54	8
Morfina 5 + arcaína 3 + salina	33,25±3,39	8
Morfina 5 + arcaína 3 + espermidina 30	37,38±4,44	8
Análise estatística	F(7, 56)= 1,172 p > 0,05	
Salina + salina + salina	35,50±2,90	8
Salina + salina + espermidina 30	34,63±3,04	8
Salina + arcaína 3 + salina	36,75±4,34	8
Salina + arcaína 3 + espermidina 30	36,38±4,33	8
Morfina 5 + salina + salina	39,75±4,47	8
Morfina 5 + salina + espermidina 30	34,50±4,87	8
Morfina 5 + arcaína 3 + salina	33,88±3,35	8
Morfina 5 + arcaína 3 + espermidina 30	37±4,48	8
Análise estatística	F(7, 56)= 0,216 p > 0,05	

RESULTADO ADICIONAL II

Parâmetros bioquímicos

A tabela 2 mostra que os níveis sanguíneos de creatinina, uréia, aspartato aminotransferase (AST) e alanina amintransferase (ALT) não foram alteradas pelos tratamentos (resultado de ANOVA de uma via na tabela). As amostras foram coletadas logo após o teste de PCL.

Tabela 2: Parâmetros bioquímicos

CONSOLIDAÇÃO	**Creatinina**	**Ureia**	**AST (U/L)**	**ALT (U/L)**
Sal x Sal x Sal	0,25±0,05	39±10	199±28	81,5±32,50
Sal x Arc x Sal	0,36±0,12	55,67±15,39	182±94	51±3,05
Mor x Sal x Sal	0,25±0,02	42,75±2,32	127,5±29,76	54,5±6,85
Mor x Arc x Sal	0,27±0,04	42,75±3,19	269,8±155,4	52,25±12,31
Análise estatística	$F_{(3, 9)}$= 0,64 p > 0,05	$F_{(3, 9)}$= 0,75 p > 0,05	$F_{(3, 8)}$= 0,34 p > 0,05	$F_{(3, 9)}$= 0,93 p > 0,05
EXPRESSÃO	**Creatinina**	**Ureia**	**AST (U/L)**	**ALT (U/L)**
Sal x Sal x Sal	0,26±0,02	46,60±4,64	217,8±41,35	53,80±1,9
Sal x Arc x Sal	0,30±0,07	35,25±1,49	196,3±49,75	54±7,71
Mor x Sal x Sal	0,26±0,02	47,17±3,15	239,5±85,51	58,67±6,51
Mor x Arc x Sal	0,28±0,03	39,50±3,33	183,2±39,02	49,83±3,36
Análise estatística	$F_{(3, 17)}$= 0,22 p > 0,05	$F_{(3, 17)}$= 2,48 p > 0,05	$F_{(3, 17)}$= 0,18 p > 0,05	$F_{(3, 17)}$= 0,55 p > 0,05

Consolidação n= 2-4 e Expressão n= 4-6
Aspartato aminotransferase (AST);
Alanina aminotransferase (ALT).

4 CONCLUSÕES

4.1 CONCLUSÕES PARCIAIS

Com os resultados do presente estudo podemos concluir que:

4.1.1- A morfina induziu PCL, enquanto que a arcaína e a espermidina não induzem PCL e nem aversão condicionada por lugar;

4.1.2- A arcaína preveniu a aquisição e reverteu a consolidação e expressão da PCL induzida por morfina;

4.1.3- A combinação de espermidina e morfina em dose sem efeito, não induziu PCL;

4.1.4- A espermidina preveniu o efeito da arcaína sobre a PCL induzida por morfina quando administrada pré-treino (aquisição), no entanto a espermidina não reverteu o efeito da arcaína quando administrada pós-treino (consolidação) e nem pré-teste (expressão);

4.1.5- Nenhum dos tratamentos alterou a atividade locomotora dos animais;

4.1.6- Nenhum dos tratamentos alterou os parâmetros bioquímicos de toxicidade renal e hepática dos animais.

4.2 CONCLUSÃO GERAL

Com os resultados do presente estudo concluímos que a arcaína reverte a PCL induzida por morfina. Uma vez que a arcaína não induz PCL e nem aversão condicionada por lugar, e não causa toxicidade renal e hepática, esta poderia ser usada para tratar a adicção causada por morfina. Porém estudos adicionais são necessários para confirmar este efeito.

5 PERSPECTIVAS

Como visto neste trabalho, o receptor NMDA pode estar envolvido na aquisição, consolidação e expressão da PCL induzida por morfina. No entanto, estes resultados apresentam efeitos a curto prazo, para se observar efeitos de longa duração e sua relevância para o estudo de mecanismos de recaídas é interessante avaliar o efeito das poliaminas (espermidina e arcaína) sobre a extinção e restabelecimento da PCL induzida por morfina.

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