UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

Douglas Marques da Silva

CONDICIONAMENTO AO LOCAL INDUZIDO POR ÁLCOOL EM RATOS: AVALIAÇÃO DA VALIDADE DE PROPÓSITO DO SEU ATUAL EMPREGO NA PESQUISA PRÉ-CLÍNICA

Porto Alegre
2024

Douglas Marques da Silva

CONDICIONAMENTO AO LOCAL INDUZIDO POR ÁLCOOL EM RATOS: AVALIAÇÃO DA VALIDADE DE PROPÓSITO DO SEU ATUAL EMPREGO NA PESQUISA PRÉ-CLÍNICA

Tese apresentada ao Programa de Pós-Graduação em Neurociências do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de Doutor em Neurociências.

Orientadora: Profa. Dra. Mirna Bainy Leal
Co-orientador: Prof. Dr. José Inácio Lemos Monteiro Carvalho

Porto Alegre
2024

CIP - Catalogação na Publicação

da Silva, Douglas Marques
Condicionamento ao local induzido por álcool em ratos: avaliação da validade de propósito do seu atual emprego na pesquisa pré-clínica / Douglas Marques da Silva. -- 2024.
179 f.
Orientadora: Mirna Bainy Leal.

Coorientador: José Inácio Lemos Monteiro Carvalho.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Neurociências, Porto Alegre, BR-RS, 2024.

1. Modelação pré-clínica. 2. Condicionamento de lugar induzido por álcool. 3. Busca por álcool induzida por pistas. 4. Fissura por álcool induzida por pistas. 5. Comportamento de procura por pistas. I. Leal, Mirna Bainy, orient. II. Carvalho, José Inácio Lemos Monteiro, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Douglas Marques da Silva

CONDICIONAMENTO AO LOCAL INDUZIDO POR ÁLCOOL EM RATOS: AVALIAÇÃO DA VALIDADE DE PROPÓSITO DO SEU ATUAL EMPREGO NA PESQUISA PRÉ-CLÍNICA

Tese apresentada ao Programa de Pós-Graduação em Neurociências do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de Doutor em Neurociências.

Aprovada em: 26 de março de 2024.

BANCA EXAMINADORA

_____

Prof. Dra. Cleopatra da Silva Planeta – Universidade Estadual Paulista

_____

Prof. Dra. Isabel Marian Hartmann de Quadros – Universidade Federal de São Paulo

_____

Prof. Dra. Rosa Maria Martins de Almeida – Universidade Federal do Rio Grande do Sul

_____

Prof. Dra. Mirna Bainy Leal – Universidade Federal do Rio Grande do Sul (orientadora)

AGRADECIMENTOS

Agradeço a Piter Kehoma Boll, 𓀠𓂸, minha mansidão, por me aguentar em meu recorrente estado de depressão e desanimo generalizado ao longo dos últimos dois anos, por estar comigo em todas as etapas deste projeto e, em todas elas, ser fundamental, emocional e laboralmente, para que eu pudesse realizá-lo: *ʻnkh-i*, eu não tenho palavras para expressar o quanto sou grato a ti por me proporcionares a felicidade que me proporcionas e sempre, sempre, sempre seres tão amável e atencioso e maravilhoso.

Agradeço à minha mãe, Ângela Maria Lobato Marques, e ao meu irmão, Paulo Miguel Marques Dionísio, por me ensinarem constantemente que tudo que se faz nessa vida, se faz por amor e segundo ética pessoal que nos reconheça como pequenos individualmente, mas que sempre preze pelo e almeje o bem, pessoal, interpessoal, transgeracional e comum.

Agradeço à Prof.ª Dra. Mirna por ter aceitado me orientar ainda no mestrado, quando lhe contatei acerca da seleção de mestrado de 2015 do PPGNEURO, e por ter renovado sua confiança em mim ao aceitar me orientar no doutorado. Também lhe agradeço por ter feito de tudo o possível para que esse projeto fosse executado da melhor maneira possível, por ter me dado toda a liberdade e confiança necessárias à realização do mesmo e por ter me acolhido tão aberta e prontamente em seu grupo de pesquisa, fazendo com que eu me sentisse com pertença, certo e seguro. Adicionalmente, lhe estendo meus agradecimentos por ser a pessoa tão maravilhosa que é e pela relação de amizade e cumplicidade construída ao longo de todos esses anos.

Agradeço ao Prof. Dr. Inácio por todos esses anos de amizade, de trabalho e pelas sempre acaloradas discussões científicas. Se me for possível dizer que adquiri repertório científico com o mínimo de consistência, em grande parte essa aquisição decorre de nossa relação, mesmo que por muitas vezes entremos em atrito: sua contribuição à minha formação acadêmica é indelével.

Agradeço ao PPGNEURO por ter me propiciado aprendizagem científica e relações interpessoais tão profícuas e significativas e por seu corpo colegiado apresentar a paciência e o apoio que por muitas vezes pós-graduandos necessitam em momentos muito difíceis. Aqui, em representação do corpo colegiado, estendo meus agradecimentos nomeadamente à Prof. Dra. Lenir Orlandi Pereira Silva e ao Prof. Dr. Lucas de Oliveira Alvares, coordenadora e vice-coordenador do PPGNEURO. Também estendo meus agradecimentos às queridas e ilustres e admiradas Prof. Denise Maria Zancan e Maria Elisa Calcagnotto, sob coordenação das quais desenvolvi valorosos e alegres trabalhos em projetos de extensão do PPGNEURO e

das quais tive a graça de ser aluno, e, em especial, às amadas e ilustres e admiradas Prof. Dra. Rosane Gomez e Prof. Dra. Eliane Dallegrave, pelo bem-querer mútuo, por serem parceiras firmes de estudos, discussões e escritos, bem como em gargalhadas e em humor, do leve ao cáustico.

Aos queridos amigos de longa data, irmãos e irmãs de escolha e coração, Felinto Maués, Silvia Letícia Maués, Júnior Silva e Giovanni Torres: obrigado por todo o apoio, compreensão, conversas, discussões pessoais e científicas, risos e choros ao longo desses muitos e muitos anos.

Reafirmo também o agradecimento às mulheres de minhas famílias paternas e materna que sempre foram e são fortalezas e enfrentam até hoje adversidades mil, além da conta, não se calam ou submetem e ainda encontram tempo e disposição para sorrirem, viverem e amarem!

Por último, agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível superior (CAPES) e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) que me concederam, cada ao seu tempo, Bolsa de estudos sem as quais eu dificilmente conseguiria cursar o doutorado.

APRESENTAÇÃO

A presente tese se encontra dividida em cinco seções. A primeira constitui introdução geral do tema de pesquisa. Na sequência, há o capítulo 1, intitulado ***Alcohol, place conditioning, and male rats: A systematic review for outcome prediction*** e publicado no jornal *Alcohol: Clinical and Experimental Research* (doi: 10.1111/acer.15092). A seguir há o capítulo 2, intitulado ***Letter to the editor: A reply to Grahame (2023) commenting on "Alcohol, place conditioning, and male rats: A systematic review of outcome prediction"***, também publicado no referido periódico (doi: 10.1111/acer.15254). Já o capítulo 3, intitulado "Estudo experimental", reporta experimentos ainda não publicados. Por último, a quinta parte constitui discussão e conclusão gerais desse trabalho.

RESUMO

Desde a década de 1970, o modelo de condicionamento de lugar (CL) vem sendo utilizado no estudo da aprendizagem componente do Transtorno por Uso de Álcool. Entretanto, não é certa sua validade de propósito nesse uso por conta da incapacidade de se predizer os resultados advindos de intervenções simples no CL induzido por álcool em ratos. Como consequência, faz-se difícil avaliar a adequação modelo-alvo (composta pelas validades de constructo, de face e de predição do modelo). Além disso, há incertezas acerca do estado das validades formais-experimentais (a saber, validades interna, operacional e de conclusão estatística) na literatura. Este trabalho é composto por três etapas de avaliação da validade de propósito do CL induzido por álcool em ratos. Na primeira (capítulo primeiro), foi realizada revisão sistemática da literatura com objetivo principal de se prever teoricamente os resultados de intervenções simples e objetivo secundário de se avaliar o estado das validades formais na literatura. Mostra-se que os resultados podem ser preditos pela interação de 4 grupos de variáveis: as que predizem a aquisição de controle condicional em aprendizagem associativa, as que preveem interferência na expressão de controle condicionado, fatores do álcool que predizem as suas propriedades farmacocinéticas e farmacodinâmicas, e fatores orgânicos e ambientais que modificam essas propriedades. Também se evidencia que o estado das validades formais na literatura é preocupantemente problemático. A partir da revisão, a segunda parte dessa tese explora a adequação modelo-alvo, tanto pela avaliação das validades de constructo, de face e de predição do modelo (capítulo segundo), bem como pela realização de experimentos para testar a predição realizada na primeira parte (capítulo terceiro). Já a terceira parte discute as evidências geradas pela revisão e pelos experimentos frente a dados de controles incondicional e condicionais de estímulos do consumo de álcool em humanos. Conclui-se que a alta sensibilidade do CL induzido por álcool a variáveis modulatórias e deletérias à aprendizagem é incompatível com os níveis de controle incondicional e condicionais exercido/induzidos pelo álcool e por pistas a ele relacionadas no Transtorno por Uso de Álcool. Portanto, a atual literatura está modelando características do uso não problemático ao levemente problemático de álcool. São recomendadas linhas de pesquisa para se sanar essa falta de validade de propósito.

Palavras-chaves: Comportamento de busca por álcool; Fissura induzida por pistas; Condicionamento de lugar álcool-induzido; Validade de propósito.

ABSTRACT

Since the 1970s, the place conditioning (CL) model has been used to study the learning component of Alcohol Use Disorder. However, its fit-for-purpose validity in this use is not certain because we do not know how to predict the results arising from simple interventions in alcohol-induced CL, especially in the case of rats. As a consequence, it is difficult to evaluate the model-target adequacy (composed of construct, face and prediction validities). Additionally, we do not know the state of formal-experimental validities (namely, internal, operational and statistical conclusion validities) in the literature. This work consists of three stages of evaluating the fit-for-purpose validity of alcohol-induced CL in rats. Firstly, in the first chapter, a systematic review of the literature was carried out with the main objective of theoretically predicting the results of simple interventions and the secondary objective of evaluating the state of formal validities in the literature. It is shown the results can be predicted by the interaction of 4 groups of variables: those predicting the acquisition of conditional control in associative learning, those predicting interference in the expression of conditioned control, alcohol factors that predict its pharmacokinetic and pharmacodynamic properties, and organic and environmental factors that modify these properties. It is also shown that the state of formal validities in the literature is worryingly problematic. Based on the review, the second part of this thesis explores the model-target adequacy, by evaluating the construct, face and prediction validities of the model (second chapter), as well as by carrying out experiments to test the prediction made in the first part (third chapter). The third part discusses the evidence generated by the review and experiments against data on unconditional and conditional stimulus controls of alcohol consumption in humans. It is concluded that the high sensitivity of alcohol-induced CL to the modulatory and deleterious effects of variables upon learning is incompatible with the levels of unconditional and conditional controls exerted/induced by alcohol and its related cues in Alcohol Use Disorder. Therefore, the current literature is modeling characteristics of nonproblematic to mildly problematic alcohol use. Lines of research are recommended to remedy this lack of fit-for-purpose validity.

Keywords: Alcohol-seeking behavior; Alcohol-craving behavior; Alcohol place conditioning; Fit-for-purpose validity.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS

ACL e CPA – Siglas a *Aversão Condicionada de Lugar* em português e inglês, respectivamente

ADH – Sigla a Álcool Desidrogenase (do inglês, *Alcohol Dehydrogenase*)

APE – Sigla a Protocolos de Condicionamento de Lugar com Pré-Exposição ao Álcool (do inglês, *Alcohol Pre-Exposure*)

AUD – Sigla a Transtorno por Uso de Álcool (do inglês, *Alcohol Use Disorder*)

BAC – Sigla a Concentração Alcoólica Sanguínea (do inglês, *Blood Alcohol Concentration*)

BWRD – Sigla a Distribuição Relativa de Água Corporal (do inglês, *Body Water Relative Distribution*)

CL e PC – Siglas a *Condicionamento de Lugar* em português e inglês, respectivamente

CF e FC – Siglas a *Falha de Condicionamento* em português e inglês, respectivamente

CREAL – Centro de Reprodução e Experimentação de Animais de Laboratório

PCL e CPP – Siglas a *Preferência Condicionada de Lugar* em português e inglês, respectivamente

H – Ratos Holtzman

HNR – Sigla a Alojamento Não Reportado (do inglês, *Housing Not Reported*)

ig – Via de administração intragástrica

ip – Via de administração intraperitoneal

ISI – Intervalo Interestímulo (do inglês, *Interstimulus Interval*)

L – Ratos Lister

L-E – Ratos Long-Evans

LPCP – Sigla a Protocolo Longo de Condicionamento (do inglês, *Long Place Conditioning Protocol*)

MDN – Sigla a Neurônios Dopaminérgicos Mesencefálicos (do inglês, *Mesencephalic Dopaminergic Neurons*)

MEOS – Sigla a Sistema Microssomal de Oxidação Alcoólica (do inglês, *Microsomal Ethanol-Oxidizing System*)

og – Via de administração orogástrica (gavagem)

SD – Ratos Sprague Dawley

W – Ratos Wistar

SPCP – Sigla a Protocolo Curto de Condicionamento (do inglês, *Short Place Conditioning Protocol*)

TBW% – Sigla a Porcentagem Total de Água Corporal (do inglês, *Total Body Water Percentage*)

UFRGS – Universidade Federal do Rio Grande do Sul

SUMÁRIO

1 INTRODUÇÃO GERAL

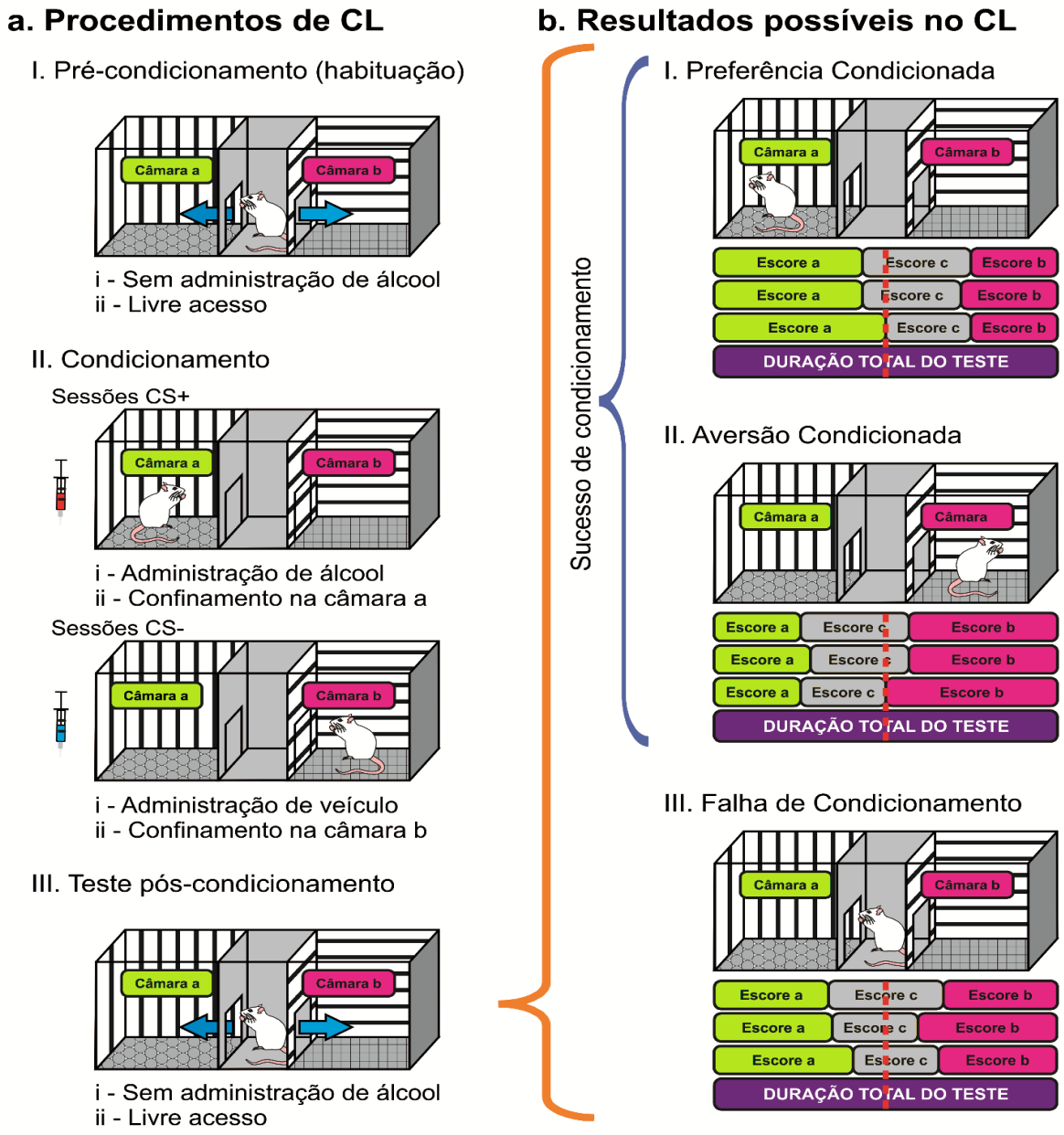
O paradigma de condicionamento de lugar (CL) é uma preparação de aprendizagem baseada nas descobertas de que, quando dada oportunidade, (a) animais escolhem se aproximar e manter contato com estímulos reforçadores primários (incondicionais), (b) escolhem se afastar e evitar estímulos aversivos primários e (c) estímulos outros podem adquirir tal controle sobre o comportamento de escolha por meio de associação prévia (intervenção de condicionamento) com os estímulos primários (WHITE *et al.*, 1987). Assim, durante a fase de condicionamento do CL, o estímulo incondicional (e.g., efeitos de droga psicoativa) é experimentado pelos animais em associação confiável com um contexto espacial (o contexto droga-pareado, chamado tecnicamente de CS+), enquanto um "estímulo incondicional falso" é apresentado com outro contexto espacial (o contexto droga-não-pareado, chamado tecnicamente de CS-), sendo que ambos os contextos compõem o aparato de CL.

Ao seguir este protocolo de condicionamento pavloviano discriminativo, espera-se que (a) o contexto pareado adquira controle secundário (i.e., condicional) sobre o comportamento de escolha, (b) esse controle seja expresso durante o teste de pós-condicionamento, quando a droga não está presente e oportunidade de escolha é dada entre os contextos pareado e não-pareado, e (c) que tal controle seja o principal fator que influenciará o comportamento de escolha durante o teste (CARR *et al*., 1989; WHITE *et al*., 1987). Consequentemente, se os animais passam relativa e significativamente mais tempo no contexto pareado, este resultado é considerado uma preferência condicionada de lugar (PCL) ao contexto pareado; caso passem relativa e significativamente mais tempo no contexto não-pareado, este resultado é considerado uma aversão condicionada de lugar (ACL) ao contexto pareado (deve ser notado que ambos PCL e ACL são instâncias de aprendizagem bem-sucedida — i.e., de condicionamento bem-sucedido — uma vez que esses resultados indicam a instalação e a expressão de controle condicional). Por outro lado, se o tempo relativo gasto pelos animais em cada um desses contextos não diferir significativamente de chance ao acaso (50%), o resultado é considerado uma falha de condicionamento (FC). A **Figura 1.1** ilustra os procedimentos componentes do CL bem como seus possíveis resultados, utilizando o álcool como pertinente exemplo de droga.

Assim definido, enquanto um modelo pré-clínico de aprendizagem, o CL segue crescendo em popularidade e aplicação (SMITH, 2020). A partir da década de 1980, após os estudos pioneiros de Beach (1957a, 1957b), Black *et al*. (1973), Kumar (1972) e Rossi e Reid (1976), o modelo se tornou uma alternativa popular ao modelo de autoadministração operante

de drogas e alcançou o *status* de um dos instrumentos mais utilizados na pesquisa pré-clínica em farmacologia (neuro)comportamental de abuso de drogas (TZSCHENTKE, 1998, 2007). Nesse caso, ele vem sendo principalmente utilizado no estudo indireto dos efeitos motivacionais positivos (recompensadores) ou negativos (aversivos) da exposição a drogas. Assim, assume-se no estudo indireto que haja paridade entre as funções incondicionais da droga e as funções condicionais dos contextos de forma que os resultados de escolhas condicionadas possam ser considerados ao estudo das propriedades incondicionais de drogas (as quais, de fato, dificilmente vem sendo estudadas diretamente no CL).

FIGURA 1.1 – Procedimentos (a) e possíveis resultados (b) do Modelo de Condicionamento de Lugar



Fonte: Elaborada pelo autor

Adicionalmente, ao longo das últimas três décadas, o CL tem sido aplicado com o mesmo objetivo para muitos outros estímulos primários (e.g., estimulação elétrica intracraniana, comida, oportunidade de cópula e acesso a rodas de corrida) e em muitas espécies diferentes, incluindo humanos (BEACH, 1957a; CHILDS; DE WIT, 2009; KUMAR, 1972; TZSCHENTKE, 2007). Simultaneamente, também vem sendo usado no exame de outros processos comportamentais básicos como a aquisição, manutenção, extinção e reinstalação dos comportamentos-alvo (i.e., preferência e aversão/esquiva) aprendidos, bem como das respectivas atividades neurobiológicas subjacentes (AGUILAR *et al*., 2009; TZSCHENTKE, 2007). Em todas essas ramificações de uso e aplicação, as características centrais do CL se conservam: o uso principal de um protocolo de condicionamento pavloviano discriminativo e a posterior testagem de preferências e aversões adquiridas para contextos nos quais ocorreram, segundo tal protocolo, as exposições aos motivadores primários.

Não obstando o sucesso de uso evidenciado na descrição precedente, esses parágrafos também servem para chamar atenção às sérias repercussões de problemas teórico-metodológicos e práticos que persistem no uso do CL, em especial no CL induzido por álcool em ratos (ver a apresentação de MARQUES, 2017). Como pode ser constatado em várias revisões da literatura de CL induzido por drogas, o corpo literário de CL induzido por álcool apresenta divergência na identificação de intervenções cujos resultados possam ser facilmente reprodutíveis, em especial quanto à obtenção de PCL (CARR *et al*., 1989; CUNNINGHAM *et al*., 2011; GRAHAME, 2013; NAASSILA, 2013; SWERDLOW *et al*., 1989; TZSCHENTKE, 1998, 2007). Essa disparidade é verificada tanto quando se compara os resultados de intervenções com álcool às feitas com outras drogas classicamente abusadas, quanto quando se compara os resultados de intervenções feitas com álcool em camundongos (intervenções consistentemente identificadas) e em ratos (intervenções não identificadas).

No caso específico do CL alcoólico com ratos, diversas explicações foram propostas a tal estado (ver a apresentação de MARQUES 2017), sem que alguma delas aumentasse nossa compreensão (i.e., identificasse variáveis relevantes a este ou aquele resultado) e nossa capacidade de predição desses mesmos resultados. Todavia, frente aos corpos experimental e teórico desenvolvidos ao longo do séc. XX pela psicologia experimental, pela etologia e pela farmacologia, o estado de incompreensão e de falta de capacidade preditiva é cientificamente inaceitável a qualquer trabalho de modelação pré-clínica em farmacologia comportamental, especialmente quando tal modelação se estende desde os anos de 1970 (BLACK *et al*., 1973). Mais que isso, a falta de capacidade preditiva nos impede de avaliar a validade de propósito

(*fit-for-purpose validity* em inglês; DENAYER *et al*., 2014) do atual uso do CL. Isto é, nos impede de avaliarmos se estamos de fato modelando aprendizagem componente de transtornos de uso substâncias no CL, em especial no CL com álcool.

Como primeira tentativa de resolver tal situação, recente revisão sistemática lógico-procedimental da literatura foi efetuada por nosso grupo de pesquisa e exposta no capítulo primeiro de minha dissertação de mestrado (MARQUES, 2017). Todavia, ainda que tal revisão consiga organizar a diversidade de resultados encontrados na literatura, ela apresenta limitações e falhas. Em primeiro, como é o caso de todo e qualquer modelo pré-clínico, o CL apresenta somente uma justificativa válida para seu uso em contexto experimental: a investigação confiável de relações causais relevantes a humanos. Nas palavras de Reiss (2019, p. 3111),

> "[i]f one is ultimately interested in learning the truth of a hypothesis of the form '*C* causes *E* in *T*[*arget system*]' (the '*T*-hypothesis'), why would one make a detour via learning the hypothesis '*C* causes *E* in *M*[*odel system*]' first (the '*M*-hypothesis')? The only sensible answer appears to be: because we can learn the *M*-hypothesis more reliably."

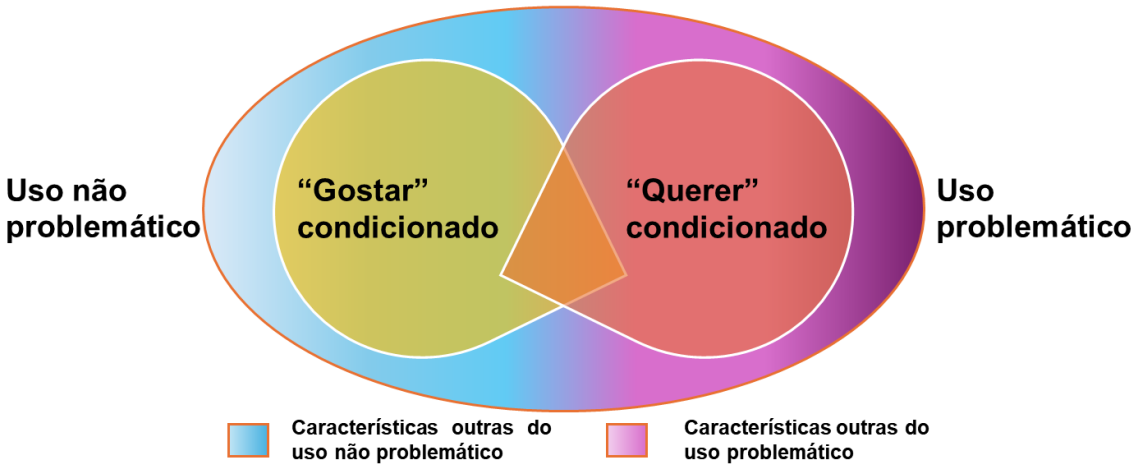
Assim o sendo, a confiança nos resultados reportados em experimentos com CL é diretamente depende das validades formais que todos os estudos experimentais devem ter — nominalmente, validades interna, de conclusão estatística e operacional (SHADISH *et al*., 2002). Frente a isso, a revisão lógico-metodológica feita no mestrado falha ao não examinar os estados de tais validades na literatura e suas repercussões sobre o risco de reportagem experimental, por um lado, e sobre a escolha adequada do método de síntese, por outro. Ainda nesse ponto, a falta de avaliação dessas validades, em especial da validade operacional dos disseminados "índices de preferência", consiste em limitação da revisão feita e acaba por contribuir para que possíveis problemas daí advindos se perpetuem na pesquisa futura no modelo.

Em segundo, o CL induzido por drogas é um modelo pré-clínico no qual se instrumentaliza achados básicos em aprendizagem associativa pavloviana com um organismo modelo sob os efeitos de uma droga para se estudar aspectos aprendidos do transtorno por uso dessa mesma droga. Frente a isso, a revisão feita em Marques (2017) falha na avaliação da adequação do organismo modelo sob os efeitos do álcool (i.e., considerada uma interpretação alométrica dos efeitos do álcool em diferentes espécies, os efeitos do álcool na espécie modelo e em humanos devem ser suficientemente similares). Similarmente, ela não alcança com a especificidade necessária a previsão teórica dos resultados segundo variáveis reconhecidas como importantes na pesquisa básica em Psicologia da aprendizagem, em

Etologia e em Farmacologia, as disciplinas que majoritariamente servem de base aos estudos em farmacologia (neuro)comportamental de abuso de drogas.

Outrossim, de acordo com a proposição teórica de sensibilização por incentivo, o uso recreativo de drogas apresenta duas motivações basais, o "querer" e o "gostar" (ROBINSON; BERRIDGE, 1993, 2000, 2001, 2008; BERRIDGE; ROBINSON, 2016). O "gostar" se refere aos aspectos hedônicos (prazerosos) de uma recompensa. No caso do álcool, esses aspectos podem ser o relaxamento e a estimulação induzidas por essa droga de acordo com a dose utilizada e o histórico de uso. Já o "querer" é referente ao intenso desejo, a "fissura", que o usuário sente em consumir álcool (MEYER *et al*., 2023). Ainda segundo essa proposição, ao longo do desenvolvimento de transtornos por uso de substâncias, o usuário recreativo experiencia um aumento acentuado na "fissura" pela droga, mesmo que não haja alteração ou ocorra diminuição no "gostar" dos efeitos de drogas diante da progressiva tolerância a esses efeitos. Deste modo, há predomínio do "gostar" no uso não problemático e predomínio do "querer" no uso problemático de drogas (**Figura 1.2**).

FIGURA 1.2 – Relações de predominância das motivações "gostar" e "querer" nos usos não problemático e problemático de drogas e de características outras desses usos



Fonte: Elaborada pelo autor

Assim o sendo, por conta da aprendizagem associativa instrumentalizada no CL e o fato de que tanto o "gostar" quanto o "querer" podem ser condicionados (i.e., vir a ser eliciados por pistas associadas ao uso da droga: CHILDRESS *et al*., 1999, CHILDS; DE WIT, 2016; SINHA *et al*., 2009), o CL induzido por drogas poderia estar modelando o condicionamento de qualquer uma dessas motivações. Contudo, é de difícil consecução a distinção experimental entre essas motivações nos resultados possíveis do CL (CUNNINGHAM *et al*., 2011) e essa distinção é importante visto que somente o "querer"

(incondicionado e condicionado) apresenta relevância clínica, tanto por compor a diagnose de transtornos por uso de substâncias quanto por ser elemento prognóstico de tratamento do Transtorno por Uso de Álcool (AUD, sigla em inglês; AMERICAN PSYCHIATRIC ASSOCIATION, 2013; MEYER *et al*., 2023; SLIEDRECHT *et al*., 2019). Nesse caso, o acesso principal a essa distinção é a avaliação da validade face dos atuais protocolos do CL álcool-induzido, i.e., a comparação das características do controle de estímulos que predomina na literatura atual de CL álcool-induzido com as características do controle de estímulos estabelecido nos usos não-problemático e problemático de álcool em humanos (**Figura 1.2**).

Dessa forma, tal como (a) a adequação modelo-alvo (a1) do organismo modelo sob a condição droga e (a2) do processo comportamental instrumentalizado (i.e., as validades de constructo, de face e de predição do modelo) e (b) a reprodutibilidade de resultados obtidos a partir de simples intervenções no modelo, (c) a predição teórica desses resultados é um passo necessário à avaliação da validade de propósito de um modelo pré-clínico como o CL induzido por drogas e fármacos abusados. É essa predição que nos permite compreender as relações de controle de estímulos em CF, CPA e CPP, segundo os níveis de variáveis preditoras, de forma a podermos comparar essas relações com aquelas que sabemos ocorrer em humanos, segundo os usos não problemático e problemático de álcool, e assim dizermos qual a relevância clínica dos protocolos majoritariamente utilizados no CL bem como de seus resultados. Em síntese, somente a partir das avaliações das validades formais podemos dizer quão seguros estamos dos resultados reportados, identificar problemas na condução/reportagem de experimentos e propor ou mesmo desenvolver soluções a serem utilizadas nos estudos experimentais vindouros. De mesmo modo, somente a partir das avaliações simultâneas dos itens *a*, *b* e *c* (em especial, *a* e *c*) anteriormente expostos, podemos dizer se um modelo pré-clínico está sendo corretamente empregado em seu propósito e, caso não esteja, identificar e sugerir necessários ajustes para que assim se faça.

Portanto, no capítulo primeiro dessa tese se apresenta revisão sistemática publicada no periódico *Alcohol: Clinical and Experimental Research* (MARQUES *et al*., 2023). O principal objetivo dessa revisão é predizer teoricamente os resultados advindos de intervenções simples no CL induzido por álcool em ratos. Além disso, em consonância com a escolha do método de síntese, nela se avalia o estado das validades formais na literatura[1] e o

[1] Nesse assunto, deve ser salientado que a deveras importante discussão acerca dos afamados "índices de preferência", ainda que identifique o problema e o analise densamente, não resolve a questão aos aparatos com mais de dois compartimentos/contextos. Ressalta-se ainda que este problema deveria ser tópico corrente de pesquisa teórico-metodológica, visto que sua importância é própria a todos os modelos que testem, de forma ou outra, preferências espaciais.

consequente risco de reportagem. Simultaneamente, se avalia a adequação do animal modelo na condição álcool, se recomenda/desenvolve resoluções aos muitos problemas formais encontrados na literatura, bem como se mostra que os argumentos em favor do uso de aparatos com viés de preferência basal e de protocolos com viés de pareamento não se sustentam teórica ou experimentalmente. Posteriormente, se recomenda o uso de varáveis que aparentam aumentar a probabilidade de obtenção de PCL em protocolos curtos de CL, bem como a investigação de outras variáveis que a revisão não pôde alcançar. Por último, se reforça o uso de protocolos de planejamento e de reportagem experimental propostos de forma a aumentar a validade interna e de conclusão estatística de pesquisa pré-clínica, bem como que permitam a revisores futuros realizar síntese estatística meta-analítica ou meta-regressional da literatura, métodos mais eficazes de síntese que os empregados em Marques et al. (2023), mas impossíveis de serem empregados segura e confiavelmente dado o atual estado da literatura com relação às validades formais.

Já o capítulo segundo (MARQUES *et al*., 2024)[2], de extensão pequena, constitui resposta ao comentário (GRAHAME, 2023) publicado acerca da revisão sistemática apresentada no capítulo primeiro (MARQUES *et al*., 2023). Originalmente, este capítulo foi planejado a ser mais extenso e centrado na análise das validades de constructo (ou de alvo na conceituação de DENAYER *et al*., 2014), de face e de predição do CL induzido por álcool. Todavia, visto as implicações deletérias que Grahame (2023) poderia gerar sobre a resolução de problemas sensíveis e extensos na literatura atual, a argumentação foi reduzida ao limite de palavras especificado pelo periódico *Alcohol: Clinical and Experimental Research* para uma resposta no estilo comentário e boa parte dessa análise argumentativa foi delegada às discussões e conclusões gerais dessa tese. Ainda assim, em Marques *et al*. (2024) se identifica a alta probabilidade de que a atual literatura de CL induzido por álcool, independentemente do emprego de ratos ou de camundongos, venha modelando somente características aprendidas próprias do uso não-problemático ao levemente problemático de álcool e não alcance, portanto, validade de propósito à modelação de características aprendidas do AUD. Também ali se identifica linhas de pesquisa experimental necessárias à resolução desses problemas de forma que a literatura vindoura possa adquirir tal validade no estudo das características aprendidas do AUD.

[2] Por conta das políticas de direitos autorais da *Alcohol: Clinical and Experimental Research* quanto a artigos publicados fora do formato *open access* e de permissões de divulgação cedidas a esses artigos, os artigos formando os capítulos 1 e 2 dessa tese estão formatados tais quais as versões aceita (CAPÍTULO 1) e submetida (CAPÍTULO 2) para publicação, acrescidas modificações de espaçamento e de justificação textuais e de numeração de figuras e tabelas, todas modificações feitas para fins de maior coesão estética e de formato da tese.

No capítulo terceiro se reporta experimentos que visam sanar problemas e limitações nos experimentos reportados em Marques (2017), elucidar lacunas na literatura bem como testar predições feitas em Marques *et al*. (2023) e, frente à análise feita em Marques *et al*. (2024), testar a validade de face de protocolos curtos de CL induzido por álcool. Por conta da pandemia de SARS-CoV2, das corretas e necessárias restrições impostas ao uso de laboratórios e de alocação de animais nos biotérios setoriais do Instituto de Ciências Básicas da Saúde da UFRGS e a parada de fornecimento de animais pelo CREAL-UFRGS (senão para uso em suas próprias dependências), não foi possível realizar investigação maior e mais complexa tal como proposta no projeto de doutorado: além da análise de dados neurobiológicos, pretendia-se, por exemplo, alcançar protocolos longos de condicionamento no projeto, bem como o uso de animais com ao menos 75 dias de idade. Decorre daí o alcance limitado em relações causais testadas no desenho experimental geral reportado. Ainda assim, discute-se com minúcia os significados dos achados no contexto da atual literatura de CL induzido por álcool em ratos, assim como a experimentos e testagens futuras, incluindo a indicação de possíveis translações.

Após esse capítulo, é apresentada a seção de discussão e conclusão gerais dessa tese. Na discussão, pela comparação dos resultados preditivos de Marques *et al*. (2023) e experimentais do capítulo 3 com os dados humanos acerca do controle de estímulos envolvidos no AUD, se argumenta de forma mais extensa que a atual literatura de CL não alcança validade de face ao estudo desse transtorno em nenhum dos fenômenos avaliados: ao máximo, alguém poderia argumentar que a atual literatura alcança o uso levemente problemático. Na conclusão geral, portanto, se reafirma que a proposição de linhas de pesquisa feita em Marques *et al*. (2024) é necessária. Também ali se aponta que os resultados reportados nessa tese, sejam os da revisão sistemática, sejam os experimentais, não implicam que, na literatura atual de CL induzido por drogas comumente abusadas por humanos, a modelação com álcool seja a única a só alcançar o uso não problemático a levemente problemático de uma droga. Por mais grave que seja essa conclusão, ela não é pertinente somente ao CL alcoólico.

2 CAPÍTULO 1:

ALCOHOL, PLACE CONDITIONING, AND MALE RATS: A SYSTEMATIC REVIEW OF OUTCOME PREDICTION

Douglas Marques[1]*, Piter Kehoma Boll[2], Rosane Gomez[1], Eliane Dallegrave[3], José Inácio Lemos[4], Mirna Bainy-Leal[1]

[1] Programa de Pós-Graduação em Ciências Biológicas: Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Sarmento Leite, 500, prédio 12101/209, 90046-900 Porto Alegre, Rio Grande do Sul, Brazil. djeser.dm@gmail.com, rosane.gomez@ufrgs.br, mirnabl@gmail.com.

[2] Programa de Pós-Graduação em Biologia, Universidade do Vale do Rio dos Sinos (UNISINOS), Avenida Unisinos, 950, 93022-000 São Leopoldo, Brazil. piterkeo@gmail.com.

[3] Departamento de Farmacociências, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Rua Sarmento Leite, 245, 90050-170 Porto Alegre, Brazil. elianedal@ufcspa.edu.br.

[4] Laboratório de Bioquímica, Fisiologia e Farmacologia, Faculdade de Medicina Veterinária, Instituto de Medicina Veterinária, Campus Universitário de Castanhal, Universidade Federal do Pará (UFPA), BR 316 (km 61), 68741-740 Castanhal, Brazil. jlemos@ufpa.br.

*Corresponding author
Douglas Marques
Universidade Federal do Rio Grande do Sul
Rua Sarmento Leite, 500, prédio 12101/209, 90046-900 Porto Alegre, Brazil
E-mail: djeser.dm@gmail.com.

ABSTRACT

Rationale Although Place Conditioning (PC) has been used to study the motivational effects of alcohol for almost 50 years, variables and situations in which alcohol induces PC in rats are still unclear, especially for short PC protocols (up to 10 conditioning trials).

Objectives To predict primary outcomes (namely, conditioning failure, conditioned place aversion (CPA), and conditioned place preference (CPP)) of alcohol-induced PC with male outbred rats.

Methods Systematic review: structured search for relevant records in PUBMED and two other sources, eligible articles defined as those conforming to all inclusion criteria, selection of experiments failing all the exclusion criteria, and analysis of procedure-outcome relations according to the background of variables affecting associative learning, alcohol interventions in rats, and PC interventions themselves.

Results We selected 192 experiments (133 short protocols, 27 long protocols, and 32 protocols with alcohol pre-exposure) from 62 articles to compose the review. Rates of conditioning failure are mainly predicted by interactions of alcohol dose and the number of habituation sessions and conditioning trials. Different conditions (housing systems) and characteristics (age and weight) of animals predict different rates of CPA and CPP: higher rates of CPA are predicted by single-housed, older, and heavier animals, while higher rates of CPP are predicted by group-housed, younger, and lighter animals.

Conclusions We recommend settings for CPP induction in short protocols, indicate the predictive analysis has broad consequences (from theoretical to translational ones) for PC in alcohol research, and specify variables needing more accurate investigation (rat sex, age, weight, strain, spatial configuration of the apparatus, and conditioning timetable). This review may improve our comprehension of results of alcohol-induced PC with rats, refining our understanding of the motivator function of alcohol and the alcohol-seeking behavior triggered by environmental contexts, and opening new research venues on their neurobiological basis.

KEYWORDS

Alcohol-seeking behavior; Ethanol; Conditioned place aversion; Conditioned place preference; Rats

2.1 INTRODUCTION

Alcohol use disorder is a chronic, relapsing disorder characterized by loss of control over alcohol use despite its many adverse consequences (Carvalho et al., 2019; Nutt et al., 2010; WHO, 2018). A main obstacle in recovering from this disorder is the compulsive alcohol-seeking behavior triggered by cues that contribute to relapse from abstinence (Sliedrecht et al., 2019). In this case, alcohol, the primary motivator, induces incentive-motivational properties on stimuli associated with its intake and, when these stimuli are presented in its absence, they may elicit sign-tracking and alcohol-craving behaviors (Colaizzi et al., 2020; Creswell and Sayette, 2022; Davis, 2013). Over the past 50 years, researchers established different animal models to study the development of alcohol-seeking and -craving behaviors triggered by cues, their repercussions over alcohol consumption, their genetic and neurobiological bases, and to test possible treatments (Anton, 1999; Black et al., 1973; Davis, 2013; Koob, 2000; Pati et al., 2019; Smith, 2020; Venniro et al., 2018).

One model is place conditioning (PC). Used in addiction research to assess the positive (rewarding) or negative (aversive) motivational effects of exposure to drugs, including alcohol, PC tests acquired preferences and aversions for contexts in which these exposures occurred (Tzschentke, 1998, 2007). As a learning model with only 3 behavioral outcomes (conditioned place preference (CPP), conditioned place aversion (CPA), and conditioning failure), PC has been used successfully with other drugs to study CPP in rodents. However, the case of alcohol-induced PC is controversial. From the late-1970s to the late-1990s alcohol produced far more conditioning failure and CPA than CPP with rats in short PC protocols (with up to 10 conditioning trials). The rare CPP results occurred after long PC protocols (with more than 10 conditioning trials), prolonged alcohol preexposure, or when alcohol was combined with morphine or food during conditioning (Bieńkowski et al., 1995, 1996; Bozarth, 1990; Cunningham, 1979; Marglin et al., 1988; Reid et al., 1985; Stewart and Grupp, 1985).

These findings are inconsistent with the outcome pattern of psychostimulants and opioids in PC with rats, which produced far more CPP outcomes (Bardo et al., 1995; Carr et al., 1989; Swerdlow et al., 1989; Tzschentke, 2007). Even more, they are inconsistent with the pattern produced by alcohol in short protocols with mice. In mice, CPP occurs easily, and we know various CPP-, CPA-, and conditioning failure-related variables, so reliable protocols for CPP and CPA acquisition and expression exist (Cunningham et al., 2006; Grahame, 2013; Naassila, 2013; Shimizu et al., 2015). This led some to propose numerous explanations regarding alcohol's rewarding and aversive effects, others to consider the use of PC to study

the rewarding effects of alcohol a “nuisance”, and yet others to regard rats as not the best model organism for alcohol-induced PC (Asin et al., 1985; Bozarth, 1990; Cunningham et al., 1993; Fidler et al., 2004; Naassila, 2013; Reid et al., 1985; Stewart and Grupp, 1981; Swerdlow et al., 1989; van der Kooy et al., 1983).

Nevertheless, from the late-1990s on, while conditioning failure remained the commonest result, CPP seems to have replaced CPA in short PC protocols with rats (Bagrov et al., 1999; Cole et al., 2003; Der-Avakian et al., 2007; Ise et al., 2013; Morales et al., 2012; Pascual et al., 2012; Patkina and Zvartal, 1998; Quertemont et al., 1998; Quertemont and De Wite, 2001). This indicates that short PC protocol settings can generate CPP for rats. However, the characteristics of these settings remain poorly understood and we still lack trustful ways to predict CPP, CPA, and conditioning failure occurrences in rats (Fidler et al., 2004; Naassila, 2013; Tzschentke, 2007).

This review aims to shed light on this prediction in male (adolescent and adult) outbred rats for alcohol-induced PC, especially for short protocols, by answering two questions. First, “What variables are differentially related to occurrences of conditioning failure and success?”, where conditioning success is defined as all CPA and CPP results (both mean that learning occurred and was expressed). Second, “What variables are differentially related to occurrences of CPA and CPP?”. To this end, we systematically searched for and selected the literature and extracted procedural covariables and outcomes of selected experiments. We then organized the procedure-outcome relations according to the background of variables known to affect the results of associative learning preparations, alcohol interventions, and PC interventions themselves. Finally, we tested whether this organization could predict the dichotomies of conditioning failure vs. conditioning success and CPP vs. CPA in rats.

2.2 MATERIALS AND METHODS

This report follows the PRISMA guidelines (Moher et al., 2009; Page et al., 2021; **Appendix S1**). In October 2019 we conducted a structured search of relevant studies in PubMed and two other sources. After excluding duplicates, 2 reviewers (DM, PB) independently evaluated article eligibility (inclusion criteria) and experiment selection (exclusion criteria). After, DM and PB extracted data independently and discussions with the last authors (JL and ML) solved disagreements. **Appendix S2** fully describes the review protocol and the reasons for the exclusion criteria used in animal sample composition. Following, to evaluate the adequacy of using the recommended statistical synthesis methods (McKenzie et al., 2019) in this review, we carefully assessed the characteristics of preference

measurement and testing (**Appendices S3**, **S13**, and **S14**) and evaluated the risk of report bias (**Appendices S4** and **S12**) in the selected literature. The first assessment revealed the widespread use of different procedures to define, operationalize, and quantify spatial preference, as well as of different experimental designs to test its change. This led the sample to ubiquitously comprise non-solvable problems of error estimation between methods to measure permanence time in the conditioning chambers (the behavioral unit operationalized into "preference indices"), between different "preference indices" themselves, between the conditioning apparatuses (in terms of potential to induce learning and its expression) themselves, and between different raw-score estimates of effect size. Given these findings and those of risk of report bias, the use of any of the recommended research synthesis methods (p-value combination, summary of effect estimates, and meta-regression) was not warranted for the objectives of the current review. We then decided to conduct a narrative synthesis aided by a categorical frequentist distribution of reported PC outcomes (**Appendix S6**), which entails vote-counting methods (McKenzie et al., 2019), to provide at least provisory answers in the proportional prediction of results (Khamis, 2005). Next, we categorized the selected experiments into (a) short (≤ 10 conditioning trials) and (b) long (> 10 trials) PC protocols conducted with drug-naïve rats and (c) PC protocols conducted with rats pre-exposed to alcohol (see **Appendix S7** for suitability of separating short and long protocols).

2.3 RESULTS OF SEARCH AND SELECTION

Our search yielded 504 records, of which 325 were original publications, and 87 articles were eligible for experiment selection (**Figure 2.1A**). Reviewers agreed on the inclusion of 85 (97.7%) of the eligible articles. Applying exclusion criteria led to a final sample of 62 articles. Exclusion agreement occurred in 58 (93.6%) of the selected articles. Although our search included the period from 1970 to 2019, all selected articles were published from 1981 to 2017. From these articles, we selected 192 experiments to compose this review (**Figure 2.1B**). **Appendices S13** and **S14** provide the database of extracted variables for all selected experiments.

2.4 DISCUSSION

2.4.1 Variables predicting conditioning failure and conditioning success in short and long PC protocols without alcohol pre-exposure

Conceptually, conditioning failures are produced by variables having non-optimal or detrimental effects on behavioral acquisition or expression. In the case of behavioral

acquisition, the theoretical and experimental analysis of associative learning (i.e., both Pavlovian and operant conditioning) predicts both negatively and positively modulating variables (Gottlieb and Begej, 2014; Lubow, 1989; Murphy and Lupfer, 2014). While the effects of many variables vary according to specific learning preparations, 3 variables have well-established effects and interactions: latent inhibition, magnitude of the primary motivator, and number of conditioning trials (**Table S6**).

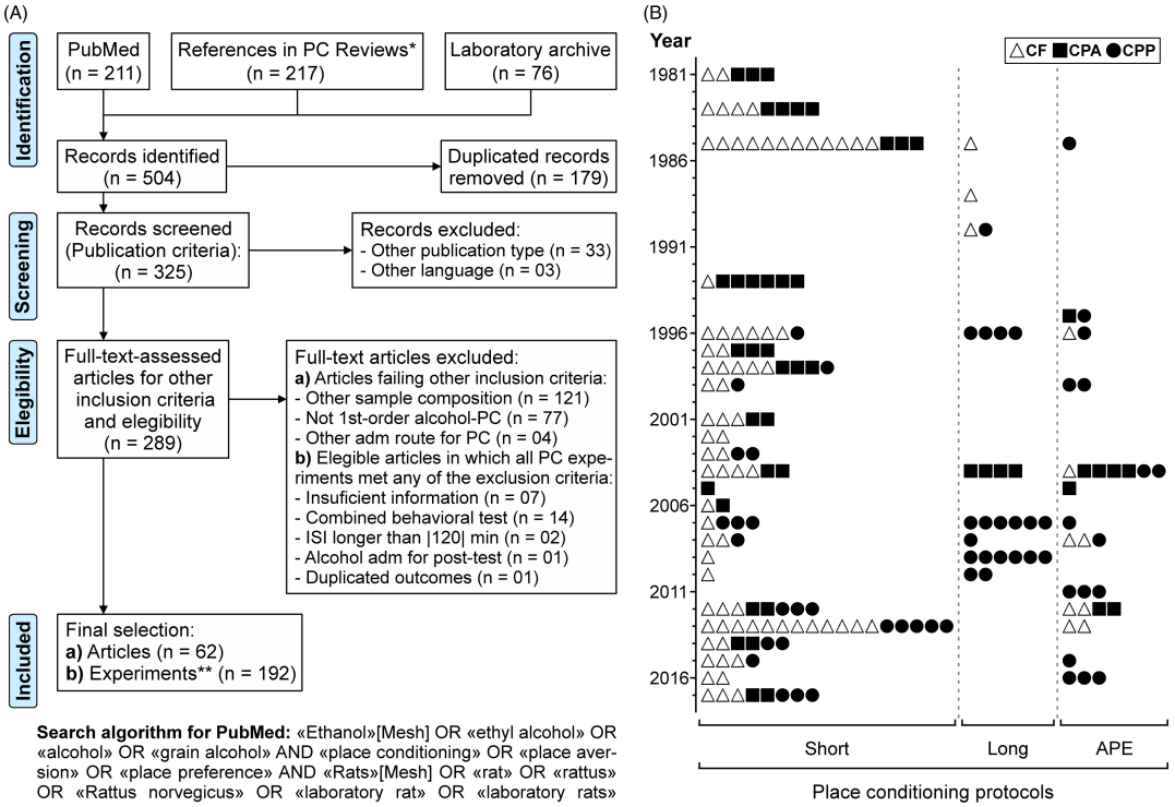


FIGURE 2.1 – PRISMA study flow diagram (A) and temporal distribution of results (B) for selected alcohol-induced PC protocols. APE, PC protocols with alcohol pre-exposure; CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference; ISI, interstimulus interval; Short, short PC protocols; Long, long PC protocols. * Search conducted in the reference lists of Schechter and Calcagnetti (1993, 1998) and Tzschentke (1998, 2007). ** Each intervention group was considered as a different experiment since both within-subject and between-subject measures have been used to test conditioning-induced changes in preference.

Latent inhibition is a detrimental effect of non-reinforced presentations (i.e., habituation sessions) of contexts/cues to be paired with the primary motivator. Accordingly, it delays acquisition of conditioned control (whether eliciting or discriminative), requiring more conditioning trials for it to happen, and decreases levels of conditioned responding (Ayres et al., 1992; Lantz, 1973; Lubow, 1989; Mellgren and Ost, 1971; Schmajuk, 2002). In turn, the magnitude of the primary motivator is positively related to conditioning success, enhancing the likelihood and speeding up the acquisition of conditioned control (i.e., acquisition occurs with fewer trials) and promoting higher conditioned responding (Morris and Bouton, 2006; Rose et al., 2009; Stahlman and Blaisdell, 2011; Webber et al., 2015). Lastly, more

conditioning trials countereffects latent inhibition and may increase the efficacy of conditioned control induced by low-magnitude primary motivators (Lubow, 1989; Morris and Bouton, 2006; Rose et al., 2009).

Considering that among drug factors (dose, solution concentration, and administration route), the dose is the main component of alcohol's magnitude as a primary motivator, we organized the outcomes of 158 short and long PC protocols according to alcohol dose, number of habituation sessions, and number of conditioning trials (**Figure 2.2**). Analysis of these 158 experiments suggests an agreement with the abovementioned predictions. In short protocols (**Figure 2.2A**), 64.5% of the 76 failures occurred for doses lower than 1.0 g/kg (intraperitoneal, ip, and intragastric, ig, routes). Thus, these doses do not seem optimal for conditioning success in short protocols. Moreover, the number (both proportional and absolute) of conditioning failures decreased as the alcohol dose range increased: 41 failure reports (82.0%) among 50 experiments using doses of 0.01-0.5 g/kg; 23 (57.5%) among 40 using 0.51-1.0 g/kg; 8 (36.4%) among 22 experiments using 1.01-1.5 g/kg; 3 (17.7%) among 17 experiments using 1.51-2.0 g/kg. Consequently, the higher the alcohol dose (motivator magnitude), the lower the conditioning failure occurrence in short protocols.

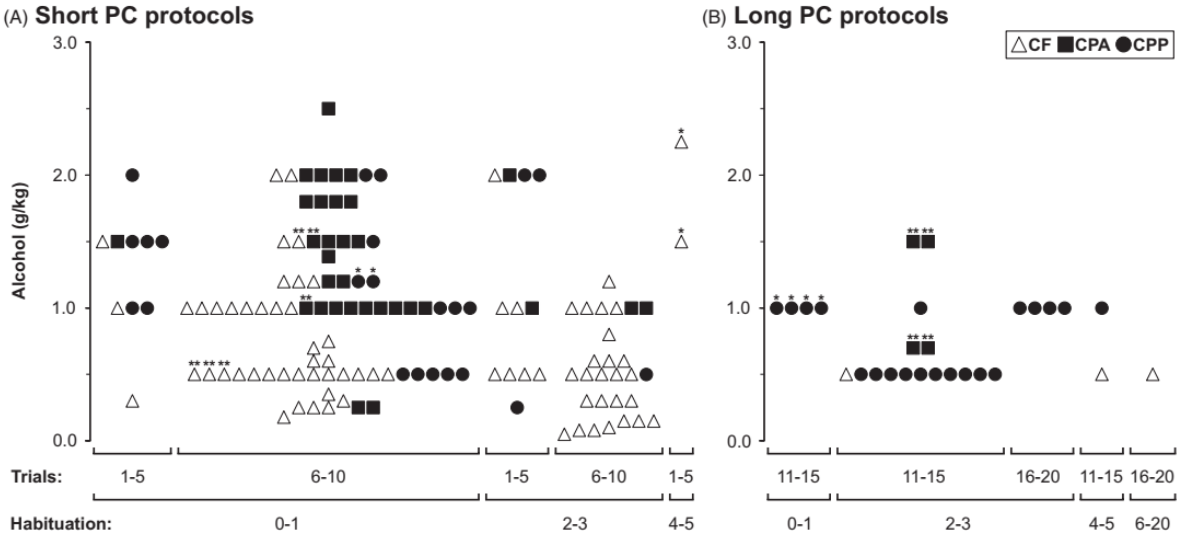


FIGURE 2.2 – Results of short (A) and long (B) alcohol-induced PC protocols without alcohol pre-exposure, organized by alcohol dose, number of conditioning trials, and number of habituation sessions. Trials, number of conditioning trials; Habituation, number of habituation sessions; CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference; *, alcohol administered by orogastric gavage; **, intragastric alcohol administration. Note: (A) does not show 2 CPAs for the doses of 3.5 and 5.0 g/kg (ig) reported by van der Kooy et al. (1983) using a short protocol with 0 habituation sessions and 8 conditioning trials.

Regarding the number of habituation sessions, the failure percentage increased in doses of 0.01-1.50 g/kg according to the number of habituation sessions (**Figure 2.2A**). The first habituation range (0-1 session) included 34 failures (61.8%) among 55 experiments, while the second (2-3 sessions) included 30 (85.7%) for 35 experiments. We found only 2 short

protocols with 4 habituation sessions, and both reported failures (100%), evidencing again that more habituation sessions increase the chances of conditioning failure. Regarding the relation between the number of habituation sessions and conditioning trials, if trials were between 1 and 5, 0-1 and 2-3 habituation sessions were associated with failure percentages of 27.3% and 58.3%, respectively. Meanwhile, if trials were between 6 and 10, 0-1 and 2-3 habituation sessions were associated with failure percentages of 50% and 89.3%, respectively. This indicates that alcohol's capacity to induce PC is highly sensitive to latent inhibition disruption in short protocols.

When the number of conditioning trials increased further, as shown for long protocols (**Figure 2.2B**), failures decreased remarkably. Specifically, when we compared short (**Figure 2.2A**) and long (**Figure 2.2B**) protocols using doses of 0.5-1.5 g/kg and 2-3 habituation sessions, long protocols presented fewer failures: 1 failure (5%) for 20 long protocols against 20 (83.3%) for 24 short protocols. Thus, latent inhibition disruption on conditioned control is mostly countered by more trials. Moreover, long protocols with 2-3 habituation sessions presented greater percentages of conditioning success even in comparison to short protocols with 0-1 sessions (for the ip dataset: 94.1% for long with 2-3 sessions; 47.3% for short with 0-1) and this effect was greater for 0.5 (ip: 90.9% for long with 2-3; 31.2% for short with 0-1; 10% for short with 2-3) than for 1.0 g/kg (ip: 100% for long with 2-3; 59% for short with 0-1; 33.3% for short with 2-3).

Given the doses used and the direction of this effect (i.e., greater for 0.5 than for 1.0 g/kg), there is marginal reason to think it entails tolerance predominantly. However, there are good reasons for sensitization: more trials elevate the efficacy of low-magnitude primary motivators (**Table S6**), a low (0.5 g/kg) but not a high (2 g/kg) alcohol dose induces behavioral sensitization with repeated administration in rats (Xu and Kang, 2017), and the direct and repeated (≥ 5 times) alcohol administration into the posterior ventral tegmental area (a brain region involved with the alcohol motivator function in short protocols: Campos-Jurado et al., 2020) causes neuronal sensitization in dopaminergic neurons (Ding et al., 2009). Thus, it is probable that all three sensitization phenomena are acting for doses of 0.5-0.75 g/kg in long protocols.

However, there is a concern in short protocols regarding latent inhibition (**Table S6**). The comparison of failure percentages of short protocols with 0 or 1 habituation session did not support its prediction, whether we considered the dose ranges of 0.01-1.0 (72.4% and 50.0% respectively), 0.01-1.5 (57.8% and 44.8% respectively) or 0.01-2.0 g/kg (50.0% and 41.7%, respectively). As the failure percentages for short protocols with 2 or 3 habituation sessions

seem to support the prediction in all these ranges (76.9% against 91.7%, respectively, in the first ones, and 73.3% against 84.6%, respectively, in the last), 2 explanations are possible for this disagreement. Either alcohol-induced PC does not conform entirely to the latent inhibition prediction or other variables negatively affecting behavioral acquisition/expression are differentially distributed in short protocols with 0 against those with 1 or more habituation sessions.

If alcohol-induced PC does conform to this prediction, what these other variables could be? For short protocols, we already know that lower doses tend to induce less efficient conditioned control (if any) that is more fragile to acquisition/expression disruptions (for dose-response relationships in alcohol-induced PC, see Der-Avakian et al., 2007; van der Kooy et al., 1983; for dose-response relationships in other drug-induced PC, see Bardo et al., 1995). Additionally, Cunningham and Niehus (1993), testing temperatures (5 ºC and 32 ºC) outside the conventional range (21-24 ºC) during conditioning, showed that 32 ºC disrupted CPA acquisition for a dose of 1.2 g/kg (ip) in a short protocol with no habituation session and 8 conditioning trials. In parallel, this temperature only reduced the levels of conditioned responding for a dose of 1.8 g/kg (ip) in the same short protocol. Moreover, Bormann and Cunningham (1998), testing different (-30, -15, -10, -5, 0, and 5 min) interstimulus intervals (ISIs), showed that CPA was acquired only in ISIs of 0 and -15 min for a dose of 1.0 g/kg (ip) in a short protocol with no habituation session and 8 conditioning trials, while all other ISIs disrupted acquisition for this alcohol dose.

As in the case of latent inhibition, all these variables affect behavioral acquisition (**Table S6**). What about those impacting behavioral expression? The PC literature indicates that higher levels of unconditioned exploratory behavior during the post-test may compete with conditioned control expression, favoring conditioning failure (Carr et al., 1989; Swerdlow et al., 1989). If so, at least 2 variables can increase exploratory behavior (**Table S7**). The first is the uneven distribution of conditioning trials for different conditioning chambers, which causes novelty-related effects for the conditioning chamber with fewer trials (Carr et al., 1988). The second is an increase in apparatus area, which increases peripheric exploration, especially locomotor activity, and avoidance of large central areas according to the open-field literature (Walsh and Cummins, 1976).

In short protocols, only 1 experiment used an uneven distribution of trials (Bie et al., 2009). As this experiment used only 1 trial for the drug-paired context, associated with 3 habituation sessions, latent inhibition and low dose (0.5 g/kg) are factors far more important for the failure reported. In long protocols, uneven distribution of trials occurred in the

experiments conducted by Marglin et al. (1988), Bozarth (1990), and López and Cantora (2010). Considering the 10 CPP reports of Zhu et al. (2007) and Bie et al. (2009) for long protocols with 3 habituation sessions, 14 evenly distributed conditioning trials, and a dose of 0.5 g/kg (ip), this variable seems to be one of the missing factors to explain the 2 failures reported by Marglin et al. (1988) and Bozart (1990) for the dose of 0.5 g/kg (ip). They used long protocols with habituation sessions (2 for Marglin et al., 1988; 5 for Bozarth, 1990) and greater exposure of animals to the drug-paired context than to the drug-unpaired one during conditioning (9 to 3 for Marglin et al., 1988; 15 to 0 for Bozarth, 1990). In fact, in these conditions, Marglin et al. (1988) only reported a CPP when the alcohol dose of 0.5 g/kg (ip) was given together with morphine (2.0 mg/kg, subcutaneously) and Bozarth (1990) only reported a CPP for a dose of 1.0 g/kg (ip), the same result obtained by López and Cantora (2010) for this dose when adapting Bozarth's protocol.

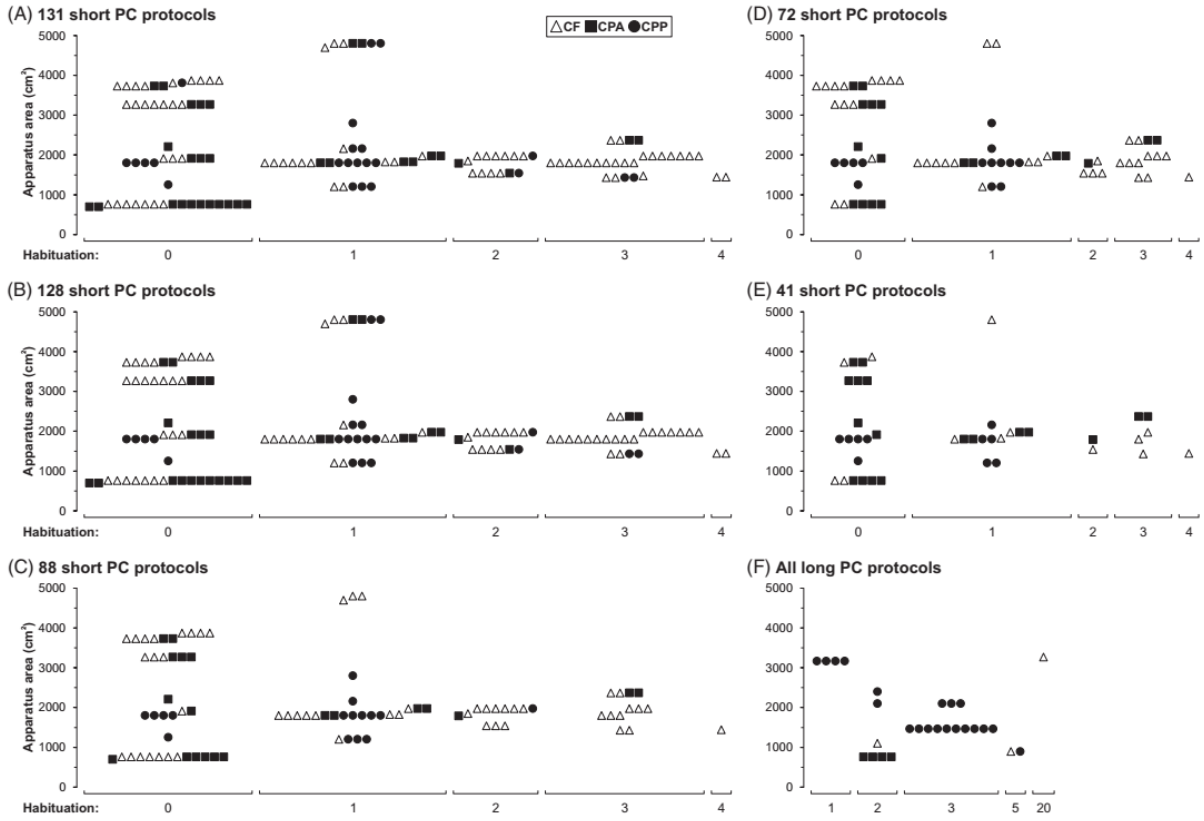


FIGURE 2.3 – Results of short (A-E) and long (F) alcohol-induced PC protocols without alcohol pre-exposure, organized by apparatus area and total number of habituation sessions. (A) 131 short PC protocols, (B) 128 short PC protocols (excluding those with less than 4 conditioning trials), (C) 88 short PC protocols (with the additional exclusion of doses lower than 0.5 or higher than 1.5 g/kg), (D) 72 short PC protocols (with additional exclusion of those with disruptive interstimulus intervals or unusual temperature during conditioning), € 41 short PC protocols (with the additional exclusion of those using alcohol doses lower than 1.0 g/kg), (F) all long PC protocols. CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference. Note: (A-E) do not include 2 CPAs for the doses of 3.5 and 5.0 g/kg (ig) reported by van der Kooy et al. (1983) using a short protocol with 0 habituation sessions.

Figure 2.3 shows the results organized according to apparatus area and the number of habituation sessions. Short protocols are shown in Figures **2.3A-E**, and Figure **2.3F** presents all long protocols. Figure **2.3A** shows 131 results for short protocols. In Figure **2.3B**, we excluded all reports with less than 4 conditioning trials as the evidence supporting conditioning success with 1 to 3 trials is tenuous at best. In Figure **2.3C**, we also excluded reports using doses lower than 0.5 or higher than 1.5 g/kg as these doses would presumably induce conditioned control too fragile and too resistant, respectively, to acquisition/expression disruptions. In Figure **2.3D**, we excluded all protocols with doses between 0.5 and 1.0 g/kg combined with the disruptive ISIs (-30, -10, -5, and 5 min) reported by Bormann and Cunningham (1998), as well as the ISI of -15 min (as only those researchers used it in the literature) and the failure involving 32 ºC reported by Cunningham and Niehus (1993). Finally, in Figure **2.3E**, we excluded all remaining reports using doses lower than 1.0 g/kg.

As shown in **Figure 2.3A**, apparatuses larger than 3000 cm^2 present a greater failure percentage (72.7% for 22 reports) than those smaller than 3000 cm^2 (33.3% for 30 reports) in short protocols with no habituation session. This difference holds even when we isolated variables with known detrimental (or non-optimal) effects on conditioned control acquisition and those too resistant to the detrimental effects, the cases of Figure **2.3B** (75.0% and 33.3%), **C** (68.8% and 38.1%), **D** (68.8% and 21.4%), and **E** (28.6% and 15.4%). Moreover, considering apparatuses with an area smaller than 3000 cm^2, the results of short protocols with 0 to 3 habituation sessions seem to follow the failure pattern expected by latent inhibition effects: Figure **2.3A** (0 habituation session: 33.3%; 1 habituation session: 41.8%; 2 habituation sessions: 73.3%; 3 habituation sessions: 84.6%), **B** (0: 33.3%; 1: 41.8%; 2: 73.3%; 3: 84.0%), **C** (0: 38.1%; 1: 39.1%; 2: 83.3%; 3: 83.3%), **D** (0: 21.4%; 1: 42.9%; 2: 80.0%; 3: 83.3%), and **E** (0: 15.4%; 1: 25.0%; 2: 50.0%; 3: 60%).

For the results of apparatuses smaller than 3000 cm^2 (**Figure 2.3A**), we made a general comparison of 0 vs. 1 vs. 2 vs. 3 habituation sessions ($F2 = 19.493$, $n = 100$, $p < 0.001$, $\varphi c = 0.437$). Following, we made post hoc comparisons (Bonferroni-corrected $\alpha = 0.0125$ for 4 multiple comparisons) of absence against presence of habituation session ($x2 = 8.129$, $df = 1$, $n = 100$, $p = 0.008$, $\varphi c = 0.285$), 0 against 1 habituation session ($x2 = 0.408$, $df = 1$, $n = 59$, $p = 0.596$, $\varphi c = 0.083$), and 2 against 3 habituation sessions ($x2 = 0.771$, $df = 1$, $n = 41$, $p = 0.434$, $\varphi c = 0.137$). As no difference was found between the last pairwise comparisons, we compared the combination of 0-1 against the combination of 2-3 habituation sessions ($x2 = 18.240$, $df = 1$, $n = 100$, $p < 0.001$, $\varphi c = 0.427$). These results confirm that more habituation sessions are associated with a higher frequency of conditioning failure. Likewise, we found a

similar pattern in statistical results for the data of Figure **2.3D** (general 0 vs. 1 vs. 2 vs. 3: F2 = 12.189, n = 53, p< 0.005, φc = 0.484; absence vs. presence: x2 = 5.811, n = 53, df = 1, p = 0.028, φc = 0.331; 0 vs. 1: x2 = 1.461, n = 36, df = 1, p = 0.292, φc = 0.201; 2 vs. 3: x2 = 0.027, n = 17, df = 1, p = 1.000, φc = 0.040; 0-1 vs. 2-3: x2 = 11.103, n = 53, df = 1, p = 0.001, φc = 0.458). This indicates that, concerning the detrimental effect, the main difference is between 0-1 and 2-3 habituation sessions.

TABLE 2.1 – Correlations of apparatus area with the number of chambers in apparatuses and number of stimulatory modalities composing the drug-paired context in short place conditioning protocols.

Habituation	**Variables in Correlation**	**No. of pairs**	**r coefficient**	**r^2**	***p*-value**
0	Apparatus area *vs*. number of stimulatory modalities	52	0.661	0.436	< **0.001**
1 to 4	Apparatus area *vs*. number of stimulatory modalities	79	0.079	0.006	0.488
0	Apparatus area *vs*. number of chambers in apparatuses	52	0.844	0.712	< **0.001**
1 to 4	Apparatus area *vs*. number of chambers in apparatuses	79	0.297	0.088	**0.008**

Note: Habituation, number of habituation sessions, no. of pairs, number of pairs composing each correlation. Boldface indicates significant results.

Conversely, although most failure reports in short protocols with 0 habituation sessions happened in apparatuses larger than 3000 cm^2, this variable cannot be considered alone. As shown in **Table 2.1**, two other variables are strongly, significantly, and positively correlated with apparatus area in these protocols: the number of stimulatory modalities (visual, tactile, and olfactory) of cues and the number of chambers (from 1 to 3) in the PC apparatus. Increases in both variables are also predicted to have detrimental effects on conditioned control acquisition (stimulatory modalities: see Bardo and Bevins, 2000; Cunningham, 1993; Cunningham et al., 2011) and expression (chambers: see **Table S7** and **Appendix S3**). Thus, the detrimental effects of these 3 variables are worrisome for conditioning success when they are combined.

As for PC protocols with alcohol pre-exposure (APE), due to space constraints, **Appendix S8** presents the analysis of the conditioning failure-success dichotomy. Briefly, the analysis shows that (a) only one APE method increased conditioning success probability in male rats, (b) this method probably involves behavioral and stress-related sensitization phenomena, and (c) it is not as efficient as long PC protocols.

2.4.2 Variables predicting conditioned place aversion and conditioned place preference in short PC protocols without APE

Once the threshold of conditioning failure-success is crossed, learning theory predicts only one aspect of the primary motivator as determining the balance between its reinforcing and aversive properties. This prediction is that the greater the motivator magnitude, the greater its chances of presenting aversive properties (Azrin and Holz, 1966; Binder et al., 2008; Davis, 2013). For drugs of abuse such as alcohol, this is expected as their effects are generally dose- and concentration-dependent, with greater toxicological effects manifesting at higher doses/concentrations according to the administration route (Bode, 1980; Davis and Riley, 2010; Kallant, 1971, 1996; Pohorecky and Brick, 1988). Hence, this prediction may explain why van der Kooy et al. (1983) reported CPAs for doses of 3.5 and 5.0 g/kg (ig, 50%, v/v) and why the dose of 1.5 (ig) only induced CPA in a concentration of 20% but resulted in failure when administered in 10%. It may also shed light on why Pascual et al. (2012) reported 2 CPAs for a dose of 1.0 g/kg (ip, 25%) as the concentration is the greatest one in short protocols using the ip route (see Table 1).

However, the prediction is unsatisfactory to distinguish CPA and CPP in other cases as both results occurred for short protocols with doses of 2.0 g/kg or lower administered through parenteral and enteral routes in varying concentrations (**Figure 2.2A**). Accordingly, many environmental and subject characteristics have been proposed and identified as modulating factors for animal's sensibility to the rewarding, aversive, and toxicological effects of alcohol (Becker et al., 2011; Bloom et al., 1982; Bode, 1980; Crabb et al., 1985, 1987; Edenberg, 2007; Horton and Mills, 1979; Hurley and Edenberg, 2012; Lad et al., 1984; Noori et al., 2014; Spanagel et al., 2014; Sturtevant and Garber, 1980, 1981; Walker and Ehlers, 2009). Relevant ones for the sample herein reviewed are the genetic (rat strain), developmental (the age-weight covariate), circadian (experimental timetables), and stress (housing system: single and group housing) modulations of alcohol biotransformation and effects (**Table S6**). Additionally, the combination of stimulus-assignment criterion (choosing which context will be paired with alcohol effects in PC) with apparatus bias (i.e., whether animals presented or not an unconditionally-preferred context in the PC apparatus) may be a relevant learning variable specific to PC (Schechter, 1992; Schechter and Krimmer, 1992).

For the combination of an unbalanced stimulus-assignment with a biased apparatus, the literature presents contradicting evidence that choosing the baseline-preferred context as the drug-paired one may favor CPA in short protocols with rats (Mucha and Iversen, 1984; Schechter, 1992; Schechter and Krimmer, 1992; Schenk et al., 1985; Spyraki et al., 1985). Nevertheless, assuming this influence is real, it would account for only 2 CPA reports (Bieńkowski et al., 1997a, 1997b) for a dose of 1.5 g/kg (ip, 10%, v/v). Thus, this

combination is insufficient to predict CPA for short protocols (as biases in stimulus-assignment and apparatuses are controversial issues in the PC literature, they are thoroughly discussed in **Appendix S10**). Of the other relevant characteristics, animal age is not reported frequently enough for analysis (spreadsheet 05 of **Appendix S13**), experimental timetables present deficits in their reports (see **Appendix S11**), and reports of animal weight are not trustful (animals' weight were mostly reported at the arriving time in laboratory facilities, without describing the time-lapse between arrival and start of the PC protocol or the weight at the beginning of the PC protocol; spreadsheet 05 of **Appendix S13**). Therefore, from now on, the analysis of the CPA-CPP distribution is centered on rat strain, especially albino ones (Holtzman, Sprague Dawley, and Wistar rats), and housing data (**Figure 2.4**).

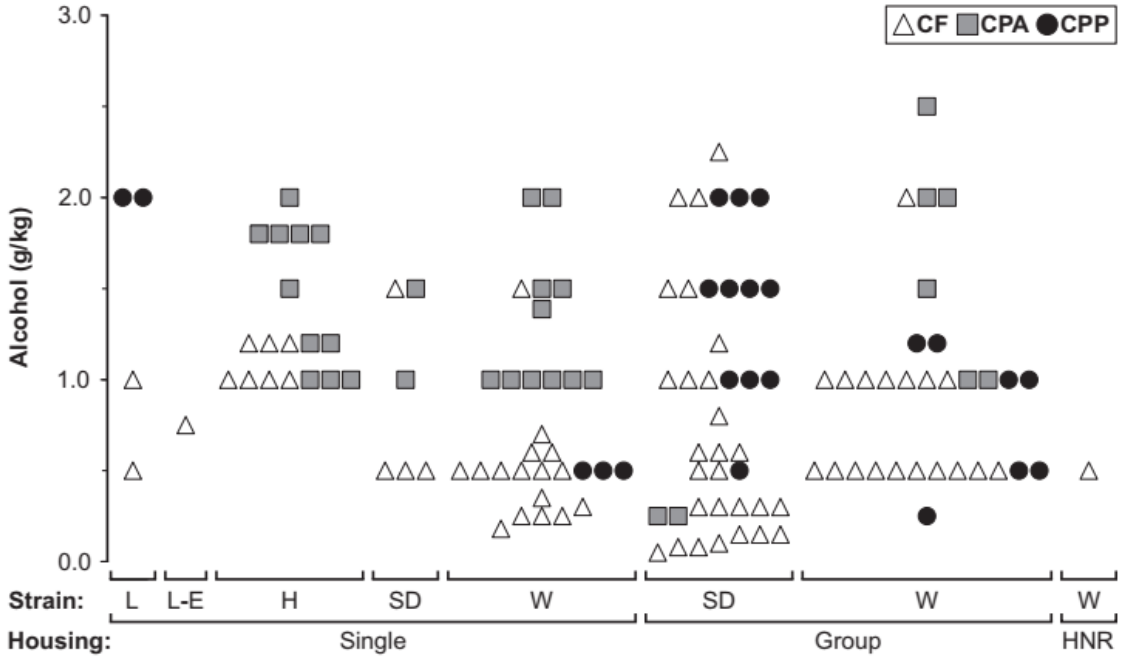


FIGURE 2.4 – Results of alcohol-induced short PC protocols without alcohol pre-exposure, organized by alcohol dose, housing system, and outbred strains of Rattus norvegicus. CF, conditioning failure; CPA, conditioned place aversion, CPP, conditioned place preference; HNR, housing not reported; L, Lister rats; L-E, Long-Evans rats; H, Holtzman rats; SD, Sprague Dawley rats; W, Wistar rats. Note: This figure does not include 2 CPAs for the doses of 3.5 and 5.0 (ig) reported by van der Kooy et al. (1983)

Examination of **Figure 2.4** suggests that housing is associated with different distributions of CPA and CPP results in Holtzman, Long-Evans, Lister, Sprague Dawley, and Wistar strains, with changes in motivational valence occurring for doses of 1.0 g/kg or higher. CPP was more frequently reported for group-housed rats and CPA for single-housed rats. Additionally, CPP has been reported in a greater dose range (0.5-2.0 g/kg, ip) for group-housed Sprague Dawley rats than for group-housed Wistar rats (0.5-1.0 g/kg, ip; 1.2 g/kg, orogastric — og). **Figure 2.4** also shows that higher doses (1.5-2.0 g/kg, ip) resulted only in

failure (1 of 4 reports) and CPA (3 of 4 reports) in group-housed Wistar rats, whereas 5 failures and 7 CPPs occurred for group-housed Sprague Dawley. Moreover, except for single-housed Sprague Dawley rats, all 3 albino strains in all housing systems presented the dose range of 1.0-2.0 g/kg, with group-housed Sprague Dawley rats and single-housed Holtzman rats presenting the most homogeneous distribution of reports in this range, while only Wistar rats (single- and group-housed) and Sprague Dawley rats (group-housed) presented the range of 0.25-2.0 g/kg. Furthermore, the dose that was more evenly distributed between the 3 albino strains is 1.0 g/kg and the range is 1.0-1.5 g/kg.

TABLE 2.2 – Comparisons of results in short PC protocols without alcohol pre-exposure for albino strains of Rattus norvegicus organized by housing system and alcohol dose ranges.

DI (g/kg)	Comparison	VC	F^2	X^2	*df*	*p*	φ_c
[0.25-0.50]	Single *vs*. Group (CF, CPA, CPP)	40	1.273	-	-	0.600	0.198
[0.25-1.00]	Single *vs*. Group (CF, CPA, CPP)	78	6.077	-	-	**0.050**	0.285
[0.25-1.50]	Single *vs*. Group (CF, CPA, CPP)	100	15.235	-	-	< **0.001**	0.388
[0.25-2.00]	Single *vs*. Group (CF, CPA, CPP)	115	21.972	-	-	< **0.001**	0.431
[0.50-1.00]	Single *vs*. Group (CF, CPA, CPP)	65	9.138	-	-	**0.008**	0.379
[0.50-1.50]	Single *vs*. Group (CF, CPA, CPP)	91	22.908	-	-	< **0.001**	0.490
[0.50-2.00]	Single *vs*. Group (CF, CPA, CPP)	102	25.133	-	-	< **0.001**	0.488
[1.00-1.50]	Single *vs*. Group (CF, CPA, CPP)	53	23.295	-	-	< **0.001**	0.637
[1.00-2.00]	Single *vs*. Group (CF, CPA, CPP)	68	31.192	-	-	< **0.001**	0.646
[1.50-2.00]	Single *vs*. Group (CF, CPA, CPP)	28	12.902	-	-	**0.001**	0.676
[0.25-0.50]	Single *vs*. Group (CF, SC)	40	-	0.339	1	0.707	0.100
[0.25-1.00]	Single *vs*. Group (CF, SC)	78	-	0.652	1	0.473	0.091
[0.25-1.50]	Single *vs*. Group (CF, SC)	100	-	0.429	1	0.544	0.066
[0.25-2.00]	Single *vs*. Group (CF, SC)	115	-	1.301	1	0.267	0.106
[0.50-1.00]	Single *vs*. Group (CF, SC)	65	-	2.042	1	0.195	0.177
[0.50-1.50]	Single *vs*. Group (CF, SC)	91	-	2.464	1	0.142	0.165
[0.50-2.00]	Single *vs*. Group (CF, SC)	102	-	2.449	1	0.164	0.155
[1.00-1.50]	Single *vs*. Group (CF, SC)	53	-	0.999	1	0.406	0.137
[1.00-2.00]	Single *vs*. Group (CF, SC)	68	-	2.485	1	0.137	0.191
[1.50-2.00]	Single *vs*. Group (CF, SC)	28	-	1.197	1	0.396	0.207

Note: Boldface indicates significant results.
Abbreviations: CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference; df, degrees of freedom; DR (g/kg), alcohol dose range; *F*2, Fisher's exact test; SC, conditioning success; VC, total number of valid cases in each comparison; *X*2, Chi-square test (exact method); *φ*c, Cramér's V.

Taking these considerations, we stratified the categorical results for albino strains according to housing systems and made comparisons to test whether housing design is associated with CPA-CPP report distribution. These comparisons were done according to different dose ranges (**Table 2.2**). There is strong evidence that housing systems are differently associated with occurrences of CPP and CPA when the dose range includes doses equal to or greater than 1.0 g/kg. Importantly, these outcomes cannot be attributed to different

rates of conditioning failure-success between housing systems: no significant difference was detected between housing systems in this regard. In other words, while single housing is associated with CPA and group housing with CPP in albino strains, these housing systems are not associated with different failure-success rates.

To evaluate the distribution of the reported results (CPP, CPA, and failures) within each albino strain stratified by housing system and different dose ranges, we implemented the following eligibility criteria for statistical comparisons: (a) each strain, according to the housing system, should present at least 6 reported results in the selected dose range and (b) each result group should present at least 1 result in the lower and 1 in the upper limit of the selected dose range (**Table 2.3**). Sprague Dawley (group-housed) and Wistar rats (single- and group-housed) were significantly different in the dose ranges of 0.25-2.0, 0.5-1.5, and 0.5-2.0 g/kg. As no significant difference was detected in the ranges of 0.25-0.5, 0.25-1.0, and 0.50-1.0, and doses lower than 1.0 g/kg were the most frequent in these ranges, these results reinforce the interpretation that the housing system was not associated with the distribution of results for doses lower than 1.0 g/kg.

Moreover, while group-housed Sprague Dawley and single-housed Wistar rats showed differences in the dose ranges of 0.5-1.5 and 0.5-2.0 g/kg, single- and group-housed Wistar rats did not. As both ranges presented doses in which housing systems have little to no association with CPA or CPP in short protocols and, as these doses were more frequent in these ranges for single-housed Wistar rats, a masking effect could be occurring in the case of single- and group-housed Wistar rats. If this were the case, a logical consequence would be that excluding doses lower than 1.0 g/kg from the analysis would not only reveal differences between single- and group-housed Wistar rats but also increment the differences between group-housed Sprague Dawley rats and single-housed Wistar rats.

Supporting this, in ranges of 1.0-1.5 and 1.0-2.0 g/kg, group- and single-housed Wistar rats presented significant differences and there was an increment in the difference between group-housed Sprague Dawley rats and single-housed Wistar rats. On the other hand, group-housed Wistar rats and single-housed Holtzman rats did not present differences in these ranges. These results might seem contradictory considering that single-housed Wistar and Holtzman rats also did not differ in these ranges. However, single-housed Holtzman rats presented a failure frequency very similar to that of group-housed Wistar rats at the dose of 1.0 g/kg and only 3 extra CPA reports than group-housed Wistar rats in the range of 1.0-1.5 g/kg. These similarities elucidate these results.

TABLE 2.3 – Comparisons of results in short PC protocols without alcohol pre-exposure, organized by albino strain of Rattus norvegicus, housing system, and alcohol dose ranges.

DI (g/kg)	Comparison	VC	F^2	X^2	*df*	*p* (overall)	*p* (multiple)	φ_c
[0.25-0.50]	Ws *vs.* SDg *vs.* Wg	37	4.147	-	-	0.361	-	0.286
[0.25-1.00]	Ws *vs.* SDg *vs.* Wg	67	3.289	-	-	0.592	-	0.164
[0.25-1.50]	Ws *vs.* SDg *vs.* Wg	81	7.907	-	-	0.090	-	0.231
[0.25-2.00]	Ws *vs.* SDg *vs.* Wg	91	11.476	-	-	**0.019**	-	0.256
[0.25-2.00]	Ws *vs.* SDg	61	11.139	-	-	-	**0.003**	0.428
[0.25-2.00]	Ws *vs.* Wg	59	3.982	-	-	-	0.148	0.264
[0.25-2.00]	SDg *vs.* Wg	62	2.063	-	-	-	0.371	0.186
[0.50-1.00]	Ws *vs.* SDg *vs.* Wg	54	7.417	-	-	0.100	-	0.281
[0.50-1.50]	SDs *vs.* Ws *vs.* SDg *vs.* Wg	74	15.810	-	-	**0.008**	-	0.334
[0.50-1.50]	SDs *vs.* Ws	28	0.931	-	-	-	0.681	0.216
[0.50-1.50]	SDs *vs.* SDg	26	7.175	-	-	-	0.017	0.592
[0.50-1.50]	SDs *vs.* Wg	32	2.542	-	-	-	0.258	0.299
[0.50-1.50]	Ws *vs.* SDg	42	12.112	-	-	-	**0.002**	0.521
[0.50-1.50]	Ws *vs.* Wg	48	5.341	-	-	-	0.087	0.339
[0.50-1.50]	SDg *vs.* Wg	46	3.021	-	-	-	0.239	0.273
[0.50-2.00]	Ws *vs.* SDg *vs.* Wg	78	18.864	-	-	**0.001**	-	0.349
[0.50-2.00]	Ws *vs.* SDg	49	17.557	-	-	-	< **0.001**	0.575
[0.50-2.00]	Ws *vs.* Wg	53	4.956	-	-	-	0.091	0.310
[0.50-2.00]	SDg *vs.* Wg	54	6.518	-	-	-	0.035	0.353
[1.00-1.50]	Hs *vs.* Ws *vs.* SDg *vs.* Wg	50	27.475	-	-	< **0.001**	-	0.533
[1.00-1.50]	Hs *vs.* Ws	23	-	4.790	1	-	0.074	0.456
[1.00-1.50]	Hs *vs.* SDg	26	13.472	-	-	-	**0.001**	0.709
[1.00-1.50]	Hs *vs.* Wg	27	4.658	-	-	-	0.124	0.429
[1.00-1.50]	Ws *vs.* SDg	23	20.186	-	-	-	< **0.001**	0.921
[1.00-1.50]	Ws *vs.* Wg	24	10.413	-	-	-	**0.004**	0.681
[1.00-1.50]	SDg *vs.* Wg	27	3.469	-	-	-	0.178	0.378
[1.00-2.00]	Hs *vs.* Ws *vs.* SDg *vs.* Wg	65	38.024	-	-	< **0.001**	-	0.530
[1.00-2.00]	Hs *vs.* Ws	30	-	3.438	1	-	0.099	0.339
[1.00-2.00]	Hs *vs.* SDg	36	23.590	-	-	-	< **0.001**	0.765
[1.00-2.00]	Hs *vs.* Wg	35	5.955	-	-	-	0.043	0.424
[1.00-2.00]	Ws *vs.* SDg	30	27.839	-	-	-	< **0.001**	0.936
[1.00-2.00]	Ws *vs.* Wg	29	10.539	-	-	-	**0.003**	0.620
[1.00-2.00]	SDg *vs.* Wg	35	7.361	-	-	-	0.019	0.464
[1.50-2.00]	Hs *vs.* Ws *vs.* SDg	22	19.354	-	-	< **0.001**	-	0.666
[1.50-2.00]	Hs *vs.* Ws	11	-	1.320	1	-	0.450	0.346
[1.50-2.00]	Hs *vs.* SDg	17	15.834	-	-	-	< **0.001**	1.000
[1.50-2.00]	Ws *vs.* SDg	16	10.776	-	-	-	**0.001**	0.876

Note: Boldface indicates results significant at $p \leq 0.05$ (overall) and at Bonferroni-corrected $\alpha = 0.0167$ and $\alpha = 0.0083$ for 3 and 6 multiple comparisons in each dose range, respectively.
Abbreviations: df, degrees of freedom; DR (g/kg), alcohol dose range; *F*2, Fisher's exact test; Hs, single-housed Holtzman rats; SDg, group-housed Sprague Dawley rats; SDs, single-housed Sprague Dawley rats; VC, total number of valid cases in each comparison; Wg, group-housed Wistar rats; Ws, single-housed Wistar rats; *X*2, Chi-square test; φc, Cramér's V.

More important, (a) the differences between group- and single-housed Wistar rats in the ranges of 1.0-1.5 and 1.0-2.0 g/kg, (b) the marked differences of group-housed Sprague

Dawley rats from single-housed Holtzman and Wistar rats in the ranges of 1.0-1.5, 1.0-2.0, and 1.5-2.0 g/kg, the lack of differences (c) between single-housed Holtzman and Wistar rats and (d) between group-housed Sprague Dawley and Wistar rats in the 1.0-1.5 and 1.0-2.0 ranges, indicate that the results shown in **Table 2.2** were not artifacts derived from single-housed Holtzman rats and support the association of single housing with a higher frequency of CPA in alcohol doses ≥ 1.0 g/kg for Holtzman and Wistar strains and most likely for Sprague Dawley rats as well. Thus, single housing may sensitize rats to the aversive properties of alcohol in short protocols using doses of 1.0 to 2.0 g/kg.

Conversely, other variables may modulate this sensitization, as indicated by the 2 CPP reports for single-housed Lister rats with an alcohol dose of 2.0 g/kg (Cole et al., 2003) and the 6 CPA reports of group-housed Wistar rats in the dose range of 1.0-2.5 g/kg (Becker et al., 2006; Bieńkowski et al., 1997a; Pascual et al., 2012; Torres et al., 2014). Although we cannot be certain, and besides the combination of stimulus-assignment criterion with apparatus bias, we suggest that at least other 2 variables are modulating CPA-CPP determination. The first one is rat strain, as Lister rats seem to be less affected by single housing, and group-housed Wistar rats could be more sensitive than group-housed Sprague Dawley rats to the aversive effects of higher alcohol doses/concentrations (ip). The other and most important is the weight/age of the animals at the beginning of the PC protocol, as most animals used in studies that resulted in CPA had heavier arrival weights and increases in the age-weight covariate cause a series of changes (less efficient alcohol distribution, greater blood alcohol concentrations, greater activity of hepatic alcohol dehydrogenase, and greater acetaldehyde accumulation in the blood) that favor the rat sensitivity to alcohol aversive effects (see **Table S6**).

As for long PC protocols without APE and protocols with APE, due to space constraints, **Appendix S9** presents the analysis of the CPP-CPA dichotomy. Briefly, it shows that most of these protocols present the same association of housing systems with CPA and CPP we found for short ones. Therefore, in their current use, they are not long enough to cause tolerance to alcohol's aversive effects in single-housed or older/heavier male albino rats.

2.4.3 Limitations

This work presents two general classes of limitations: those posed by the selected literature and those proper to the theoretical analysis done. In the first case, they are (a) the characteristics of preference measurement and testing, (b) the consistency of data reported, and (c) the risk of report bias. **Appendix S3** discusses preference measurement and testing,

which imply the most serious limitations: (i) the prevalence of different measurement methods, “preference indices”, and experimental designs in the literature, (ii) lack of trustful agreement comparisons between methods and instruments measuring the principal behavioral unit, (iii) the impossibility of estimating error between different “preference indices” for most experiments selected, (iv) the impossibility of estimating effect sizes to the same raw-score metric, and (v) the consequential limitation of using a categorical frequentist distribution analysis of reported PC outcomes. **Appendices S10** and **S11** discuss the most serious cases of lack of consistency in data reported, and section 1 of **Appendix S12** describes the findings of report bias risk: a median risk prevalence in the whole literature and its worrisome increase for the 2010s. In turn, the theoretical limitation is discussed in section 2 of **Appendix S12**.

2.5 CONCLUSIONS

Over the 4 last decades, several explanations have been proposed for the low reproducibility of results in alcohol-induced PC interventions with rats. Although many of these considerations certainly have an explaining role for individual results of these interventions, their predictive power is not enough to organize the outcome patterns present in this literature. In contrast, the results of alcohol-induced PC with male outbred rats may be organized by the interaction of 4 general groups of variables: those predicting conditioned control acquisition in associative learning processes, those predicting interference of exploratory behavior on conditioned control expression according to the general PC literature, drug agent factors predicting pharmacokinetic and pharmacodynamic properties of alcohol, and animal and environmental factors modifying these properties of alcohol. Accordingly, conditioning failure and success are better predicted by the set of the 3 first classes of variables, while CPA and CPP are better predicted by the last 2.

In short PC protocols, alcohol dose (motivator magnitude) seems to be the most reliable predictor of conditioning failure and success, with doses lower than 1.0 g/kg accumulating most of the failures reported in the reviewed literature. Thus, these doses do not have an optimal effect on conditioned control for short protocols, making such control more susceptible to variables disrupting its acquisition (e.g., latent inhibition and unforeseen interactions of stimuli in compound contexts) and expression (e.g., competing exploratory behavior derived from the spatial organization of the apparatus and differential exposure to conditioning contexts). Then, for more effective conditioned control and a countereffect on these disruptions, a greater contact history with alcohol is necessary for these doses. This is

shown in long PC protocols and for the specific method of APE developed by Bieńkowski et al. (1995) and later adapted by others (see **Appendix S8**).

Conversely, while drug factors must be considered for the lower and upper marginal prediction of CPA and CPP in short protocols, these results are better predicted by conditions and characteristics of outbred rats used. For albino rats, single housing is associated with greater frequency of CPA and group housing is associated with greater frequency of CPP, both in the range of 1-2 g/kg (10-20% v/v), regardless of the PC protocol being short, long, or with APE (see **Appendix S9**). Moreover, when one considers the arrival weights of the animals, there is some evidence in the selected literature that adult animals tend to be more sensitive to the presumable effects of single housing (or to the aversive effects of alcohol) than adolescent rats in alcohol-induced PC. This is supported by the extensive literature on changes in alcohol metabolism and effects in rats according to changes in the age-weight covariate (see **Table S6**).

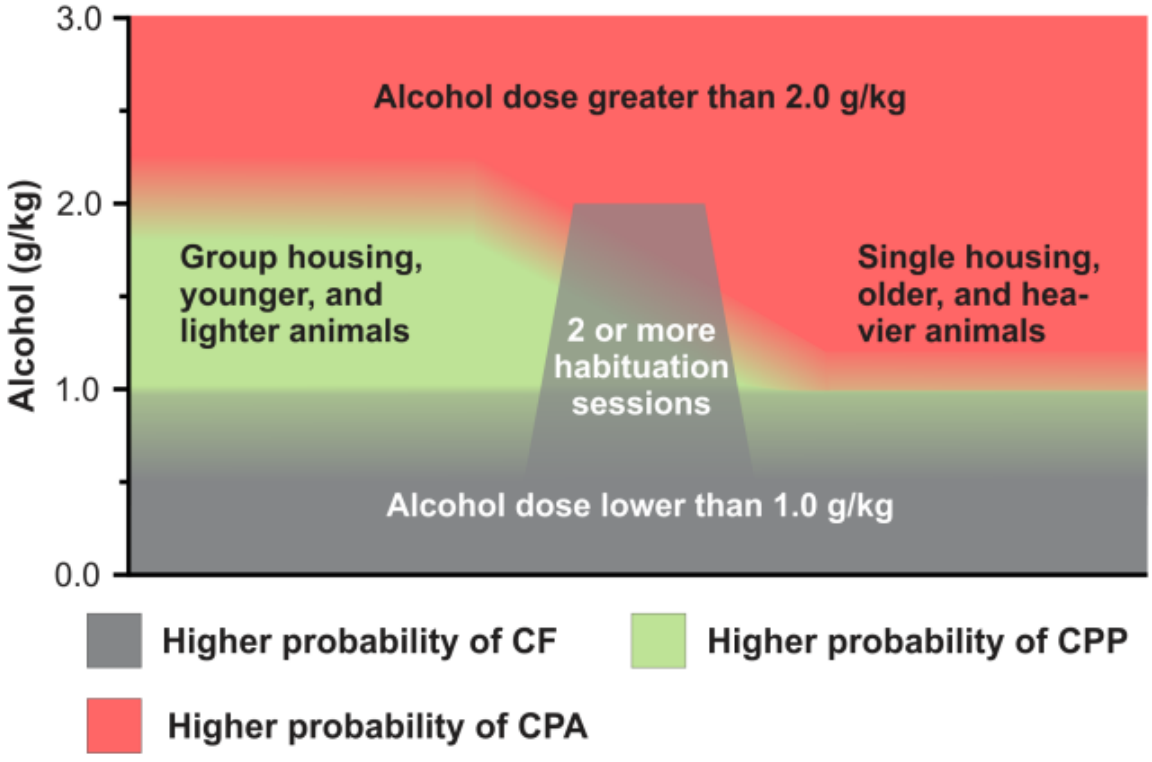


FIGURE 2.5 – Distribution of expected result probabilities of alcohol-induced short PC protocols in outbred male albino rats, according to changes in alcohol dose, number of habituation sessions, housing system, and age and weight of animals. Detailed characteristics of animals, procedures, and apparatuses are given in the text.

Accordingly, **Figure 2.5** illustrates the expected outcome probability of short PC protocols in which animals, procedures, and apparatuses have the following characteristics: male albino rats of outbred strains subjected to 4 to 10 conditioning trials, trials evenly distributed for the

alcohol-paired and -unpaired contexts, contexts formed by tactile and/or visual cues, conducted in an apparatus with 1 to 3 chambers and area up to 3000 cm2, with an alcoholic solution concentration of 10-20% (v/v) administered (ip) in an ISI of 0 min. As shown, according to changes in alcohol dose, number of habituation sessions, housing system, and, most likely, in the age/weight of the animals, the probabilities of conditioning failure, CPP, and CPA are expected to change dramatically in such short PC protocols.

Therefore, for CPP acquisition and expression in the aforesaid short protocols, the following recommendations are given: use of group-housed adolescent rats (40-60 days old) or rats weighting 150-250 g at the beginning of the PC protocol; preferable use of alcohol doses between 1 and 2 g/kg, decreasing the solution concentration (%, v/v) for higher doses (1.5 at 12.5-17.5%; 2 g/kg at 12.5-15%); for doses of 0.5-0.9 g/kg, one should use a concentration of no less than 15% and no less than 8-10 conditioning trials (preferably, use 12 or more trials); avoid using more than 1 habituation session (avoid using any for doses of 0.5-0.9 g/kg in short protocols); avoid using the combination of a biased apparatus with the stimulus-assignment criterion of the baseline-preferred context (if possible, use an unbiased apparatus with the balanced stimulus-assignment: for reasons and definitions, see **Appendices S3** and **S10**); preferable use of one- or two-chamber apparatuses for doses of 0.5-0.9 g/kg in short protocols.

Alongside these practical recommendations, the predictive organization also has theoretical, instrumental, and translational consequences for the conduction, analysis, and use of alcohol-induced PC with rats in alcohol research. Some are discussed in **Table S8**. In all, these implications indicate that alcohol-induced PC with outbred rats is a valuable tool for solving theoretical impasses proper of PC, generating new questions for and instrumentalizations of PC in alcohol research, as well as for understanding different vulnerabilities, developments, phenotypes, and the neurobiology of alcohol use disorders.

Given the relevance of these implications, it is important to highlight that predictive analysis provides only circumstantial evidence and, as so, is vulnerable to spurious associations. While for some variables (e.g., latent inhibition, motivator magnitude, and number of conditioning trials) this causes minor concerns, this is not the case for others, such as housing system: as the general evidence of stress effects on alcohol-related variables is equivocal (see **Table S6**), the detected association may be a fortuitous consequence of younger-lighter and older-heavier animals being majorly group- and single-housed, respectively (see spreadsheet 05 of **Appendix S13**), a possibility supported by Philpot et al. (2003) and Andaloussi et al. (2021). Moreover, this review does not reach other variables that

previous studies point as relevant for alcohol effects, because these variables were either erratically reported in the selected literature or excluded during the selection process due to methodological constraints.

Then, for all these variables more investigation is needed for confirming and identifying their effects on alcohol-induced PC. These are the differences between males and females (a point not evaluated in this review), circadian modulation, effects of different spatial configurations of PC apparatuses on exploratory behavior, the presumable differences between outbred strains, especially between pigmented and albino ones, and, particularly, the relations of housing system and animals' age-weight with alcohol pharmacokinetics and pharmacodynamics.

Simultaneously with the necessary improvement in experimental design and report quality (we recommend researchers follow the ARRIVE guidelines: Kilkenny et al., 2010; Percie du Sert et al., 2000) and the solution of problems pointed in **Appendix S3**, the testing of these variables may not just remedy and unveil the "nuisance" that alcohol-induced PC with rats is generally considered to be. Most importantly, it may lead us to better understand the motivator function of alcohol, the alcohol-seeking behavior triggered by environmental contexts, and their neurobiological basis.

FUNDING SOURCE: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil (CNPq) – Grant Numbers "454963/2014-9", "131694/2015-4", and "14905/2019-0".

CONFLICT OF INTEREST: The authors declare they have no conflict of interest.

SUPPORTING INFORMATION: The online version contains supplementary material.

ORCID

Douglas Marques https://orcid.org/0000-0001-5571-4172
Piter Kehoma Boll https://orcid.org/0000-0002-3029-0841
Rosane Gomez https://orcid.org/0000-0002-0868-8394
Eliane Dallegrave https://orcid.org/0000-0001-6586-2080
José Inácio Lemos https://orcid.org/0000-0001-7872-4920
Mirna Bainy-Leal https://orcid.org/0000-0001-7205-6669

REFERENCES

Andaloussi, Z.I.L., Lauer, W., Zulu, S.S., Taghzouti, K. & Abboussi, O. (2021) Acute cannabidiol treatment attenuates ethanol-induced place preference and reduces agressivity in group-housed male rats. *Pharmacology, Biochemistry, and Behavior*, 211, 173290. Available from: https://doi.org/10.1016/j.pbb.2021.173290

Anton, R.F. (1999) What is craving? *Alcohol Research & Health*, 2, 165–173.

Asin, K.E., Wirtshafter, D. & Tabakoff, B. (1985) Failure to establish a conditioned place preference with ethanol in rats. *Pharmacology, Biochemistry, and Behavior*, 22, 169–173. Available from: https://doi.org/10.1016/0091-3057(85)90372-7

Ayres, J.J.B., Philbin, D., Cassidy, S. & Belling, L. (1992) Some parameters of latent inhibition. *Learning and Motivation*, 23, 269–287. Available from: https://doi.org/10.1016/0023-9690(92)90009-B

Azrin, N.H. & Holz, W.C. (1966) Punishment. In: Honig, W.K. (Ed.) *Operant behavior: areas of research and application*. New York: AppletonCentury-Crofts, pp. 380–447.

Bagrov, Y.Y., Dmitrieva, N.I., Manusova, N.B., Zvartau, E.E., Patkina, N.A. & Bagrov, A.Y. (1999) Involvement of endogenous digitalislike factors in voluntary selection of alcohol by rats. *Life Sciences*, 64, PL219–PL225. Available from: https://doi.org/10.1016/s0024-3205(99)00131-9

Bardo, M.T. & Bevins, R.A. (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology*, 153, 31–43. Available from: https://doi.org/10.1007/s002130000569

Bardo, M.T., Rowlett, J.K. & Harris, M.J. (1995) Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neuroscience and Biobehavioral Reviews*, 19, 39–51. Available from: https://doi.org/10.1016/0149-7634(94)00021-r

Becker, A., Schmitz, M. & Grecksch, G. (2006) Kindling modifies morphine, cocaine and ethanol place preference. *Experimental Brain Research*, 168, 33–40. Available from: https://doi.org/10.1007/ s00221005-0081-3

Becker, H.C., Lopez, M.F. & Doremus-Fitzwater, T. (2011) Effects of stress on alcohol drinking: a review of animal studies. *Psychopharmacology*, 218, 131–156. Available from: https://doi.org/10.1007/s0022 1005-0081-3

Bie, B., Zhu, W. & Pan, Z.Z. (2009) Ethanol-induced delta-opioid receptor modulation of glutamate synaptic transmission and conditioned place preference in central amygdala. *Neuroscience*, 160, 348–358. Available from: https://doi.org/10.1016/j.neuroscience.2009.02.049

Bieńkowiski, P., Iwinska, K., Piasecki, J. & Kostowski, W. (1997) 5,7-dihydroxytryptamine lesion does not affect ethanol-induced conditioned taste and place aversion in rats. *Alcohol*, 14, 439– 443. Available from: https://doi.org/10.1016/s0741-8329(96) 00191-7

Bieńkowski, P., Kuca, P. & Kostowski, W. (1995) Conditioned place preference after prolonged pre-exposure to ethanol. *Polish Journal of Pharmacology*, 47, 189–191.

Bieńkowski, P., Kuca, P., Piasecki, J. & Kostowski, W. (1996) Low dose of ethanol induces conditioned place preference in rats after repeated exposures to ethanol or saline injections. *Alcohol and Alcoholism*, 31, 547–553. Available from: https://doi.org/10.1093/oxfordjour nals.alcalc.a008190

Bieńkowski, P., Kuca, P., Piasecki, J. & Kostowski, W. (1997) 5-HT3 receptor antagonist, tropisetron, does not influence ethanol-induced conditioned taste aversion and conditioned place aversion. *Alcohol*, 14, 63–69. Available from: https://doi.org/10.1016/s0741 -8329(96)00108-5

Binder, M.D., Hirokawa, N. & Windhorst, U. (2008) Aversive stimulus. In: Binder, M.D., Hirokawa, N. & Windhorst, U. (Eds.) *Encyclopedia of neuroscience*. Berlin-Heidelberg: Springer, p. 296.

Black, R.W., Albiniak, T., Davis, M. & Schumpert, J. (1973) A preference in rats for cues associated with intoxication. *Bulletin of the Psychonomic Society*, 2, 423–424. Available from: https://doi.org/10.3758/BF03334436

Bloom, F., Lad, P., Pittiman, Q. & Rogers, G. (1982) Blood alcohol levels in rats: non-uniform yields from intraperitoneal doses based on body weight. *British Journal of*

Pharmacology, 75, 251–254. Available from: https://doi.org/10.1111/j.1476-5381.1982.tb087 80.x

Bode, J.C. (1980) Alcohol and the gastrointestinal tract. In: Frick, P., von Harnack, G.-A., Martini, G.A. & Prader, A. (Eds.) *Advances in internal medicine and pediatrics*, Vol. 45. New York: Springer-Verlag, pp. 1–75.

Bormann, N.M. & Cunningham, C.L. (1998) Ethanol-induced conditioned place aversion in rats: effects of interstimulus interval. *Pharmacology, Biochemistry, and Behavior*, 59, 427–432. Available from: https://doi.org/10.1016/S0091-3057(97)00455-3

Bozarth, M.A. (1990) Evidence for the rewarding effects of ethanol using the conditioned place preference method. *Pharmacology, Biochemistry, and Behavior*, 35, 485–487. Available from: https://doi.org/10.1016/0091-3057(90)90191-j

Campos-Jurado, Y., Martí-Prats, M.J.A., Polache, A., Granero, L. & Hipólito, L. (2020) Dose-dependent induction of CPP or CPA by intra-pVTA ethanol: role of mu opioid receptors and effects of NMDA receptors. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 100, 109875. Available from: https://doi.org/10.1016/j.pnpbp.2020.109875

Carr, G.D., Fibiger, H.C. & Phillips, A.G. (1989) Conditioned place preference as a measure of drug reward. In: Liebman, J.M. & Cooper, S.J. (Eds.) *Topics in experimental psychopharmacology: Vol. 1. The Neuropharmacological basis of reward*. Oxford: Clarendon Press/ Oxford University Press, pp. 264–319.

Carr, G.D., Phillips, A.G. & Fibiger, H.C. (1988) Independency of amphetamine reward from locomotor stimulation demonstrated by conditioned place preference. *Psychopharmacology*, 94, 221–226. Available from: https://doi.org/10.1007/BF00176849

Carvalho, A.F., Heiling, M., Perez, A., Probst, C. & Rehm, J. (2019) Alcohol use disorders. *Lancet*, 394, 781–792. Available from: https://doi. org/10.1016/S0140-6736(19)31775-1

Colaizzi, J.M., Flagel, S.L., Joyner, M.A., Gearhardt, A.N., Stewart, J.L. & Paulus, M.P. (2020) Mapping sign-tracking and goal-tracking onto human behaviors. *Neuroscience and Biobehavioral Reviews*, 111, 84–94. Available from: https://doi.org/10.1016/j.neubiorev.2020.01.018

Cole, J.C., Sumnall, H.R., O'Shea, E. & Marsden, C.A. (2003) Effects of MDNA exposure on the conditioned place preference produced by other drugs of abuse. *Psychopharmacology (Berlin)*, 166, 383–390. Available from: https://doi.org/10.1007/s00213-002-1374-x

Crabb, D.W., Bosron, W.F. & Li, T.-K. (1987) Ethanol metabolism. *Pharmacology & Therapeutics*, 34, 59–73. Available from: https:// doi.org/10.1016/0163-7258(87)90092-1

Crabb, D.W., Morzorati, S.L., Simon, J.R. & Li, T.K. (1985) Central nervous system control of alcohol dehydrogenase activity in rats. *Life Sciences*, 37, 2381–2387. Available from: https://doi.org/10.1016/0024-3205(85)90105-5

Creswell, K.G. & Sayette, M.A. (2022) How laboratory studies of cigarette craving can inform the experimental alcohol craving literature. *Alcoholism, Clinical and Experimental Research*, 46, 344–358. Available from: https://doi.org/10.1111/acer.14773

Cunningham, C.L. (1979) Flavor and location aversions produced by ethanol. *Behavioral and Neural Biology*, 27, 362–367. Available from: https://doi.org/10.1016/S0163-1047(79)92440-3

Cunningham, C.L. (1993) Pavlovian drug conditioning. In: van Haaren, F. (Ed.) *Techniques in the behavioral and neural sciences: Vol. 10. Methods in behavioral pharmacology*. Amsterdan: Elsevier, pp. 349–381.

Cunningham, C.L., Gremel, C.M. & Groblewski, P.A. (2006) Druginduced conditioned place preference and aversion in mice. *Nature Protocols*, 1, 1662–1670. Available from: https://doi.org/10.1038/nprot.2006.279

Cunningham, C.L., Groblewski, P.A. & Voorhees, C.M. (2011) Place conditioning. In: Olmstead, M.C. (Ed.) *Neuromethods: Vol. 53. Animal models of drug addiction*. New York: Springer, pp. 167–189.

Cunningham, C.L. & Niehus, J.S. (1993) Drug-induced hypothermia and conditioned place aversion. *Behavioral Neuroscience*, 107, 468–479. Available from: https://doi.org/10.1037/073 5-7044.107.3.468

Cunningham, C.L., Niehus, J.S. & Noble, D. (1993) Species difference in sensitivity to ethanol's hedonic effects. *Alcohol*, 10, 97–102. Available from: https://doi.org/10.1016/0741-8329(93)90087-5

Davis, C.M. (2013) Animal models of drug abuse: place and taste conditioning. In: Conn, P.M. (Ed.) *Animal models for the study of human disease*. Boston: Academic Press, pp. 681–707.

Davis, C.M. & Riley, A.L. (2010) Conditioned taste aversion learning: implications for animal models of drug abuse. *Annals of the New York Academy of Sciences*, 1187, 247–275. Available from: https://doi.org/10.1111/j.1749-6632.2009.05147.x

Der-Avakian, A., Bland, S.T., Rozeske, R.R., Tamblyn, J.P., Hutchinson, M.R., Watkins, L.R. et al. (2007) The effects of a single exposure to uncontrollable stress on the subsequent conditioned place preference responses to oxycodone, cocaine, and ethanol in rats. *Psychopharmacology*, 191, 909–917. Available from: https://doi.org/10.1007/s00213-006-0678-7

Ding, Z.-M., Rodd, Z.A., Engleman, E.A. & McBride, W.J. (2009) Sensitization of ventral tegmental area dopamine neurons to the stimulating effects of ethanol. *Alcoholism, Clinical and Experimental Research*, 33, 1571–1581. Available from: https://doi.org/10.1111/j.1530-0277.2009.00985.x

Edenberg, H.J. (2007) The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol Research & Health*, 30, 5–13.

Fidler, T.L., Bakner, L. & Cunningham, C.L. (2004) Conditioned place aversion induced by intragastric administration of ethanol in rats. *Pharmacology, Biochemistry, and Behavior*, 77, 731–743. Available from: https://doi.org/10.1016/j.pbb.2004.01.010

Gottlieb, D.A. & Begej, E.L. (2014) Principles of Pavlovian conditioning: description, content, function. In: McSweeney, F.K. & Murphy, E.S. (Eds.) *The Wiley Blackwell Handbook of operant and classical conditioning*. Oxford: John Wiley & Sons, pp. 1–25.

Grahame, N.J. (2013) Mice and alcohol. In: Miller, P.M. (Ed.) *Comprehensive addictive behaviors and disorders: Vol. 2. Biological research on addiction*. Boston: Academic Press, pp. 11–19.

Horton, A.A. & Mills, D.J. (1979) Developmental patterns of alcohol dehydrogenase and aldehyde dehydrogenases in homogenates and subcellular fractions of rat liver. *Mechanisms of Aging and Development*, 11, 363–370. Available from: https://doi.org/10.1016/0047-6374(79)90011-3

Hurley, T.D. & Edenberg, H.J. (2012) Genes encoding enzymes involved in ethanol metabolism. *Alcohol Research: Current Reviews*, 34, 339–344.

Ise, Y., Mori, T., Katayama, S., Nagase, H. & Suzuki, T. (2013) Rewarding effects of ethanol combined with low doses of morphine through dopamine D1 receptors. *Journal of Nippon Medical School*, 80, 34– 41. Available from: https://doi.org/10.1272/jnms.80.34

Kalant, H. (1996) Pharmacokinetics of ethanol: absorption, distribution, and elimination. In: Begleiter, H. & Kissin, B. (Eds.) *The pharmacology of alcohol and alcohol dependence*. New York: Oxford University Press, pp. 15–58.

Kalant, H. (1971) Absorption, diffusion, distribution, and elimination of ethanol: effects on biological membranes. In: Kissin, B. & Begleiter, H. (Eds.) *The biology of alcoholism: Vol. 1. Biochemistry*. Boston: Springer, pp. 1–62.

Khamis, H.J. (2005) Measures of association. In: Armitage, P. & Colton, T. (Eds.) *Encyclopedia of biostatistics*. Hoboken: Wiley & Sons, pp. 1–6.

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. & Altman, D.G. (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biology*, 8, e1000412. Available from: https://doi.org/10.1371/journal.pbio.1000412

Koob, G.F. (2000) Animal models of craving for ethanol. *Addiction*, 95, S73–S81. Available from: https://doi.org/10.1080/0965214005 0111663

Lad, P., Shoemaker, W.J. & Leffert, H.L. (1984) Developmental changes in rat liver alcohol dehydrogenase. *Developmental Biology*, 105, 526– 529. Available from: https://doi.org/10.1016/0012-1606(84)90310 -5

Lantz, A.E. (1973) Effect of number of trials, interstimulus interval, and dishabituation on subsequent conditioning in a CER paradigm. *Animal Learning & Behavior*, 1, 273–277. Available from: https://doi.org/10.3758/BF03199250

López, M. & Cantora, R. (2010) Opposite effects of ethanol on taste and place conditioning in rats. *Revista Iberoamericana de Psicologia y Salud*, 1, 207–221.

Lubow, R.E. (1989) *Problems in the behavioural sciences: Vol. 9. Latent inhibition and conditioned attention theory*. Cambridge: Cambridge University Press.

Marglin, S.H., MacKechnie, D.K., Mattie, M.E., Hui, Y. & Reid, L.D. (1988) Ethanol with small doses of morphine establishes a conditioned place preference. *Alcohol*, 5, 309–313. Available from: https://doi.org/10.1016/0741-8329(88)90071-7

McKenzie, J.E., Brennan, S.E., Ryan, R.E., Thomson, H.J. & Johnston, R.V. (2019) Summarizing study characteristics and preparing for synthesis. In: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J. et al. (Eds.) *Cochrane handbook for systematic reviews of interventions*. The Cochrane Collaboration/Wiley Blackwell: Hoboken, pp. 229–240.

Mellgren, R.L. & Ost, J.W.P. (1971) Discriminative stimulus preexposure and learning of an operant discrimination in the rat. *Journal of Comparative and Physiological Psychology*, 77, 179–187. Available from: https://doi.org/10.1037/h0031663

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G. & The PRISMA Group. (2009) Preferred reporting items for systematic reviews and metaanalyses: the PRISMA statement. *PLoS Medicine*, 6, e1000097. Available from: https://doi.org/10.1371/journal.pmed.1000097

Morales, M., Varlinskaya, E.I. & Spear, L.P. (2012) Evidence for conditioned place preference to a moderate dose of ethanol in adult male Sprague-Dawley rats. *Alcohol*, 46, 643–648. Available from: https://doi.org/10.1016/j.alcohol.2012.06.001

Morris, R.W. & Bouton, M.E. (2006) Effects of unconditioned stimulus magnitude on the emergence of conditioned responding. *Journal of Experimental Psychology: Animal Behavior Processes*, 32, 371–385. Available from: https://doi.org/10.1037/0097-7403.32.4.371

Mucha, R.F. & Iversen, S.D. (1984) Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: a procedural examination. *Psychopharmacology*, 82, 241–247. Available from: https://doi.org/10.1007/BF00427782

Murphy, E.S. & Lupfer, G.J. (2014) Basic principles of operant conditioning. In: McSweeney, F.K. & Murphy, E.S. (Eds.) *The Wiley Blackwell Handbook of operant and classical conditioning*. Oxford: John Wiley & Sons, pp. 167–194.

Naassila, M. (2013) Alcohol and rats. In: Miller, P.M. (Ed.) *Comprehensive addictive behaviors and disorders: Vol. 2. Biological research on addiction*. Boston: Academic Press, pp. 21–30.

Noori, H.R., Helisnki, S. & Spanagel, R. (2014) Cluster and meta-analyses on factors influencing stress-induced alcohol drinking and relapse in rodents. *Addiction Biology*, 19, 225–232. Available from: https://doi.org/10.1111/adb.12125

Nutt, D.J., King, L.A. & Phillips, L.D. (2010) Drug harm in the UK: a multicriteria decision analysis. *Lancet*, 376, 1558–1565. Available from: https://doi.org/10.1016/S0140-6736(10)61462-6

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D. et al. (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, 372, n71. Available from: https://doi.org/10.1136/bmj.n71

Pascual, M., Do Couto, B.R., Alfonso-Loeches, S., Aguilar, M.A., Rodriguez-Arias, M. & Guerri, C. (2012) Changes in histone acetylation in the prefrontal cortex of ethanol-exposed adolescent rats are associated with ethanol-induced place conditioning. *Neuropharmacology*, 62, 2309–2319. Available from: https://doi.org/10.1016/j.neuropharm.2012.01.011

Pati, D., Pina, M.M. & Kash, T.L. (2019) Ethanol-induced conditioned place preference and aversion differentially alter plasticity in the bed nucleus of the stria terminalis. *Neuropsychopharmacology*, 44, 1843–1854. Available from: https://doi.org/10.1038/s4138 6-019-0349-0

Patkina, N.A. & Zvartau, E.E. (1998) Caffeine place conditioning in rats: comparison with cocaine and ethanol. *European Neuropsychopharmacology*, 8, 287–291. Available from: https://doi.org/10.1016/s0924-977x(97)00086-2

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M. et al. (2020) The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biology*, 18, e3000410. Available from: https://doi.org/10.1371/journal.pbio.3000410

Philpot, R.M., Badanich, K.A. & Kirstein, C.L. (2003) Place conditioning: age-related changes in the rewarding and aversive effects of alcohol. *Alcoholism, Clinical and Experimental Research*, 27, 593– 599. Available from: https://doi.org/10.1097/01.ALC.00000 60530.71596.D1

Pinkston, J.N. & Soliman, K.F.A. (1979) Effect of light and fasting on the circadian variation of ethanol metabolism in the rat. *Journal of Interdisciplinary Cycle Research*, 10, 185–193. Available from: https://doi.org/10.1080/09291017909359669

Pohorecky, L.A. & Brick, J. (1988) Pharmacology of ethanol. *Pharmacology & Therapeutics*, 36, 335–427. Quertemont, E. & De Witte, P. (2001) Conditioned stimulus preference after acetaldehyde but not ethanol injections. *Pharmacology, Biochemistry, and Behavior*, 68, 449–454. Available from: https://doi.org/10.1016/s0091-3057(00)00486-x

Quertemont, E., Goffaux, V., Vlaminck, A.-M., Wolf, C. & De Witte, P. (1998) Oral taurine supplementation modulates ethanolconditioned stimulus preference. *Alcohol*, 16, 201–206. Available from: https://doi.org/10.1016/s0741-8329(98)00002-0

Reid, L.D., Hunter, G.A., Beaman, C.M. & Hubbell, C.L. (1985) Toward understanding ethanol's capacity to be reinforcing: a conditioned place preference following injections of ethanol. *Pharmacology, Biochemistry, and Behavior*, 22, 483–487. Available from: https://doi.org/10.1016/0091-3057(85)90051-6

Rose, J., Schmidt, R., Grabemann, M. & Güntürkun, O. (2009) Theory meets pigeons: the influence of reward-magnitude on discrimination-learning. *Behavioural Brain Research*, 198, 125–129. Available from: https://doi.org/10.1016/j.bbr.2008.10.038

Schechter, M.D. (1992) Locomotor activity but not conditioned place preference is differentially affected by moderate dose of ethanol administered to P and NP rats. *Alcohol*, 9, 185–188. Available from: https://doi.org/10.1016/0741-8329(92)90050-k

Schechter, M.D. & Krimmer, E.C. (1992) Differences in response to the aversive properties and activity effects of low dose ethanol in LAS and HAS selectively bred rats. *Psychopharmacology*, 107, 564–568. Available from: https://doi.org/10.1007/BF02245271

Schechter, M.D. & Calcagnetti, D.J. (1993) Trends in place preference conditioning with a cross-indexed bibliography; 1957–1991. *Neuroscience & Biobehavioral Reviews*, 17(1), 21–41. Available from: https://doi.org/10.1016/s0149-7634(05)80228-3

Schechter, M.D. & Calcagnetti, D.J. (1998) Continued trends in the conditioned place preference literature from 1992 to 1996, inclusive, with a cross-indexed bibliography. *Neuroscience & Biobehavioral Reviews*, 22(6), 827–846. Available from: https://doi.org/10.1016/ s0149-7634(98)00012-8

Schenk, S., Ellison, F., Hunt, T. & Amit, Z. (1985) An examination of heroin conditioning in preferred and nonpreferred environments and in differentially housed mature and immature rats. *Pharmacology, Biochemistry, and Behavior*, 22, 215–220. Available from: https://doi.org/10.1016/0091-3057(85)90380-6

Schmajuk, N.A. (2002) *Latent inhibition and its neural substrates*. New York: Springer.

Shimizu, C., Oki, Y., Mitani, Y., Nakamura, T. & Nabeshima, T. (2015) Factors affecting ethanol-induced conditioned place preference and locomotor sensitization in mice. *Biological and Pharmaceutical Bulletin*, 38, 1935–1945. Available from: https://doi.org/10.1248/ bpb.b15-00626

Sliedrecht, W., de Waart, R., Witikiewitz, K. & Roozen, H.G. (2019) Alcohol use disorder relapse factors: a systematic review. *Psychiatry Research*, 278, 97–115. Available from: https://doi.org/10.1016/j.psychres.2019.05.038

Smith, M.A. (2020) Nonhuman animal models of substance use disorders: translational value and utility to basic science. *Drug and Alcohol Dependence*, 206, 107733. Available from: https://doi.org/10.1016/j.drugalcdep.2019.107733

Spanagel, R., Noori, H.R. & Heilig, M. (2014) Stress and alcohol interactions: animal studies and clinical significance. *Trends in Neurosciences*, 37, 219–227. Available from: https://doi.org/10.1016/j.tins.2014.02.006

Spyraki, C., Kazandjian, A. & Varonos, D. (1985) Diazepam-induced place preference conditioning: appetitive and antiaversive properties. *Psychopharmacology*, 87, 225–232. Available from: https://doi.org/10.1007/BF00431813

Stahlman, W.D. & Baisdell, A.P. (2011) The modulation of operant variation by the probability, magnitude, and delay of reinforcement. *Learning and Motivation*, 42, 221–236. Available from: https://doi.org/10.1016/j.lmot.2011.05.001

Stewart, R.B. & Grupp, L.A. (1981) An investigation of the interaction between the reinforcing properties of food and ethanol using the place preference paradigm. *Progress in NeuroPsychopharmacology*, 5, 609–613. Available from: https://doi.org/10.1016/0364-7722(81)90057-6

Stewart, R.B. & Grupp, L.A. (1985) Some determinants of the motivational properties of ethanol in the rat: concurrent administration of food and social stimuli. *Psychopharmacology*, 87, 43–50. Available from: https://doi.org/10.1007/BF00431776

Sturtevant, R.P. & Garber, S.L. (1980) Light-dark and feeding regimens affect circadian phasing of blood-ethanol decay rates. *Pharmacology, Biochemistry, and Behavior*, 13, 637–642. Available from: https://doi.org/10.1016/0091-3057(80)90005-2

Sturtevant, R.P. & Garber, S.L. (1981) Meal timing dominates the lighting regimen as a synchronizer of the circadian blood ethanol clearance rate rhythm in rats. *The Journal of Nutrition*, 111, 2000–2005. Available from: https://doi.org/10.1093/jn/111.11.2000

Swerdlow, N.R., Gilbert, D. & Koob, G.F. (1989) Conditioned drug effects on spatial preference: critical evaluation. In: Boulton, A.A., Baker, G.B. & Greenshaw, A.J. (Eds.) *Neuromethods: Vol. 13. Psychopharmacology*. Clifton: Humana Press, pp. 399–446.

Torres, O.V., Walker, E.M., Beas, B.S. & O'Dell, L.E. (2014) Female rats display enhanced rewarding effects of ethanol that are hormone dependent. *Alcoholism, Clinical and Experimental Research*, 38, 108– 115. Available from: https://doi.org/10.1111/acer.12213

Tzschentke, T.M. (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress, and new issues. *Progress in Neurobiology*, 56, 613–672. Available from: https://doi.org/10.1016/s0301-0082(98)00060-4

Tzschentke, T.M. (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addiction Biology*, 12, 227–462. Available from: https://doi.org/10.1111/j.1369-1600.2007.00070.x

Van der Kooy, D., O'shaughnessy, M., Mucha, R.F. & Kalant, H. (1983) Motivational properties of ethanol in naive rats as studied by place conditioning. *Pharmacology, Biochemistry, and Behavior*, 19, 441– 445. Available from: https://doi.org/10.1016/0091-3057(83)90117-x

Venniro, M., Caprioli, D. & Shaham, Y. (2018) Novel models of drug relapse and craving after voluntary abstinence. *Neuropsychopharmacology*, 44, 234–235. Available from: https://doi.org/10.1038/s41386-018-0196-4

Walker, B.M. & Ehlers, C.L. (2009) Age-related differences in the blood alcohol levels of Wistar rats. *Pharmacology, Biochemistry, and Behavior*, 91, 560–565. Available from: https://doi.org/10.1016/j.pbb.2008.09.017

Walsh, R.N. & Cummins, R.A. (1976) The open-field test: a critical review. *Psychological Bulletin*, 83, 482–504. Available from: https://doi.org/ 10.1037/0033-2909.83.3.482

Webber, E.S., Chambers, N.E., Kostek, J.A., Mankin, D.E. & Cromwell, H.C. (2015) Relative reward effects on operant behavior: incentive contrast, induction and variety effects. *Behavioural Processes*, 116, 87–99. Available from: https://doi.org/10.1016/j.beproc.2015.05.003

WHO. (2018) *Global status report on alcohol and health 2018*. Geneva: World Health Organization.

Xu, S. & Kang, U.G. (2017) Characteristics of ethanol-induced behavioral sensitization in rats: molecular mediators and cross-sensitization between ethanol and cocaine. *Pharmacology, Biochemistry, and Behavior*, 160, 47–54. Available from: https://doi.org/10.1016/j.pbb.2017.08.001

Zhu, W., Bie, B. & Pan, Z.Z. (2007) Involvement of non-NMDA glutamate receptors in central amygdala in synaptic actions of ethanol and ethanol-induced reward behavior. *The Journal of Neuroscience*, 27, 289–298. Available from: https://doi.org/10.1523/JNEUROSCI.3912-06.2007

3 CAPÍTULO 2:

LETTER TO THE EDITOR: A REPLY TO GRAHAME (2023) COMMENTING ON "ALCOHOL, PLACE CONDITIONING, AND MALE RATS: A SYSTEMATIC REVIEW OF OUTCOME PREDICTION"

Douglas Marques[1]*, Piter Kehoma Boll[2], Rosane Gomez[1], Eliane Dallegrave[3], José Inácio Lemos[4], Mirna Bainy-Leal[1]

[1] Programa de Pós-Graduação em Ciências Biológicas: Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Sarmento Leite, 500, prédio 12101/209, 90046-900 Porto Alegre, Rio Grande do Sul, Brazil. djeser.dm@gmail.com, rosane.gomez@ufrgs.br, mirnabl@gmail.com.

[2] Programa de Pós-Graduação em Biologia, Universidade do Vale do Rio dos Sinos (UNISINOS), Avenida Unisinos, 950, 93022-000 São Leopoldo, Brazil. piterkeo@gmail.com.

[3] Departamento de Farmacociências, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Rua Sarmento Leite, 245, 90050-170 Porto Alegre, Brazil. elianedal@ufcspa.edu.br.

[4] Laboratório de Bioquímica, Fisiologia e Farmacologia, Faculdade de Medicina Veterinária, Instituto de Medicina Veterinária, Campus Universitário de Castanhal, Universidade Federal do Pará (UFPA), BR 316 (km 61), 68741-740 Castanhal, Brazil. jlemos@ufpa.br.

*Corresponding author
Douglas Marques
Universidade Federal do Rio Grande do Sul
Rua Sarmento Leite, 500, prédio 12101/209, 90046-900 Porto Alegre, Brazil
E-mail: djeser.dm@gmail.com.

Although we appreciate the commentary (Grahame, 2023) on our review (Marques et al., 2023), it surprised us because we did not expect a discussion stating that our work fails to provide predictive generalizations for humans. Reflecting on this, perhaps we did not emphasize enough the limitations in the literature of alcohol place conditioning (PC) for the fulfillment of this goal. So, to bluntly present our perspective, while we do believe that preclinical research on alcohol PC can generate relevant insights for clinical researchers, we do not think the current literature consistently generalizes to alcohol use disorder (AUD). Although this is the primary objective of any preclinical model of AUD, for it to happen in alcohol PC, we need research that is better theorized, planned, executed, and reported.

For preclinical models, external validity refers to making reliable predictive generalizations to humans based on the experimental study of a model system. As indicated by Grahame (2023), there may be a tension between internal and external validity in many research contexts. However, this is not the case for preclinical models because their sole intrinsically valid justification is to reliably investigate a human-relevant causal relationship (Reiss, 2019). Consequently, the confidence of generalizations provided by a preclinical model hinges on the formal validities that experimental studies must have — namely internal, statistical-conclusion, and operational validities (Shadish et al., 2002).

Moreover, drug PC is a particular preclinical model: It instrumentalizes basic findings on associative learning with a model organism under a drug condition to study learned aspects of drug use disorders. Therefore, deriving suitable generalizations requires not only evaluating the literature for the formal validities or the adequacy of the model organism in the drug condition (Table S6 provides the model organism evaluation in our review; Grahame cited Table S6 as Table 1), it also involves evaluating the model in a fit-for-purpose manner, and here are essential (a) the model–target adequacy of the instrumentalized behavioral process (i.e., the construct, face, and predictive validities of the behavioral model) and (b) the theoretical prediction and experimental reproducibility of outcomes from simple interventions in the model.

Considering all of this, in the first paragraphs of our review, we did not make a justification for alcohol PC. After indicating the relevant learned aspect of AUD to be modeled, we defined alcohol PC in a fit-for-purpose manner: to "(…) study the development of alcohol-seeking and -craving behaviors triggered by cues, their repercussions over alcohol consumption, their genetic and neurobiological bases, and to test possible treatments" (Marques et al., 2023). From this definition, one can derive the use of PC to indirectly study alcohol's primarily rewarding or aversive effects that Grahame (2023) alluded to, and we cited

in the second paragraph of our review. In any case, whether by our definition or its derivation, alcohol PC faces validation challenges for the study of AUD in both formal and fit-for-purpose validities.

Regarding the adequacy of the behavioral process, German and Fields' (2007) elegant analysis suggests sign-tracking behavior as the learning basis of preclinical drug PC in short protocols (≤10 conditioning trials). While linked to drug abuse/addiction vulnerability (Collaizzi et al., 2020), sign-tracking may not be a valid construct for cue-induced drug-seeking and -craving behaviors in humans as it only makes animals approach the conditioned stimulus, not the unconditioned one (alcohol; see Burns & Domjan, 1996). Thus, given the "liking" and "wanting" motivations in drug use, their respective predominancies in non-problematic and problematic drug use, and the "wanting" motivation basis and goal-tracking features of drug-craving/seeking behaviors, sign-tracking seems more related to the "liking" motivation (Burns & Domjan, 1996; Childress et al., 1999; Meyer et al., 2023; Robinson & Berridge, 1993). This suggests that current research on alcohol PC is mostly modeling non-problematic alcohol use.

Accordingly, evidence coming from our review indicates the preclinical literature is face-valid for modeling non-problematic to mildly problematic alcohol use. This is shown by (a1) the high prevalence of conditioning failure for doses <1.0 g/kg in short PC protocols, (a2) the evident sensitization for these doses in long protocols and in one short protocol with alcohol preexposure, (b1) the high rate of conditioned place aversion (CPA) in short protocols for doses of 1.0–2.0 g/kg, and (b2) the marginal evidence for tolerance to the aversive effects of these doses in long protocols and those with alcohol preexposure. Likewise, although mice seem better to model a different phenotype in human alcohol use, and we need a systematic review with this rodent in alcohol PC, this conclusion is also pertinent for this species (see items 5 and 6 of Table S8 in our review; Grahame cited Table S8 as Table 6).

In agreement, studies on alcohol PC with humans show that preclinical ones are valid for conditioning the "liking" motivation (Childs & de Wit, 2016). Moreover, if it is true that proximal and contextual cues of alcohol do not differ in their craving-inducing capacities (Heck et al., 2023), the findings of Field and Duka (2002) and Lutz and Childs (2021) also indicate that short "Pavlovian"/PC protocols used with humans (and rodents) are sufficient for cues to induce conditioned "liking" and may even potentiate seeking/craving behaviors induced by alcohol itself, but not for conditioning "wanting" (as a side note, for individuals without AUD, this potentiation is probably relevant for the "night of debauchery" cited by Grahame). Meanwhile, we know of no preclinical experiment showing that, for example, the

conditioned place preference (CPP) outcome is predictive-valid for cue-induced alcohol-seeking and -craving behaviors.

Additionally, the indirect study of the reinforcing effects of alcohol, as advocated in the literature, assumes a valence parity between unconditioned and conditioned motivation (the primary reward will instate CPP and not CPA). This parity assumption has not received experimental attention in preclinical drug PC (at least, we know none). Meanwhile, studies of alcohol PC with nondependent drinkers favor the existence of such a parity (see Childs & de Wit, 2016; Lutz & Childs, 2021), but the study of alcohol cues in individuals with AUD indicates a valence change (from rewarding to aversive) in the conditioned "liking" motivation: Alcohol cues simultaneously elicit alcohol craving ("wanting" motivation) and anxiety ("liking" motivation) in these individuals (Fox et al., 2007; Sinha et al., 2009; see also Townshend & Duka, 2007). For humans, this suggests the parity in the "liking" motivation is valid only for nonproblematic to mildly problematic alcohol use. For rodents, we do not know.

Therefore, while the preclinical literature may help us understand how problematic alcohol use starts (see Table S8 in our review), it adds very little to the understanding of the reinforcing properties of alcohol in AUD. After all, we are primarily concerned with the environmental and biological factors allowing, accompanying, and leading alcohol cues to trigger alcohol-seeking/craving behaviors, not merely with variables sufficient for alcohol to induce someone to like or dislike an alcohol cue to the point of approaching or avoiding it. For this, it seems we need longer PC protocols (>20 conditioning trials) than those sampled in our review or protocols with an alcohol preexposure phase as long as or longer than that used by Reid et al. (1985). Additionally, we need to evaluate whether, for rats (or mice), the "wanting" motivation may be conditioned and if the assumed parity for the "liking" motivation is true (a possible limit of the model organism).

On the other hand, although the prediction of outcomes in alcohol PC has utilitarian consequences for preclinical researchers, it is primarily a necessary feature of model validation in a fit-for-purpose manner. Accordingly, it must generate predictive generalizations for humans, but, as already argued, the confidence of these generalizations depends on the methodological (formal) consistency of the literature. In a systematic review with a statistical synthesis, the method used to test the prediction indicates such consistency: better methods demand more consistency in terms of estimating systematic errors in internal validity (e.g., the many randomization procedures needed in PC), operational validity (i.e., estimating how interchangeable are the so-called "preference indices"), and measurement

agreement (i.e., whether different measuring systems give accurate, reliable, and interchangeable measurements). Also, they require using the same or interchangeable raw-score effect-size metrics for the synthesis.

As shown with excruciating details in Appendix S3 of our review, the literature has undetermined measurement agreement, serious problems in the operational validity of "preference indices", and a worrisome low internal validity. On top of that, currently one cannot assume that raw-score effect-size metrics of experimental (with a control group as a comparator) and semi-experimental designs (using a theoretical mean as a comparator) have the same vulnerability to different sources of systematic error. Similarly, one cannot transform change-score metrics (coming from a repeated measure in a before–after experimental design) to raw-score ones because data reports allowing such a transformation are faulty. Additionally, the literature presents a relevant risk of report bias that has increased during the last decade (Appendix S12 of our review).

So fragile and fragmentary is the literature in these aspects that the only way we found to test the prediction of outcomes was by using a nonrecommended synthesis method (vote counting). Here, we must correct Grahame (2023) and emphasize that our work cannot be considered a meta-analysis or a meta-regression synthesis by any statistical criteria. Accordingly, compared to a meta-analysis, the method has a much smaller power (the unit of analysis is the number of animals in the first and the number of experiments in the second), is less accurate and more vulnerable to spurious associations (as it transforms continuous dependent variables into categorical ones), and does not distinguish the differential relevance (i.e., weight) that single experiments have in the results of the synthesis.

Furthermore, when deriving inclusion and exclusion criteria from the PICO formulation, the method requires more stringent exclusion criteria to select populations and interventions for the review sample because it is even less trustful when there is a very small number of experiments with some populations or interventions in comparison to others (e.g., populations: male vs. *female*, adolescent/adult vs. *infant rats*, outbred vs. *inbred* vs. *selected* vs. *transgenic* strains; interventions: first-order vs. *second-order* Pavlovian conditioning, Pavlovian vs. *operant* conditioning). This is particularly true for rat populations where consistent differences are reported, preventing them from being seen as a continuum of phenotypic variation (the cases of adolescent/adult vs. *infant rats* and male vs. *female* ones in the alcohol condition). Additionally, the lack of studies on relevant confounding effects that the unintentional cosegregation of traits might have on inbred/selected/transgenic strains also poses a challenge.

In other words, given the power limits of the method and its consequent inadequacy for many ad hoc and post hoc comparisons, the population exclusion criteria for all inbred/selected/transgenic strains, infant rats, and outbred females served to prevent us from making false claims (based on invalid premises or false positive and negative results in the synthesis) for these same populations in a learning model with alcohol. In front of this, we will not further discuss Grahame's critiques of the exclusion criteria we adopted because the critiques consist of nonsequitur arguments, except for one observation: The literature has a problematic lack of experiments with female rats.

Consequently, using this method demands authors of a review to (a) state clearly that the prediction of results constitutes a provisory answer, (b) indicate and even devise future solutions to the problems found, (c) indicate where spurious associations are likely and which other variables need more testing (because they were too inconsistently reported to be tested or excluded from the review), (d) recommend detailed and consistent investigation of the predicting variables, and (e) refrain from proposing many generalizations to humans, even when considering them applicable only to nonproblematic or mildly problematic alcohol use. All these points may be found throughout our review, from the “Materials and Methods” section to Appendix S12.

Simultaneously, reviewers must provide the database of extracted variables from the analyzed experiments (our Appendices S13 and S14). When reviewers used better methods, this is done to facilitate broader syntheses. However, we did this hoping that better works may come into reality, allowing someone to estimate the impact of these systematic errors in the studies we reviewed and unifying reviewed and new studies under a meta-regression synthesis. If all of this happens, our work will have fulfilled all of its roles, being duly superseded. Thus, our review is not meant to be valid in the long run. Quite the contrary: It must become obsolete as soon as possible.

In conclusion, it is no wonder our work is secondarily focused on *how* to conduct and report preclinical alcohol PC experiments, while it adds little to research on AUD. Surely Grahame (2023) is right in affirming that the public is likely to fund only useful preclinical research. After all, this is the primordial reason we use preclinical models: the reliable investigation of causal relationships relevant to humans. It is, thus, the responsibility of preclinical researchers to produce trustworthy research so that it may be adequately useful. Otherwise, while some of us mount grand propagandas and merchandise, we will continue to submerge preclinical research in the crisis of reproducibility in which it already is deeply submerged, continue to profusely generate and perpetuate contradictory discourse over causal

relationships under scientific investigation, continue to fail in generating reliable translations for humans and, ultimately, producing research that has little to no relevance for important human phenomena but undoubtedly feeds the "Valley of death" (Seyhan, 2019). And just as Grahame (2023) pointed out, at the end of the day, the public funds our work.

Funding Source: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil (CNPq) – Grant Numbers "454963/2014-9", "131694/2015-4", and "14905/2019-0".

Conflict of interest: The authors declare they have no conflict of interest.

Data availability statement: This commentary has no original data.

Orcid
Douglas Marques https://orcid.org/0000-0001-5571-4172
Piter Kehoma Boll https://orcid.org/0000-0002-3029-0841
Rosane Gomez https://orcid.org/0000-0002-0868-8394
Eliane Dallegrave https://orcid.org/0000-0001-6586-2080
José Inácio Lemos https://orcid.org/0000-0001-7872-4920
Mirna Bainy-Leal https://orcid.org/0000-0001-7205-6669

REFERENCES

Burns, M. & Domjan, M. (1996) Sign tracking versus goal tracking in the sexual conditioning of male Japanese quail (*Coturnix japonica*). *Journal of Experimental Psychology: Animal Behavior Processes*, 22, 297–306. Available from: https://doi.org/10.1037/0097-7403. 22.3.297

Childress, A.R., Mozley, P.D., McElgin, B.A., Fitzgerald, J., Reivich, M. & O'Brien, C.P. (1999) Limbic activation during cue-induced cocaine craving. *The American Journal of Psychiatry*, 156, 11–18. Available from: https://doi.org/10.1176/ajp.156.1.11

Childs, E. & de Wit, H. (2016) Alcohol-induced place conditioning in moderate social drinkers. *Addiction*, 111, 2157–2165. Available from: https://doi.org/10.1111/add.13540

Collaizzi, J.M., Flagel, S.B., Joyner, M.A., Gearhardt, A.N., Stewart, J.L. & Paulus, M.P. (2020) Mapping sign-tracking and goal-tracking onto human behaviors. *Neuroscience & Biobehavioral Reviews*, 111, 84–94. Available from: https://doi.org/10.1016/j.neubiorev.2020. 01.018

Field, M. & Duka, T. (2002) Cues paired with a low dose of alcohol acquire conditioned incentive properties in social drinkers. *Psychopharmacology*, 159, 325–334. Available from: https://doi. org/10.1007/s00213-001-0923-z

Fox, H.C., Bergquist, K.L., Hong, K.-I. & Sinha, R. (2007) Stress-induced and alcohol cue-induced craving in recently abstinent alcoholdependent individuals. *Alcoholism: Clinical and Experimental Research*, 31, 395–403. Available from: https://doi.org/10.1111/j. 1530-0277.2006.00320.x

German, P.W. & Fields, H.L. (2007) How prior reward experience biases exploratory movements: a probabilistic model. *Journal of Neurophysiology*, 97, 2083–2093. Available from: https://doi.org/ 10.1152/jn.00303.2006

Grahame, N.J. (2023) Commentary on “alcohol, place conditioning, and male rats: a systematic review for outcome prediction”. *Alcohol: Clinical and Experimental Research*, 47, 1649–1652. Available from: https://doi.org/10.1111/acer.15154

Heck, M., Loréa, C., Quertemont, E. & Simon, J. (2023) Effects of proximal, contextual, and social alcohol cues on craving in an immersive virtual environment. 19th ESBRA conference. Available from: https://www.researchgate.net/publication/373659079_Effects_of_proximal_contextual_and_social_alcohol_cues_on_craving_in_an_immersive_virtual_environment

Lutz, J.A. & Childs, E. (2021) Alcohol conditioned contexts enhance positive subjective alcohol effects and consumption. *Behavioural Processes*, 187, 104340. Available from: https://doi.org/10.1016/j.beproc.2021.104340

Marques, D., Boll, P.K., Gomez, R., Dallegrave, E., Lemos, J.I. & BainyLeal, M. (2023) Alcohol, place conditioning, and male rats: a systematic review of outcome prediction. *Alcohol: Clinical and Experimental Research*, 47, 1039–1054. Available from: https://doi.org/10.1111/ acer.15092

Meyer, J.S., Farrar, A.M., Biezonski, D. & Yates, J.R. (2023) Drug misuse and addiction. In: Meyer, J.S., Farrar, A.M., Biezonski, D. & Yates, J.R. (Eds.) *Psychopharmacology: drugs, the brain, and behavior*, 4th edition. Massachusetts, USA: Oxford University Press, pp. 252–287.

Reid, L.D., Hunter, G.A., Beaman, C.M. & Hubbell, C.L. (1985) Toward understanding ethanol's capacity to be reinforcing: a conditioned place preference following injections of ethanol. *Pharmacology, Biochemistry, and Behavior*, 22, 483–487. Available from: https://doi.org/10.1016/0091-3057(85)90051-6

Reiss, J. (2019) Against external validity. *Synthese*, 196, 3103–3121. Available from: https://doi.org/10.1007/s11229-018-1796-6

Robinson, T.E. & Berridge, K.C. (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Research Reviews*, 18, 247–291. Available from: https://doi.org/10.1016/ 0165-0173(93)90013-P

Seyhan, A.A. (2019) Lost in translation: the valley of death across preclinical and clinical divide – identification of problems and overcoming obstacles. *Translational Medicine Communications*, 4, 18. Available from: https://doi.org/10.1186/s41231-019-0050-7

Shadish, W.R., Cook, T.D. & Campbell, D.T. (2002) *Experimental and quasiexperimental designs for generalized causal inference*. Houghton: Mifflin and Company.

Sinha, R., Fox, H.C., Hong, K.A., Bergquist, K., Bhagwagar, Z. & Siedlarz, K.M. (2009) Enhanced negative emotion and alcohol craving, and altered physiological responses following stress and cue exposure in alcohol dependent individuals. *Neuropsychopharmacology*, 34, 1198–1208. Available from: https://doi.org/10.1038/npp.2008.78

Townshend, J.M. & Duka, T. (2007) Avoidance of alcohol-related stimuli in alcohol-dependent individuals. *Alcoholism: Clinical and Experimental Research*, 31, 1349–1357. Available from: https://doi.org/10.1111/j. 1530-0277.2007.00429.x

5 DISCUSSÕES E CONCLUSÕES GERAIS

5.1 DISCUSSÕES

No segundo capítulo dessa tese, a partir das evidências disponíveis, argumentou-se brevemente que a atual literatura pré-clínica de CL com álcool é válida somente à modelação da aprendizagem ocorrendo no uso não-problemático ao levemente problemático de álcool (MARQUES *et al*., 2024). Ainda que ali essa argumentação inclua a avaliação das validades de constructo, de face e preditiva do processo comportamental instrumentalizado no CL, ela é insuficiente ao entendimento próprio de quão distante a atual literatura aparenta se encontrar dessa modelação, visto que Marques *et al*. (2024) foi publicado no limitado formato de comentário. Assim, frente aos dados da revisão apresentada no capítulo primeiro, os experimentos reportados no capítulo terceiro e dos dados de controle de estímulos no uso de álcool em humanos, essa seção é destinada à avaliação mais extensa da validade de face da atual literatura de CL induzido por álcool.

A premissa geral dessa avaliação é o fato de que ao longo do desenvolvimento do Transtorno por Uso de Álcool (AUD, sigla e inglês) há um progressivo aumento do controle exercido pelo álcool, estímulo motivador e reforçador primário, e de estímulos a ele relacionados sobre os comportamentos dos indivíduos (HASIN *et al*., 1990; VAILLANT, 2009; VAILLANT; HILLERSTURMHÖFEL, 1996). No AUD, esses controles incondicional e condicionais são exacerbados e generalizados ao ponto de haver perda por estímulos outros da capacidade de competirem com esses controles, bem como severa redução/perda de controle voluntário sobre a procura por álcool, a frequência de ingestão e a quantidade de álcool ingerido, a despeito das muitas consequências negativas que tudo isso gera ao indivíduo (CARVALHO *et al*., 2019).

Esse nível de controle comportamental pelo álcool e estímulos a ele relacionados é incompatível com (a) a falta de propriedades motivacionais/reforçadoras de doses baixas de álcool, (b) a falta de tolerância aos efeitos aversivos de doses mais altas, bem como com (c) a alta sensibilidade a influências deletérias ou modulatórias de variáveis outras sobre motivações e comportamentos eliciados, reforçados e condicionados(as) pelo álcool. Pelo contrário, a existência desse nível de controle por esse estímulo primário e por pistas a ele relacionadas exige que a falta de função de doses baixas, a falta de tolerância à aversividade de doses mais altas e as influências modulatórias e deletérias de outras variáveis sobre esse controle já tenham sido superadas em larga margem nos indivíduos com AUD. E ainda assim,

são justamente essas características incompatíveis com o AUD que são encontradas no CL induzido por álcool, como exposto nos capítulos primeiro e terceiro dessa tese.

Usuários de drogas em abstinência, por exemplo, relatam frequentemente que única exposição a dose baixa da droga anteriormente consumida resulta em maior viés de atenção e de aproximação a pistas relacionadas à droga, em aumento da fissura e em comportamento de procura pela droga, três fenômenos comportamentais que, quando sob controle de doses baixas, são conceituados como instâncias de *priming* (DE WIT, 1996)[6]. *Priming* tem sido demonstrado em laboratório tanto em usuários dependentes de álcool quanto em animais não humanos, mas o efeito é inconsistentemente relatado em usuários sociais não-problemáticos, especialmente em usuários leves (KIRK; DE WIT, 2000; FIELD *et al*., 2004).

Essa inconsistência aparenta ser especialmente decorrente das diferentes sensibilidades individuais ainda modularem fortemente os efeitos motivacionais do álcool em usuários leves (KIRK; DE WIT, 2000; FIELD *et al*., 2004), algo que também está de acordo com as diferentes trajetórias de consumo identificadas por Sylvestre *et al*. (2020). Assim, esses usuários não estão suficientemente sensibilizados aos efeitos motivacionais/reforçadores de doses baixas de álcool[7] a ponto delas eliciarem um efeito *priming* (KIRK; DE WIT, 2000). Ou seja, *priming* induzido por doses baixas depende dessas doses terem adquirido função incondicional a ponto de eliciarem ou elevarem a probabilidade desses comportamentos. No capítulo primeiro, entretanto, mostrou-se que doses baixas de álcool possuem baixíssima chance de apresentarem função incondicional em protocolos curtos de CL. Já no capítulo terceiro se demonstrou isso experimentalmente. De fato, os exemplos de *priming* que podem ser achados na literatura pré-clínica de CL advêm do uso de protocolos compostos de pré-exposição e de condicionamento, os quais utilizam a mesma dose baixa de álcool tanto na pré-exposição e no condicionamento, quanto na testagem do efeito *priming* (e.g., GAWEL *et al*., 2016).

---

[6] Notar que desses fenômenos comportamentais, os que mais se assemelham ao tipo de preferência aprendida no CL são o viés de atenção e de aproximação a pistas relacionadas ao álcool. Isso também é contundente com a argumentação apresentada em Marques *et al*. (2024).

[7] Notar que o uso da expressão "baixa dose de álcool" para doses menores que 1.0 g/kg não é adequado em comparações alométricas entre espécies, como indicado pelos dados alcoolêmicos de ratos (BLOOM *et al*., 1982; ROINE *et al*., 1991) e humanos (WILKINSON *et al*., 1977). Enquanto certamente essas doses são baixas para ratos (dadas as concentrações alcoólicas sanguíneas que geram), o mesmo não pode ser dito para humanos: uma pessoa de 70 kg que ingira 0,25, 0,5, ou 0,75 g/kg consome em verdade 17,5, 35, ou 52,5 g de álcool puro, respectivamente. Além disso, pelas unidades padrão de consumo alcoólico da Islândia e do Reino Unido (8 g), da China, França, Irlanda e Espanha (10 g), e dos Estados Unidos (14 g), a ingestão de 0,5 g/kg para essa pessoa é o equivalente ao consumo de ~ 4,4, 3,5 e 2,5 vezes as respectivas unidades padrão (ALCOHOL RESEARCH: CURRENT REVIEWS EDITORIAL STAFF, 2018). Portanto, o paralelo feito entre espécies modelo e alvo para doses menores que 1.0 g/kg é muito mais um indicativo que uma comparação alométrica direta.

Ademais, o estudo do controle condicional previamente instalado (i.e., da fissura induzida por pistas relacionadas ao álcool, bem como do viés de atenção e do viés de aproximação a essas pistas) em pessoas com diferentes severidades de consumo de álcool também aponta a incompatibilidade da atual literatura de CL quanto ao controle exacerbado e generalizado do álcool no AUD. Pesquisas com usuários não-dependentes indicam que a magnitude do viés de atenção é positivamente associada à quantidade de álcool que é regularmente consumida (JONES *et al*., 2003) e, quando são comparados usuários leves e pesados, apenas os pesados apresentam significativo viés de atenção e de aproximação às pistas relacionadas ao álcool (FIELD *et al*., 2007; TOWNSHEND; DUKA, 2001). Adicionalmente, quando a latência de aproximação é comparada à latência de afastamento de estímulos relacionados ao álcool, usuários pesados apresentam menor latência de aproximação, mas não os leves (FIELD *et al*., 2011). Da mesma forma, em usuários pesados, o consumo único de baixa dose de álcool (0,3 g/kg), em comparação ao consumo de placebo, apresenta efeito *priming* para viés de atenção e de aproximação a pistas relacionadas ao álcool, bem como para fissura por essa droga (SCHOENMAKERS *et al*., 2008). Todos esses dados indicam que baixas doses de álcool possuem propriedades motivacionais em usuários pesados e apontam que protocolos curtos de CL estão modelando aprendizagem própria ao consumo não problemático de álcool.

Adicionalmente, parece então razoável que as doses de 0.2-0.8 g/kg sejam capazes de instalar propriedades motivacionais em pistas a elas pareadas segundo protocolos curtos de condicionamento em humanos com histórico de consumo de álcool. Interessante notar aqui que tal instalação ocorre a partir da dose de 0,8 em usuários moderados e da dose de 0,2 em usuários pesados (CHILDS; DE WIT, 2016; FIELD; DUKA, 2002; LUTZ; CHILDS, 2021). Também aparenta ser razoável afirmar que isso seja válido a ratos naïve submetidos a protocolos longos de CL, bem como a ratos pré-expostos ao álcool e submetidos a protocolos curtos, mas não a animais naïve submetidos a protocolos curtos (GAWEL *et al*., 2016; MARQUES *et al*., 2023). Decorre então a generalização de que, devido ao histórico prolongado de consumo/administração, os usuários e os animais já se encontram sensibilizados a efeitos motivacionais e reforçadores incondicionais de doses baixas.

Similarmente, a alta sensibilidade aos efeitos de inibição latente em protocolos curtos de CL alcoólico, como evidenciado em Marques (2017), em nossa revisão (MARQUES *et al*., 2023) e no capítulo terceiro, é incompatível com a modelação de características aprendidas no AUD. Caso a atual literatura de fato estivesse realizando tal modelação, seria esperado, segundo a análise teórica pavloviana e o controle exacerbado do álcool no AUD, que somente

grande número de sessões de habituação tivesse efeito deletério sobre a aquisição de comportamentos condicionados como ACL e PCL. Entretanto apenas uma sessão de habituação é suficiente ao decréscimo da probabilidade de sucesso de condicionamento em protocolos curtos.

O mesmo pode ser dito acerca das influências deletérias da distribuição desigual de sessões de condicionamento entre contextos álcool-pareado e álcool-não-pareado que resulte em menos sessões no contexto não-pareado e, portanto, em diferencial novidade entre as câmaras de condicionamento. Caso características aprendidas do AUD estivessem sendo modeladas na atual literatura de CL, essas influências não deveriam ser detectadas em protocolos curtos ou longos de condicionamento, mesmo para doses de 0,5 g/kg no último caso, como o foram por Marglin *et al*. (1988) e Bozarth (1990). Pelo contrário, elas já deveriam ter sido superadas visto que novidade ambiental pode ter função motivadora e reforçadora incondicional a ratos (CARR *et al*., 1988) e que em humanos motivadores/reforçadores incondicionais outros (comida, interação social, sexo etc.) progressivamente perdem a capacidade de competir eficazmente com o álcool ao longo do desenvolvimento de AUD (PEUGH; BELENKO, 2001; BARVE *et al*., 2017).

Outrossim, na introdução do capítulo terceiro, foi exposto que existe um trade-off de relações causais entre ciclos circadianos/relógios biológicos e a motivação para o consumo e o próprio consumo de álcool na medida em que o AUD é desenvolvido. Tanto em humanos com AUD quanto em animais experimentais, a ingestão crônica de álcool está associada a problemas dramáticos e perturbações generalizadas dos ciclos sono-vigília e outros ritmos biológicos diários (humanos: BROWER, 2003; KUHLWEIN *et al*., 2003; SANO *et al*., 1993; animais: EHLERS; SLAWECKI, 2000; MUKHERJEE; SIMASKO, 2009; WASIELEWSKI; HOLLOWAY, 2001). Importante ressaltar que modificações ocorrem tanto nos *pacemakers* e *pacekeepers* encefálicos (por exemplo, no núcleo hipotalâmico supraquiasmático), quanto nos periféricos (alterações morfométricas circadianas nos hepatócitos e padrões circadianos de expressão e de atividade das enzimas hepáticas álcool desidrogenase e do sistema microssomal de oxidação do álcool: KIRILLOV *et al*., 2021; STURTEVANT; GARBER, 1984).

Assim, no AUD, bem como em outros transtornos por uso de drogas, o controle fisiológico e comportamental exercido pela droga leva a modificações e disfunções severas nos relógios e ciclos biológicos que são incompatíveis com larga influência de ciclos biológicos intactos sobre os efeitos das drogas ao ponto dessas perderem efeitos incondicionais capazes de condicionar respostas à apresentação de outros estímulos.

Entretanto, é justamente essa alta influência circadiana que tem sido detectada para outras drogas (LI *et al*., 2014; WEBB *et al*., 2009) e para o álcool em protocolos curtos de CL, como pode ser visto em Marques (2017) e no capítulo terceiro dessa tese.

FIGURA 5.1 – Características dos controles de estímulos induzidos no CL alcoólico e presentes no uso pesado e no Transtorno por Uso de Álcool

Características do controle de estímulos no MCL álcool-induzido segundo protocolos curtos	Características do controle de estímulos no uso pesado de álcool e no Transtorno por Uso de Álcool
- Competição do comportamento exploratório (eliciado por novidade, área do aparato e nº de câmaras) com o controle condicionado (CAPÍTULO 1) - Falta de propriedades motivacionais de doses baixas de álcool (CAPÍTULO 1) - Falta de tolerância aos efeitos aversivos de doses mais altas (CAPÍTULOS 1 e 2) - Modulação circadiana dos efeitos condicionados do álcool segundo o esperado para relógios biológicos intactos (CAPÍTULO 3) - Alta sensibilidade a influências deletérias ou modulatórias de variáveis outras sobre motivações e comportamentos condicionados(as) pelo álcool (CAPÍTULOS 1, 2 e 3)	- Perda da capacidade por estímulos ecologicamente relevantes de competirem com o controle condicionado (PEUGH; BELENKO, 2001; BARVE *et al.*, 2017) - Sensibilidade aos efeitos incondicionais de doses baixas (*priming*: SCHOENMAKERS *et al.*, 2008) - Tolerância aos efeitos aversivos de doses altas/uso continuado (CARVALHO *et al.*, 2019) - Mudança/ruptura prolongada dos padrões circadianos de sono-vigília e de "fissura" (BROWER, 2003; KUHLWEIN *et al.*, 2003; SANO *et al.*, 1993) - Alta insensibilidade às consequências negativas que uso provoca (CARVALHO *et al.*, 2019) - Severa redução/perda de controle voluntário sobre a procura por álcool, a frequência de ingestão e a quantidade de álcool ingerido (CARVALHO *et al.*, 2019; SYLVESTRE *et al.*, 2020)

Fonte: Elaborada pelo autor.

Portanto, a análise dessas variáveis todas e suas relações com a probabilidade de se obter condicionamento bem-sucedido em protocolos curtos de CL induzido por álcool consistentemente aponta que a atual literatura não está modelando aprendizagem própria ao AUD (**Figura 5.1**).

5.2 CONCLUSÕES

A partir da análise preditiva do capítulo primeiro e da testagem experimental relatada no capítulo terceiro, há três argumentações conclusivas a serem feitas acerca da literatura pré-clínica atual de CL. A primeira se refere ao fato de que, apesar das falhas formais que predominam na literatura — e sem que isso justifique a manutenção dessas falhas —, os corpos teórico-experimentais básicos da Psicologia, da Farmacologia e da Etologia são suficientes à predição dos resultados de intervenções simples no CL induzido por álcool em ratos. Nesse sentido, tal como identificado por Kalant (1989) no estudo de sensibilização e tolerância na farmacologia comportamental, a falha de predição teórica prevalente na literatura de CL alcoólico está mais ligada à falta de comunicação eficiente entre farmacólogos, psicólogos e etólogos que a faltas nessas disciplinas científicas. Aqui deve ser dito que se há tributo digno a um trabalho científico, este é o reconhecimento da consistência

de seu legado e este ensaio não traz algo novo a estes corpos. Pelo contrário, é deles que deriva a capacidade de identificarmos variáveis relevantes aos resultados de CL com álcool em ratos a ponto de conseguirmos predizê-los. Isto, portanto, aponta que necessitamos formar farmacólogos comportamentais que de fato consigam entender e usar de maneira unificada os conceitos postos por essas disciplinas.

O segundo argumento é referente ao significado da predição de resultados de CL induzido por álcool quanto à modelação de aprendizagem própria ao AUD. Dada a alta sensibilidade dos resultados de CL álcool-induzido a variáveis modulatórias e deletérias, a predição indica que há alta incompatibilidade da modelação feita no CL com as características aprendidas e mesmo fisiológicas do AUD, transtorno do qual se objetiva modelar essas características no CL alcoólico. No capítulo segundo, a partir dessa indicação, mostrou-se que quando consideramos o conjunto de validades de constructo, face e predição, há outros dados relevantes indicando falta de validade de propósito no CL álcool-induzido. Já no terceiro, testou-se a alta sensibilidade dos protocolos curtos às variáveis indicadas no capítulo primeiro e os testes foram confirmatórios em seus resultados tanto da predição feita, quanto dessa interpretação. A partir disso, na discussão geral precedente, se expandiu a análise da validade de face da literatura. Todas essas análises são contundentes em mostrar que não estamos modelando comportamentos característicos desse transtorno.

Assim, como apontado em Marques *et al*. (2024), protocolos mais longos de condicionamento, i.e., com mais de 20 sessões de condicionamento, aparentam ser necessários. Adicionalmente, também são necessárias novas linhas de pesquisa que unam o CL à avaliação dos efeitos que o condicionamento de pistas possui sobre a livre ingestão de álcool em animais modelos. Isto também foi apontado em Marques *et al*. (2024).

Por outro lado, o terceiro argumento conclusivo é o de que os resultados reportados nessa tese não colocam em xeque somente a validade de propósito dos correntes estudos de CL feitos com álcool. De fato, eles também apontam que intervenções realizadas com outras drogas no CL que visem modelar outros transtornos por uso de drogas também não possuem validade de propósito nesta modelação. Consideremos, por exemplo, a metanálise de Bardo *et al*. (1995) para CL induzido por anfetamina, cocaína, morfina e heroína em ratos. Nela se detectou altas influências das variáveis inibição latente, número e duração das sessões de condicionamento e presença/ausência de sessões com salina em protocolos curtos de condicionamento com essas drogas. Para a modelação dos respectivos transtornos por uso de drogas, as influências de todas essas variáveis deveriam estar senão ausentes ao menos em muito diminuídas a ponto de serem, em muitos casos, negligenciáveis em resultados e

análises. Testar se esse realmente é o caso para essas drogas é imperativo, portanto, ao delineamento de pesquisa pré-clínica no CL que de fato seja confiável quanto às suas generalizações com implicações clínicas a humanos.

REFERÊNCIAS

ABEL, E. L.; YORK, J. L. Age-related differences in response to ethanol in the rat. **Psychobiology**, USA, v. 7, p. 391–395, 1979. Disponível em: https://doi.org/10.3758/BF03326662

AGUILAR, M. A. *et al.* Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. **Brain Research Reviews**, USA, v. 59, p. 253–277, 2009. Disponível em: https://doi.org/10.1016/j.brainresrev.2008.08.002

AL AMERI, M. *et al*. The histone deacetylase (HDAC) inhibitor valproic acid reduces ethanol consumption and ethanol-conditioned place preference in rats. **Brain Research**, USA, v. 1583, p. 122–131, 2014. Disponível em: http://dx.doi.org/10.1016/j.brainres.2014.07.051

ALCOHOL RESEARCH: CURRENT REVIEWS EDITORIAL STAFF. Drinking patterns and their definitions. **Alcohol Research Current Reviews**, USA, v. 39, p. e1–e2, 2018. Disponível em: https://arcr.niaaa.nih.gov/volume/39/1/drinking-patterns-and-their-definitions

AMERICAN PSYCHIATRIC ASSOCIATION. **Diagnostic and Statistical Manual of Mental Disorders**, 5th editon. USA: American Psychological Association, 2013.

ANDALOUSSI, Z. *et al*. Acute cannabidiol treatment attenuates ethanol-induced place preference and reduces agressivity in group-housed male rats. **Pharmacology, Biochemistry & Behavior**, USA, v. 211, artigo 173290, 2021. Disponível em: https://doi.org/10.1016/j.pbb.2021.173290

ANDREWS, J. S. *et al*. Performance of four different rat strains in the autoshaping, two-object discrimination, and swim maze tests of learning and memory. **Physiology & Behavior**, USA, v. 57, p. 785-790, 1995. Disponível em: https://doi.org/10.1016/0031-9384(94)00336-X

BAHI, A. Viral-mediated knockdown of mGLUR7 in the Nucleus Accumbens mediates excessive alcohol drinking and increased ethanol-elicited conditioned place preference in rats. **Neuropsychopharmacology**, USA, V. 38, p. 2109–2119, 2013. Disponível em: https://doi.org/10.1038/npp.2012.122

BAHI, A.; DREYER, J.-L. Striatal modulation of BDNF expression using microRNA124a-expressing lentiviral vectors impairs ethanol-induced conditioned-place preference and voluntary alcohol consumption. **European Journal of Neuroscience**, England, v. 38, p. 2328–2337, 2013. Disponível em: https://doi.org/10.1111/ejn.12228

BAHI, A.; DREYER, J.-L. Lentiviral vector-mediated dopamine D3 receptor modulation in the rat brain impairs alcohol intake and ethanol-induced conditioned place preference. **Alcohol: Clinical and Experimental Research**, USA, v. 38, p. 2369–2376, 2014. Disponível em: https://doi.org/10.1111/acer.12503

BARDO, M. T. *et al*. Conditioned place preference using opiate and stimulant drugs: A meta-analysis. **Neuroscience & Biobehavioral Reviews**, USA, v. 19, p. 39–51, 1995. Disponível em: https://doi.org/10.1016/0149-7634(94)00021-r

BARVE, S. *et al*. Development, prevention, and treatment of alcohol-induced organ injury: The role of nutrition. **Alcohol Research Current Reviews**, USA, v. 38, p. 289–302, 2017.

BEACH, H. D. Morphine addiction in rats. **Canadian Journal of Psychology**, Canada, v. 11, p. 104–112, 1957a. Disponível em: https://doi.org/104-12. 10.1037/h0083703

BEACH, H. D. Some effects of morphine on habit function. **Canadian Journal of Psychology**, Canada, 11:93–198, 1957b. Disponível em: https://doi.org/10.1037/h0083702

BERRIDGE, K. C.; ROBINSON, T. E. Liking, wanting, and the incentive-sensitization theory of addiction. **American Psychologist**, USA, v. 71, p. 670–679, 2016. Disponível em: https://doi.org/10.1037/amp0000059

BLACK, R. W. *et al*. A preference in rats for cues associated with intoxication. **Bulletin of the Psychonomic Society**, USA, v. 2, p. 423–424, 1973. Disponível em: https://doi.org/10.3758/BF03334436

BLOOM, F. *et al.* Blood alcohol levels in rats: Non-uniform yields from intraperitoneal doses based on body weight. **British Journal of Pharmacology**, England, v. 75, p. 251–254, 1982. Disponível em: https://doi.org/10.1111/j.1476-5381.1982.tb08780.x

BODE. J. C. Alcohol and the gastrointestinal tract. *In* Frick, P.; von Harnack, G.-A.; Martini, G. A.; Prader, A. (ed). **Advances in internal medicine and pediatrics** (v. 45). New York: Springer-Verlag, 1980, p. 1–75.

BORMANN, N. M.; CUNNINGHAM, C. L. The effects of naloxone on expression and acquisition of ethanol place conditioning in rats. **Pharmacology, Biochemistry & Behavior**, v. 58, p. 975–982, 1997. Disponível em: https://doi.org/10.1016/s0091-3057(97)00304-3

BOZARTH, M. A. Evidence for the rewarding effects of ethanol using the conditioned place preference method. **Pharmacology, Biochemistry & Behavior**, USA, v. 35, p. 485–487, 1990. Disponível em: https://doi.org/10.1016/0091-3057(90)90191-j

BRASIL. Lei nº 11.794 de 08/10/2008. **Diário Oficial da União**, p. 1, col. 1, 2008. Disponível em: https://legis.senado.leg.br/norma/582216/publicacao/15745691. Acessado em: 20 de junho de 2017.

BROWER, K. J. Insomnia, alcoholism and relapse. **Sleep Medicine Reviews**, USA, v. 7, p. 523–539, 2003. https://doi.org/10.1016/s1087-0792(03)90005-0

BURGESS, H. J. *et al*. Sleep and circadian differences between light and heavy adult alcohol drinkers. **Alcohol: Clinical and Experimental Research**, USA, v. 46, p. 1181–1191, 2022. Disponível em: https://doi.org/10.1111/acer.14872

BUSSE, G. D. *et al.* The effects of alcohol preexposure on cocaine, alcohol. **Pharmacology, Biochemistry & Behavior**, USA, v. 81, p. 459–465, 2005. Disponível em: https://doi.org/10.1016/j.pbb.2005.04.003

BUSSE, G. D.; RILEY, A. L. Modulation of cocaine-induced place preferences by alcohol. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, USA, v. 26, p. 1373–1381, 2002. Disponível em: https://doi.org/10.1016/s0278-5846(02)00302-0

CARR, G. D. *et al.* Conditioned place preference as a measure of drug reward. *In*: Liebman, J. M.; Cooper, S. J. (ed). **Topics in experimental psychopharmacology**: the neuropharmacological basis of reward. Oxford: Clarendon Press/Oxford University Press, 1989, p. 264–319.

CARVALHO, A. F. *et al.* Alcohol use disorders. **Lancet**, England, v. 394, p. 781–792, 2019. Disponível em: https://doi.org/10.1016/S0140-6736(19)31775-1

CASTAÑEDA, T. A. *et al.* Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: Modulation by light. **Journal of Pineal Research**, USA, v. 36, p. 177–185, 2004. Disponível em: https://doi.org/10.1046/j.1600-079x.2003.00114.x

CHILDRESS, A. R. *et al.* Limbic activation during cue-induced cocaine craving. **American Journal of Psychiatry**, USA, v. 156, p. 11–18, 1999. Disponível em: https://doi.org/10.1176/ajp.156.1.11

CHILDS, E.; DE WIT, H. Amphetamine-induced place preference in humans. **Biological Psychiatry**, USA, v. 65, p. 900–904, 2009. Disponível em: https://doi.org/10.1016/j.biopsych.2008.11.016

CHILDS, E.; DE WIT, H. Alcohol-induced place conditioning in moderate social drinkers. **Addiction**, USA, v. 111, p. 2157–2165, 2016. Disponível em: https://doi.org/10.1111/add.13540

CONCEA. Resolução normativa nº 13 de 26/09/2013. **Diário Oficial da União**, seção 1, p. 5, 2013. Disponível em: https://antigo.mctic.gov.br/mctic/export/sites/institucional/institucional/concea/arquivos/legislacao/resolucoes_normativas/Resolucao-Normativa-CONCEA-n-13-de-20.09.2013-D.O.U.-de-26.09.2012-Secao-I-Pag.-5.pdf. Acessado em: 20 de junho de 2017.

CONCEA. Resolução normativa nº 32 de 08/09/2016. **Diário Oficial da União**, seção 1, p. 5, 2016. Disponível em: https://www.gov.br/mcti/pt-br/composicao/conselhos/concea/arquivos/arquivo/legislacao/resolucao-normativa-no-32-de-06-de-setembro-de-2016.pdf. Acessado em: 20 de junho de 2017.

COSSIO-BOLAÑOS, M. *et al.* Curvas de referencia para valorar el crescimento físico de ratas machos Wistar. **Nutricion hospitalaria**, Espanha, v. 28, p. 2151–2156, 2013. Disponível em: https://dx.doi.org/10.3305/nh.2013.28.6.6659

CRABB, D. W. *et al*. Central nervous system control of alcohol dehydrogenase activity in rats. **Life Sciences**, USA, v. 37, p. 2381–2387, 1985. Disponível em: https://doi.org/10.1016/0024-3205(85)90105-5

CUNNINGHAM, C. L. *et al.* Place conditioning. *In*: Olmstead, M. C. (ed). **Neuromethods:** animal models of drug addiction. New York: Springer, 2011, p. 167–189.

DE CASTRO, J. M. Social, circadian, nutritional, and subjective correlates of the spontaneous pattern of moderate alcohol intake of normal humans. **Pharmacology, Biochemistry & Behavior**, USA, v. 35, p. 923-931, 1990. Disponível em: https://doi.org/10.1016/0091-3057(90)90380-z

DENAYER T. *et al.* Animal models in translational medicine: Validation and prediction. **New Horizons in Translational Medicine**, USA, v. 2, p. 5–11, 2014. Disponível em: http://dx.doi.org/10.1016/j.nhtm.2014.08.001

DE WIT, H. Priming effects with drugs and other reinforcers. **Experimental and Clinical Psychopharmacology**, USA, v. 4, p. 5–10, 1996. Disponível em: https://doi.org/10.1037/1064-1297.4.1.5

DI CHIARA, G.; IMPERATO, A. Preferential stimulation of dopamine release in the nucleus accumbens by opiates, alcohol, and barbiturates: Studies with transcerebral dialysis in freely moving rats. **Annals of the New York Academy Sciences**, USA, v. 473, p. 367–381, 1986. Disponível em: https://doi.org/10.1111/j.1749-6632.1986.tb23629.x

EHLERS, C. L.; SLAWECKI, C. J. Effects of chronic ethanol exposure on sleep in rats. **Alcohol**, USA, v. 20, p. 173–179, 2000. Disponível em: https://doi.org/10.1016/s0741-8329(99)00077-4

ERIKSSON, C. J. P. Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. **Biochemical Pharmacology**, USA, v. 22, p. 2283–2292, 1973. Disponível em: https://doi.org/10.1016/0006-2952(73)90009-9

FERRIS, M. J. *et al.* Dopamine transporters govern diurnal variation in extracellular dopamine tone. **Proceedings of the National Academy of Sciences of the United States of America**, USA, v. 111, p. E2751–E2759, 2014. Disponível em: https://doi.org/10.1073/pnas.1407935111

FIELD. Andy. **Discovering statistics using SPSS**. 5 ed. London: Sage, 2018.

FIELD, M.; DUKA, T. Cues paired with a low dose of alcohol acquire conditioned incentive properties in social drinkers. **Psychopharmacology**, USA, v. 159, p. 325–334, 2002. Disponível em: https://doi.org/10.1007/s00213-001-0923-z

FIELD, M. *et al.* Attentional biases for alcohol cues in heavy and light social drinkers: The role of initial orienting and maintained attention. **Psychopharmacology**, USA, v. 176, p. 88–93, 2004. Disponível em: https://doi.org/10.1007/s00213-004-1855-1

FIELD, M. *et al.* Experimental manipulation of attentional biases in heavy drinkers: do the effects generalise? **Psychopharmacology**, USA, v. 192, p. 593–608, 2007. Disponível em: https://doi.org/10.1007/s00213-007-0760-9

FIELD, M. *et al.* Alcohol approach tendencies in heavy drinkers: Comparison of effects in a relevant stimulus-response compatibility task and an approach/avoidance Simon task. **Psychology of Addictive Behaviors**, USA, v. 25, p. 697–701, 2011. Disponível em: https://doi.org/10.1037/a0023285

GAWEL, K. *et al.* Acquisition and reinstatement of ethanol-induced conditioned place preference in rats: Effects of the cholinesterase inhibitors donepezil and rivastigmine. **Journal of Psychopharmacology**, England, v. 30, p. 1–12, 2016. Disponível em: https://doi.org/10.1177/0269881116642539

GRAHAME, N. J. Mice and alcohol. *In*: Miller, P. M. (ed). **Comprehensive addictive behaviors and disorders**: biological research on addiction. USA: Academic Press, 2013, p. 11–19.

GRAHAME, N. J. Commentary on "Alcohol, place conditioning, and male rats: A systematic review for outcome prediction". **Alcohol: Clinical and Experimental Research**, USA, v. 47, p. 1649-1652, 2023. Disponível em: https://doi.org/10.1111/acer.15154

HASIN, D. S. *et al.* The natural history of alcohol abuse: Implications for definitions of alcohol use disorders. **American Journal of Psychiatry**, USA, v. 147, p. 1537–1541, 1990. Disponível em: https://doi.org/10.1176/ajp.147.11.1537

HAUKOOS, J. S.; LEWIS, R. L. Advanced statistics: Bootstrapping confidence intervals for statistics with "difficult" distributions. **Academic Emergency Medicine**, USA, v. 12, p. 360–365, 2005. Disponível em: https://doi.org/10.1197/j.aem.2004.11.018

KALANT, H. Drug tolerance and sensitization. *In*: Goudie, A. J.; Emmett-Oglesby, M. W. (ed). **Psychoactive drugs**: tolerance and sensitization. New York: Springer, 1989, p. 547–577.

KALANT, H. Pharmacokinetics of ethanol: absorption, distribution, and elimination. *In*: Begleiter, H.; Kissin, B. (ed). **The pharmacology of alcohol and alcohol dependence**. New York: Oxford University Press, 1996, p. 15–58.

KALANT, H. Pharmacological interactions of aging and alcohol. *In*: Gomberg, E. L.; Hegedus, A. M.; Zucker, R. A. (ed). **Alcohol problems and aging**. Maryland: National Institute of Health, 1998, p. 99–116.

KEELEY, R. J. *et al.* Strain and sex differences in brain and behaviour of adult rats: Learning and memory, anxiety and volumetric estimates. **Behavioural Brain Research**, England, v. 288, p. 118-131, 2015. Disponível em: https://doi.org/10.1016/j.bbr.2014.10.039

KIRILLOV, Y. A. *et al.* The effect of chronic alcohol intoxication on the daily rhythm of some micromorphometric parameters of rat hepatocytes. **Journal of Pharmacy and Nutrition Sciences**, USA, v. 11, p. 1–12, 2021. Disponível em: https://doi.org/10.29169/1927-5951.2021.11.01

KIRK, J. M.; DE WIT, H. Individual differences in the priming effect of ethanol in social drinkers. **Journal of Studies on Alcohol**, USA, v. 61, p. 64–71, 2000. Disponível em: https://doi.org/10.15288/jsa.2000.61.64

KOIVISTO, T.; ERIKSSON, C. J. P. Hepatic aldehyde and alcohol dehydrogenase in alcohol-preferring and alcohol-avoiding rat lines. **Biochemical Pharmacology**, USA, v. 48, p. 1551–1558, 1994. Disponível em: https://doi.org/10.1016/0006-2952(94)90199-6

KOIVULA, T. *et al.* Liver Aldehyde and alcohol dehydrogenase activities in rat strains genetically selected for their ethanol preference. **Biochemical Pharmacology**, USA, v. 24, p. 1807–1811, 1975. Disponível em: https://doi.org/10.1016/0006-2952(75)90461-x

KUHLWEIN, E. *et al.* Abnormal nocturnal melatonin secretion and disordered sleep in abstinent alcoholics. **Biological Psychiatry**, USA, v. 54, p. 1437–1443, 2003. Disponível em: https://doi.org/10.1016/s0006-3223(03)00005-2

KUMAR, R. Morphine dependency in rats: Secondary reinforcement from environmental stimuli. **Psychopharmacologia**, Germany, v. 25, p. 332–338, 1972. Disponível em: https://doi.org/10.1007/BF00421972

JONES, B. T. *et al.* A flicker paradigm for inducing change blindness reveals alcohol and cannabis information processing biases in social users. **Addiction**, USA, v. 98, p. 235–244, 2003. Disponível em: https://doi.org/10.1046/j.1360-0443.2003.00270.x

LE BOURHIS, B.; MÉNORET, Y. Evacuation gastrique des aliments et de l'éthanol administrés seuls ou conjointement. **Reproduction Nutrition Développement**, France, v. 20, p. 1173–1179, 1980.

LI, S.-X. *et al.* Glycogen synthase kinase-3β in the ventral tegmental area mediates diurnal variations in cocaine-induced conditioned place preference in rats. **Addiction Biology**, USA, v. 19, p. 996-1005, 2014. Disponível em: https://doi.org/10.1111/adb.12068

LILLIE, L. E. *et al.* Reference values for young normal Sprague-Dawley rats: Weight gain, hematology and clinical chemistry. **Human & Experimental Toxicology**, USA, v. 15, p. 612–616, 1996. Disponível em: https://doi.org/10.1177/096032719601500802

LIVY, D. J. *et al.* Blood ethanol concentration profiles: A comparison between rats and mice. **Alcohol**, USA, v. 29, p. 165–171, 2003. Disponível em: https://doi.org/10.1016/s0741-8329(03)00025-9

LUBOW, Robert. **Problems in the behavioural sciences** (v. 9): latent inhibition and conditioned attention theory. Cambridge: Cambridge University Press, 1989.

LUTZ, J. A.; CHILDS, E. Alcohol conditioned contexts enhance positive subjective alcohol effects and consumption. **Behavioural Processes**, England, v. 187, article 104340, 2021. Disponível em: https://doi.org/10.1016/j. beproc.2021.104340

MADRID, J. A. *et al.* Feeding behavior and entrainment limits in the circadian system of the rat. **American Journal of Physiology**, USA, v. 275, p. R372–383, 1998. Disponível em: https://doi.org/10.1152/ajpregu.1998.275.2.R372

MARGLIN, S. H. *et al.* Ethanol with small doses of morphine establishes a conditioned place preference. **Alcohol**, USA, v. 5:, p. 309–313, 1988. Disponível em: https://doi.org/10.1016/0741-8329(88)90071-7

MARQUES, D. **Condicionamento ao local induzido por etanol em ratos: Revisão metodológica e estudo experimental**. 2017. Dissertação (Mestrado em Neurociências) – Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, 2017.

MARQUES, D. *et al.* Alcohol, place conditioning, and male rats: A systematic review of outcome prediction. **Alcohol: Clinical and Experimental Research**, USA, v. 47, p. 1039-1054, 2023. Disponível em: https://doi.org/10.1111/acer.15092

MARQUES, D. *et al.* Letter to the editor: A reply to Grahame (2023) commenting on "Alcohol, place conditioning, and male rats: A systematic review of outcome prediction". **Alcohol: Clinical and Experimental Research**, USA, v. 48, p. 246–249, 2024. Disponível em: https://doi.org/10.1111/acer.15254

MAYHUGH, R. E. *et al.* Differing patterns of stress and craving across the day in moderate-heavy alcohol consumers during their typical drinking routine and imposed period of alcohol abstinence. **PLoS ONE**, USA, v. 13, artigo e0195063, 2018. Disponível em: https://doi.org/10.1371/journal.pone.0195063

MCBRIDE, W. J. *et al.* Localization of brain reinforcement mechanisms: Intracranial self-administration and intracranial place-conditioning studies. **Behavioural Brain Research**, England, v. 101, p. 129–152, 1999. Disponível em: https://doi.org/10.1016/S0166-4328(99)00022-4

MEYER, J. S. *et al.* Drug misuse and addiction. *In*: Meyer, J. S.; Farrar, A. M.; Biezonski, D.; Yates, J. R. (ed). **Psychopharmacology**: drugs, the brain, and behavior. 4 ed. Massachusetts: Oxford University Press, 2023, p. 252–287.

MEYREL, M. *et al.* Alterations in circadian rhythms following alcohol use: A systematic review. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, USA, v. 99, artigo 109831, 2020. Disponível em: https://doi.org/10.1016/j.pnpbp.2019.109831

MORALES, M. *et al.* Evidence for conditioned place preference to a moderate dose of ethanol in adult male Sprague-Dawley rats. **Alcohol**, USA, v. 46, p. 643–648, 2012. Disponível em: https://doi.org/10.1016/j.alcohol.2012.06.001

MUKHERJEE, S.; SIMASKO, S. M. Chronic alcohol treatment in rats alters sleep by fragmenting periods of vigilance cycling in the light period with extended wakenings. **Behavioural Brain Research**, England, v. 198, p. 113–124, 2009. Disponível em: https://doi.org/10.1016/j.bbr.2008.10.026

NAASSILA, M. Alcohol and rats. *In*: Miller, P. M. (ed). **Comprehensive addictive behaviors and disorders**: biological research on addiction. USA: Academic Press, 2013, p. 21–30.

NAKAGAWA, H.; OKUMURA, N. Coordinated regulation of circadian rhythms and homeostasis by the suprachiasmatic nucleus. **Proceedings of the Japan Academy (Series B): Physical and Biological Sciences**, v. 86, p. 391–408, 2010. Disponível em: https://doi.org/10.2183/pjab.86.391

NISHIGUCHI, M. *et al.* Different blood acetaldehyde concentration following ethanol administration in a newly developed high alcohol preference and low alcohol preference rat model system. **Alcohol and Alcoholism**, England, v. 37, p. 9–12, 2002. Disponível em: https://doi.org/10.1093/alcalc/37.1.9

NRC. **Guidelines for the care and use of mammals in neuroscience and behavioral research**. New York: National Academy Press, 2003.

NRC. **Guide for the care and use of laboratory animals**. 8 ed. New York: National Academy Press, 2010.

NURMI, M. *et al.* Brain ethanol in AA, ANA, and Wistar rats monitored with one-minute microdialysis. **Alcohol**, USA, v. 11, p. 315-32, 1994. Disponível em: https://doi.org/10.1016/0741-8329(94)90098-1

PEUGH, J.; BELENKO, S. Alcohol, drugs and sexual function: A review. **Journal of. Psychoactive Drugs**, England, v. 33, p. 223–232, 2001. Disponível em: https://doi.org/10.1080/02791072.2001.10400569

PIERCE, R. C.; KUMARESAN, V. The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? **Neuroscience & Biobehavioral Reviews**, USA, v. 30, p. 215–238, 2006. https://doi.org/10.1016/j.neubiorev.2005.04.016

PHILPOT, R. M. *et al.* Place conditioning: Age-related changes in the rewarding and aversive effects of alcohol. **Alcohol: Clinical and Experimental Research**, USA, v. 27, p. 593–599, 2003. Disponível em: https://doi.org/10.1097/01.ALC.0000060530.71596.D1

QUERTEMONT, E. Genetic polymorphism in ethanol metabolism: Acetaldehyde contribution to alcohol abuse and alcoholism. **Molecular Psychiatry**, England, v. 9, p. 570–581, 2004. Disponível em: https://doi.org/10.1038/sj.mp.4001497

REISS, J. Against external validity. **Synthese**, USA, v. 196, p. 3103–3121, 2019. Disponível em: https://doi.org/10.1007/s11229-018-1796-6

ROBINSON, T. E.; BERRIDGE, K. C. The neural basis of drug craving: an incentive-sensitization theory of addiction. **Brain Research Reviews**, USA, v. 18, p. 247–291, 1993. Disponível em: https://doi.org/10.1016/0165-0173(93)90013-P

ROBINSON, T. E.; BERRIDGE, K. C. The psychology and neurobiology of addiction: An incentive-sensitization view. **Addiction**, USA, v. 95(Suppl. 2), p. S91–S117, 2000. Disponível em: https://doi.org/10.1080/09652140050111681

ROBINSON, T. E.; BERRIDGE, K. C. Incentive-sensitization and addiction. **Addiction**, USA, v. 96, p. 103–114, 2001. Disponível em: https://doi.org/10.1046/j.1360-0443.2001.9611038.x

ROBINSON, T. E.; BERRIDGE, K. C. The incentive sensitization theory of addiction: Some current issues. **Philosophical Transactions of the Royal Society (B): Biological Sciences**, England, v. 363, p. 3137–3146, 2008. Disponível em: https://doi.org/10.1098/rstb.2008.0093

ROINE, R. P. *et al.* Effect of concentration of ingested ethanol on blood alcohol levels. **Alcohol: Clinical and Experimental Research**, USA, v. 15, p. 734–738, 1991. Disponível em: https://doi.org/10.1111/j.1530-0277.1991.tb00589.x

ROSENWASSER, A. M. Circadian clock genes: non-circadian roles in sleep, addiction, and psychiatric disorders? **Neuroscience & Biobehavioral Reviews**, USA, v. 34, p. 1249-1255, 2010. Disponível em: https://doi.org/10.1016/j.neubiorev.2010.03.004

ROSSI, N.A.; REID, L. D. Affective states associated with morphine injections. **Psychobiology**, USA, v. 4, p. 269–274, 1976. Disponível em: https://doi.org/10.3758/BF03332869

SANO, H. *et al.* Circadian variation in plasma 5-hydroxyindoleacetic acid level during and after alcohol withdrawal: phase advances in alcoholic patients compared with normal subjects. **Acta Psychiatrica Scandinavica**, Scandinavia, v. 87, p. 291–296, 1993. Disponível em: https://doi.org/10.1111/j.1600-0447.1993.tb03374.x

SENGUPTA, P. The laboratory rat: Relating its age with human's. **International Journal of Preventive Medicine**, v. 4,:p. 624–630, 2013.

SHADISH, W. R. *et al.* **Experimental and quasiexperimental designs for generalized causal inference**. Houghton: Mifflin and Company, 2002.

SHOAIB, M. *et al.* Strain differences in the rewarding and dopamine-releasing effects of morphine in rats. **Psychopharmacology**, USA, v. 117, p. 240–247, 1995. Disponível em: https://doi.org/10.1007/BF02245193

SCHOENMAKERS, T. *et al.* Effects of a low dose of alcohol on cognitive biases and craving in heavy drinkers. **Psychopharmacology**, USA, v. 197, p. 169–178, 2008. Disponível em: https://doi.org/10.1007/s00213-007-1023-5

SHULTZ, J. *et al.* Retardation of ethanol absorption by food in the stomach. **Journal of Studies on Alcohol**, USA, v. 41, p. 861–870, 1980. Disponível em: https://doi.org/10.15288/jsa.1980.41.861

SINHA, R. *et al.* Enhanced negative emotion and alcohol craving and altered physiological responses following stress and cue exposure in alcohol dependent individuals. **Neuropsychopharmacology**, USA, v. 34, p. 1198–1208, 2009. Disponível em: https://doi.org/10.1038/npp.2008.78

SLEIDRECHT, W. *et al.* Alcohol use disorder relapse factors: A Systematic review. **Psychiatry Research**, USA, v. 278, p. 97–115, 2019. Disponível em: https://doi.org/10.1016/j.psychres.2019.05.038

SMITH, M. A. Nonhuman animal models of substance use disorders: Translational value and utility to basic science. **Drug and Alcohol Dependence**, USA, v. 206, article 107733, 2020. Disponível em: https://doi.org/10.1016/j.drugalcdep.2019.107733

SPITERI, N. Circadian patterning of feeding, drinking and activity during diurnal food access in rats. **Physiology & Behavior**, USA, v. 28, p. 139–147, 1982. Disponível em: https://doi.org/10.1016/0031-9384(82)90115-9

STURTEVANT, R. P.; GARBER, S. L. Light-dark and feeding regimens affect circadian phasing of blood-ethanol decay rates. **Pharmacology, Biochemistry & Behavior**, USA, v. 13, p. 637–642, 1980. Disponível em: https://doi.org/10.1016/0091-3057(80)90005-2

STURTEVANT, R. P.; GARBER, S. L. Meal timing dominates the lighting regimen as a synchronizer of the circadian blood ethanol clearance rate rhythm in rats. **The Journal of Nutrition**, USA, v. 111, p. 2000–2005, 1981. Disponível em: https://doi.org/10.1093/jn/111.11.2000

STURTEVANT, R. P.; GARBER, S. L. Circadian rhythms of alcohol dehydrogenase and MEOS in the rat. **Experimental Biology and Medicine**, USA, v. 175, p. 299–303, 1984. Disponível em: https://doi.org/10.3181/00379727-175-3-rc2

SWERDLOW, N. R. *et al.* Conditioned drug effects on spatial preference: critical evaluation. *In*: Boulton, A. A.; Baker, G. B.; Greenshaw A. J. (ed). **Neuromethods**: psychopharmacology. Clifton: Humana Press, 1989, p. 399–446.

SYLVESTRE, M.-P. *et al.* Discrete time measures versus trajectories of drinking frequency across adolescence as predictors of binge drinking in young adulthood: a longitudinal investigation. **British Medical Journal Open**, England, v. 10, article e035939, 2020. Disponível em: https://doi.org/10.1136/bmjopen-2019-035939

TOWNSHEND, J. M.; DUKA, T. Attentional bias associated with alcohol cues: Differences between heavy and occasional social drinkers. **Psychopharmacology**, USA, v. 157, p. 67–74, 2001. Disponível em: https://doi.org/10.1007/s002130100764

TZSCHENTKE, T. M. Measuring reward with the Conditioned place preference paradigm: A comprehensive review of drug effects, recent progress, and new issues. **Progress in Neurobiology**, USA, v. 56, p. 613–672, 1998. Disponível em: https://doi.org/10.1016/s0301-0082(98)00060-4

TZSCHENTKE, T. M. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. **Addiction Biology**, USA, v. 12, p. 227–462, 2007. Disponível em: https://doi.org/10.1111/j.1369-1600.2007.00070.x

VAILLANT, George. **The natural history of alcoholism revisited**. Massachusetts: Harvard University Press, 2009.

VAILLANT, G. E.; HILLER-STURMHÖFEL, S. The natural history of alcoholism. **Alcohol Health & Research World**, USA, v. 20, p. 152–161, 1996.

WALKER, B. M.; EHLERS, C. L. Age-related differences in the blood alcohol levels of Wistar rats. **Pharmacology, Biochemistry & Behavior**, USA, v. 91, p. 560–565, 2009. Disponível em: https://doi.org/10.1016/j.pbb.2008.09.017

WASIELEWSKI, J. A.; HOLLOWAY, F. A. Alcohol's interactions with circadian rhythms. A focus on body temperature. **Alcohol Research & Health**, USA, v. 25, p. 94-100, 2001.

WEBB, I. C. *et al.* Diurnal variations in natural and drug reward, mesolimbic tyrosine hydroxylase, and clock gene expression in the male rat. **Journal of Biological Rhythms**, USA, v. 24; p. 465–476, 2009. Disponível em: https://doi.org/10.1177/0748730409346657

WEBBER, E. S. *et al.* Relative reward effects on operant behavior: Incentive contrast, induction and variety effects. **Behavioural Processes**, England, v. 116, p. 87–99, 2015. Disponível em: https://doi.org/10.1016/j.beproc.2015.05.003

WHITE, N. M. *et al.* Operationalizing and measuring the organization of drugs on behavior. *In*: Bozarth, M. A. (ed). **Methods of assessing the reinforcing properties of abused drugs** (Bozarth MA Ed). New York: Spring-Verlag, 1987, p. 591–617.

WILKINSON, P. K. *Et al.* Pharmacokinetics of ethanol after oral administration in the fasting state. **Journal of Pharmacokinetics and Biopharmaceutics,** USA, v. 5, p. 207–224, 1977. Disponível em: https://doi.org/10.1007/BF01065396

APÊNDICES AO CAPÍTULO 1

SEÇÃO METODOLÓGICA SUPLEMENTAR: APÊNDICES S1 A S7

Appendix S1: PRISMA Checklist (Page et al., 2021)

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Manuscript (Title)
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Manuscript (Abstract)
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Manuscript (Introduction)
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Manuscript (Introduction)
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Manuscript (Discussion), Appendices S2, S3, S6, and S7
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Appendix S2
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Appendix S2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Appendix S2
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Appendices S2 and S14
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Appendix S2
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Appendices S2, S13, and S14

Section and Topic	Item #	Checklist item	Location where item is reported
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Appendices S4 and S12
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Appendices S2, S3, and S14
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Appendices S2 and S3
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Appendices S2, S3, S13, and S14
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Appendices S2, S3, S13, and S14
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Appendices S3 and S4
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Manuscript (Discussion), Appendices S3, S7, S8, S9, S10, S11, and S12
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Appendix S3
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Appendices S4, S12, and S13
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Appendices S3, S4, S12, and S14
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Manuscript (Results), Appendices S13 and S14
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	None
Study characteristics	17	Cite each included study and present its characteristics.	Manuscript (Discussion), Appendices S3, S13, and S14
Risk of bias	18	Present assessments of risk of bias for each included study.	Appendices S12

Section and Topic	Item #	Checklist item	Location where item is reported
in studies			and S13
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Appendices S3 and S14
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Appendices S12 and S13
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Manuscript (Discussion), Appendices S7, S8, S9, and S12
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Manuscript (Discussion), Appendices S3, S7, S10, S11, and S12
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	None
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Appendix S12
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Appendices S3 and S14
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Manuscript (Discussion and Conclusions), Appendices S8, S9, S10, and S11
	23b	Discuss any limitations of the evidence included in the review.	Manuscript (Discussion and Limitations), Appendices S3, S10, S11, and S12
	23c	Discuss any limitations of the review processes used.	Manuscript (Limitations), Appendices S3 and S12
	23d	Discuss implications of the results for practice, policy, and future research.	Manuscript (conclusions)

Section and Topic	Item #	Checklist item	Location where item is reported
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Appendix S2
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Appendix S2
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Appendix S2 and S5
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Manuscript (Funding Source)
Competing interests	26	Declare any competing interests of review authors.	Manuscript (Conflict of Interest)
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Manuscript (Supporting information)

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

Appendix S2: Search strategy, screening, and selection of articles and experiments, and data extraction procedure

Originally, as the review planning and execution begun before 2020, its protocol was based on the PRISMA statement and checklist of 2009 (Moher et al., 2009). However, due to journal demands, post-review changes were made in the protocol report so that it could occur according to the PRISMA statement and checklist of 2020 (Page et al., 2021). For the reader, just the adapted protocol and the PRISMA checklist of 2020 are presented (**Appendix S1**).

The systematic review protocol has not been registered.

Search

In October of 2019 we conducted a systematic literature search in (a) PubMed, (b) the reference lists of comprehensive and indexing reviews of PC (Schechter and Calcagnetti, 1993, 1998; Tzschentke, 1998, 2007), and in (c) our laboratory archive. Last search/consult occurred in October (5th) for PUBMED and August (15th) for the other two sources. We used the same filters of language (reports published in English, Portuguese, and Spanish) and date of publication (reports published from January 1970 to September 2019) for all search processes:

PubMed

We identified articles by the algorithm: ((("Ethanol"[Mesh] OR "Ethyl Alcohol" OR "Alcohol" OR “Grain Alcohol”) **AND** ("place conditioning" OR "place aversion" OR "place preference") **AND** ("Rats"[Mesh] OR "Rat" OR "Rattus" OR "Rattus norvegicus" OR "Laboratory Rat" OR "Laboratory Rats"))).

Reference lists of PC reviews

We identified articles in references by screening titles for “alcohol” OR “ethanol” OR “acetaldehyde” OR “sasolinol”.

Laboratory archive

We identified articles by screening titles and abstracts according to the algorithm: ((“ethanol” OR “alcohol”) **AND** (“place conditioning” OR "place aversion" OR "place preference" OR “spatial preference” OR “spatial aversion”)).

Eligibility criteria and selection of articles and experiments

After the exclusion of duplicates, 2 reviewers (DM and PB) independently conducted assessment for article eligibility (inclusion criteria) and experiment selection (exclusion criteria). We conducted screening for eligible articles in a stepwise manner, in which titles and abstracts were screened first for attendance of the publication inclusion criteria (**Table S1**). If the record conformed to these criteria, we subsequently screened the “Materials and

methods" for attendance of the sample composition and intervention inclusion criteria. Thus, we defined eligible articles as those conforming to all these inclusion criteria. Next, we conducted selection of PC experiments from eligible articles by a full-text screening (again, in a step-wise manner). We defined an experiment as each independent experimental group since both within-subject and between-subject measures have been used to quantify relative change in the primary behavioral unit (i.e., relative change of permanence time in the conditioning contexts) in the PC literature. This experiment definition has also been used by Bardo et al. (1995). An experiment was excluded if it attended any of the pertinent 20 exclusion criteria (**Table S1)**. An eligible article was excluded if all its reported experiments attended any of these criteria. Disagreements between reviewers were solved by discussing with the last authors (JL, ML).

Data extraction procedure and extracted data

After the experiment/article selection phase, the first two and the last two authors read a random sample of 15 selected articles and stablished the data categories to be extracted through discussion. Following, the first two authors conducted the data extraction independently according to piloted forms. In cases where there was a discrepancy between the two independent extractions, a new reading of the referring data was performed, and extraction correction performed. **Table S2** provides the list of variables extracted.

Table S1 Inclusion and exclusion criteria for article and experiment selection

Criterion class	Inclusion criteria	Exclusion criteria[1]
Publication type	Peer-reviewed articles reporting original experimental research on alcohol-induced PC	- Review articles, congress abstracts, editorials, book chapters
Publication date	Articles in journal volumes and/or issues published from 1 January 1970 to 30 September 2019	- Exclusion of articles published before 1 January 1970 or after 30 September 2019
Publication language	Articles published in English, Portuguese, and Spanish	- Exclusion of articles published in other languages
Sample composition*	Outbred male rats with an age of at least 42 post-natal days and/or weighting at least 135 g at the beginning of the alcohol-induced PC protocol, drug-naïve or pre-exposed to no other drug/substance/agent but alcohol	- Experiments addressing alcohol-induced PC in other species - Experiments addressing alcohol-induced PC in selected, isogenic, or transgenic rat strains - Experiments addressing alcohol-induced PC in outbred rat samples composed only of females or of males and females indiscriminately - Experiments addressing ethanol-induced PC in samples composed of male rats weighting less than 135 g or aged less than 42 post-natal days at the beginning of the PC protocol
Intervention	Alcohol-induced PC conducted according to the "absolute measure PC procedure"[2] and according to 1st-order conditioning, by parenteral (intraperitoneal, ip) and enteral (orogastric gavage, og; intragastric intubation, ig) passive administration of alcohol during conditioning	- Experiments addressing alcohol-induced PC but implementing other behavioral models/tests before or concomitantly with the PC protocol - Experiments addressing the effects of alcohol on PC induced by other drugs, substances, or agents - Experiments addressing the effects of other drugs, substances, or agents on alcohol-induced PC, except for experiments in which animals were pre-exposed to alcohol or sham-treated with other drugs, substances, or agents** - Experiments addressing PC induced by alcohol-other-drug solution or concomitant administration/presentation of other drugs, substances, or agents with aversive or reinforcing properties (e.g., food) - Experiments in which 2 or more animals were placed simultaneously in the same conditioning chamber or the same PC apparatus - Experiments addressing alcohol-induced PC by routes of administration other than parenteral (ip) and enteral (og and ig) for a passive administration of alcohol for the conditioning phase - Experiments addressing alcohol-induced PC instated by 2nd order conditioning - Experiments addressing alcohol-induced PC exclusively instated by instrumental conditioning or voluntary/forced consumption of alcohol during conditioning trials - Experiments addressing alcohol-induced PC protocols with interstimulus intervals longer than |120| minutes - Experiments addressing alcohol-induced PC in which alcohol administration happened for the post-conditioning test
Report of methods and procedures		- Reports of alcohol-induced PC experiments in rats in which insufficient or non-solvable contradicting information is given on animals' age/weight, sex, conditioning procedures, PC apparatus, drug factors (dose, solution concentration, and adm. route)[3].
Outcome		- When the experiment of alcohol-induced PC was composed of more than one PC procedure

(conditioning trials divided into blocks by test sessions) for the same group of animals, exclusion of data from the second procedure onwards, except when the data of post-conditioning tests are reported only as a joint mean (in these cases, procedures were considered as one until the last test composing the joint mean and were included in the review under such consideration)***

- When the experiment of alcohol-induced PC had more than one conditioning test (one immediately following the other), exclusion of data from the second test onwards, except when the data of post-conditioning tests are reported only as a joint mean (in these cases, the tests were considered as one until the last test composing the joint mean and were included in the review under such consideration)***
- Exclusion of secondary behavioral outcomes (e.g., number of visits to the drug-paired context, locomotor activity) from review***
- Exclusion from review of experiments with duplicated outcomes
- Exclusion from review of experiments for which no information is given on the centrality and dispersion statistical measures of the "preference measure" or on the statistical test used to evaluate such preference or on the results of statistical assessment of changes in preference[3].

---

1 As an eligible article may report more than one PC experiment, exclusion criteria were applied primarily to PC experiments (the exceptions are the exclusion criteria of publication type, date, and language, which were directly screened in the front page, titles, and abstracts of records found). In this way, an experiment was excluded if it attended any of the last 20 exclusion criteria. On the other hand, an article was excluded if (a) it attended any of three first exclusion criteria or (b) if all of its reported experiments attended any of the last 20 exclusion criteria. For other cases, articles were included in the review but only in respect to experiments not attending any of the exclusion criteria. **2** "Absolute measure PC procedure" is defined as "place conditioning studies [in which, for the intervention group], one context is paired with the treatment the rewarding effect of which is intended to be examined, and another context is paired with a neutral, 'control' treatment" (Tzschentke, 2007, p. 242). **3** For these cases, when the article was published from 2000 onwards, we tried to contact the article authors (or, where appropriate, the manufactures of the PC apparatuses) via e-mail requesting the missing information/clarification of the contradiction detected. When the article was published before the 2000s or when we did not receive replies to our contacts, we excluded the experiment/article from the review. * In the case of sample composition: **(a)** the age-weight exclusion criterion was established based on (1) the ontogeny of the hepatic alcohol and aldehyde dehydrogenases (ADH and ALDH, respectively) and of the microsomal ethanol oxidizing system (MEOS) (in their specific activities, ADH and ALDH attain stability at the 5th postnatal week, while MEOS attains it at the 6th postnatal week: Alcorn et al., 2007; Horton and Mills, 1979; Lad et al., 1984; De Zwart et al., 2008), (2) the onset of puberty and attainment of sexual maturation (for males, mean age is ~42 postnatal days and range is 36 to 60 days: Bolton et al., 2015; Sengupta, 2011; 2013), (3) the stages of normal brain development in the rat (stages started and completed before 42 postnatal days: cell proliferation and migration, apoptosis, pruning, synaptogenesis; stage started before and completed after 42 postnatal days: myelination) (Babikian et al., 2010; Winzer-Serhan, 2008), and (4) the smallest weight reported for 42-days-old outbred males rats (Cossio-Bolaños et al., 2013; Lillie et al., 1996). This criterion led to the exclusion of all experiments reported in 12 articles (Degoulet et al., 2016; Miller et al., 1990; Miranda-Morales et al., 2014; Molina et al., 1996; 2007; Nizhnikov et al., 2006; 2014; Pautassi et al., 2002; 2006; 2008; 2012a; 2012b); **(b)** the non-outbred rat strain exclusion criterion was established based on the data that selection of different strains for high (UchB, P, AA, HAD, sP strains) and low (UchA, ANA, NP, LAD, snP strains) alcohol drinking phenotypes, for example, leads to differential segregation of other behavioral phenotypes (e.g., learning capacity, stress sensibility, high depressive- and anxiety-like behaviors, and so on) even within the same alcohol drinking phenotype (i.e., between high-drinking strains themselves and between low-drinking strains themselves), that otherwise are assumed to be normally distributed in outbred populations (Ciccocioppo, 2013; Ciccocioppo, Panocka, Froldi, Colombo et al., 1999; Li et al., 1993; 2001). This criterion led to the exclusion of all experiments reported in 11 articles (Acevedo et al., 2013; Ciccocioppo Panocka, Froldi et al., 1999; Ciccocioppo Panocka, Polidori et al., 1999; Colombo et al., 1990; Fadda et al., 1999; Gauvin et al., 2000; Quintanilla and Tampier, 2011; Schechter, 1992; Schechter and Krimmer, 1992; Stewart et al., 1996; Xu et al., 2012); **(c)** the exclusion of rat samples composed only of females or of males and females indiscriminately was established on basis that, in comparison to males, female rats show (1) different neurohormonal regulation of body weight, energy balance, body fat distribution, and ADH activity, leading them to have (2) a 1.5 to 2.5 more active **(**ADH, (3) faster alcohol clearance rates for mature females (immature and older females do not present

differences in outbred strains), (4) a 180° phase shift in the chronovariation of ADH activity, (5) different heterotopy of alcohol metabolism in the liver (which may imply different susceptibility to alcoholic liver injury), (6) different influences of the age-weight covariate and body water relative content on alcohol distribution (and probably on acetaldehyde distribution if it accumulates in the blood), as well as (7) influence of the estrous cycle on alcohol distribution to peripheric tissue (Applegate et al., 1982; Collins et al., 1975; Cortright et al. 1997; Harada et al., 1998; Kim et al., 2003; Maly and Sasse, 1985; Mayes and Watson, 2004; Nedungadi and Clegg, 2009; Robinson et al., 2002; Salsano et al., 1990; Shi and Clegg, 2009; Simon et al., 2001; Tagliaferro et al., 1986). Lastly, adult female rats also seem to present (8) a different sensibility to the motivator function of alcohol in two-bottle choice and learning experiments, as well as (9) different negative affects and neuronal activity after acute and prolonged alcohol abstinence (Juárez and Barrios De Tomasi, 1999; Lancaster and Spiegel, 1992; Lancaster et al., 1996; Li et al., 2019; Schramm-Sapyta et al., 2014). This criterion led to the exclusion of all experiments reported in 8 articles — 5 using outbred (Fu et al., 2016; Roma and Riley, 2005) and non-outbred (de Carvalho et al., 2010; Quintanilla et al., 2014; 2016) females and 3 using outbred males and females indiscriminately (Barbier et al., 2009; Lee et al., 1998; Philpot et al., 2003) — as well as to the exclusion of 14 PC experiments with outbred females — 12 (8 for normal and 4 for ovariectomized adults) reported by Torres et al. (2014) and 2 reported by Nentwig et al. (2017). ** This criterion does not include drugs (anesthetics, anti-inflammatory, and antibiotics) used for surgical operations. *** These exclusion criteria are referent to exclusion of information from the review, not to experiment/article exclusion per se. **Note 1**: The search process also returned records reporting no alcohol-induced PC interventions. However, given the use of a stepwise-check procedure, attendance to any of the sample composition exclusion criteria was checked before attendance to any of the intervention exclusion criteria. For these reasons, some articles excluded due to sample composition may not include PC interventions. In the same fashion, an article/experiment whose exclusion is identified according to one exclusion criterion of the sample composition may also attend other exclusion criteria (whether or not these other criteria also belong to the sample composition class). **Note 2:** In the case of the sample composition and intervention classes, the pertinence evaluation of exclusion criteria (as well as of their proximal reasons) must take into account (1) the requirement that these criteria be specified in advance during the composition of the protocol, (2) the characteristics, proper use, limits, and analysis unit of the synthesis method chosen (see **Appendices S3** and **S6**), (3) the predominant characteristics of rats and interventions composing the staggering majority of the drug-PC experiments (for an example of strain and sex, see Bardo et al., 1995), and (4), because we are dealing with a learning model for alcohol, the consistence of differences as well as confounding factors in alcohol-related responses/variables, associative learning, and general behavioral profile reported for the age-weight covariate, strain, and sex.

Table S2 Description of data extracted

Class of Data	Data description
Article-related data	Article citation, publication date, and country of research.
Measurement characteristics of behavioral preference	Statements of PC group allotment randomization, statement of baseline score matching, statement of randomization of associative conditions (for balanced stimulus assignment), preference index, experimental design (one-sample with theoretical mean, one-sample with repeated measures or independent-groups design), type of independent control group (pharmacological or psychological), time dimension of preference change isolation or testing (before-after or after conditioning), measurement method of the principal behavioral unit, measurement method description.
Report quality items	Statement of compliance with regulatory requirements, statement of compliance with the ARRIVE guidelines (Kilkenny et al., 2010), sample size calculation method, statement regarding possible conflict of interests, randomization of allocation to the PC intervention, exact sample size description for the PC intervention, and blinded or automated assessment of the PC primary outcome.
Animal-related data	Rat strain, sex, age (days), weight (g), time to start PC from rats' arrival in laboratory facilities (days), type of housing (single *vs.* group + number of animals per cage), light-dark cycle (hours/hours + time of lights on).
APE intervention	Full description of the alcohol pre-exposure procedure and the temporal gap between its end and the beginning of the alcohol-induced PC experiment.
PC intervention covariables	PC timetable (for conditioning and post-conditioning phases), apparatus dimensions (Length x Width x Depth, cm), conditioning chamber dimensions (Length x Width x Depth, cm), apparatus area (cm^2), apparatus' number of chambers (for the conditioning phase), apparatus bias statement, reason for bias statement, baseline report, bias measurement, animal exclusion "to correct" bias, cue stimulatory modality, route of alcohol administration (intraperitoneal, orogastric gavage, intragastric), alcohol solution concentration (v/v, %), volume of alcohol solution administered (ml/kg), alcohol dose administered (g/kg), number of habituation sessions to the PC apparatus without to-be-conditioned cues, number of habituation sessions to the PC apparatus with to-be-conditioned cues, number of baseline test sessions, number of conditioning trials, interphasic interval (hours) between the last habituation session to the apparatus and the first conditioning trial, habituation session duration (min), baseline session duration (min), conditioning trial duration (min), post-conditioning test duration (min), cue-drug-pairing assignment distribution (balanced, unbalanced, combined), cue-drug-pairing assignment criteria (none, baseline preferred chamber, baseline non-preferred chamber, fixed assignment), conditioning trial order (balanced order, vehicle-alcohol order, alcohol-vehicle order), interstimulus interval (min), figures and tables showing the primary PC outcomes, textual descriptions of statistical evaluations of the PC primary outcome.
PC primary outcome	PC primary outcome is defined as the relative change in preference as indicated by the behavioral unit, i.e., relative change (or difference) in permanence time in the conditioning chambers during the post-conditioning test in the intervention group in reference to a control measure (theoretical mean or repeated measure) or control group (independent measure). This outcome was extracted both categorically (i.e., as CF, CPA, and CPP) and according to statistical measures of centrality (mean) and dispersion (standard deviation and/or error). For the categorical extraction, categories were defined by the significance and sign of the change (or difference) detected in the statistical test: a) CF = no statistically significant change (or difference) detected in the post-conditioning test of the intervention group in comparison with a control measure (theoretical mean or repeated measure) or control group (independent measure procedure) b) CPA = statistically significant decrease (negative change/difference) in the post-conditioning test of the intervention group in comparison with a control measure (theoretical mean or repeated measure) or control group (independent measure procedure) c) CPP = statistically significant increase (positive change/difference) in the post-conditioning test of the intervention group in comparison with a control measure (theoretical mean or repeated measure) or control group (independent measure procedure) Statistical measures of centrality and dispersion were extracted from texts, tables, and figures. In the case of figures, we used the Web Plot Digitizer (version 4.5) to conduct extraction. We also extracted the number of animals composing the intervention (for all experimental designs) and the control (for the

	independent-groups design) groups.
Other intervention covariables	**Non-exposure interventions:** Restriction of food or water consumption, surgical operations (anesthetic, anti-inflammatory, and antibiotic agents not considered). **Covariables of exposures to vehicles and pharmacologically inactive agents happening before the PC protocol:** route of administration, solution concentration administered (v/v %), solution volume administered (ml/kg), dose administered (g/kg), compound administered, number of exposure sessions, exposure session duration (min), intertrial interval between exposure sessions (hours), interphasic interval between last exposure session and first habituation session to the PC apparatus (hours), interphasic interval between last exposure session and first conditioning session of the PC protocol (hours). **Covariables of exposures to vehicles and pharmacologically inactive agents happening during the PC protocol:** route of administration, solution concentration administered (v/v %), solution volume administered (ml/kg), dose administered (g/kg), compound administered, number of exposure sessions, exposure session duration (min), intertrial interval between exposure sessions (hours), interphasic interval between exposure sessions and habituation sessions of the PC protocol (min), interphasic interval between exposure sessions and conditioning sessions of the PC protocol (min), interphasic interval between the exposure session and the post-conditioning test of the PC protocol (min).

PC, place conditioning; APE, alcohol pre-exposure; CPA, conditioned place aversion; CPP, conditioned place preference; CF, conditioning failure.

Appendix S3: Assessing the measurement characteristics of behavioral preference and its change in the reviewed literature

To decide which type of research synthesis (McKenzie et al., 2019) we would use, we assessed the measurement characteristics of the selected literature (spreadsheet 02 of **Appendix S13**). This assessment requires some definitions beforehand for the reader to understand it. Thus, we first talk about the requirements to measure a change in behavioral preference between two spatial options and, in front of these requirements, of how some choices made by researchers constitute problems for statistical synthesis. Afterward, we talk about the sources of error in measuring the principal behavioral unit present in the reviewed literature of ethanol-induced place conditioning (PC) in rats.

Although researchers do not agree on which associative learning process (Pavlovian or operant) is primarily responsible for PC (Huston et al., 2013), few would argue that an experiment of drug-induced PC (following the "absolute measure procedure"[8]) is not a test of learned choice/preference behavior in reference to the drug-paired and drug-unpaired contexts. If this is so, attendance to three measurement requirements is necessary for this conception.

The first requirement is proper to the measurement definition of a preference or choice between two simultaneously available and interconnected spatial options (i.e., the drug-paired and drug-unpaired chambers): since the scores in the spatial options are interdependent, a measure of spatial preference is a relative measurement. This means that a proper preference measure in PC is not given by a behavioral score referencing only the drug-paired chamber, for example, but by the relation of this score with the one obtained in the drug-unpaired chamber. In this case, a readily interpretable and accurate formula for this interdependence-based relative property is $\boldsymbol{a/(a+b)}$, where $\boldsymbol{a}$ is the score in one conditioning chamber (the drug-paired chamber) and $\boldsymbol{b}$ is the score in the other conditioning chamber (the drug-unpaired chamber). Yet, one could argue for a ratio relation $\boldsymbol{\log a/b}$ or a subtraction relation $\boldsymbol{a-b}$. These, however, would not be accurate because the first formula does not state interdependence and the second treats the scores as non-interdependent ones (when they are not). Therefore, for example, the $\boldsymbol{a-b}$ relation not only tends to increase the range of residuals but also (and counterintuitively) increases the chances of statistically detecting a significant preference

[8] "Absolute measure PC procedure" is defined as "place conditioning studies [in which, for the intervention group], one context is paired with the [drug] treatment the rewarding effect of which is intended to be examined, and another context is paired with a neutral, 'control' treatment" (Tzschentke, 2007, p. 242).

(say, when testing apparatus bias in a one-sample *t*-test) for one chamber to the other when there is none.

The second requirement is the behavioral unit per se to be quantified and operationalized in a preference measure. In the early investigation of drug-induced PC, researchers used discrete choice trials between the spatial options to test preference, either before and/or after conditioning, and therefore used the number of entries in each conditioning chamber as the principal behavioral unit (Beach, 1957a; 1957b). Since the 1970s, however, the preference test of PC offers the animals the continuous possibility of choosing to enter, stay in, and exit each apparatus chamber, either before and/or after conditioning (Black et al., 1973; Kumar, 1972; Rossi and Reid, 1976). Accordingly, most researchers using the continuous test have adopted the "total time spent in the conditioning chambers" as the principal behavioral unit.

For a preference measure, this adoption came with two advantages for one- and two-chamber apparatuses: the total interdependency of scores ***a*** and ***b*** (i.e., decreases in one quantity necessarily produce equivalent increases in the other) and the consequential equality between ***(a+b)*** and the total duration of the PC test. These properties allowed the preference measure of ***a/(a+b)*** to be simplified without loss/change of information by virtually any other partial or nonspecific measure of preference between the conditioning chambers. For independent-groups designs, this is true for "score ***a***", "score ***a*** divided by the total time of test", and even ***a-b***, but it is not for ***log a/b***. This is shown in the simulated data in **Table S3**. Again, this is the case for these apparatuses because the times spent in both contexts are totally interdependent. In front of this, these different quantities and data transformations have proliferated as the now the so-called "preference indices" in the drug-induced PC literature as a whole (Bardo et al., 1995; Carr et al., 1989; Cunningham et al., 2003) and in the alcohol-induced PC literature herein reviewed.

Table S3 Simulated data for independent-groups post-test pharmacological design in an unbiased 2-chamber apparatus

Groups and comparisons	**Score a**	**Score b**	**a/(a+b)**	**a-b**	**log a/b**	**a/total time**
Mean scores (± SD) of control group (n = 12)	492.105 ± 53.199	407.895 ± 53.199	0.547 ± 0.059	84.211 ± 106.399	0,083 ± 0.107	0.547 ± 0.059
Mean scores (± SD) of intervention group (n = 12)	656.596 ± 63.166	243.404 ± 63.166	0.729 ± 0.070	413.192 ± 126.331	0.443 ± 0.159	0.729 ± 0.070
t-test between-group design (pharmacological control)	$T_{(22)}$ = 6.900, ***p* < .001**, 95% CI [115.049, 213.931]	-	$t_{(22)}$ = 6.900, ***p* < .001**, 95% CI [.128, .238]	$t_{(22)}$ = 6.900, ***p* < .001**, 95% CI [230.099, 427.863]	$t_{(22)}$ = 6.498, ***p* < .001**, 95% CI [.245, .475]	$t_{(22)}$ = 6.900, ***p* < .001**, 95% CI [.128, .238]

The total post-test time is 15 min (900 seconds; scores ***a*** and ***b*** given in seconds). The data simulation (randomly normally distributed) was done for a CPP outcome in ***a/(a+b)***. Data applies to unbiased one- and two-chamber apparatuses. Statistical comparisons presented were done for a 2-tailed *t*-test for independent-groups post-test pharmacological design. For this design, the *t*-test value indicates that only the "preference index" ***log a/b*** presents an error to ***a/(a+b)***. The "preference index" ***a-b*** presents an error to ***a/(a+b)*** in a repeated post-test design (simulations not shown).

Table S4 Simulated data for independent-groups post-test pharmacological design in an unbiased 3-chamber apparatus

Groups and comparisons	**Score a**	**Score b**	**a/(a+b)**	**a-b**	**log a/b**	**a/total time**
Control group (n = 12) mean scores (± SD)	328.446 ± 71.169	307.175 ± 72.213	0.517 ± 0.069	21.271 ± 90.601	0,031 ± 0.123	0.365 ± 0.079
Intervention group 1 (n = 12) mean scores (± SD)	458.808 ± 67.487	159.725 ± 62.819	0.745 ± 0.093	299.083 ± 103.972	0.497 ± 0.245	0.510 ± 0.075
Intervention group 2 (n = 12) mean scores (± SD)	445.785 ± 56.027	228.511 ± 59.030	0.663 ± 0.066	217.274 ± 84.344	0.303 ± 0.137	0,495 ± 0.062
Intervention group 3 (n = 12) mean score (± SD) in seconds	434.577 ± 94.240	307.808 ± 119.445	0.591 ± 0.134	126.770 ± 200.374	0.169 ± 0.248	0.483 ± 0.105
Statistical comparison in *t*-test for between-group design (intervention 1 – control; pharmacological control)	t_{22} = 4.604, ***p* < .001**	-	t_{22} = 6.834, ***p* < .001**	t_{22} = 6.978, ***p* < .001**	t_{22} = 5.892, ***p* < .001**	t_{22} = 4.604, ***p* < .001**
Statistical comparison in *t*-test for between-group design (intervention 2 – control; pharmacological control)	t_{22} = 4.488, ***p* < .001**	-	t_{22} = 5.291, ***p* < .001**	t_{22} = 5.485, ***p* < .001**	t_{22} = 5.095, ***p* < .001**	t_{22} = 4.488, ***p* < .001**
Statistical comparison in *t*-test for between-group design (intervention 3 – control; pharmacological control)	t_{22} = 3.113, ***p* = .005**	-	t_{22} = 1.698, *p* = .104	t_{22} = 1.662, *p* = .111	t_{22} = 1.734, *p* = .097	t_{22} = 3.113, ***p* = .005**

The total post-test time is 15 min (900 seconds; scores ***a*** and ***b*** given in seconds). The data simulations (randomly normally distributed) were done for a CPP outcome (first two intervention groups) and a conditioning failure (last one) in ***a/(a+b)*** according to three scenarios of coupling between scores ***a*** and ***b*** in the intervention group (coupling is defined in footnote 5). Data applies to unbiased three-chamber apparatuses. Statistical comparisons presented were done for a 2-tailed *t*-test for independent-groups post-test pharmacological design. For this design, the *t*-test values indicate that all other "preference indices" present an error to ***a/(a+b)***, with the score ***a*** and "score **a**/total time" presenting an equally worrisome error in all scenarios. Confidence intervals may be obtained by the methods shown in Altman and Bland (2011).

Although useful, the interchangeability between these measures for those apparatuses is an inconspicuous trap for apparatuses with more than two chambers. As said before, drug-induced PC is concerned with (a change of) preference between the drug-paired and the drug-unpaired chambers (i.e., with a preference between two options). Therefore, PC deals with only three courses of action in terms of preference[9]: preference for the drug-paired chamber over the drug-unpaired one (conditioned place preference, CPP, after conditioning), preference for the drug-unpaired chamber over the drug-paired one (conditioned place aversion, CPA, after conditioning), and preference for neither (conditioning failure, after conditioning). Given this limitation of PC and the current characteristics of its tests, an apparatus with more than two chambers introduces error in any preference measure of PC based on the total time spent in the conditioning chambers. This is the case since any additional chamber is also an option and influences the time animals may spend in the conditioning chambers, turning the scores in these chambers only partially interdependent[10].

9 Note that for *n* number of options, animals have at least *n+1* courses of action in terms of preference.

10 On the one hand, the theoretical level of relative error introduced is greater for preference measures that do not use the aforementioned ratio relation (i.e., if the ratio relation ***a/(a+b)*** is the proper measure of preference between two options and PC only deals with three courses of action, it follows that it is the preference measure the least affected by the error

Due to the introduction of this error, the relative measure of preference (i.e., ***a/(a+b)***), although related to the other "preference indices", is not interchangeable with them, strictly speaking, in apparatuses with three or more chambers. This is shown in the simulated data in **Table S4**. Thus, although a three-chamber apparatus is a better option for what has been called "time analysis" or "sojourn time analysis" (German and Fields, 2007; Huston et al., 2013; Krauth, 1992), for these apparatuses the "score ***a***", the relations ***a-b*** and ***log a/b***, as well as other ratio relations (e.g., "score ***a*** divided by the total time of test") are not measuring the same preference construct as ***a/(a+b)*** in total time because the scores in the conditioning chambers are partially (instead of totally) interdependent.

The third requirement derives from the fact that PC is a test of learned preference behavior, i.e., it aims to test possible conditioning-induced changes in preference for one conditioning chamber over the other. Given the use of group-based (parametric) inferential statistics, verifying such a change is only possible in an experimental design that tests the change against a control measure of preference (i.e., against a comparator). This not only stipulates the origin of the control measure (a theoretical mean, a repeated measure, an independent control group) but also stipulates in which temporal dimension (posttest *vs.* posttest-pretest) the experimental design isolates and tests the preference change and, in the case of drugs used as the primary motivator, the type of independent control group (pharmacological control groups *vs.* psychological control groups: for definitions of the latter, see Cunningham, 1993). For short PC protocols, the selected literature presents four general classes of experimental designs (**Figure S1**): independent-groups posttest design (with pharmacological and psychological control subclasses), independent-groups posttest-pretest design, single-group with repeated measures design (with posttest-pretest and posttest-posttest subclasses[11]), and single-group posttest with a theoretical mean as a control measure.

As one can see, this is a complex scenario of variables affecting the decisions that researchers must take for measuring preference and its possible change in PC. Nevertheless, after recognizing this difficulty, these decisions pose challenges for a research synthesis of the

---

introduced by a three-chamber apparatus, for example). On the other hand, when comparing ***a/(a+b)*** in different unbiased three-chamber apparatus, the level of the absolute error seems to depend on the total number of chambers, the apparatus' area, the level of experience that animals have with the additional chambers over the PC protocol, and the spatial relations between all chambers in the apparatus (the variables known to affect exploratory behavior, i.e., the other determinant of time spent in the conditioning chambers relevant for this review).

11 In the posttest-posttest subclass of the single-group with repeated measures design, one tests the difference between scores ***a*** and ***b*** or between ratios of these scores (e.g., "***a***/total test time" – "***b***/total test time" and so on) in the posttest. However, as we discussed earlier, scores ***a*** and ***b*** are interdependent in PC. Due to this, these tests are invalid in the single-group with repeated measures design (just as it is invalid their use to isolate the preference change in a design of single-group with a theoretical mean). In this case, calculating the *M* and *SD* of ***a*** (or ***b***, for that matter) is possible for a one- or two-chamber apparatus. Thus, for these apparatuses, one can tentatively compare the posttest ***a*** parameters to a theoretical mean in a one-sample *t*-test. For a three-chamber apparatus, this problem is unsolvable (see ahead).

selected literature. For such a synthesis, in the case of different "preference indices" that are not interchangeable with ***a/(a+b)***, the best solution would be to estimate the mean (*M*) and standard deviation (SD) of ***a/(a+b)*** (or of a measure interchangeable with it) and use them instead of the other "indices". Considering the above discussion, for one- or two-chamber apparatuses the only problematic "indices" would be those of ***a-b*** and ***log a/b***. For ***a-b*** it is possible to estimate both *M* and SD of ***a*** as these apparatuses cause ***(a+b)*** to be equal to the total test time and the SD of ***a-b*** is twice the SD of ***a*** or ***b*** (given that these SDs are equal). For ***log a/b*** only the *M* of ***a*** may be estimated in this way. In this case, the second approach would be to estimate the error ***log a/b*** has to ***a/(a+b)***. The respective *t*-test values of **Table S3** may be used to estimate this error according to the metanalytical method of Rosenthal (1991) and corrections done for a research synthesis (the same is also true for ***a-b*** in other experimental designs).

Otherwise, although the best solution is the same as that for one- or two-chamber apparatuses, the case is more complex for three-chamber apparatuses. Forthright, it is necessary to say that we do not know if it is possible to solve the problems posed by different "indices". On the one hand, all possible solutions (from the best one — calculation of ***a/(a+b)*** *M* and SD — to the estimation of absolute and relative errors) demand raw data availability. For example, for the same apparatus, the zero- and the first-order correlation coefficients between ***a*** and ***b*** would be necessary to estimate the absolute error introduced by the third chamber. As ***a*** and ***b*** are interdependent, these coefficients may be estimated only according to the method shown in Bland and Altman (1995a, 1995b). To our knowledge, this may be calculated only from raw individual data.

On the other hand, the resolution demands some stability in the aforementioned absolute and relative errors between the different "indices". In this case, there is the argument that the absolute error introduced by the third chamber is dependent on the spatial relations of a three-chamber apparatus. Consequently, (absolute and relative) error calculations for a three-chamber apparatus do not apply to other three-chamber apparatuses if these spatial relations are not the same (or at least very similar) between these apparatuses (not to mention the probable influence of habituation sessions to the apparatuses). Even more, we do not even know whether the absolute error is stable throughout the same preference test or across

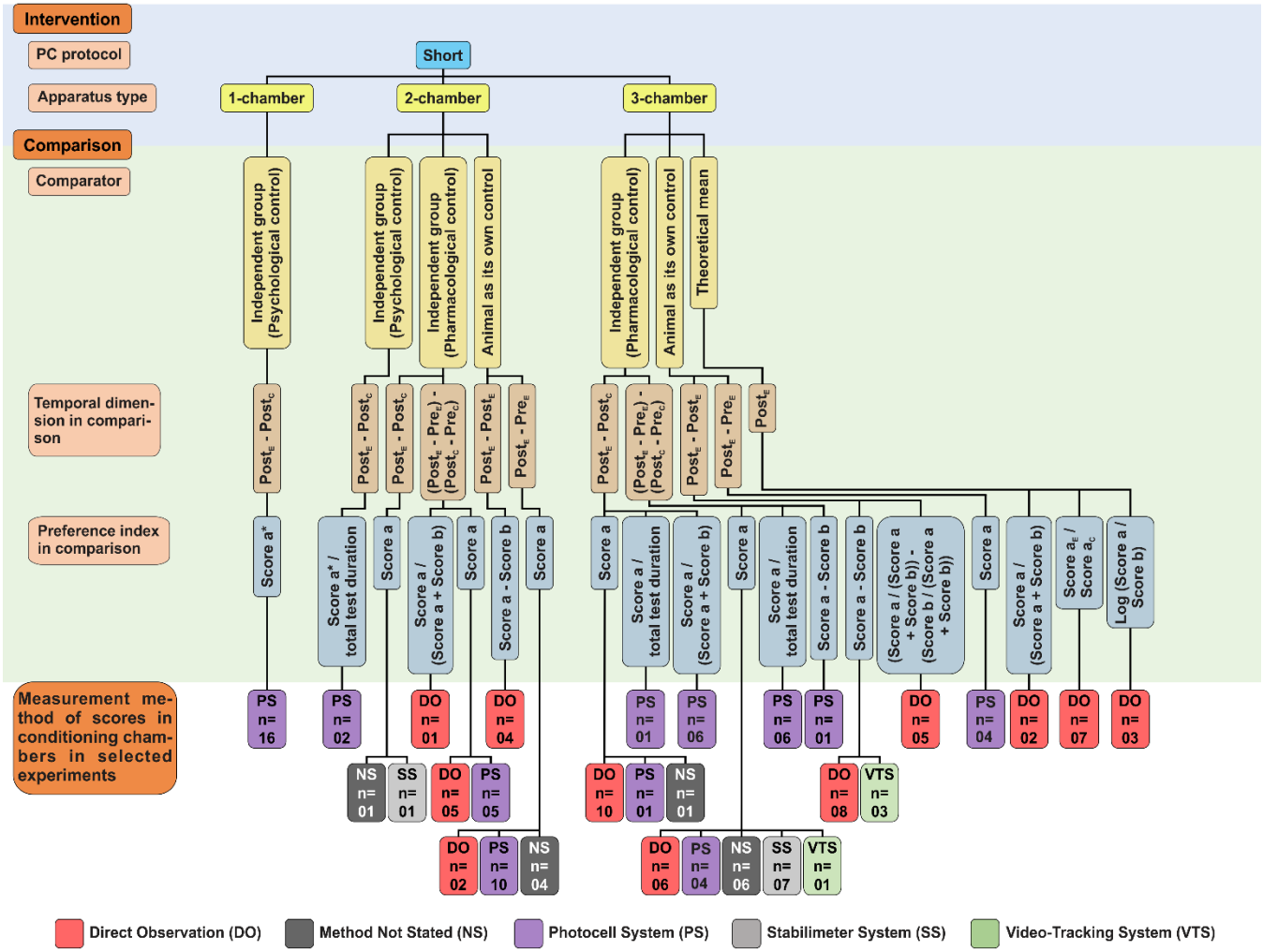


Figure S1 Characteristics of measurements and tests of behavioral preference and its change in short protocols of alcohol-induced PC of the reviewed literature.

Post$_E$, posttest score of the experimental group; **Post**$_C$, posttest score of the control group; **Pre**$_E$, pretest score of the experimental group; **Pre**$_C$, pretest score of the control group; **score a**, temporal score in the alcohol-paired conditioning chamber; **score b**, temporal score in the alcohol-unpaired conditioning chamber; **score a**$_E$ and **score a**$_C$, temporal score in the alcohol-paired conditioning chamber of experimental and control groups, respectively. ***** In the case of the psychological control used for one- or two-chamber apparatuses, as alcohol is paired to a conditioning chamber for both experimental and control groups, the definition of "score a" is given by the alcohol-paired chamber in the experimental group, which is the inverse one to that of the control group. Then, in this independent control procedure, one is in truth comparing "score a" of the experimental group with the "score b" of the control group, following a reverse contingency learning control design. **Note 1:** Determination of independent-groups or single-group designs was done not according to the presence of a control group in the experiment, but by comparator researchers used in statistical tests. Accordingly, the experiments reported by Bahi (2013) and Barbier et al. (2008) were considered as single-group designs. **Note 2**: Characteristics of all PC protocols are given in **Appendix S13** (spreadsheet 02).

different preference tests in a three-chamber apparatus[12]. This is the reason why **Table S4** presents statistical comparisons according to three different scenarios of "stability" (i.e., coupling) in the simulated data, one for each hypothetical intervention group. Moreover, complicating further the issue of stability, the relative error between different "indices" varies not just according to apparatus type but may also vary according to the experimental design (as argued for ***a-b*** in one- or two-chamber apparatuses). This then poses a considerable threat to construct validity (Valentine, 2009) of "preference indices" in the case of three-chamber apparatuses as only 13 experiments (from a total of 82) with these apparatuses used the ***a/(a+b)*** preference measure or other "indices" that permit its calculation.

As for experimental designs, each of the designs present in the literature differently tests conditioning-induced changes and controls for different sources of potential bias[13] (i.e., systematic error). This diversity has consequences for the comparability of effect size estimates in a research synthesis as each design-associated effect size is distinctly defined (in terms of *M* contrasts and SDs). Consequently, they may use different metrics (raw-score *vs.* change-score metrics), are more or less vulnerable to different sources of bias, and estimate the treatment effect with more or less precision (**Table S5**). In the case of different metrics (i.e., d_{IGRS1}, d_{IGRS2}, d_{OSRS}, d_{IGCS}, d_{RMCS}, d_{RMRS}: each effect size estimate/metric is mathematically defined in **Table S5**), effect sizes from alternate designs are comparable only if these SDs are the same or can be transformed into a common parameter. As a raw-score metric is preferable over the change-score one (Morris and DeShon, 2002), transforming change-score metrics to raw-score ones is required. This can be achieved directly or

12 The excellent and insightful sojourn time analysis of German and Fields (2007) for an unbiased three-chamber apparatus (in which all chambers have the same area and are serially organized) suggests that the error is stable for the pre-conditioning test. For the post-conditioning one, however, coupling seems to progressively happen between scores ***a*** and ***b*** if learning (of a morphine-induced CPP, in this case) took place during conditioning. If this coupling is a general phenomenon of drug-induced PC, then the interdependency of scores ***a*** and ***b*** increases progressively in the post-test. This may seem inconsequential at first consideration. However, it has consequences for the level of error that a "preference index" such as "score ***a***" would tend to present when evaluating preference change (in a between-group design with after dimension and pharmacological control), for example.

13 Morris and DeShon (2002) identify the sources of bias as the selection effect (α), the relationship of pre and posttest scores (β), the time effect (γ), and the error in estimation of the population standard deviation (σ). α equals the population difference between the group pretest mean and the population grand mean, where the population grand mean refers to the pretest mean of the common population from which animals are sampled, before any selection, treatment, or other events occur. If animals are randomly assigned to experimental and control conditions, the expected value of α is 0 (in alcohol-induced PC, this random assignment refers to the animal allocation in experimental and control groups as well as to the allotment of alcohol-paired and -unpaired chambers). β is the slope of the relationship between pre- and posttest scores. If pre- and posttest scores have equal variances, β is the within-group correlation (ρ) between the pretest and posttest. Consequently, posttest scores will be influenced to some degree by the individual's pretest score. γ reflects any factors that might systematically alter scores between the pretest and posttest but are unrelated to the treatment. As one can see, β is an especial case of γ. Accordingly, both β and γ may differ across groups in independent-group designs. Error in the estimation of σ happens according to a subject by treatment interaction, which may inflate or deflate posttest variance. Consequently, any effect size that uses posttest standard deviations is susceptible to this form of bias.

indirectly[14]. For the 35 experiments conducted in two-chamber apparatus, this transformation would be necessary for 27 but is only achievable for 10. For the 82 conducted with three-chamber apparatuses, disregarding the validity problem posed by "preference indices", it would be necessary for 35 but is only feasible for 17 of them (i.e., according to the "preference index" used in each case).

Table S5 Study design, effect size metric and estimate, and susceptibility to potential sources of bias*

Study design	Effect size metric	Effect size estimate	Potential bias in estimate of treatment effect			Potential bias in estimate of σ
			Selection effect (α)	Time effect (γ)	Differential time effect (γ_E-γ_C)	Subject *vs.* treatment interaction
Single-group posttest-pretest	Raw-score (d_{RMRS})	$\frac{M_{post,\,E} - M_{pre,\,E}}{SD_{pre,\,E}}$		✓		
	Change-score (d_{RMCS})***	$\frac{M_{post,\,E} - M_{pre,\,E}}{SD_{D,\,E}}$		✓		✓
Single-group posttest-theoretical mean**	Raw-score (d_{OSRS})	$\frac{M_{post,\,E} - M_{pop}}{SD_{post,\,E}}$	✓			✓
Independent-groups posttest	Raw-score (d_{IGRS1})	$\frac{M_{post,\,E} - M_{post,\,C}}{SD_{P,\,post}}$	✓		✓	✓
Independent-groups posttest-pretest	Raw-score (d_{IGRS2})	$\frac{(M_{post,\,E} - M_{pre,\,E})}{SD_{pre,\,E}} - \frac{(M_{post,\,C} - M_{pre,\,C})}{SD_{pre,\,C}}$			✓	
	Change-score (d_{IGCS})	$\frac{(M_{post,\,E} - M_{pre,\,E})}{(SD_{D,\,E})} - \frac{(M_{post,\,C} - M_{pre,\,C})}{(SD_{D,\,C})}$			✓	✓

$M_{post,\,E}$, experimental group posttest mean; $M_{pre,\,E}$, experimental group pretest mean; M_{pop}, estimated population mean; $SD_{pre,\,E}$, experimental group pretest standard deviation; $SD_{post,\,E}$, experimental group posttest standard deviation; $M_{post,\,C}$, control group posttest mean; $M_{pre,\,C}$, control group pretest mean; $SD_{pre,\,C}$, control group pretest standard deviation; $SD_{post,\,C}$, control group posttest standard deviation; $SD_{P,\,post}$, posttest pooled standard deviation; $SD_{D,\,E}$, the standard deviation of posttest-pretest score difference in the experimental group; $SD_{D,\,C}$, the standard deviation of posttest-pretest score difference in the control group; $\boldsymbol{\gamma_E}$-$\boldsymbol{\gamma_C}$, different time effect between experimental and control groups; **σ**, population standard deviation; ✓, relevant sources of bias for the design-associated estimate of effect size. *, adapted from Morris and DeShon (2002). **, the theoretical mean value may be 0, the theoretical chance level (i.e., 50%), or a theoretical 100% level. ***, Lakens (2013) remarks that, as this estimate is conservative, it is preferable to use $d_{av} = (M_{post}\text{-}M_{pre})/((SD_{pre}+SD_{post})/2)$ whenever possible.

Conversely, for the reliable use of these different raw-score metrics in the same synthesis, one needs to assume with strong certainty that the potential sources of bias do not differently impact the raw-score effect size estimates coming from different designs. This assumption, unfortunately, is not warranted for the reviewed literature. For example, according to **Table S5**, the d_{IGRS1} and d_{OSRS} are both vulnerable to the selection effect (α) bias. Here, one may assume α = 0 only when the requirement of group allocation randomization is fulfilled for multigroup studies (notwithstanding if each experimental group is compared to a

14 Directly if the study gives (i) the pretest and posttest *M* and (ii) permits the calculation of pretest SD. In this case, one directly uses the d_{RMRS} or the d_{IGRS} metrics given in **Table S5**. Indirectly, one transforms the d_{RMCS} into a d_{RMRS} (or a d_{IGCS} into a d_{IGRS}) by using the formula $d_{RS} = d_{CS}\sqrt{2(1 - \rho)}$, where ρ is the withing-group correlation between pretest and posttest scores. For each group composing the experimental design, this correlation is given by the formulas $\rho = (SD_{pre}^2 + SD_{post}^2 - SD_D^2)/(2 \times SD_{pre} \times SD_{post})$, $\rho = 1 - (SD_D^2/SD_P^2)$, or $\rho = ((SD_{pre}^2 \times t^2 + SD_{post}^2 \times t^2) - ((M_{post} - M_{pre}) \times n)))/(2 \times SD_{pre}^2 \times SD_{post}^2 \times t^2)$. In this last formula, *t* is the result of one-sample *t* test with repeated measures (Becker, 1988; Dunlap et al., 1996; Higgins et al., 2019; Morris and DeShon, 2002). As SD_D is not derivable from the difference of SD_{pre} and SD_{post} (nor SD_{pre} and SD_{post} are derivable from SD_D; Higgins et al., 2019), and no study gives all of these SDs, indirect estimation of ρ is not possible.

population parameter or a control group). Additionally, as the "absolute measure PC procedure" includes two associative conditions (i.e., drug-paired and drug-unpaired contexts) for each group, the use of a randomized counterbalance allotment of these conditions[15] is also necessary for this assumption to be true. For the 88 and 45 short PC experiments to which d_{IGRS1} and d_{OSRS} estimates are respectively pertinent, group assignment randomization (for multigroup studies) and randomized counterbalancing of conditions were reported in only 27 and 19 experiments, respectively, with only 12 of these reporting both. Even more, d_{IGRS1} and d_{OSRS} could be calculated for only 41 and 39 of these experiments, respectively. For the other 47 and 6 experiments, only other metrics could be calculated and only for some of them: d_{IGCS} for 38 experiments using independent-groups designs and d_{RMCS} for 6 experiments using single-group designs (**Fig. S2** and **S3**).

Otherwise, in all considerations done until now for a research synthesis, two assumptions are at play. The first assumption is that apparatuses with one, two, or three chambers always have an equal potential to induce learning and allow its expression. The above discussion of "preference indices" clearly shows that this assumption is not warranted for three-chamber apparatuses concerning one- or two-chamber apparatuses. This conclusion is supported, one may tentatively say, by the metanalytic results of Bardo et al. (1995) when testing number of apparatus chambers (i.e., two- or three-chamber apparatuses) for different drugs. Meanwhile, the tour-de-force studies of Cunningham et al. (2006) and Cunningham and Zerizef (2014) with isogenic mice suggest that this assumption is not warranted between one- and two-chamber apparatuses.

The second assumption is that methods and instruments used to measure the principal behavioral unit (**Figure S1**) are free from systematic error of observer bias (or instrument bias for automated methods), are reproducible (reliable), have a fair accuracy, present fair inter-rating with each other, and do not cause feedback to the measurand (i.e., the animal's behavior; this problem is potentially present, for example, in the "tilt cage" instrument and the direct observation method). Accordingly, aside from the feedback problem, all other are problems of measurement agreement: **a)** Observer (or instrument) bias and accuracy are rela-

[15] As the accepted solution for treatment allocation is the use of independent randomization (Armitage et al., 2002), the best available procedure is to (i) stipulate first that half of the animals of each experimental group will go to one associative condition (drug-chamber 1 and vehicle-chamber 2) and half to the other associative condition (drug-chamber 2 and vehicle-chamber 1) and, afterwards, (ii) assort each condition to each animal randomly and independently. This procedure is called "balanced stimulus assignment" in the PC literature. Although this literature mostly discusses stimulus assignment and its criteria in relation to apparatus preference bias (see **Appendix S10**), here stimulus assignments is related to the assumption of conditioning equipotentiality, i.e., the assumption that any conditioning cues have equal conditioning potential for a given primary motivator. However, since the studies of Garcia and Koelling (1966; 1967) we know this assumption is unwarranted. Therefore, conditioning chambers of the same apparatus may present different potentials even when apparatus preference bias is absent. Due to this, it is unwise to use an unbalanced stimulus assignment (for definitions, see **Appendix S10**).

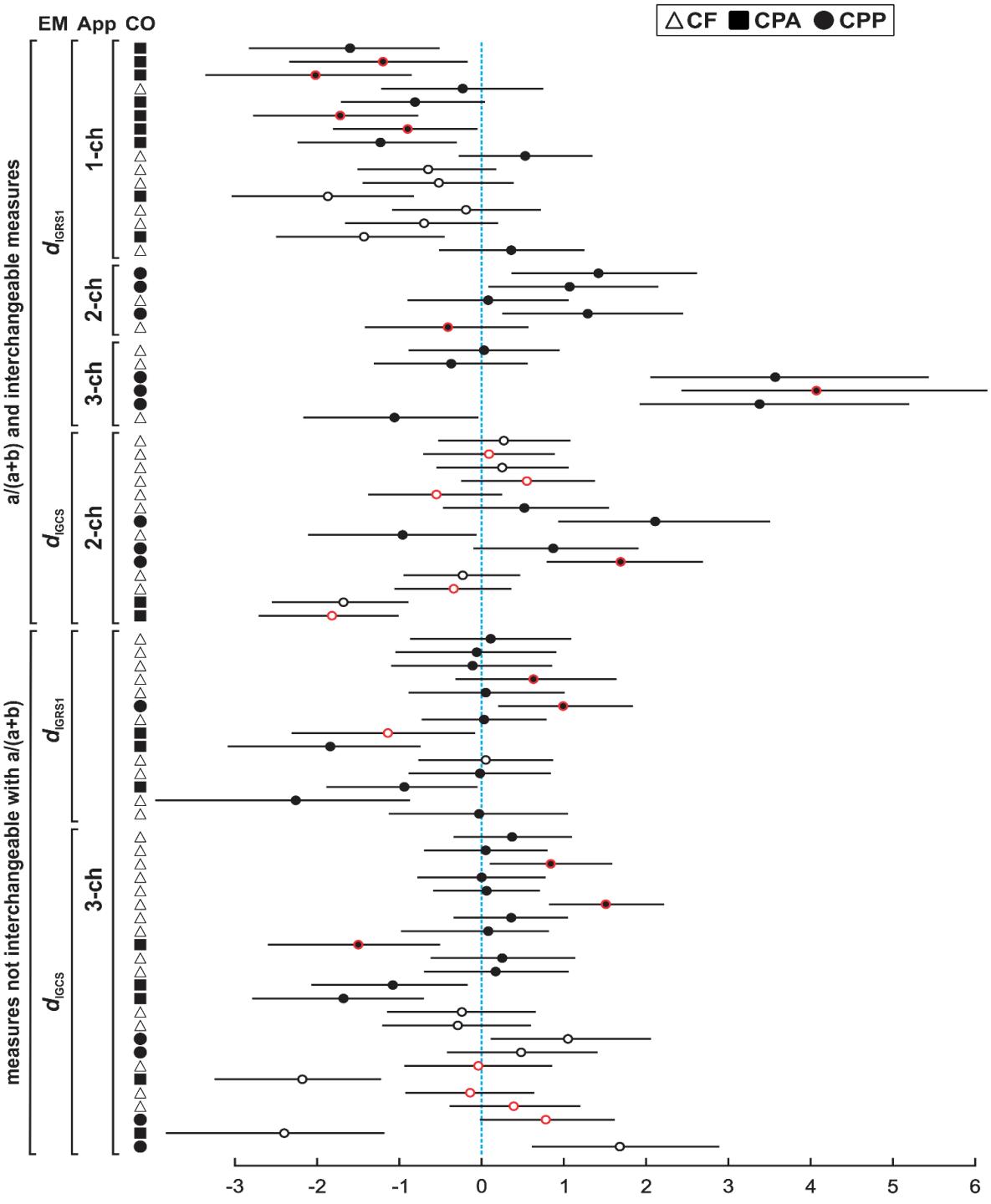


Figure S2 Effect size estimates (with 95% CI) for independent-groups designs in short PC protocols, arranged according to preference measures, estimate metrics, and number of chambers in apparatuses. CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference; EM, estimate metrics; App, apparatus type; CO, categorical outcome (extracted according to the definitions of Table S2); 1-ch, one-chamber apparatus; 2-ch, two-chamber apparatus; 3-ch, three-chamber apparatus. Effect size point estimates of black-filled circles were calculated from exact sample sizes and meet the assumption of equal variance between control and experimental groups. Point estimates of black-open circles were calculated from range sample sizes (by the mean of the range) and, in this condition, meet the assumption of equal variance between groups. Red-haloed and black-filled point estimates were calculated from exact sample sizes but do not meet the assumption of equal variance. Red-haloed open circles were calculated from range sample sizes (by the mean of the range) and do not meet the assumption of equal variance. Note 1: Regardless of the metric, all point estimates are presented according to the Hedges' correction for small sample sizes (Lakens, 2013), while all the 95% CIs were calculated for the respective uncorrected point estimates, following the recommendation of Cumming (2012). Note 2: Effect size estimates (with 95% CI) for independent-groups designs of all PC protocols are given in Appendix S14 (spreadsheet 01).

ted to the agreement between independent measurements done by different (and blinded) observers (or different instruments of the same type) on the same quantity, for the same animals, under similar conditions; **b)** Reproducibility and accuracy are related to the agreement between independent measurements done by the same observer (or the same instrument) for the same quantity, for the same animals, under similar conditions; **c)** Inter-rating and accuracy are related to the agreement between independent measurements done by different methods (e.g., direct observation *vs.* photocell *vs.* video tracking *vs.* stabilimeter) on the same quantity, for the same animals, under similar conditions. Here, the method of Altman and Bland (1983; Bland and Altman, 1986, 1990, 1995c, 2003, 2007) is the most trustful to estimate all these classes of measurement agreement and their respective errors[16].

Problems of systematic error in instrument accuracy, reliability, and feedback to the animal's behavior are known to occur in the "tilt cage" stabilimeter instrument (e.g., issues in animal weight sensibility, carryover momentum, and cage mobility). On the other hand, problems of observer bias, accuracy, and reliability are present in the direct observation method (for discussion of these two methods, see Robbins, 1977). From what we know, none of these measurement techniques for the principal behavioral unit has ever been probed by the Altman and Bland (1983) method for these problems. For example, blinding and inter-rating the scoring of two independent observers have been recommended as solutions for observer bias and accuracy problems of the direct observation method (Robbins, 1977). However, in reports of direct observation in the reviewed literature, blinding is rarely stated and whenever inter-rating for different observers happened, it was done by a correlation analysis, which only tells how strongly two measurements are associated and not their level of agreement or relative error (Altman and Bland, 1983; Bland and Altman, 1986; Müller and Büttner, 1994). Thus, we know of no trustful estimation of agreement error for these methods, whether in the literature of PC or related behavioral literature.

Meanwhile, concerning automatic data collection via photocell and video tracking instruments, few would argue these are not reliable methods of data collection. Nonetheless, reliability per se does not imply accuracy (Bonate, 2000). So, it does not mean that different photocell systems or different video tracking systems have fair intraclass measurement agree-

16 Note that Pearson's correlation coefficient (r) does not indicate good agreement between two measures. As stated by Müller and Büttner (1994), "[f]or example, we may observe a correlation coefficient of nearly 1 when one measure is approximately twice a second measure. The strong correlation allows nearly perfect prediction of one measure from the other, but the actual agreement [between the two measures] is non-existent. Good agreement is only obtained when the pairs of readings closely follow the line of equality. Pearson's r may be quite misleading in judging agreement" (p. 2465). Meanwhile, linear regression is not adequate for this estimation except for one case: satisfied the condition that the sample size is great enough, linear regression can be used to estimate the relative error between two measurements of the same phenomenon when these measurements do not have the same unit (see Bland and Altman, 2003).

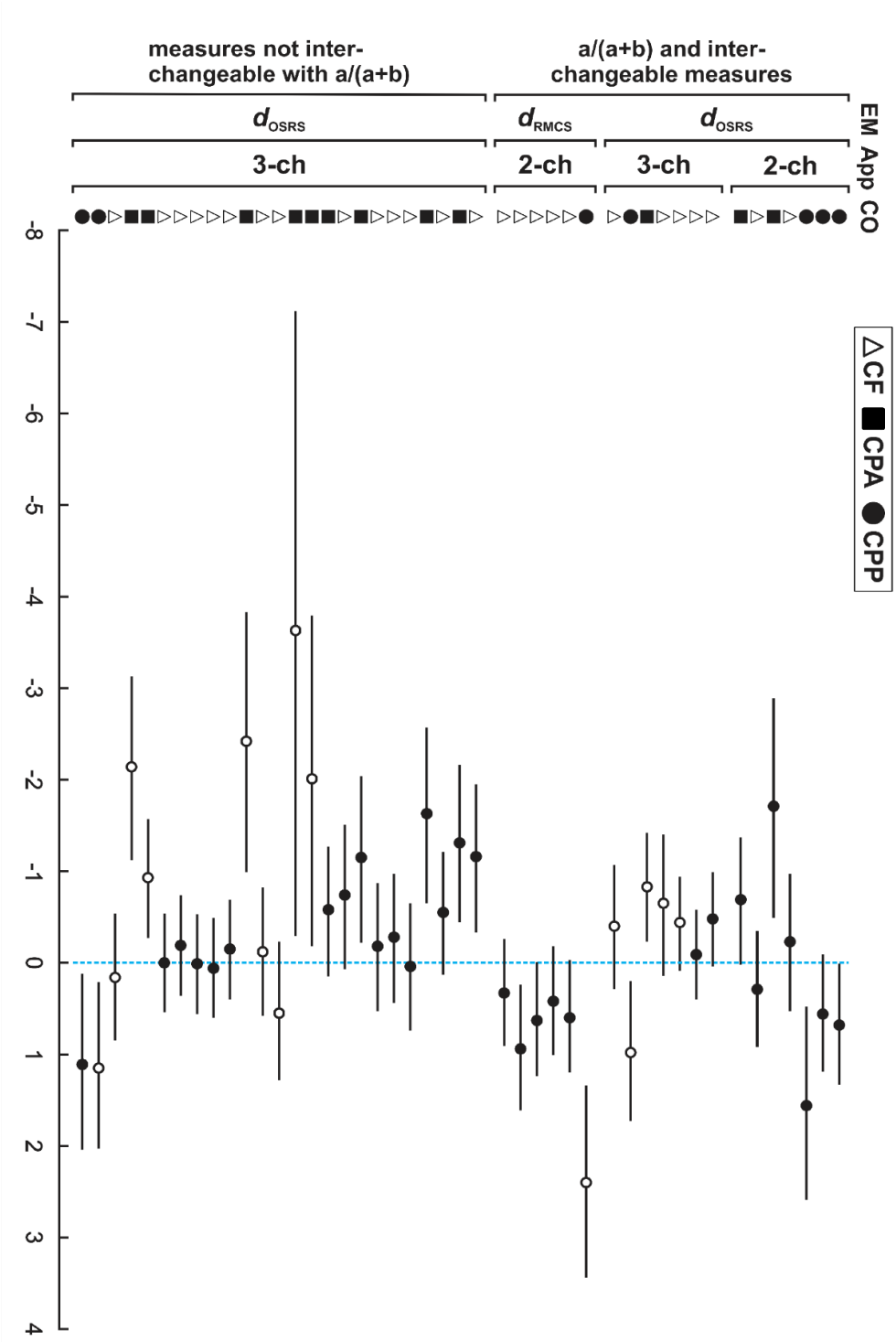


Figure S3 Effect size estimates (with 95% CI) for single-group designs in short PC protocols, arranged according to preference measures, estimate metrics, and number of chambers in apparatuses. CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference; EM, estimate metrics; App, apparatus type; CO, categorical outcome (extracted according to the definitions of Table S2); 2-ch, two-chamber apparatus; 3-ch, three-chamber apparatus.. Effect size point estimates of black-filled circles were calculated from exact sample sizes. Point estimates of black-open circles were calculated from range sample sizes (by the mean of the range). Note 1: For the d_{RMCS} metric, all point estimates are presented according to the Hedges' correction for small sample sizes (Lakens, 2013), while all the 95% CIs were calculated for the respective uncorrected point estimates, following the recommendation of Cumming (2012). For the d_{OSRS} metric, both the point estimate and the 95% CI were calculated from and for the uncorrected point estimate. Note 2: Effect size estimates (with 95% CI) for single-group designs of all PC protocols are given in Appendix S14 (spreadsheet 02).

ments between themselves. Similarly, it does not mean that these methods have fair interclass agreements with the other instruments or methods, and "[h]ow far apart measurements can be

without causing difficulties will be a question of [researcher] judgment" (Bland and Altman, 1986, p. 308). For these two methods, this is especially true for different photocell systems for three reasons: (i) these systems are immobile, (ii) the number of photocell units and their spatial organization change irregularly from apparatus to apparatus, and, according to these variables, (iii) the definitions of an animal entering, remaining, and leaving a chamber may be quite different from the definitions used in direct observation and video tracking systems, for example (very similar considerations are also true for a "tilt cage" instrument).

That said, from what we know, there is no trustful agreement estimation of these different methods and instruments in the behavioral literature proper of PC or related to it (e.g., open-field test). All "agreement comparisons" of these methods that we know of used linear regression or Pearson's correlation coefficient, whether comparing direct observation and a photocell system (Kršiak et al., 1970) or different video-tracking systems between themselves and with a photocell system (Bailoo et al., 2010)[17], for example. Therefore, for this review, the agreement error between measurements of the behavioral unit may not be estimated, and properly informed judgment may not occur.

In front of the widespread presence of these non-solvable problems of error estimation and those related to the report quality issue (see **Appendix S12**) in the reviewed literature, a research synthesis according to any of the recommended methods (p-value combination, summary of effect estimates, and meta-regression: McKenzie et al., 2019) could be conducted only on data from one-chamber apparatus. Hardly, however, such a research synthesis would be suitable to answer the questions posed in this study. We then decided to conduct a categorical frequentist distribution of the principal outcome (principal outcome categorized as CPA, CPP, and conditioning failure according to the definitions of **Table S2**), which entails the use of vote counting methods to provide at least provisory answers in the proportional prediction of results (Khamis, 2005). Admittedly, this is an inaccurate analysis (Altman, 2005; McKenzie et al., 2019; Naggara et al., 2011) in comparison with the other synthesis methods, but one that acknowledges the problems present in the literature without an unjustifiable suggestion of precision. Nevertheless, as one can see in **Fig. S2** and **S3**, the set of categorical outcomes present a somewhat fair agreement with the sets of effect size estimates (with their respective 95% CIs) that we were able to calculate.

[17] The study of Bailoo et al. (2010), even though using correlation coefficient to estimate agreement, has two advantages over all the other ones we found: it stablishes the real values to be measured by the automated systems (video-tracking and photocell systems) and provides these values together with the measurements. For this study then the problem may be considered as both a calibration problem in relation to the real value (for all tracking systems) and as a problem of relative error for the different video-tracking systems, one in respect to the other, under several conditions.

Appendix S4: Procedures of risk of bias assessment

We assessed the risk of report bias through a 7-item list of report quality indicators adapted by Carneiro et al. (2018) from the CAMARADES checklist (Sena et al., 2007). The 7-item list used for report quality assessment included (a) compliance with regulatory requirements, (b) compliance with the ARRIVE guidelines (Kilkenny et al., 2010), (c) sample size calculation methods, (d) statement regarding possible conflict of interests, (e) randomization of group allocation to the PC intervention, (f) exact sample size description for the PC intervention, and (g) blinded or automated assessment of the principal PC outcomes. Evaluation of attendance to items *a*, *b*, *c*, and *d* was article-wise and to items *e*, *f*, and *g* was PC experiment-wise. In the first case, the evaluation was done by a 1-point score for each of the items for each article. In the second one, for each item, a 1-point score was given to each selected PC experiment.

Additionally, as our systematic search included a period of 50 years, regulatory requirements differ temporally on national grounds (Vasbinder and Locke, 2016), and the ARRIVE guidelines were published in July 2010, we did not expect *a* and *b* criteria to apply to every article in the same fashion. To circumvent these problems, we extracted the month and year of "article publication date" and "country of research" for each article, and the requirement of criterion *a* was considered adequate for articles published from 1990 onwards, as Canada, China, European Union, Japan, and the USA already had some regulatory measure in effect from 1984 to 1988 (Felsmann et al., 2014; Griffin and Locke, 2016; Kilkenny et al., 2010; Mohr et al., 2016; Ogden et al., 2016; Olsson et al., 2016; Retnam et al., 2016; Timoshanko et al., 2016; Vasbinder and Locke, 2016). Meanwhile, the requirement of criterion *b* was considered adequate for articles published from July 2010 onwards.

Lastly, scores in PC-wise criteria were used to determine the mean aggregate risk of bias, which was calculated by a simple mean formula.

Appendix S5: Definition of apparatus bias and evaluation of its report

For the extraction of apparatus bias, following the preference measurement definition provided in **Appendix S3**, we considered that apparatus bias is defined by the results of the formula for measuring baseline preference:

$(t_0CC1/(t_0CC1+ t_0CC2))*100\%$,

where: t_0CC1 = baseline time in conditioning chamber/context 1; t_0CC2 = baseline time in conditioning chamber/context 2.

Thus, whenever, the baseline preference is strong and consistent across animals composing a sample, i.e., if they spend, on average, significantly more time than 50% in a specific conditioning chamber over the other, it is considered an unconditioned preference, and the apparatus is considered biased. Whenever the result of this formula does not differ significantly from 50%, an unconditioned preference is not considered present, an unbiased apparatus is used, and the individual discrepancy in the baseline preference is a simple instance of biological and stimulatory variation (individual differences). Reasons for this conception are given in **Appendix S10**.

However, although we adopted this definition of preference bias beforehand, the use of a random sample of 15 selected articles to establish the data categories to be extracted forced us to deal with the problem of bias report reliability, an evaluation that was not programmed in our review protocol. As we noticed a great variation in bias reports, we decided to evaluate this variation in the selected literature by extracting (a) researchers' statement of apparatus bias and (b) what reason did researchers provide for reporting or not the (in)existence of bias. We also evaluated whether researchers reported the mean scores (± SE or SD) or range of the baseline test for conditioning chambers/contexts (even if this report was done just in figures and/or tables), (c) if bias was evaluated according to the formula we presented or by another measure, and (d) if it was reported after exclusion of animals solely based on exclusion criteria related to the baseline scores. This assessment is shown in **Appendix S13** (spreadsheet 04) and further developed in **Appendix S10**. As one can see, the report of bias in the selected literature is not reliable for the statistical evaluation of this PC covariable.

Appendix S6: Statistical analysis

We organized the categorical outcomes as conditioning failure, CPA, and CPP when analyzing variables related to CPA and CPP distributions. When analyzing variables related to the distribution of conditioning failure and success, we combined CPA and CPP occurrences to produce the frequency data of conditioning success. When considering the distribution of outcomes according to experimental setting covariables, we performed statistical analysis of the frequency distributions using a chi-square test (X^2, exact method, two-tailed) for 2x2 contingency tables or Fisher-Freeman-Halton Exact Test (F^2, two-tailed) for larger tables (Mehta and Hilton, 1993). We conducted multiple (post hoc) comparisons with the chi-square test or Fisher-Freeman-Halton Exact Test (both exact method, two-tailed) where appropriate. For post hoc analysis, pairwise comparisons of collapsed marginal frequencies were included only when we considered conditioning failure-success distribution. For all frequency distribution tests, effect sizes were expressed with Cramér's V (φ_c). φ_c presents significant bias for tables larger than 2x2 and Bergsma (2013) has proposed a correction. As all analyses were performed in IBM SPSS (package 20), this correction is not reported because the statistical package does not perform it. Lastly, we used the Pearson product-moment correlation coefficient (r, two-tailed) to test the existence of associations between the PC intervention covariables themselves. We set $\alpha = 0.05$ for all common and overall comparisons and used Bonferroni correction for multiple comparisons (MacDonald and Gardner, 2000; McDonald, 2014).

Appendix S7: Evaluation of the classification of place conditioning protocols without ethanol preexposure for a categorical frequentist distribution analysis of procedure-outcome relations

The general literature of PC has considered protocols with up to 10 conditioning trials as a "short period of conditioning" and/or as enough for PC instatement (Bieńkowski et al., 1996; Carr et al., 1989; Cunningham et al., 2011). Meanwhile, previous reviews of the literature have indicated that a greater frequency of conditioning success, generally CPP, is obtained with longer periods of conditioning (with more than 10 conditioning trials) (Swerdlow et al., 1989; Tzschentke, 1998, 2007). We evaluated the distribution of results obtained in these protocols to ascertain if their separation was adequate for posterior analysis. Thus, to determine possible differences between short PC protocols (10 or less conditioning sessions, SPCP) and long PC protocols (11 or more conditioning sessions, LPCP) on the distribution of results, we compared Wistar rats in LPCP with Wistar rats in SPCP (F^2 = 39.175, p = < 0.001, φ_c = 0.703) and with Sprague Dawley rats in SPCP (F^2 = 27.881, p = < 0.001, φ_c = 0.648).

Moreover, since no article reported LPCP with Holtzman rats, only 3 articles reported 6 experiments with LPCP in Sprague Dawley rats (Fidler et al., 2004; Marglin et al., 1988; Spina et al., 2010), and 9 articles reported 19 experiments with LPCP in Wistar rats, 16 of which with intraperitoneal (ip) administration (Biała and Langwiński, 1996a, 1996b; Bie et al., 2009; López and Cantora, 2010; Stewart and Grupp, 1985; Zhu et al., 2007), we performed these comparisons in the ip dataset: Wistar rats in SPCP *vs.* Wistar rats in LPCP: F^2 = 32.491, p = < 0.001, φ_c = 0.706; Sprague Dawley rats in SPCP *vs.* Wistar rats in LPCP: F^2 = 22.392, p = < 0.001, φ_c = 0.614. Lastly, since LPCP in Wistar rats have been reported using only ethanol doses of 0.50 and 1.00 g/kg, we constrained the SPCP ip dataset to this dose range and performed new comparisons: Wistar rats in SPCP *vs.* Wistar rats in LPCP (F^2 = 18.553, p = < 0.001, φ_c = 0.658) and Sprague Dawley rats in SPCP *vs.* Wistar rats in LPCP (F^2 = 17.186, p = < 0.001, φ_c = 0.711).

These results confirm that LPCP is associated with a greater occurrence of conditioning success (especially CPP) and lower occurrence of conditioning failure in Wistar rats. As we also found a significant difference when comparing SPCP in Sprague Dawley rats with LPCP in Wistar rats, this result suggests that LPCP may mask differences between Sprague Dawley and Wistar rats in SPCP if SPCP and LPCP are not separated in a frequency distribution

analysis of categorical outcomes. Thus, the separation of SPCPs and LPCPs is adequate for this review.

Referências aos apêndices S1-S7

Acevedo, M.B., Nizhnikov, M.E., Spear, N.E., Molina, J.C., Pautassi, R.M. 2013. Ethanol-induced locomotor activity in adolescent rats and the relationship with ethanol-induced conditioned place preference and conditioned taste aversion. Devel. Psychobiol. 55(4), 429–442. DOI: https://doi.org/10.1002/dev.21048

Alcorn, J., Elbarby, F.A., Allouh, M.Z., McNamara, P.J. 2007.Evaluation of the assumptions of an ontogeny model of rat hepatic cytochrome P450 activity. DMD 35, 2225–2231. DOI: https://doi.org/10.1124/dmd.107.017590

Altman, D.G. 2005. Categorizing continuous variables. In Armitage, P., Colton, T. (Eds.), Encyclopedia of biostatistics (2nd ed.). Wiley & Sons.

Altman, D.G., Bland, J.M. 1983. Measurement in medicine: The analysis of method comparison studies. Stat. 32, 307–317. DOI: https://doi.org/10.2307/2987937

Altman, D.G., Bland, J.M. 2011. How to obtain the confidence interval from a P value. B.M.J. 343, d2090. DOI: https://doi.org/10.1136/bmj.d2090

Applegate, E.A., Upton, D.E., Stern, J.S. 1982. Food intake, body composition and blood lipids following treadmill exercise in male and female rats. Physiol. Behav. 28(5), 917–920. DOI: https://doi.org/10.1016/0031-9384(82)90214-1

Armitage, P., Berry, G., Matthews, J.N.S. 2002. Statistical methods in medical research (4° Ed.). UK, Oxford, Blackwell Publishing.

Babikian, T., Prins, M.L., Cai, Y., Barkhoudarian, G., Hartonian, I., Hovda, D.A., Giza, C.C. 2010. Molecular and Physiological Responses to juvenile traumatic brain injury: Focus on growth and metabolism. Dev. Neurosci. 32, 431–441. DOI: https://doi.org/10.1159/000320667

Bahi, A. 2013. Viral-mediated knockdown of mGluR7 in the nucleus accumbens mediates excessive alcohol drinking and increased ethanol-elicited conditioned place preference in rats. Neuropsychopharmacol. 38, 2109–2019. DOI: https://doi.org/10.1038/npp.2012.122

Bailoo, J.D., Bohlen, M.O., Wahlsten, D. 2010. The precision of video and photocell tracking systems and the elimination of tracking errors with infrared backlighting. J. Neurosci. Meth. 188, 45–52. DOI: https://doi.org/10.1016/j.jneumeth.2010.01.035

Barbier, E., Houchi, H., Warnault, V., Pierrefiche, O., Daoust, M., Naassila, M. 2009. Effects of prenatal and postnatal maternal ethanol on offspring response to alcohol and psychostimulants in long evans rats. Neuroscience 161(2), 427–440. DOI: https://doi.org/10.1016/j.neuroscience.2009.03.076

Barbier, E., Pierrefiche, O., Vaudry, D., Vaudry, H., Daoust, M., Naassila, M. 2008. Long-term alterations in vulnerability to addiction to drugs of abuse and in brain gene expression after early life ethanol exposure. Neuropharmacol 55, 1199–1211. DOI: https://doi.org/10.1016/j.neuropharm.2008.07.030

Bardo, M.T., Rowlett, J.K., Harris, M.J. 1995. Conditioned place preference using opiate and stimulant drugs: A meta-analysis. Neurosci. Biobehav. Rev. 19, 39–51. DOI: https://doi.org/10.1016/0149-7634(94)00021-r

Beach, H.D. 1957a. Morphine addiction in rats. Can. J. Psychol. 11, 104–112. DOI: https://doi.org/104-12. 10.1037/h0083703

Beach, H.D. 1957b. Some effects of morphine on habit function. Can. J. Psychol. 11, 193–198. DOI: https://doi.org/10.1037/h0083702

Becker, B. J. 1988. Synthesizing standardized mean-change measures. Br. J. Math. Stat. Psychol., 41, 257–278. DOI: https://doi.org/10.1111/j.2044-8317.1988.tb00901.x

Bergsma, W. 2013. A bias-correction for Cramér's V and Tschuprow's T. J. Kor. Stat. Soc. 42, 323–328. DOI: https://doi.org/10.1016/j.jkss.2012.10.002

Biała, G., Langwiński, R. 1996a. Effects of calcium channel antagonists on the reinforcing properties of morphine, ethanol and cocaine as measured by place conditioning. J. Physiol. Pharmacol. 47, 497–502.

Biała, G., Langwiński, R. 1996b. Rewarding properties of some drugs studied by place preference conditioning. Pol. J. Pharmacol. 48, 425–430.

Bie, B., Zhu, W., Pan, Z.Z. 2009. Ethanol-induced delta-opioid receptor modulation of glutamate synaptic transmission and conditioned place preference in central amygdala. Neurosci. 160, 348–358. DOI: https://doi.org/10.1016/j.neuroscience.2009.02.049

Bieńkowski, P., Kuca, P., Piasecki, J., Kostowski, W. 1996. Low dose of ethanol induces conditioned place preference in rats after repeated exposures to ethanol or saline injections. Alcohol Alcohol. 31, 547–553. DOI: https://doi.org/10.1093/oxfordjournals.alcalc.a008190

Black, R.W., Albiniak, T., Davis, M., Schumpert, J. 1973. A preference in rats for cues associated with intoxication. Bull. Psychon. Soc. 2, 423–424. DOI: https://doi.org/10.3758/BF03334436

Bland, J.M., Altman, D.G. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet, 327, P307–310. https://doi.org/10.1016/S0140-6736(86)90837-8

Bland, J.M., Altman, D.G. 1990. A note on the use of the intraclass correlation coefficient in the evaluation of agreement between two methods of measurement Comput. Biol. Med. 20, 337–340. DOI: https://doi.org/10.1016/0010-4825(90)90013-f

Bland, J.M., Altman, D.G. 1995a. Statistics notes. Calculating correlation coefficient with repeated observations: Part 1 — correlation within subjects. BMJ 310, 446. DOI: https://doi.org/10.1136/bmj.310.6977.446

Bland, J.M., Altman, D.G. 1995b. Statistics notes. Calculating correlation coefficient with repeated observations: Part 2 — correlation between subjects. BMJ 310, 633. DOI: https://doi.org/10.1136/bmj.310.6980.633

Bland, J.M., Altman, D.G. 1995c. Comparing methods of measurement: why plotting difference against standard method is misleading. Lancet 346, 1085–1087. DOI: https://doi.org/10.1016/s0140-6736(95)91748-9

Bland, J.M., Altman, D.G. 2003. Applying the right statistics: analyses of measurement studies. Ultrasound Obstet Gynecol. 22, 85–93. DOI: https://doi.org/10.1002/uog.122

Bland, J.M., Altman, D.G. 2007. Agreement between methods of measurement with multiple observations per individual. J. Biopharm. Stat. 17, 571–582. DOI: https://doi.org/10.1080/10543400701329422

Bolton, M.M., Heaney, C.F., Murtishaw, A.S., Sabbagh, J.J., Magcalas, C.M., Kinney, J.W. 2015. Postnatal alterations in GABAB receptor tone produce sensorymotor gating and protein level differences in adulthood. Int. J. Dev. Neurosci. 41, 17–27. DOI: https://doi.org/10.1016/j.ijdevneu.2014.10.001

Bonate, P.L. 2000. Analysis of pretest-posttest designs. Chapman & Hall/CRC Press, USA.

Carneiro, C.F.D., Moulin, T.C., Macleod, M.R., Amaral, O.B. 2018. Effect size and statistical power in the rodent fear conditioning literature – A systematic review. PLoS ONE 13, e0196258. DOI: https://doi.org/10.1371/journal.pone.0196258

Carr, G.D., Fibiger, H.C., Phillips, A.G. 1989. Conditioned place preference as a measure of drug reward. In: Liebman, J.M., Cooper, S.J. (Eds.), Topics in experimental

psychopharmacology (1): The neuropharmacological basis of reward. Clarendon Press/Oxford University Press, Oxford, pp. 264–319.

Cortright, R.N., Chandler, M.P., Lemon, P.W.R., DiCarlo, S.E. 1997. Daily exercise reduces fat, protein and body mass in male but not female rats. Physiol. Behav. 62(1), 105–111. DOI: https://doi.org/10.1016/S0031-9384(97)00148-0

Cossio-Bolaños, M., Campos, R.G., Vitoria, R.V., Fogaça, R.T.H., de Arruda, M. 2013. Curvas de referencia para valorar el crescimento físico de ratas machos Wistar. Nutr. Hosp. 28(6), 2151–2156. DOI: https://dx.doi.org/10.3305/nh.2013.28.6.6659

Ciccocioppo, R. 2012. Genetically selected alcohol preferring rats to model human alcoholism. In: Sommer, W., Spanagel, R. (eds) Behavioral Neurobiology of Alcohol Addiction. Current Topics in Behavioral Neurosciences, vol 13. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-28720-6_199

Ciccocioppo, R., Panocka, I., Froldi, R., Quitadamo, E., Massi, M. 1999. Ethanol induces conditioned place preference in genetically selected alcohol-preferring rats. Psychopharmacol. 141(3), 235–241. DOI: https://dx.doi.org/10.1007/s002130050830

Ciccocioppo, R., Panocka, I., Froldi, R., Colombo, G., Gessa, G.L., Massi, M. 1999. Antidepressant-like effect of ethanol revealed in the forced swimming test in Sardinian alcohol-preferring rats. Psychopharmacol. (Berl), 144, 151–157. DOI: https://doi.org/10.1007/s002130050988

Ciccocioppo, R., Panocka, I., Polidori, C., Regoli, D., Massi, M. 1999. Effect of nociceptin on alcohol intake in alcohol-preferring rats. Psychopharmacol. (Berl.) 141(2), 220–224. DOI: https://doi.org/10.1007/s002130050828

Collins, A.C., Yeager, T.N., Lebsack, M.E., Panter, S.S. 1975. Variations in alcohol metabolism: Influence of sex and age. Pharmacol. Biochem. Behav. 3, 973–978. DOI: https://doi.org/10.1016/0091-3057(75)90004-0

Colombo, G., Kuzmin, A., Fadda, F., Pani, L., Gessa, G. L. 1990. Conditioned place preference induced by ethanol in a rat line selected for ethanol preference. Pharmacol. Res. 22(Suppl. 3), 48. DOI: https://doi.org/10.1016/s1043-6618(09)80021-2

Cumming, G. 2012. Understanding the New Statistics: Effect sizes, Confidence Intervals, and Meta-Analysis. New York, NY: Routledge.

Cunningham, C.L. 1993. Pavlovian drug conditioning. In: Van Haaren, F. (Ed.), Techniques in the behavioral and neural sciences (10): Methods in behavioral pharmacology. Elsevier, Amsterdan, pp. 349–381.

Cunningham, C.L., Ferree, N.K., Howard, M.A. 2003. Apparatus bias and place conditioning with ethanol in mice. Psychopharmacol. 170, 409–422. DOI: https://doi.org/10.1007/s00213-003-1559-y

Cunningham, C.L., Groblewski, P.A., Voorhees, C.M. 2011. Place conditioning. In: Olmstead, M.C. (Ed.) Neuromethods (53): Animal models of drug addiction. Springer, New York, pp. 167–189.

Cunningham, C.L., Patel, P., Milner, L. 2006. Spatial location is critical for conditioning place preference with visual but not tactile stimuli. Behav. Neurosci. 120, 1115–1132. DOI: https://doi.org/10.1037/0735-7044.120.5.1115

Cunningham, C.L., Zerizef, C.L. 2014. Effects of combining tactile with visual and spatial cues in conditioned place preference. Pharmacol. Biochem. Behav. 124, 443–450. DOI: http://dx.doi.org/10.1016/j.pbb.2014.07.012

Degoulet, M., Stelly, C.E., Ahn, K.-C., Morikawa, H. 2016. L-type Ca2+ channel blockade with antihypertensive medication disrupts VTA synaptic plasticity and drug-associated contextual memory. Mol. Psychiat. 21(3), 394–402. DOI: https://doi.org/10.1038/mp.2015.84

De Carvalho, C.R., Pandolfo, P., Pamplona, F.A., Takahashi, R.N. 2010. Environmental enrichment reduces the impact of novelty and motivational properties of ethanol in spontaneously hypertensive rats. Behav. Brain Res. 208(1), 231–236. DOI: https://doi.org/10.1016/j.bbr.2009.11.043

De Zwart, L., Scholten, M., Monbaliu, J.G., Annaert, P.P., Van Houdt, J.M., Van den Wyngaert, I., et al. 2008. The ontogeny of drug metabolizing enzymes and transporters in the rat. Reprod. Toxicol. 26, 220–230. DOI: https://doi.org/10.1016/j.reprotox.2008.09.010

Dunlap, W.P., Cortina, J.M., Vaslow, J.B., Burke, M.J. 1996. Meta-analysis of experiments with matched groups or repeated measures designs. Psychol. Meth. 1, 170–177. DOI: https://doi.org/10.1037/1082-989X.1.2.170

Fadda, F., Colombo, G., Gessa, G. L. 1990. Ethanol-induced place preference in naive alcohol-preferring rats. Pharmacol. Res. 22(Suppl. 2), 184.

Felsmann, M.Z., Szarek, J., Felsmann, M., Strzyzewska, E. 2014. Protection of animals used in experiments in Polish law– history, present day and perspective: A review. Veter. Med. 59, 117–123. DOI: https://doi.org/10.17221/7378-VETMED

Fidler, T.L., Bakner, L., Cunningham, C.L. 2004. Conditioned place aversion induced by intragastric administration of ethanol in rats. Pharmacol. Biochem. Behav. 77, 731–743. DOI: https://doi.org/10.1016/j.pbb.2004.01.010

Fu, R., Chen, X., Zuo, W., Li, J., Kang, S., Zhou, L.-H., … Ye, J.-H. 2016. Ablation of μ opioid receptor-expressing GABA neurons in rostromedial tegmental nucleus increases ethanol consumption and regulates ethanol-related behaviors. Neuropharmacol. 107, 58–67. DOI: https://doi.org/10.1016/j.neuropharm.2016.02.027

Garcia, J., Koelling, R.A. 1966. Relation of cue to consequence in avoidance learning. Psychon. Sci. 4, 123–124. DOI: https://doi.org/10.3758/BF03342209

Garcia, J., Koelling, R.A. 1967. A comparison of aversions induced by X rays, toxins, and drugs in the rat. Radiat. Res. Suppl. 7, 439–450.

Gauvin, D.V., Baird, T.J., Briscoe, R.J. 2000. Differential development of behavioral tolerance and the subsequent hedonic effects of alcohol in AA and ANA rats. Psychopharmacol. 151(4), 335–343. DOI: https://doi.org/10.1007/s002130000477

German, P.W., Fields, H.L. 2007. How prior reward experience biases exploratory movements: A probabilistic model. J. Neurophysiol. 97, 2083–2093. DOI: https://doi.org/10.1152/jn.00303.2006

Griffin, G., Locke, P. 2016. Comparison of Canadian and US laws, regulations, policies, and systems of oversight for animals in research. ILAR J. 57, 271–284. DOI: https://doi.org/10.1093/ilar/ilw037

Harada, S., Tachiyashiki, K., Iamizumi, K. 1998. Effect of sex hormones on rat liver cytosolic dehydrogenase activity. J. Nutr. Sci. Vitaminol. 44, 625–639. DOI: https://doi.org/10.3177/jnsv.44.625

Horton, A.A., Mills, D.J. 1979. Developmental patterns of alcohol dehydrogenase and aldehyde dehydrogenases in homogenates and subcellular fractions of rat liver. Mech. Ageing Dev. 11, 363–370. DOI: https://doi.org/10.1016/0047-6374(79)90011-3

Higgins, J.P.T., Li, T., Deeks, J.J. 2019. Choosing effect measures and computing estimates of effect. In: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A. (Eds.), Cochrane Handbook for Systematic Reviews of Interventions (2nd ed.). The Cochrane Collaboration/Wiley Blackwell, Hoboken, NJ, pp. 142–176.

Huston, J.P, Silva, M.A.S., Topic, B. Müller, C.P. 2013. What's conditioned in conditioned place preference? Trends Pharmacol. Sci. 34, 162–166. DOI: http://dx.doi.org/10.1016/j.tips.2013.01.004

Khamis, H.J., 2005. Measures of association. In Armitage, P., Colton, T. (Eds.), Encyclopedia of biostatistics (2nd ed.). Wiley & Sons.

Juárez, J., Barrios De Tomasi, E. 1999. Sex differences in alcohol drinking patterns during forced and voluntary consumption in rats. Alcohol 19(1), 15-22. DOI: http://dx.doi.org/10.1016/s0741-8329(99)00010-5

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G. 2010. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS Biol 8, e1000412. DOI: https://doi.org/10.1371/journal.pbio.1000412

Kim, Y.C., Kim, S.Y., Sohn, Y.R. 2003. Effect of age increase on metabolism and toxicity of ethanol in female rats. Life Sci. 74, 509–519. DOI: https://doi.org/10.1016/j.lfs.2003.07.009

Krauth, J. 1992. Parametric analysis of sojourn times in conditioned place preference experiments. J. Neurosci. Methods 42, 219–227. DOI: https://doi.org/10.1016/0165-0270(92)90101-i

Kršiak, M., Steinberg, H., Stolerman, I.P. 1970. Uses and limitations of photocell activity cages for assessing effects of drugs. Psychopharmacol. (Berl.) 17, 258–274. DOI: https://doi.org/10.1007/BF00402085

Kumar, R. 1972. Morphine dependency in rats: Secondary reinforcement from environmental stimuli. Psychopharmacol. (Berl.) 25, 332–338. DOI: https://doi.org/10.1007/BF00421972

Lad, P.J., Shoemaker, W.J., Leffert, H.L. 1984. Developmental changes in rat liver alcohol dehydrogenase. Dev. Biol. 105, 526–529. DOI: https://doi.org/10.1016/0012-1606(84)90310-5

Lancaster, F.E., Brown, T.D., Coker, K.L., Elliot, J.A., Wren, S.B. 1996. Sex differences in alcohol preference and drinking patterns emerge during early postpubertal period in Sprague-Dawley rats. Alcohol. Clin. Exp. Res. 20(6), 1043–1049. DOI: https://doi.org/10.1111/j.1530-0277.1996.tb01945.x

Lancaster, F.E., Spiegel, K.S. 1992. Sex differences in pattern of drinking. Alcohol 9(5), 415–420. DOI: https://doi.org/10.1016/0741-8329(92)90041-8

Lee, J.S., Crawford, J., Spear, N.E. 1998. Characteristics and consequences of free-feeding ethanol ingestion during the first two postnatal weeks of the rat. Alcohol. Clin. Exp. Res. 22(8), 1615–1622. DOI: https://doi.org/10.1111/j.1530-0277.1998.tb03957.x

Li, J., Chen, P., Han, X., Zuo, W., Mei, Q., Bian, E.Y., Umeugo, J., Ye, J. 2019. Differences between male and female rats in alcohol drinking, negative affects, and neuronal activity after acute and prolonged abstinence. Int. J. Physiol. Pathophysiol. Pharmacol. 11(4), 163–176.

Li, T.-K., Lumeng, L., Doolittle, D.P. 1993. Selective breeding for alcohol preference and associated responses. Behav. Genet. 23(2), 163–170. DOI: https://doi.org/10.1007/BF0106742

Li, T.-K., Spanagel, R., Colombo, G., McBride, W.J., Porrino, L.J., Suzuki, T., Rodd-Henricks, Z.A. Alcohol reinforcement and voluntary ethanol consumption. Alcohol. Clin. Exp. Res. 25(5), 117S–126S. DOI: https://doi.org/10.1097/00000374-200105051-00021

Lillie, L.E., Temple, N.J., Florence, L.Z. 1996. Reference values for young normal Sprague-Dawley rats: Weight gain, hematology and clinical chemistry. Human Exp. Toxicol. 15, 612–616. DOI: https://doi.org/10.1177/096032719601500802

López, M., Cantora, R. 2010. Opposite effects of ethanol on taste and place conditioning in rats. Rev. Iberoam. Psicol. Salud. 1, 207–221.

MacDonald, P.L., Gardner, R.C. 2000. Type I error rate comparisons of post hoc procedures for I J chi-square tables. Educ. Psychol. Meas. 60, 735–754. DOI: https://doi.org/10.1177/00131640021970871

Maly, I.P., Sasse, D. 1985. Microquantitative determination of the distribution patterns of alcohol dehydrogenase activity in the liver of rat, guinea-pig and horse. Histochem. 83, 431–436. DOI: https://doi.org/10.1007/BF00509205

Marglin, S.H., MacKechnie, D.K., Mattie, M.E., Hui, Y., Reid, L.D. 1988. Ethanol with small doses of morphine establishes a conditioned place preference. Alcohol 5, 309–313. DOI: https://doi.org/10.1016/0741-8329(88)90071-7

Mayes, J.S., Watson, G.H. 2004. Direct effects of sex steroid hormones on adipose tissues and obesity. Obesity Rev. 5, 197–216. DOI: https://doi.org/10.1111/j.1467-789x.2004.00152.x

McDonald, J.H. 2014. Handbook of biological statistics. Sparky House Publishing, Maryland.

McKenzie, J.E., Brennan, S.E., Ryan, R.E., Thomson, H.J., Johnston, R.V. 2019. Summarizing study characteristics and preparing for synthesis. In: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A. (Eds.), Cochrane Handbook for Systematic Reviews of Interventions (2nd ed.). The Cochrane Collaboration/Wiley Blackwell, Hoboken, NJ, pp. 229–240.

Mehta, C.R., Hilton, J.F. 1993. Exact power of conditional and unconditional tests: Going beyond the 2x2 contingency table. Am. Stat. 47, 91–98. DOI: http://doi.org/10.1080/00031305.1993.10475946

Miller, J.S., Molina, J.C., Spear, N.E. 1990. Ontogenetic differences in the expression of odor-aversion learning in 4- and 8-day-old rats. Devel. Psychobiol. 23(4), 319–330. DOI: http://doi.org/10.1002/dev.420230404

Miranda-Morales, R.S., Nizhnikov, M.E., Waters, D.H., Spear, N.E. 2014. New evidence of ethanol's anxiolytic properties in the infant rat. Alcohol 48(4), 367–374. DOI: https://doi.org/10.1016/j.alcohol.2014.01.007

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., The PRISMA Group 2009. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 6(7), e1000097. DOI: https://doi.org/10.1371/journal.pmed.1000097

Mohr, B.J., Fakoya, F.A., Hau, J., Souilem, O. Anestidou, L. 2016. The governance of animal care and use for scientific purposes in Africa and the Middle East. ILAR J. 57, 333–346. DOI: http://doi.org/10.1093/ilar/ilw035

Molina, J.C., Bannoura, M.D., Chotro, M.G., McKinzie, D.L., Arnold, H.M., Spear, N.E. 1996. Alcohol-mediated tactile conditioned aversions in infanct rats: devaluation of conditioning through alcohol-sucrose associations. Neurobiol. Learn. Mem. 66, 121–132. DOI: https://doi.org/10.1006/nlme.1996.0053

Molina, J.C., Pautassi, R.M., Truxell, E., Spear, N. 2007. Differential motivational properties of ethanol during early ontogeny as a function of dose and postadministration time. Alcohol 41, 41–55. DOI: https://doi.org/10.1016/j.alcohol.2007.01.005

Morris, S.B., DeShon, R.P. 2002. Combining effect size estimates in meta-analysis with repeated measures and independent-groups designs. Psychol. Meth. 7, 105–125. DOI: http://doi.org/10.1037//1082-989X.7.1.105

Müller, R., Büttner, P. 1994. A critical discussion of intraclass correlation coefficients. Stat. Med. 13, 2465–2476. DOI: http://doi.org/10.1002/sim.4780132310

Naggara, O., Raymond, J., Guolbert, F., Roy, D., Weill, A., Altman, D.G. 2011. Analysis by categorizing or dichotomizing continuous variables is inadivisible: An example from the natural history of unruptured aneurysms. Am. J. Neuroradiol. 32, 437–440. DOI: http://doi.org/10.3174/ajnr.A2425

Nedungadi, T.P., Clegg, D.J. 2009. Sexual dimorphism in body fat distribution and risk of cardiovascular diseases. J. Cardiovasc. Trans. Res. 2, 321–327. DOI: https://doi.org/10.1007/s12265-009-9101-1

Nentwig, T.B., Myers, K.P., Grisel, J.E. 2017. Initial subjective reward to alcohol in Sprague-Dawley rats. Alcohol 58, 19–22. DOI: https://doi.org/10.1016/j.alcohol.2016.11.005

Nizhnikov, M.E., Molina, J.C., Varlinskaya, E.I., Spear, N.E. 2006. Prenatal ethanol exposure increases ethanol reinforcement in neonatal rats. Alcohol. Clin. Exp. Res. 30(1), 34–45. DOI: https://doi.org/10.1111/j.1530-0277.2006.00009.x

Nizhnikov, M.E., Pautassi, R.M., Carter, J.M., Landin, J.D., Varlinskaya, E.I., Bordner, K.A., … Spear, N.E. 2014. Brief prenatal ethanol exposure alters behavioral sensitivity to the kappa opioid receptor agonist (U62,066E) and antagonist (Nor-BNI) and reduces kappa opioid receptor expression. Alcohol. Clin. Exp. Res. 38(6), 1630–1638. DOI: https://doi.org/10.1111/acer.12416

Ogden, B.E., Pang, W., Agui, T., Lee, B.H. 2016. Laboratory animal laws, regulations, guidelines. ILAR J. 57, 301–311. DOI: http://doi.org/10.1093/ilar/ilw018

Olsson, A.S., Da Silva, S.P., Townend, D., Sandoe, P. 2016. Protecting animals and enabling research in the European Union: An overview of development and implementation of directive 2010/63/EU. ILAR J. 57, 347–357. DOI: https://doi.org/10.1093/ilar/ilw029

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffman, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., et al. 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BJM 372, 71. DOI: https://doi.org/10.1136/bmj.n71

Pautassi, R.M., Godoy, J.C., Spear, N.E., Molina, J.C. 2002. Early responsiveness to stimuli paired with different stages within the state of alcohol intoxication. Alcohol. Clin. Exp. Res. 26(5): 644–654. DOI: https://doi.org/10.1111/j.1530-0277.2002.tb02586.x

Pautassi, R.M., Molina, J.C., Spear, N. 2008. Infant rats exhibit aversive learning mediated by ethanol's orosensory effects but are positively reinforced by ethanol's post-ingestive effects. Pharmacol. Biochem. Behav. 88: 393–402. DOI: https://doi.org/10.1016/j.pbb.2007.09.012

Pautassi, R.M., Nizhnikov, M.E., Fabio, M.C., Spear, N.E. 2012a. Early maternal separation affects ethanol-induced conditioning in a nor-BNI insensitive manner, but does not alter ethanol-induced locomotor activity. Pharmacol. Biochem. Behav. 100(3), 630–638. DOI: https://doi.org/10.1016/j.pbb.2011.11.005

Pautassi, R.M., Nizhnikov, M.E., Spear, N.E., Molina, J.C. 2012b. Prenatal ethanol exposure leads to greater ethanol-induced appetitive reinforcement. Alcohol 46(6), 585–593. DOI: https://doi.org/10.1016/j.alcohol.2012.05.004

Pautassi, R.M., Sanders, S., Miller, S., Spear, N., Molina, J.C. 2006. Early ethanol's anxiolytic effects assessed through an unconditional stimulus revaluation procedure. Alcohol. Clin. Exp. Res. 30(3): 448–459. DOI: https://doi.org/10.1111/j.1530-0277.2006.00049.x

Philpot, R.M., Badanich, K.A., Kirstein, C.L. 2003. Place conditioning: age-related changes in the rewarding and aversive effects of alcohol. Alcohol. Clin. Exp. Res. 27(4), 593–599. DOI: https://doi.org/10.1097/01.ALC.0000060530.71596.D1

Quintanilla, M.E., Rivera-Meza, M., Berrios-Cárcamo, P.A., Bustamante, D., Buscaglia, M., Morales, P., … Israel, Y. 2014. Salsolinol, free of isosalsolinol, exerts ethanol-like motivational/sensitization effects leading to increases in ethanol intake. Alcohol 48(6), 551–559. DOI: https://doi.org/10.1016/j.alcohol.2014.07.003

Quintanilla, M.E., Rivera-Meza, M., Berríos-Cárcamo, P., Cassels, B.K., Herrera-Marschitz, M., Israel, Y. 2016. (R)-Salsolinol, a product of ethanol metabolism, stereospecifically

induces behavioral sensitization and leads to excessive alcohol intake. Addict. Biol. 21(6), 1063–1071. DOI: https://doi.org/10.1111/adb.12268

Quintanilla, M. E., Tampier, L. 2011. Place conditioning with ethanol in rats bred for high (UChB) and low (UChA) voluntary alcohol drinking. Alcohol, 45(8), 751–762. DOI: https://doi.org/10.1016/j.alcohol.2011.06.002

Retnam, L., Chatikavanij, P., Kunjara, P., Paramastri, Y.A., Goh, Y.M., Hussein, F.N., Mutalib, A.R., Poosala, S. 2016. Laws, regulations, guidelines and standards for animal care and use for scientific purposes in countries of Singapore, Thailand, Indonesia, Malaysia, and India. ILAR J., 57, 312–323. DOI: https://doi.org/10.1093/ilar/ilw038

Robbins, T.W. 1977. A critique of the methods available for the measurement of spontaneous motor activity. In: Iversen, L.L., Iversen, S.D., Snyder, S.H. (Eds.), Handbook of psychopharmacology (7): Principles of behavioral pharmacology. Plenum Press, New York, pp. 37–82.

Robinson, D.L., Brunner, L.J., Gonzales, R.A. 2002. Effect of gender and estrous cycle on the pharmacokinetics of ethanol in the rat brain. Alcohol. Clin. Exp. Res. 26(2), 165–172. DOI: https://doi.org/10.1111/j.1530-0277.2002.tb02521.x

Roma, P.G., Riley, A.L. 2005. Apparatus bias and the use of light and texture in place conditioning. Pharmacol. Biochem. Behav. 82(1), 163–169. DOI: https://doi.org/10.1016/j.pbb.2005.08.004

Rosenthal, R. 1991. Applied social research methods series (6): Meta-analytic procedures for social research. Sage Publications, USA.

Rossi, N. A., Reid, L.D. 1976. Affective states associated with morphine injections. Psychobiol. 4, 269–274. DOI: https://doi.org/10.3758/BF03332869

Salsano, F., Maly, I.P., Sasse, D. 1990. The circadian rhythm of the intra-acinar profiles of alcohol dehydrogenase activity in rat liver: A microquantitative study. Histochem. J. 22, 395–400. DOI: https://doi.org/10.1007/BF01003458

Schechter, M.D. 1992. Locomotor activity but not conditioned place preference is differentially affected by a moderate dose of ethanol administered to P and NP rats. Alcohol 9(3), 185–188. DOI: https://doi.org/10.1016/0741-8329(92)90050-k

Schechter, M.D., Calcagnetti, D.J. 1993. Trends in place preference conditioning with a cross-indexed bibliography; 1957-1991. Neurosci. Biobehav. Rev. 17, 21–41. DOI: https://doi.org/10.1016/S0149-7634(05)80228-3

Schechter, M.D., Calcagnetti, D.J. 1998. Continued trends in the conditioned place preference literature from 1992 to 1996, inclusive, with a cross-indexed bibliography. Neurosci. Biobehav. Rev. 22, 827–846. DOI: https://doi.org/10.1016/s0149-7634(98)00012-8

Schechter, M.D., Krimmer, E.C. 1992. Differences in response to the aversive properties and activity effects of low dose ethanol in LAS and HAS selectively bred rats. Psychopharmacol. (Berl.) 107(4), 564–568. DOI: https://doi.org/10.1007/BF02245271

Schramm-Sapyta, N.L., Francis, R., MacDonald, A., Keistler, C., O'Neill, Khun, C.M. 2014. Effect of sex on ethanol consumption and conditioned taste aversion in adolescent and adult rats. Psychopharmacol. 231, 1831–1839. DOI: https://doi.org/10.1007/s00213-013-3319-y

Sena, E., Van der Worp, H.B., Howells, D., Macleod, M. 2007. How can we improve the pre-clinical development of drugs for stroke? Trends Neurosci. 30, 433–439. DOI: https://doi.org/10.1016/j.tins.2007.06.009

Sengupta, P. 2011. A scientific review of age determination for a laboratory rat: How old is it in comparison with human age? Biomed. Int. 2, 81–89.

Sengupta, P. 2013. The laboratory rat: Relating its age with human's. Int. J. Prevent. Med. 4(6), 624–630.

Simon, F.R., Fortune, J., Iwahashi, M., Sutherland, E. 2002. Sexual dimorphic expression of ADH in rat liver: Importance of the hypothalamic-pituitary-liver axis. Am. J. Physiol. Gastrointest. Liver. Physiol. 283, G646–G655. DOI: https://doi.org/10.1152/ajpgi.00438.2001

Shi, H., Clegg, D.J. 2009. Sex differences in the regulation of body weight. Physiol. Behav. 97, 199–204. DOI: https://doi.org/10.1016/j.physbeh.2009.02.017

Spina, L., Longoni, R., Vinci, S., Ibba, F., Peana, A.T., Muggironi, G., Spiga, S., Acquas, E. 2010. Role of dopamine D_1 receptors and extracellular signal regulated kinase in the motivational properties of acetaldehyde as assessed by place preference conditioning. Alcohol. Clin. Exp. Res. 34, 607–616. DOI: https://doi.org/10.1111/j.1530-https://doi.org/0277.2009.01129.x

Stewart, R.B., Grupp, L.A. 1985. Some determinants of the motivational properties of ethanol in the rat: Concurrent administration of food and social stimuli. Psychopharmacol. (Berl) 87, 43–50. DOI: https://doi.org/10.1007/BF00431776

Stewart, R.B., Murphy, J.M., McBride, W.J., Lumeng, L., Li, T.-K. 1996. Place conditioning with alcohol in alcohol-preferring and -nonpreferring rats. Pharmacol. Biochem. Behav. 53(3), 487–491. DOI: https://doi.org/10.1016/0091-3057(95)02102-7

Swerdlow, N.R., Gilbert, D., Koob, G.F. 1989. Conditioned drug effects on spatial preference: Critical evaluation. In: Boulton, A.A., Baker, G.B., Greenshaw, A.J. (Eds.), Neuromethods (13): Psychopharmacology. Humana Press, Clifton, pp. 399–446.

Tagliaferro, A.R., Davis, J.R., Truchon, S., Van Hamont, N. 1986. Effects of dehydroepiandrosterone acetate on metabolism, body weight and composition of male and female rats. J. Nutr. 116(10), 1977–1983. DOI: https://doi.org/10.1093/jn/116.10.1977

Torres, O.V., Walker, E.M., Beas, B.S., O'Dell, L.E. 2014. Female rats display enhanced rewarding effects of ethanol that are hormone dependent. Alcohol. Clin. Exp. Res. 38(1), 108–115. DOI: https://doi.org/10.1111/acer.12213

Timoshanko, A.C., Marston, H., Lidbury, B.A. 2016. Australian regulation of animal use in science and education: A critical appraisal. ILAR J. 57, 324–332. DOI: https://doi.org/10.1093/ilar/ilw015

Tzschentke, T.M. 1998. Measuring reward with the Conditioned place preference paradigm: A comprehensive review of drug effects, recent progress, and new issues. Prog. Neurobiol. 56, 613–672. DOI: https://doi.org/10.1016/s0301-0082(98)00060-4

Tzschentke, T.M. 2007. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict. Biol. 12, 227–462. DOI: https://doi.org/10.1111/j.1369-1600.2007.00070.x

Valentine, J.C. 2009. Judging the quality of primary research. In: Cooper, H., Hedges, L.V., Valentine, J.C. (Eds.), The Handbook of Research Synthesis and Meta-Analysis (2nd ed.). Russell Sage Foundation, USA, pp. 129–146.

Vasbinder, M.A., Locke, P. 2016. Introduction: Global laws, regulations, and standards for animal in research. ILAR J. 57, 261–265. DOI: https://doi.org/10.1093/ilar/ilw039

Winzer-Serhan, U.W. 2008. Long-term consequences of maternal smoking and developmental chronic nicotine exposure. Front. Biosci. 13, 636–649. DOI: https://doi.org/10.2741/2708

Xu, Y., Liu, X., Zhang, X., Zhang, G., Zhang, R., Liu, T., Hao, W. 2012. Histone acetylation of the htr3a gene in the prefrontal cortex of Wistar rats regulates ethanol-seeking behavior. Neural Regen. Res. 7(13), 1021–1028. DOI: https://doi.org/10.3969/j.issn.1673-5374.2012.13.009

Zhu, W., Bie, B., Pan, Z.Z. 2007. Involvement of non-NMDA glutamate receptors in central amygdala in synaptic actions of ethanol and ethanol-induced reward behavior. J. Neurosci. 27, 289–298. DOI: https://doi.org/10.1523/JNEUROSCI.3912-06.2007

SEÇÃO DE DISCUSSÃO SUPLEMENTAR AO CAPÍTULO 1: TABELAS COMPLEMENTARES S6 A S8

Table S6 Associative learning-, alcohol-, and animal-related variables affecting the acquisition of alcohol-induced PC and the primary motivator valence in alcohol-naïve rats

Variable	Physiological and behavioral effect of changes in variable parameters
Latent inhibition	Decreases chances of successful conditioning, delays acquisition of conditioned control, and decreases levels of conditioned responding. Variables influencing latent inhibition are number of habituation sessions, total duration of the habituation phase (aggregate of all habituation sessions), interphasic interval between habituation and conditioning phases, motivator magnitude, and number of conditioning trials. Increases in the first 2 variables favor latent inhibition, while increases in the last 3 challenge it (Ayres et al.,1992; Lubow, 1989; Fanselow, 1990; Rodríguez and Alonso, 2004).
Multiple cues	May increase/decrease chances of conditioning success according to the learning preparation, stimulatory interactions, and the phylogenetic characteristics of the organism (Fanselow, 1999; Mucha et al., 1982). Using olfactory modality in compounds demands greater control of its presentation concerning the visual and tactile modalities: animals may experience the olfactory stimulus before the others, causing blocking effects and unspecific conditioned control (Schmajuk, 2010; Vezina and Stewart, 1987a).
Interstimulus interval (ISI)	May increase/decrease chances of conditioning success according to the learning preparation, cue and motivator properties, and the phylogenetic characteristics of the organism (see Bormann and Cunningham, 1998). In front of an algebraic summation hypothesis of PC (White et al., 1987), the significance of ISIs for alcohol-induced PC must be taken with care. If the algebraic interpretation is valid, ISIs that have detrimental effects on CPA acquisition could promote CPP acquisition and vice-versa (within the limits imposed by alcohol pharmacokinetics and pharmacodynamics).
Alcohol dose (g/kg)	Lower motivator magnitude decreases chances of conditioning success and favors conditioned control more susceptible to disruptions when fewer conditioning trials are used (more conditioning trials increase the efficacy of conditioned control induced by primary motivators of low magnitude). Greater motivator magnitude speeds up behavioral acquisition and increases chances of conditioning success. Motivators of too great magnitude tend to be aversive and favor CPA over CPP (Azrin and Holz, 1966; Binder et al., 2008; Morris and Bouton, 2006; Rose et al., 2009).
Alcohol concentration (%, v/v) and adm. route	For the ip route, under similar conditions of blood volume and irrigation, total body water percentage (TBW%), and body water relative distribution (BWRD), administering the same dose in different concentrations does not affect the total of dose absorbed but determines different maximum blood alcohol concentrations (BACs) as absorption kinetics is slower for low-concentration solutions (see Linakis and Cunningham, 1979; Roine et al., 1991). For the og and ig, concentration and feeding state of the animals affect both the total amount of dose to be absorbed and absorption time (Roine et al., 1991). Moreover, concentrations higher than 20% slow down absorption kinetics and decrease the maximum BAC achievable for various reasons — including local tissue damage, which may cause pain to the animal, favoring CPA acquisition — (see Bode, 1980; Kalant, 1971, 1996; Roine et al., 1991).
Alcohol distribution	For both humans and rats, main variables affecting alcohol distribution are TBW% and the lean/fat mass ratio (known to affect BWRD) (Cederbaum, 2012; Ernst et al., 1976; Kalant, 2000). For rats, TBW% reductions and BWRD changes occur according to the age-weight covariate, leading the same dose to cause greater BACs in older/heavier rats than in younger/lighter ones. For example, Bloom et al. (1982) studied BACs in 72 naïve rats (age range of 36-100 days) divided into 7 weight groups (means of 135, 182, 247, 286, 311, 357, and 419 g). Each rat within each weight group received a different dose (1, 2, and 3 g/kg, ip, 20%) and BACs were measured in tail blood. From the lighter to the heavier group, maximum BACs varied from ~7 to ~23 mM, from ~33 to ~52 mM, and from ~65 to ~70 mM for 1, 2, and 3 g/kg, respectively (see also Ernst et al., 1976; Walker and Ehlers, 2009).

Alcohol biotransformation and blood accumulation of acetaldehyde	For the valence of alcohol motivator function, the blood accumulation of free acetaldehyde is considered a strong aversive stimulus (Eriksson, 1980, 2001; Li, 2000). In accordance, acetaldehyde production, blood accumulation, and elimination are the best predictors of susceptibility to alcohol use disorder in humans and the probability of lesser/greater alcohol intake in rats selected for alcohol drinking (humans: Bierut et al., 2012; Edenberg, 2007; Hurley and Edenberg, 2012; Israel et al., 2011; Li et al., 2001; Neumark et al, 2004; rats: Eriksson, 1973; Koivula et al., 1975; Koivisto and Eriksson, 1994; Nishiguchi et al., 2002; Quintanilla et al., 2005a, 2005b, 2006; Rivera-Meza et al., 2010; Sapag et al., 2003). For UchA rats submitted to PC, the lower threshold for aversive effects seems to be an acetaldehyde concentration of 20-30 µM in the arterial blood and may be surpassed transiently 5-10 min after a dose of 1 g/kg (ip) is given (Quintanilla and Tampier, 2011). Considering that about 90% of alcohol metabolism is hepatic (mainly via a two-step enzymatic process: in the first, alcohol dehydrogenase — ADH — and the microsomal ethanol-oxidizing system — MEOS — oxidize alcohol to acetaldehyde; in the second, aldehyde dehydrogenase — ALDH — irreversibly oxidizes acetaldehyde to acetate), three characteristics and conditions of rats must be considered for the balance between the production (by ADH and MEOS) and elimination (by ALDH) of acetaldehyde: the age-weight covariate, circadian rhythms, and stress.
The age-weight covariate	For humans, onset of alcohol use in late-infancy/early-adolescence is a risk factor for posterior alcohol misuse (Aiken et al., 2018; Kraus et al., 2000; Sylvestre et al., 2020; but see Kuntsche et al., 2016). For rats aged 35 to 180 days, age-weight increase causes a series of changes that progressively favor acetaldehyde buildup: **a)** Given that the alcohol Km of ADH is lower (0.5-1 mM) than that of MEOS (9-13 mM) and that 1 g/kg of alcohol can generate BACs significantly greater than 10 mM in older rats, with 2 g/kg attaining BACs of ~50 mM (see above), this BAC range not only saturates ADH activity for longer in older/heavier animals. It also recruits the activity of MEOS in the oxidation of alcohol to acetaldehyde, may cause the MEOS to nearly attain its maximum activity at BACs of ~50 mM (Teschke, 2019), and may extend both saturations for longer (see the BAC kinetics in Bloom et al., 1982; other important factors for these kinetics in Braggins and Crow, 1981; Gershman, 1975; Tottmar and Marchner, 1975); **b)** The specific activities of hepatic ADH and ALDH attain stability in the 5th post-natal week (Horton and Mills, 1979; Lad et al., 1984). However, the total activity of ADH increases with age-weight: for rats weighting ~180, ~280, and ~420 g (~42, ~60-70, and ~100 days old, respectively), ADH has an overall increase of ~40%, with more than half (25%) occurring between the weights of 280 and 420g (Bloom et al., 1982; see also Coldwell et al., 1971; Lad et al., 1984). Meanwhile, the total activity of ALDH does not increase much (~10%) for rats 60 to 180 days old (Danh et al., 1983; Horton and Mills, 1979); **c)** Eriksson and Sippel (1977) showed that BACs (from ~10 to ~47 mM) produced by doses of 0.75, 1.5, and 3 g/kg (og, 18.75%) generated acetaldehyde concentrations of ~17 to ~160 µM in cerebral blood (a proxy for arterial blood) (for limitations in this study, see Kilanmaa and Virtanen, 1978; see acetaldehyde data of Tottmar and Marchner, 1975); **d)** Using Wistar rats with different ages (~40, ~70, ~100, and ~130 days old) at the beginning of a free-choice protocol (10 days of 5% alcohol v/v, followed by 40 days of 10% v/v), Amir (1978) showed the mean alcohol intake decreased with age: during the last 20 days of free-choice, groups 40, 70, 100, and 130 drank ~3.81, ~3.85, ~2.98, and ~2.02 ml/kg/day, respectively, a total reduction of ~47% (see also Wood and Armbrecht, 1982).
Circadian rhythms in the 12:12 light (L) and dark (D) cycle	In humans, alcohol consumption and craving phenomenon present circadian rhythms (Hiller-Sturmhöfel and Kulkosky, 2001; Hisler et al., 2020). In rats, consumption, BACs, blood clearance rates, and ADH activity present circadian rhythms with strikingly similar zeniths and nadirs (late period/end of the D and L phases, respectively) (Boyle et al., 1997; García-Burgos et al., 2009; Pinkston and Soliman, 1979; Soliman and Walker, 1979; Sturtevant and Garber, 1980, 1981, 1984). For the L phase, this indicates a greater probability of acetaldehyde accumulation in the morning than in the afternoon. Also, circadian rhythms happen for the blood-brain barrier permeability and in glutamate, GABA, and dopamine (DA) release in the dorsal and ventral striatum (Castañeda et al., 2004; Cuddapah et al., 2019; Ferris et al., 2014). As the phasic release of DA in the ventral striatum is implicated in the reinforcing effects of many drugs of abuse, including alcohol (Di Chiara and Imperato, 1986; McBride et al., 1999; Yim and Gonzales, 2000), the circadian variation in the striatum release of DA must be considered. The zenith of the tonic release occurs ~5-6 hours into the D phase and the nadir occurs ~5-6 hours into the L phase, whereas the zenith of the phasic release occurs ~6-7 hours into the L phase and the nadir occurs ~6-7 hours into the D phase (Ferris et al., 2014). For the L phase, this points to a greater sensibility for alcohol reinforcement in the afternoon than in the morning.

Stressful stimuli and conditions	In rats, stress effects on alcohol motivator function are equivocal and depend not only on stress type (e.g., foot shock, single housing, social defeat, restrain), duration, variability, intensity, and temporal patterns, but also on the behavioral preparation (operant self-administration *vs.* free-choice *vs.* PC), animal age, and alcohol dose (for reviews of operant and free-choice preparations, see Becker et al., 2011; Noori et al., 2014; Spanagel et al., 2014). For PC, Bieńkowski et al. (1996) showed that 20 daily ip administrations of saline prior to a short protocol could facilitate CPP induction by a dose of 0.5 g/kg but not by 1 g/kg (both 10%, ip) for group-housed Wistar rats. Matsuzawa, Suzuki, and Misawa (1998), Matsuzawa, Suzuki, Misawa, and Nagase (1998), and Matsuzawa et al. (1999a, 1999b, 1999c) showed that applying conditioned fear stress before short protocols using very low doses (0.15 and 0.3 g/kg, 20%, ip) seems to enhance both conditioned control acquisition and appetitive properties of alcohol for group-housed Sprague Dawley rats. Funk et al. (2004) showed that uncontrollable shock and social defeat immediately before each conditioning trial did not affect the dose of 0.6 g/kg (CF) but abolished CPA for a dose of 1 g/kg for single-housed Wistar rats. Der-Avakian et al. (2007) showed that one session of inescapable shock before the conditioning phase did not affect a dose of 0.3 g/kg (CF), abolished CPP for 1 g/kg, and decreased CPP for 2 g/kg in group-housed Sprague Dawley rats.

PC, place conditioning; CPA, conditioned place aversion; CPP, conditioned place preference; CF, conditioning failure; ip, intraperitoneal route of administration; og, orogastric gavage route of administration; ig, intragastric route of administration. **Note:** The isozymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) oxidize other alcohols and aldehydes, respectively, with ontogeny, specific and total activities, and Km varying for each substrate (see Yoon et al., 2006). Additionally, ADH is capable of both alcohol oxidation and acetaldehyde reduction, with different ontogeny, specific and total activities, and Km for these reactions (compare ADH data on acetaldehyde reduction in Horton and Mills, 1979 with the ADH data on alcohol oxidation in Lad et al., 1984). Lastly, although the ontogeny data of the microsomal ethanol-oxidizing system — MEOS — point stability of its specific activity at the 6th post-natal week, these data are not for ethyl alcohol as a substrate (Alcorn et al., 2007; De Zwart et al., 2008). For these reasons, when composing this table, we used only data in which ethyl alcohol was the substrate for ADH and MEOS and acetaldehyde was the substrate for ALDH.

Table S7 Variables affecting the expression of alcohol-induced PC in rats during the post-conditioning test phase

Variable	Behavioral effect of changes in variable parameters
Distribution of trials	Uneven distribution of conditioning trials for the conditioning chambers causes differences in exposure to these chambers during conditioning. Great differences in exposure may increase exploratory behavior in the chamber with less exposure (generally the drug-unpaired chamber) during the post-test, an increase due to (relative) novelty effects. In this case, exploratory behavior would compete with conditioned control expression for the drug-paired chamber. If this is so, it may generate CF and even an artifact CPA. In the alternative case (i.e., less exposure to the drug-paired chamber), it may generate an artifact CPP (see Carr et al., 1988, 1989).
Apparatus spatial relations: Area	Open-field data indicates that apparatus area positively influences exploratory behavior (Walsh and Cummings, 1976). If this is the case for PC apparatuses in post-conditioning tests, greater exploratory behavior would compete with conditioned control expression, favoring CF. Importantly, an increase in apparatus area is not just predicted to interfere with conditioned control expression from supposed relationships between open-field and PC apparatuses themselves. It is also predicted to have such influence from how conditioned choice behavior has been operationalized in drug-induced PC studies, i.e., time spent in the drug-paired and -unpaired contexts during the post-test. As such, this operationalization implies that choice behavior depends on exploratory behavior. Consequently, variables modulating exploratory behavior during the post-test may also modulate conditioned choice behavior by increasing or decreasing conflict between the exploratory drive and the conditioned control. This has implications not just to apparatus area, but to any spatial configuration of the PC apparatus, such as the effects of number of chambers on the magnitude of opioid- and psychostimulant-induced PC shown by Bardo et al. (1995) (see below). Even more, it establishes the need for differentiating, in PC, drugs that induce conditioned locomotion in outbred rats from those that rarely do so, such as alcohol.
Apparatus spatial relations: Number of chambers	Magnitudes of cocaine-, heroin- and morphine-induced PC are greater in 3-chamber apparatuses than in 2-chamber ones (Bardo et al., 1995). Although significant increases in magnitude are restricted to heroin and cocaine, all these drugs are known to induce conditioned locomotion in outbred rats (Brown and Fibiger, 1992; Cervo and Samanin, 1996; Gold et al., 1988; Hotsenpiller and Wolf, 2002; Vezina and Stewart, 1987a, 1987b; Zakharova et al., 2009). The alcohol-induced PC literature herein reviewed presents 1- to 3-chamber apparatuses. In the latter, a central chamber is used as a “start-box” during habituation and post-conditioning test (Carr et al., 1989). As animals are less exposed to this chamber and it has different dimensions from the conditioning contexts, it has novelty- and spatial-related influences on exploratory behavior. Thus, it may generate patterns of exploratory behavior that compete with conditioned control, rendering its expression ineffective or unspecific. Consequently, dissimilar to the results reported by Bardo et al. (1995) for opioid- and stimulant-induced PC, more chambers would be deleterious to alcohol-induced PC, decreasing expression of conditioned control. Moreover, this would be more pronounced in larger apparatuses because alcohol rarely induces conditioned locomotion in outbred rats.

PC, place conditioning; CPA, conditioned place aversion; CPP, conditioned place preference; CF, conditioning failure.

Table S8 Inferences from the predictive organization of results of alcohol PC with male outbred rats

Inferences	Discussion
Theoretical: Associative learning in drug PC*	**Rationale:** Both operant and classical conditioning have been proposed to occur in drug PC (Huston et al., 2013). In both instances, delays in learning are evident after unreinforced exposure (habituation) to the to-be-conditioned stimulus, but the increased number of such exposures does not enhance further this disruption on operant learning (see Mellgren and Ost, 1971; Lipp, 1999; Lubow, 1989). **Evidence:** Previous analyses of acquisition and expression of morphine PC support the predominance of classical conditioning (German and Fields, 2007; Spiteri et al., 2000). Similarly, latent inhibition disruption in alcohol PC is more akin to that of classical conditioning (see section **4.1**) and further learning delay is also shown by the conditioning failure for a long protocol (16 trials) with 20 habituation sessions (Stewart and Grupp, 1985) compared to the 10 CPP for long protocols (14 trials) with 3 sessions (Bie et al., 2009; Zhu et al., 2007).
Theoretical: Sensitivity in alcohol motivation	**Comparison:** Outbred albino strains *vs.* alcohol-selected strains in short protocols. **Evidence:** Outbred rats are highly sensitive to latent inhibition disruptions: two or more habituation sessions severely increase the probability of conditioning failure (dose ≤ 1.0 g/kg, ≤ 20% v/v). In contrast, alcohol-selected strains seem to resist latent inhibition in these settings (Schechter and Krimmer, 1992; Schechter, 1992; Ciccocioppo et al., 1999; Quintanilla and Tampier, 2011)**. **Experimental question:** For these rats, compared to outbred ones, is conditioned control also more resistant to extinction and easier to reinstate in alcohol PC?
Instrumental: Sensitivity in alcohol motivation	**Direct experimental comparison:** Outbred albino *vs.* alcohol-selected strains in alcohol PC. **Instrumentalization:** Once confirmed, the latent inhibition difference may be used to identify distinct response patterns in brain substrates related to latent inhibition (e.g., the nucleus accumbens core: Kutlu et al., 2022) or to the formation "drug-biased memories", i.e., on how greater sensitivity to alcohol effects makes associated environmental stimuli more salient, lessening disruptions. If conditioned control is also more resistant to extinction and easier to reinstate for these rats, instrumentalization can also be done for these cases in the study of "drug-biased memories" and their brain substrates.
Translational: Age-dependent vulnerability	**Comparison:** Younger *vs.* older outbred rats in short protocols. **Evidence (rats):** The instances of successful conditioning (CPP and CPA) are better predicted by the animals' characteristics (age-weight covariate) and conditions (housing system). Age-dependence in alcohol PC agrees with the age-dependence of alcohol reinforcement (Amir, 1978) and interactions of reinforcement and stress in rats (Noori et al., 2014; Spanagel et al., 2014). **Evidence (humans):** Onset of alcohol use in late infancy/early adolescence is a risk factor for alcohol misuse, binge drinking, alcohol-related problems, and alcoholism (Aiken et al., 2018; Chou and Pickering, 1992; Kraus et al., 2000; Sylvestre et al., 2020; but see Kuntsche et al., 2016). **Translation (model):** As mice are less affected by age in these cases (see Noori et al., 2014; Song et al., 2007), rats would be a better model for this vulnerability in PC.
Translational: Early induction of Incentive motivation	**Comparison:** Outbred rats *vs.* outbred mice in short protocols. **Evidence:** Successful conditioning results indicate that conditioned control may be induced early in a contact history with alcohol, and this early induction majorly depends on the use of moderate to high doses of alcohol (≥ 1 g/kg). Additionally, considering an allometric interpretation of drug effects in different species, a similar conclusion is pertinent for mice: successful conditioning is easier in these protocols with moderate to high doses of alcohol (≥ 2 g/kg). However, there are differences. For rats, valence seems to be mainly dependent on animal age-weight and, for doses above 2 g/kg, it is likely biased toward an aversive valence. For mice, valence does not seem to be modulated by age-weight and seems less sensitive to high doses of alcohol (CPP has been shown for 4 g/kg: Risinger and Oakes, 1996; Song et al., 2007). Second, CPP may reach greater magnitudes in mice than in rats. **Translation (questions for human research):** Does early induction also happen for humans? Is it also dependent on using moderate to higher doses of alcohol? Are there different human phenotypes in this early induction (e.g., one dependent on age and alcohol dose, with a lower response magnitude; another less dependent on age, more related to high doses, and with higher response magnitude)? If all of this is true, what are the implications of this early induction in different human phenotypes for the natural history, treatment, and prognosis of alcoholism? Although some human data might suggest positive answers to some of these questions (Sylvestre et al., 2020), the data are not for the development of incentive motivation.
Translational: Late induction of incentive motivation	Late induction of conditioned control in alcohol PC. **Comparison:** Outbred rats *vs.* outbred mice in short and long protocols. **Evidence:** For rats, the different ratios of conditioning success/failure of short and long protocols with 0.5-0.75 g/kg indicate that these doses can also instate conditioned control in PC, but a longer acquisition phase is required (i.e., sensitization-dependence). Considering an allometric interpretation of drug effects in different species, data with mice point to the same conclusion (e.g., 1.0 g/kg: Risinger and Oakes, 1996). **Translation (questions for human research):** This seems to agree with the escalating trajectories in the frequency of alcohol consumption in humans (Sylvestre et al., 2020) and questions similar to those for early induction may be posed.

PC, place conditioning; CPA, conditioned place aversion; CPP, conditioned place preference. *, This item refers to drug PC experiments conducted according to the "absolute measure PC procedure" (Tzschentke, 2007) in which the drug is delivered by the experimenter. **, A priori, it is not possible to discharge influences of a different phase of the circadian cycle in Ciccocioppo et al. (1999) and of sex in Quintanilla and Tampier (2011).

Referências às tabelas complementares

Aiken, A., Clare, P.J., Wadolowski, M., Hutchinson, D., Najman, J.M., Slade, T. et al. (2017) Age of alcohol initiation and progression to binge drinking in adolescence: a prospective cohort study. *Alcoholism, Clinical and Experimental Research*, 42, 100–110. Available from: https://doi. org/10.1111/acer.13525

Alcorn, J., Elbarbry, F.A., Allouh, M.Z. & McNamara, P.J. (2007) Evaluation of the assumptions of an ontogeny model of rat hepatic cytochrome p450 activity. *Drug Metabolism and Disposition*, 35, 2225–2231. Available from: https://doi.org/10.1124/dmd.107.017590

American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed. American Psychological Association, Washington, D.C.

Amir, S. (1978) Brain and liver aldehyde dehydrogenase activity and voluntary ethanol consumption by rats: relations to strain, sex, and age. *Psychopharmacology*, 57, 97–102. Available from: https://doi. org/10.1007/BF00426964

Ayres, J.J.B., Philbin, D., Cassidy, S. & Belling, L. (1992) Some parameters of latent inhibition. *Learning and Motivation*, 23, 269–287. Available from: https://doi.org/10.1016/0023-9690(92)90009-B

Azrin, N.H. & Holz, W.C. (1966) Punishment. In: Honig, W.K. (Ed.) *Operant behavior: areas of research and application*. New York: AppletonCentury-Crofts, pp. 380–447.

Bardo, M.T., Rowlett, J.K. & Harris, M.J. (1995) Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neuroscience and Biobehavioral Reviews*, 19, 39–51. Available from:
https://doi.org/10.1016/0149-7634(94)00021-r

Becker, H.C., Lopez, M.F. & Doremus-Fitzwater, T. (2011) Effects of stress on alcohol drinking: a review of animal studies. *Psychopharmacology*, 218, 131–156. Available from: https://doi. org/10.1007/s00221-005-0081-3

Bie, B., Zhu, W. & Pan, Z.Z. (2009) Ethanol-induced delta-opioid receptor modulation of glutamate synaptic transmission and conditioned place preference in central amygdala. *Neuroscience*, 160, 348–358. Available from: https://doi.org/10.1016/j.neuroscience.2009.02.049

Bieńkowski, P., Kuca, P., Piasecki, J. & Kostowski, W. (1996) Low dose of ethanol induces conditioned place preference in rats after repeated exposures to ethanol or saline injections. *Alcohol and Alcoholism*, 31, 547–553. Available from: https://doi.org/10.1093/oxfordjour nals.alcalc.a008190

Bierut, L.J., Goate, A.M., Breslau, N., Johnson, E.O., Bertelsen, S., Fox, L. et al. (2012) *ADH1B* is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. *Molecular Psychiatry*, 17, 445–450. Available from: https://doi. org/10.1038/mp.2011.124

Binder, M.D., Hirokawa, N. & Windhorst, U. (2008) Aversive stimulus. In: Binder, M.D., Hirokawa, N. & Windhorst, U. (Eds.) *Encyclopedia of neuroscience*. Berlin-Heidelberg: Springer, pp. 296

Bloom, F., Lad, P., Pittiman, Q. & Rogers, G. (1982) Blood alcohol levels in rats: non-uniform yields from intraperitoneal doses based on body weight. *British Journal of Pharmacology*, 75, 251–254. Available from: https://doi.org/10.1111/j.1476-5381.1982.tb08780.x

Bode, J.C. (1980) Alcohol and the gastrointestinal tract. In: Frick, P., von Harnack, G.-A., Martini, G.A. & Prader, A. (Eds.) *Advances in internal medicine and pediatrics*, Vol. 45. New York: Springer-Verlag, pp. 1–75.

Bormann, N.M. & Cunningham, C.L. (1998) Ethanol-induced conditioned place aversion in rats: effects of interstimulus interval. *Pharmacology, Biochemistry, and Behavior*, 59, 427–432. Available from: https://doi.org/10.1016/S0091-3057(97)00455-3

Boyle, A.E., Smith, B.R. & Amit, Z. (1997) A descriptive analysis of the structure and temporal pattern of voluntary ethanol intake within an acquisition paradigm. *Journal of Studies on Alcohol*, 58, 382–391. Available from: https://doi.org/10.15288/jsa.1997.58.382

Braggins, T.J. & Crow, K.E. (1981) The effects of high ethanol doses on rates of ethanol oxidation in rats. *European Journal of Biochemistry*, 119, 633–640. Available from: https://doi. org/10.1111/j.1432-1033.1981.tb05654.x

Brown, E.E. & Fibiger, H.C. (1992) Cocaine-induced conditioned locomotion: absence of associated increases in dopamine release. *Neuroscience*, 48, 621–629. Available from: https://doi. org/10.1016/0306-4522(92)90406-r

Carr, G.D., Fibiger, H.C. & Phillips, A.G. (1989) Conditioned place preference as a measure of drug reward. In: Liebman, J.M. & Cooper, S.J. (Eds.) *Topics in experimental psychopharmacology: Vol. 1. The Neuropharmacological basis of reward*. Oxford: Clarendon Press/ Oxford University Press, pp. 264–319.

Carr, G.D., Phillips, A.G. & Fibiger, H.C. (1988) Independency of amphetamine reward from locomotor stimulation demonstrated by conditioned place preference. *Psychopharmacology*, 94, 221–226. Available from: https://doi.org/10.1007/BF00176849

Castañeda, T.A., de Prado, B.M., Prieto, D. & Mora, F. (2004) Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. *Journal of Pineal Research*, 36, 177–185. Available from: https://doi. org/10.1046/j.1600-079x.2003.00114.x

Cederbaum, A.I. (2012) Alcohol metabolism. *Clinics in Liver Disease*, 16, 667–685. Available from: https://doi.org/10.1016/j.cld.2012.08.002

Cervo, L. & Samanin, R. (1996) Effects of dopaminergic and glutamatergic receptor antagonists on the establishment and expression of conditioned locomotion to cocaine in rats. *Brain Research*, 731, 31–38. Available from: https://doi.org/10.1016/0006-8993(96)00455-6

Chou, S.P. & Pickering, R.P. (1992) Early onset of drinking as a risk factor for lifetime alcohol-related problems. *British Journal of Addiction*, 87, 1199–1204. Available from: https://doi. org/10.1111/j.1360-0443.1992.tb02008.x

Ciccocioppo, R., Panocka, I., Froldi, R., Quitadamo, E. & Massi, M. (1999) Ethanol induces conditioned place preference in genetically selected alcohol-preferring rats. *Psychopharmacology*, 141, 235–241. Available from: https://doi.org/10.1007/s002130050830

Coldwell, B.B., Solomonraj, G., Trenholm, H.L. & Wiberg, G.S. (1971) The gas chromatographic estimation of ethanol, acetaldehyde and aceton in ethanol metabolism studies. *Clinical Toxicology*, 4, 99–113. Available from: https://doi.org/10.3109/15563657108990152

Cuddapah, V.A., Zhang, S.L. & Sehgal, A. (2019) Regulation of the blood–brain barrier by circadian rhythms and sleep. *Trends in Neurosciences*, 42, 500–510. Available from: https://doi. org/10.1016/j.tins.2019.05.001

Danh, H.C., Benedetti, M.S. & Dostert, P. (1983) Age-Related Changes in Aldehyde Dehydrogenase Activity of Rat Brain, Liver, and Heart. *Journal of Neurochemistry*, 41(3), 618–622. Available from: https://doi.org/10.1111/j.1471-4159.1983.tb04786.x

De Zwart, L., Scholten, M., Mobaliu, J.G., Annaret, P.P., van Houdt, J.M., van den Wyngaert, I. et al. (2008) The ontogeny of drug metabolizing enzymes and transporters in the rat.

Reproductive Toxicology, 26, 220–230. Available from: https://doi.org/10.1016/j.reprotox.2008.09.010

Der-Avakian, A., Bland, S.T., Rozeske, R.R., Tamblyn, J.P., Hutchinson, M.R., Watkins, L.R. et al. (2007) The effects of a single exposure to uncontrollable stress on the subsequent conditioned place preference responses to oxycodone, cocaine, and ethanol in rats. *Psychopharmacology*, 191, 909–917. Available from: https://doi.org/10.1007/s00213-006-0678-7

Di Chiara, G. & Imperato, A. (1986) Preferential stimulation of dopamine release in the nucleus accumbens by opiates, alcohol, and barbiturates: studies with transcerebral dialysis in freely moving rats. *Annals of the New York Academy of Sciences*, 473, 367–381. Available from: https://doi.org/10.1111/j.1749-6632.1986.tb23629.x

Edenberg, H.J. (2007) The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol Research & Health*, 30, 5–13.

Eriksson, C.J.P. (1973) Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochemical Pharmacology*, 22, 2283–2292. Available from: https:// doi.org/10.1016/0006-2952(73)90009-9

Eriksson, C.J.P. (1980) The aversive effect of acetaldehyde on alcohol drinking behavior in the rat. *Alcoholism, Clinical and Experimental Research*, 4, 107–111. Available from: https://doi. org/10.1111/j.1530-0277.1980.tb04799.x

Eriksson, C.J.P. (2001) The role of acetaldehyde in the actions of alcohol (update 2000). *Alcoholism, Clinical and Experimental Research*, 25, 15S–32S. Available from: https://doi.org/10.1097/00000374- 200105051-00005

Eriksson, C.J.P. & Sippel, H.W. (1977) The distribution and metabolism of acetaldehyde in rats during ethanol oxidation-I. The distribution of acetaldehyde in liver, brain, blood and breath. *Biochemical Pharmacology*, 26, 241–247. Available from: https://doi.org/10.1016/0006-2952(77)90310-0

Ernst, A.J., Dempster, J.P., Yee, R., Dennis, C. & Nakano, L. (1976) Alcohol toxicity, blood alcohol concentration and body water in young and adult rats. *Journal of Studies on Alcohol*, 37, 347–356. Available from: https://doi.org/10.15288/jsa.1976.37.347

Fanselow, M.S. (1990) Factors governing one-trial contextual conditioning. *Animal Learning & Behavior*, 18, 264–270. Available from: https://doi.org/10.3758/BF03205285

Fanselow, M.S. (1999) Learning theory and neuropsychology: configuring their disparate elements in the hippocampus. *Journal of Experimental Psychology. Animal Behavior Processes*, 25, 275–283. Available from: https://doi.org/10.1037/0097-7403.25.3.275

Ferris, M.J., España, R.A., Locke, J.L., Konstantopoulos, J.K., Rose, J.H., Chen, R. et al. (2014) Dopamine transporters govern diurnal variation in extracellular dopamine tone. *Proceedings of the National Academy of Sciences of the United States of America*, 111, E2751–E2759. Available from: https://doi.org/10.1073/pnas.14079 35111

Funk, D., Vohra, S. & Lê, A.D. (2004) Influence of stressors on the rewarding effects of alcohol in Wistar rats: studies with alcohol deprivation and place conditioning. *Psychopharmacology (Berlin)*, 176, 82– 87. Available from: https://doi.org/10.1007/s00213-004-1859-x

García-Burgos, D., González, F., Manrique, T. & Gallo, M. (2009) Patterns of ethanol intake in preadolescent, adolescent, and adult Wistar rats under acquisition, maintenance, and relapse-like conditions. *Alcoholism, Clinical and Experimental Research*, 33, 722–728. Available from: https://doi.org/10.1111/j.1530-0277.2008.00889.x

German, P.W. & Fields, H.L. (2007) How prior reward experience biases exploratory movements: a probabilistic model. *Journal of Neurophysiology*, 97, 2083–2093. Available from: https://doi. org/10.1152/jn.00303.2006

Gershman, H. (1975) Acetaldehyde-ethanol exchange in vivo during ethanol oxidation. *Archives of Biochemistry and Biophysics*, 158, 327–330. Available from: https://doi.org/10.1016/0003-9861(75)90257-X

Gold, L.H., Swerdlow, N.R. & Koob, G.F. (1988) The role of mesolimbic dopamine in conditioned locomotion produced by amphetamine. *Behavioral Neuroscience*, 102, 544–552. Available from: https://doi. org/10.1037//0735-7044.102.4.544

Hiller-Sturmhöfel, S. & Kulkosky, P. (2001) Chronobiological regulation of alcohol intake. *Alcohol Research & Health*, 25, 141–148.

Hisler, G., Rothenberger, S.D., Clark, D.B. & Hasler, B.P. (2020) Isthere a 24-hour rhythm in alcohol craving and does it vary by sleep/circadian timing? *Chronobiology International*, 38, 109–121. Available from: https://doi.org/10.1080/07420528.2020.1838532

Horton, A.A. & Mills, D.J. (1979) Developmental patterns of alcohol dehydrogenase and aldehyde dehydrogenases in homogenates and subcellular fractions of rat liver. *Mechanisms of Aging and Development*, 11, 363–370. Available from: https://doi. org/10.1016/0047-6374(79)90011-3

Hotsenpiller, G. & Wolf, M.E. (2002) Conditioned locomotion is not correlated with behavioral sensitization to cocaine: an intra-laboratory multi-sample analysis. *Neuropsychopharmacology*, 27, 924–929. Available from: https://doi.org/10.1016/S0893-133X(02)00370-6

Hurley, T.D. & Edenberg, H.J. (2012) Genes encoding enzymes involved in ethanol metabolism. *Alcohol Research: Current Reviews*, 34, 339–344.

Huston, J.P., Silva, M.A.S., Topic, B. & Müller, C.P. (2013) What's conditioned in conditioned place preference? *Trends in Pharmacological Sciences*, 34, 162–166. Available from: https://doi.org/10.1016/j. tips.2013.01.004

Israel, Y., Rivera-Meza, M., Quintanilla, M.E., Sapag, A. & Tampier, L. (2011) Acetaldehyde burst protection of ADH1B*2 against alcoholism: an additional hormesis protection against esophageal cancers following alcohol consumption? *Alcoholism, Clinical and Experimental Research*, 35, 806–810. Available from: https://doi. org/10.1111/j.1530-0277.2010.01403.x

Kalant, H. (1996) Pharmacokinetics of ethanol: absorption, distribution, and elimination. In: Begleiter, H. & Kissin, B. (Eds.) *The pharmacology of alcohol and alcohol dependence*. New York: Oxford University Press, pp. 15–58.

Kalant, H. (1971) Absorption, diffusion, distribution, and elimination of ethanol: effects on biological membranes. In: Kissin, B. & Begleiter, H. (Eds.) *The biology of alcoholism: Vol. 1. Biochemistry*. Boston: Springer, pp. 1–62.

Kalant, H. (2000) Effects of food and body composition on blood alcohol curves. *Alcoholism, Clinical and Experimental Research*, 24, 413–414.

Kilanmaa, K. & Virtanen, P. (1978) Ethanol and acetaldehyde levels in cerebrospinal fluid during ethanol oxidation in the rat. *Neuroscience Letters*, 10, 181–186. Available from: https://doi. org/10.1016/0304-3940(78)90032-0

Koivisto, T. & Eriksson, C.J.P. (1994) Hepatic aldehyde and alcohol dehydrogenase in alcohol-preferring and alcohol-avoiding rat lines. *Biochemical Pharmacology*, 48, 1551–1558. Available from: https:// doi.org/10.1016/0006-2952(94)90199-6

Koivula, T., Koivusalo, M. & Lindros, K.O. (1975) Liver aldehyde and alcohol dehydrogenase activities in rat strains genetically selected for their ethanol preference. *Biochemical Pharmacology*, 24, 1807–1811. Available from: https://doi.org/10.1016/0006-2952(75)90461-x

Kraus, L., Bloomfield, K., Augustin, R. & Reese, A. (2000) Prevalence of alcohol use and the association between onset of use and alcoholrelated problems in a general population

sample in Germany. *Addiction*, 95, 1389–1401. Available from: https://doi.org/10.1046/j.1360-0443.2000.95913899.x

Kuntsche, E., Rossow, I., Engels, R. & Kuntsche, S. (2016) Is 'age at first drink' a useful concept in alcohol research and prevention? We doubt that. *Addiction*, 111, 957–965. Available from: https://doi. org/10.1111/add.12980

Kutlu, M.G., Zachry, J.E., Melugin, P.R., Tat, J., Cajigas, S., Isiktas, A.U. et al. (2022) Dopamine signaling in the nucleus accumbens core mediates latent inhibition. *Nature Neuroscience*, 25, 1071–1081. Available from: https://doi.org/10.1038/s41593-022-01126-1

Lad, P., Shoemaker, W.J. & Leffert, H.L. (1984) Developmental changes in rat liver alcohol dehydrogenase. *Developmental Biology*, 105, 526–529. Available from: https://doi.org/10.1016/0012-1606(84)90310-5

Li, T.-K. (2000) Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. *Journal of Studies on Alcohol*, 61, 5–12. Available from: https://doi.org/10.15288/jsa.2000.61.5

Li, T.-K., Yin, S.-J., Crabb, D.W., O'Connor, S. & Ramchandani, V.A. (2001) Genetic and environmental influences on alcohol metabolism in humans. *Alcoholism, Clinical and Experimental Research*, 25, 136–144. Available from: https://doi.org/10.1111/j.1530-0277.2001.tb021 38.x

Linakis, J.K. & Cunningham, C.L. (1979) Effects of concentration of ethanol injected intraperitoneally on taste aversion, body temperature, and activity. *Psychopharmacology*, 64, 61–65. Available from: https://doi.org/10.1007/BF00427346

Lipp, O.V. (1999) The effect of stimulus specificity and number of preexposures on latent inhibition in an instrumental trials-to-criterion task. *Australian Journal of Psychology*, 51, 77–81. Available from: https://doi.org/10.1080/00049539908255339

Lubow, R.E. (1989) *Problems in the behavioural sciences: Vol. 9. Latent inhibition and conditioned attention theory*. Cambridge: Cambridge University Press

Matsuzawa, S., Suzuki, T. & Misawa, M. (1998) Conditioned fear stress induces ethanol-associated place preference in rats. *European Journal of Pharmacology*, 341, 127–130. Available from: https://doi. org/10.1016/S0014-2999(97)01456-8

Matsuzawa, S., Suzuki, T., Misawa, M. & Nagase, H. (1998) Involvement of μ- and δ-opioid receptors in the ethanol-associated place preference in rats exposed to foot shock stress. *Brain Research*, 803, 169–177. Available from: https://doi.org/10.1016/S0006-8993(98)00679-9

Matsuzawa, S., Suzuki, T., Misawa, M. & Nagase, H. (1999a) Different roles of μ-, δ- and κ-opioid receptors in ethanol-associated place preference in rats exposed to conditioned fear stress. *European Journal of Pharmacology*, 368, 9–16. Available from: https://doi. org/10.1016/S0014-2999(99)00008-4

Matsuzawa, S., Suzuki, T., Misawa, M. & Nagase, H. (1999b) Roles of 5-HT3 and opioid receptors in the ethanol-induced place preference in rats exposed to conditioned fear stress. *Life Sciences*, 64, PL241–PL249. Available from: https://doi.org/10.1016/S0024-3205(99)00144-7

Matsuzawa, S., Suzuki, T., Misawa, M. & Nagase, H. (1999c) Involvement of dopamine D1 and D2 receptors in the ethanol-associated place preference in rats exposed to conditioned fear stress.*Brain Research*, 835, 298– 305. Available from: https://doi.org/10.1016/S0006-8993(99)01606-6

McBride, W.J., Murphy, J.M. & Ikemoto, S. (1999) Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning

studies. *Behavioural Brain Research*, 101, 129–152. Available from: https://doi.org/10.1016/ S0166-4328(99)00022-4

Mellgren, R.L. & Ost, J.W.P. (1971) Discriminative stimulus preexposure and learning of an operant discrimination in the rat. *Journal of Comparative and Physiological Psychology*, 77, 179–187. Available from: https://doi.org/10.1037/h0031663

Morris, R.W. & Bouton, M.E. (2006) Effects of unconditioned stimulus magnitude on the emergence of conditioned responding. *Journal of Experimental Psychology: Animal Behavior Processes*, 32, 371–385. Available from: https://doi.org/10.1037/0097-7403.32.4.371

Mucha, R.F., van der Kooy, D., O'Shaughnessy, M. & Bucenieks, P. (1982) Drug reinforcement studied by the use of place conditioning in rat. *Brain Research*, 243, 91–105. Available from: https://doi. org/10.1016/0006-8993(82)91123-4

Neumark, Y.D., Friedlander, Y., Durst, R., Leitersdorf, E., Jaffe, D., Ramchandani, V.A. et al. (2004) Alcohol dehydrogenase polymorphisms influence alcohol-elimination rates in a male Jewish population. *Alcoholism, Clinical and Experimental Research*, 28, 10–14. Available from: https://doi.org/10.1097/01.ALC.00001 08667.79219.4D

Nishiguchi, M., Kinoshita, H., Mostofa, J., Taniguchi, T., Ouchi, H., Minami, T. et al. (2002) Different blood acetaldehyde concentration following ethanol administration in a newly developed high alcohol preference and low alcohol preference rat model system. *Alcohol and Alcoholism*, 37, 9–12. Available from: https://doi.org/10.1093/ alcalc/37.1.9

Noori, H.R., Helisnki, S. & Spanagel, R. (2014) Cluster and meta-analyses on factors influencing stress-induced alcohol drinking and relapse in rodents. *Addiction Biology*, 19, 225–232. Available from: https:// doi.org/10.1111/adb.12125

Pinkston, J.N. & Soliman, K.F.A. (1979) Effect of light and fasting on the circadian variation of ethanol metabolism in the rat. *Journal of Interdisciplinary Cycle Research*, 10, 185–193. Available from: https://doi.org/10.1080/09291017909359669

Quintanilla, M.E., Israel, Y., Sapag, A. & Tampier, L. (2006) The UChA and UChB rat lines: metabolic and genetic differences influencing ethanol intake. *Addiction Biology*, 11, 310–323. Available from: https:// doi.org/10.1111/j.1369-1600.2006.00030.x

Quintanilla, M.E. & Tampier, L. (2011) Place conditioning with ethanol in rats bred for high (UChB) and low (UChA) voluntary alcohol drinking. *Alcohol*, 45, 751–762. Available from: https://doi.org/10.1016/j. alcohol.2011.06.002

Quintanilla, M.E., Tampier, L., Sapag, A. & Israel, Y. (2005) Polymorphism in the mitochondrial aldehyde dehydrogenase gene (*Aldh2*) determine peak blood acetaldehyde levels and voluntary ethanol consumption in rats. *Pharmacogenetics and Genomics*, 15, 427– 431. Available from: https://doi.org/10.1097/01213011-20050 6000-00009

Quintanilla, M.E., Tampier, L., Valle-Prieto, A., Sapag, A. & Israel, Y. (2005) Complex I regulates mutant mitochondrial aldehyde dehydrogenase activity and voluntary ethanol consumption in rats. *The FASEB Journal*, 19, 36–42. Available from: https://doi.org/10.1096/ fj.04-2172com

Risinger, F.O. & Oakes, R.A. (1996) Dose- and conditioning trial-dependent ethanol-induced conditioned place preference in Swiss-Webster mice. *Pharmacology, Biochemistry, and Behavior*, 55, 117–123. Available from: https://doi.org/10.1016/0091-3057(96)00069-x

Rivera-Meza, M., Quintanilla, M.E., Tampier, L., Mura, C.V., Sapag, A. & Israel, Y. (2010) Mechanism of protection against alcoholism by an alcohol dehydrogenase polymorphism: development of an animal model. *The FASEB Journal*, 24, 266–274. Available from: https://doi. org/10.1096/fj.09-132563

Rodríguez, G. & Alonso, G. (2004) Latent inhibition as a function of US intensity in a two-stage CER procedure. *Psicológica*, 25, 207–216.

Roine, R.P., Gentry, T., Lim, R.T., Jr., Baraona, E. & Lieber, C.S. (1991) Effect of concentration of ingested ethanol on blood alcohol levels. *Alcoholism, Clinical and Experimental Research*, 15, 734–738. Available from: https://doi.org/10.1111/j.1530-0277.1991.tb005 89.x

Rose, J., Schmidt, R., Grabemann, M. & Güntürkun, O. (2009) Theory meets pigeons: the influence of reward-magnitude on discrimination-learning. *Behavioural Brain Research*, 198, 125–129. Available from: https://doi.org/10.1016/j.bbr.2008.10.038

Sapag, A., Tampier, L., Valle-Prieto, A., Quintanilla, M.E., Moncada, C. & Israel, Y. (2003) Mutations in mitochondrial aldehyde dehydrogenase (ALDH2) change cofactor affinity and segregate with voluntary alcohol consumption in rats. *Pharmacogenetics*, 13, 509– 515. Available from: https://doi.org/10.1097/00008571-20030 8000-00009

Schechter, M.D. (1992) Locomotor activity but not conditioned place preference is differentially affected by moderate dose of ethanol administered to P and NP rats. *Alcohol*, 9, 185–188. Available from: https://doi.org/10.1016/0741-8329(92)90050-k

Schechter, M.D. & Krimmer, E.C. (1992) Differences in response to the aversive properties and activity effects of low dose ethanol in LAS and HAS selectively bred rats. *Psychopharmacology*, 107, 564–568. Available from: https://doi.org/10.1007/BF02245271

Schmajuk, N.A. (2010) *Mechanisms in classical conditioning: a computational approach*. Cambridge: Cambridge University Press

Soliman, K.F.A. & Walker, C.A. (1979) Diurnal rhythm of ethanol metabolism in the rat. *Experientia*, 15, 808–809. Available from: https://doi. org/10.1007/BF01968264

Song, M., Wang, X.-Y., Zhao, M., Wang, X.-Y., Zhai, H.-F. & Lu, L. (2007) Role of stress in acquisition of alcohol-conditioned place preference in adolescent and adult mice. *Alcoholism, Clinical and Experimental Research*, 31, 2001–2005. Available from: https://doi. org/10.1111/j.1530-0277.2007.00522.x

Spanagel, R., Noori, H.R. & Heilig, M. (2014) Stress and alcohol interactions: animal studies and clinical significance. *Trends in Neurosciences*, 37, 219–227. Available from: https://doi. org/10.1016/j.tins.2014.02.006

Spiteri, T., Le Pape, G. & Agmo, A (2000) What is learned during place preference conditioning? A comparison of food- and morphine-induced reward. *Psychobiology*, 28, 367–382. Available from: https://doi. org/10.3758/BF03331994

Stewart, R.B. & Grupp, L.A. (1985) Some determinants of the motivational properties of ethanol in the rat: concurrent administration of food and social stimuli. *Psychopharmacology*, 87, 43–50. Available from: https://doi.org/10.1007/BF00431776

Sturtevant, R.P. & Garber, S.L. (1980) Light-dark and feeding regimens affect circadian phasing of blood-ethanol decay rates. *Pharmacology, Biochemistry, and Behavior*, 13, 637–642. Available from: https:// doi.org/10.1016/0091-3057(80)90005-2

Sturtevant, R.P. & Garber, S.L. (1981) Meal timing dominates the lighting regimen as a synchronizer of the circadian blood ethanol clearance rate rhythm in rats. *The Journal of Nutrition*, 111, 2000–2005. Available from: https://doi.org/10.1093/jn/111.11.2000

Sturtevant, R.P. & Garber, S.L. (1984) Circadian rhythms of alcohol dehydrogenase and MEOS in the rat. *Proceedings of the Society for Experimental Biology and Medicine*, 175, 299–303. Available from: https://doi.org/10.3181/00379727-175-3-rc2

Sylvestre, M.-P., Wellman, R.J., Ahun, M.N., Datta, G. & Jutras-Aswad, L.J.O. (2020) Discrete time measures versus trajectories of drinking frequency across adolescence as

predictors of binge drinking in young adulthood: a longitudinal investigation. *BMJ Open*, 10, e035939. Available from: https://doi.org/10.1136/bmjop en-2019-035939

Teschke, R. (2019) Microsomal ethanol-oxidizing system (MEOS): success over 50 years and an encouraging future. *Alcoholism, Clinical and Experimental Research*, 43, 386–400. Available from: https:// doi.org/10.1111/acer.13961

Tottmar, O. & Marchner, H. (1975) Characteristics of the acetaldehyde oxidation in rat liver, and the effect of antabuse, 4-methylpyrazole and unknown dietary factors on the hepatic output of acetaldehyde. In: Lindros, K.O. & Eriksson, C.J.P. (Eds.) *The role of acetaldehyde in the actions of ethanol: Satellite Symposium to the Sixth International Congress of Pharmacology*. The Finnish Foundation for Alcohol Studies: Helsinki, pp. 47–66.

Tzschentke, T.M. (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addiction Biology*, 12, 227–462. Available from: https://doi. org/10.1111/j.1369-1600.2007.00070.x

Vezina, P. & Stewart, J. (1987a) Conditioned locomotion and place preference elicited by tactile cues paired exclusively with morphine in an open field. *Psychopharmacology*, 91, 375–380. Available from: https://doi.org/10.1007/BF00518195

Vezina, P. & Stewart, J. (1987b) Morphine conditioned place preference and locomotion: the effects of confinement during training. *Psychopharmacology*, 93, 257–260. Available from: https://doi. org/10.1007/BF00179944

Walker, B.M. & Ehlers, C.L. (2009) Age-related differences in the blood alcohol levels of Wistar rats. *Pharmacology, Biochemistry, and Behavior*, 91, 560–565. Available from: https://doi.org/10.1016/j. pbb.2008.09.017

Walsh, R.N. & Cummins, R.A. (1976) The open-field test: a critical review. *Psychological Bulletin*, 83, 482–504. Available from: https:// doi.org/10.1037/0033-2909.83.3.482

White, N.M., Messier, C. & Carr, G.D. (1987) Operationalizing and measuring the organization of drugs on behavior. In: Bozarth, M.A. (Ed.) *Methods of assessing the reinforcing properties of abused drugs*. New York: Spring-Verlag, pp. 591–617.

Wood, W.G. & Armbrecht, H.J. (1982) Behavioral effects of ethanol in animals: age differences and age changes. *Alcoholism, Clinical and Experimental Research*, 6, 3–12. Available from: https://doi. org/10.1111/j.1530-0277.1982.tb05376.x

Yim, H.J. & Gonzales, R.A. (2000) Ethanol-induced increases in dopamine extracellular concentration in rat nucleus accumbens are accounted for by increased release and not uptake inhibition. *Alcohol*, 22, 107–115. Available from: https://doi.org/10.1016/ s0741-8329(00)00121-x

Yoon, M., Madden, M.C. & Barton, H.A. (2006) Developmental expression of aldehyde dehydrogenase in rat: a comparison of liver and lung development. *Toxicological Sciences*, 89, 386–398. Available from: https://doi.org/10.1093/toxsci/kfj045

Zakharova, E., Leoni, G., Kichko, I. & Izenwasser, S. (2009) Differential effects of methamphetamine and cocaine on conditioned place preference and locomotor activity in adult and adolescent male rats. *Behavioural Brain Research*, 198, 45–50. Available from: https://doi. org/10.1016/j.bbr.2008.10.019

Zhu, W., Bie, B. & Pan, Z.Z. (2007) Involvement of non-NMDA glutamate receptors in central amygdala in synaptic actions of ethanol and ethanol-induced reward behavior. *The Journal of Neuroscience*, 27, 289–298. Available from: https://doi.org/10.1523/JNEUR OSCI.3912-06.2007

SEÇÃO DE DISCUSSÃO SUPLEMENTAR AO CAPÍTULO 1: APÊNDICES S8 A S12

Appendix S8: Variables predicting conditioning failure and conditioning success in alcohol-induced PC protocols with alcohol preexposure

Figure S4 shows results reported for PC protocols with alcohol pre-exposure (APE), organized according to alcohol dose, number of habituation sessions and conditioning trials (**Figure S4a**), and apparatus area and number of habituation sessions (**Figure S4b**). Of these results, 28 are from short and 4 from long PC protocols, all associated with some APE method. The comparison of short protocols without (**Figure 2a** and **3a**) and with APE (**Figure S4**) shows that results of the latter do not conform to the prediction of motivator magnitude for conditioning success. In short protocols without APE, alcohol doses of 0.5 and 1.0 g/kg generated conditioning failure percentages of 79.3% and 46.9%, respectively. When we controlled failures for apparatus areas smaller than 3000 cm^2, percentages were 70.0% and 46.2%, respectively. In short protocols with APE, these same doses generated failure percentages of 22.2% and 28.6%, respectively.

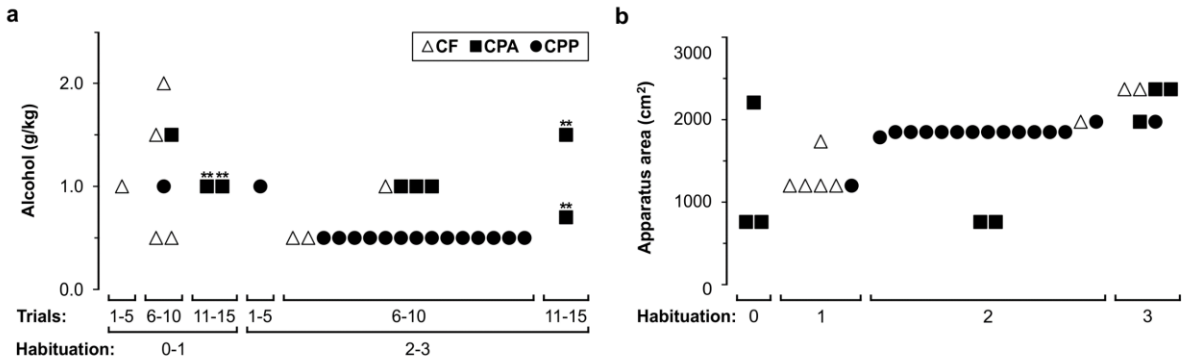


Figure S4 Results of alcohol-induced PC protocols with alcohol pre-exposure, organized by alcohol dose, total number of conditioning trials, habituation sessions (a), and apparatus area (b). Trials, number of conditioning trials; Habituation, number of habituation sessions; CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference; *, alcohol administered by orogastric gavage; **, intragastric alcohol administration.

Additionally, the comparison of number of habituation sessions in these short protocols with APE (**Figure S4a**) suggests that they do not conform to latent inhibition predictions either since we found a greater percentage of conditioning failure in those with 0-1 habituation session (71.4% of 7 reports) than in those with 2-3 habituation sessions (14.3% of 21 reports). This may not be attributed to the effects of larger apparatus since all reports came from apparatus smaller than 3000 cm^2 (**Figure S4b**). Closer inspection of these results of short protocols with APE (**Figure S4a**) suggests that these prediction failures are mainly derived from results reported for an alcohol dose of 0.5 g/kg. Clearly, the used APE methods

generated greater conditioning success rates for this dose in short PC protocols. This then raises the question “which APE methods?”.

Table S9 Description of alcohol pre-exposure methods used before alcohol-induced PC protocols

Citation	Alcohol pre-exposure procedure
Reid et al. (1985)	35 days before the PC protocol, for the first 26 days, rats were daily given 60 min of limited access to a 6% alcohol solution (concentration unity not reported) followed by a 180 min period of limited access to water. Alcohol intakes averaged 8 to 11 ml/day. Except for the 26 days, subjects had ad libitum access to water.
Bieńkowski et al., (1995, 1996)*	For 20 days immediately preceding the PC protocol, rats were daily given one intraperitoneal administration of alcohol (10% v/v). The volume administered corresponded to a dose of 0.5 g/kg for one experimental group and of 1.0 g/kg for the other.
Biała and Kotlińska (1999)	For 15 days immediately preceding the PC protocol, rats were daily given one intraperitoneal administration of alcohol (0.5 g/kg, 10% v/v).
Fidler et al. (2004)	19 days before the PC protocol, for the first 15 days, rats were daily given one intragastric infusion of alcohol (1.5 g/kg, 10% v/v, 18.75 ml/kg). Infusions were given by hand over approximately 30 seconds.
Fidler et al. (2004)	4 days before the PC protocol, for the first two 2 days, rats were daily given one intragastric infusion of alcohol (1.0 g/kg, 10% v/v, 12.5 ml/kg). For one experimental group, the duration of each infusion was 600 seconds. For the other, it was 30 seconds.
Kotlińska et al. (2004, 2007, 2011), Gibula-Bruzda et al. (2015) and Gawel et al. (2016)	For 15 days immediately preceding the PC protocol, rats were daily given one intraperitoneal administration of alcohol (0.5 g/kg, 12.5% v/v).
Sable et al. (2004)	22 days before the PC protocol, for the first 14 days, rats were daily given 60 min of limited access to a 6% (v/v) alcohol solution followed by a 180 min period of limited access to water. During the last 4 days, intakes averaged around 1.267 ± 0.03 g/kg for the 60-min period. Except for the 14 days, subjects had ad libitum access to water.
Busse et al. (2005)	20 days before the PC protocol, for the first 17 days, rats were given a single intraperitoneal administration of alcohol (1.50 g/kg, 15% v/v) every fourth day for a total of 5 administrations.
Barbier et al. (2008)	Non-pregnant female rats received alcohol (10% v/v) as the sole drinking fluid for 4 weeks before mating. After successful mating, females were maintained on the 10.0% alcohol solution throughout gestation and lactation. Male offspring were used for PC protocols at 60 days of age.
Pascual et al. (2012)	28 days before the PC protocol, for the first 14 days, rats were given a single intraperitoneal administration of alcohol (3.0 g/kg, 25% v/v) in a pattern where injections (8 doses) were given on two consecutive days with 2-day gaps without injections.
Alaux-Cantin et al. (2013)	35 days before the PC protocol, for the first 14 days, rats were given a single intraperitoneal administration of alcohol (3.0 g/kg, 20% v/v) in a pattern where injections (8 doses) were given on two consecutive days with 2-day gaps without injections.

* Bienkowski et al. (1995) also administered alcohol intraperitoneally 90 min after each drug-unpaired context trial of the short PC protocol. For each experimental group, these injections were done in the same doses, concentrations, and volumes as those used for the alcohol pre-exposure period.

Table S9 shows the descriptions of APE methods. Many of these methods are adaptations of previous ones reported in the literature. As in many uses of these methods the experimental design included PC protocols without APE, a direct comparison is possible for

the evaluation of APE effects on conditioning failure-success in PC. Here, only one of the APE methods has resulted in a greater probability of conditioning success than the equivalent short and long PC protocols without APE. This method is the one developed by Bieńkowski et al. (1995), refined in Bieńkowski et al. (1996) and later adapted by Kotlińska and collaborators (Biała and Kotlińska, 1999; Gawel et al., 2016; Gibula-Bruzda et al., 2015; Kotlińska et al., 2004; 2007; 2011). The other 4 methods do not seem to have changed the chances of conditioning success.

In addition, although the general literature of PC has discussed the APE method of Bieńkowski et al. (1995; 1996) in terms of sensitization/tolerance to alcohol effects (Tzschentke, 1998), these parameters may only be confirmed for the alcohol of dose of 0.5 g/kg (Bieńkowski et al., 1995; 1996; Gawel et al., 2016). Indeed, while 0.5 g/kg resulted in CPP, 1.0 g/kg resulted only in CPA and conditioning failure reports (Bieńkowski et al., 1995; 1996). Moreover, the sensitization caused by this method seems to be partially dependent on stress effects according to the tests done by Bieńkowski et al. (1996) (**Table S6**). Considering these different results and remarks, this method seems to sensitize, via stress and drug history, outbred animals to an alcohol dose of 0.5 g/kg, counteracting variables negatively affecting PC installation and/or expression, as one can see in **Figure S4**. Furthermore, if a sensitization/tolerance to alcohol is also present in long protocols without APE, then these protocols are more effective than those with APE to induce conditioning success. Although we did not find different distributions of conditioning failure-success between long protocols and those with APE ($x^2 = 1.862$, df = 1, n = 59, $p = 0.200$, $\varphi_c = 0.178$), this is true whether one considers the total number of alcohol administration sessions presumably necessary for such sensitization/tolerance to occur (much smaller for long protocols) or the range of alcohol doses to which the animals, presumably, become sensitized or tolerant (much greater for long protocols).

Appendix S9: Variables predicting conditioned place aversion and conditioned place preference in long PC protocols without APE and in PC protocols with ape

Regarding long PC protocols without APE and PC protocols with APE, **Figure S5** presents data according to the housing system, rat strain, and alcohol dose. As can be seen (**Figure S5a**), 3 failures have been reported for the dose of 0.5 g/kg in long PC protocols without APE (Bozarth, 1990; Marglin et al., 1988; Stewart and Grupp, 1985). Previously, we pointed out 2 variables related to these failures: latent inhibition derived from habituation sessions and unconditioned exploratory behavior derived from apparatus area, number of chambers, and differential exposure to conditioning contexts during conditioning.

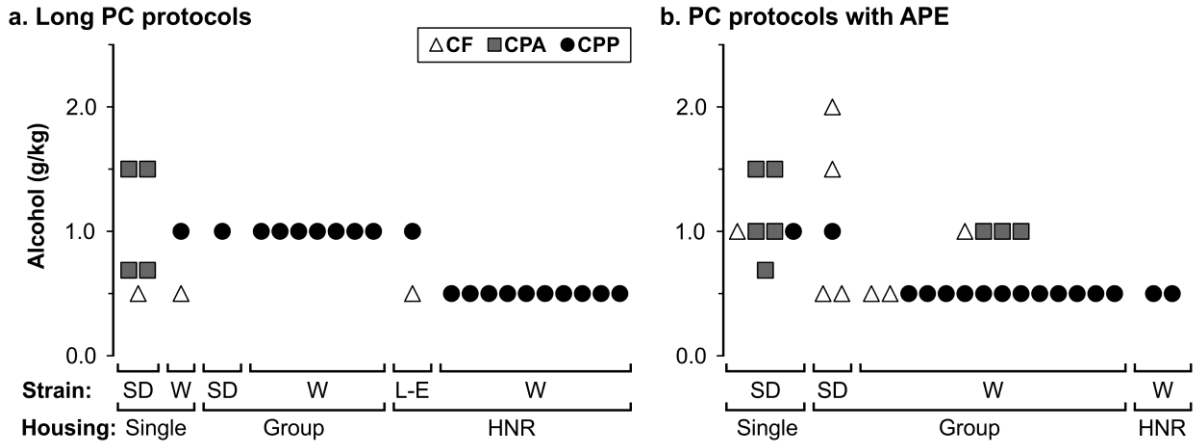


Figure S5 Results of alcohol-induced long PC protocols without alcohol pre-exposure (a) and PC protocols with alcohol pre-exposure (b), organized by alcohol dose, housing system, and outbred strains of *Rattus norvegicus*. CF, conditioning failure; CPA, conditioned place aversion, CPP, conditioned place preference; HNR, housing not reported; L-E, Long-Evans rats; SD, Sprague Dawley rats; W, Wistar rats.

In the cases of larger doses, long protocols are associated with CPP instatement at the dose of 1.0 g/kg (ip and og) in group-housed Wistar and Sprague Dawley rats (Biała and Langwiński, 1996a, 1996b; Diana et al., 2008; Melis et al., 2007; Peana et al., 2009; Spina et al., 2010). However, Fidler et al. (2004) reported 4 CPAs for doses of 0.7 and 1.5 g/kg (ig) in single-housed Sprague Dawley rats, and López and Cantora (2010) reported a CPP for a dose of 1.0 g/kg (ip) in single-housed Wistar rats. As both studies used single-housed animals, the discrepancy in their results for the doses of 0.7 and 1.0 g/kg is not easily explainable only by the strain and housing variables analyzed in this section. The water-deprivation schedule implemented by López and Cantora (2010) may have had some unpredicted effect on their result. On the other hand, considering the hypothesis that a history of contact with alcohol may generate sensitization of the rewarding effects and/or tolerance to the aversive effects of alcohol (Tzschentke 1998), the discrepant reports for the doses of 1.0 and 1.5 g/kg indicate that such history must be quite long for single-housed animals as alcohol doses increase.

As for PC protocols with APE (**Figure S5b**), these protocols are not associated with an increase in the probability of CPP instatement nor with a decrease in the probability of CPA instatement in single-housed Sprague Dawley rats (Busse et al., 2005; Fidler et al., 2004) at the dose range of 0.7-1.5 g/kg (ip and ig). The exception is the CPP report of Reid et al. (1985) with a dose of 1.0 g/kg (ip). As Reid et al. (1985) used a longer APE protocol with single-housed Sprague Dawley rats than other research groups (see **Table S9**), the reported CPP may also implicate that a much longer history of contact with alcohol is needed to counter single housing effects on the valence of alcohol motivator function. Also, an inspection of **Appendix S13** (spreadsheet 05) suggests that, if this countereffect is real, it may be modulated by the total duration of single housing and animal age/weight, i.e., the longer the period of single housing before the implemented PC protocols and the heavier/older the rats, the longer the necessary PC protocol and/or APE method to counter single-housing effects. If so, this is another relevant factor for the previously mentioned discrepant results of Fidler et al. (2004) and López and Cantora (2010) with doses of 1.0 (ip), 0.7, and 1.5 (ig) g/kg in long PC protocols.

However, one must consider that Reid et al. (1985) used a rather unusual method to quantify the time animals spent in conditioning chambers during the post-conditioning test. Using a 3-chamber apparatus, those researchers considered the middle of the central chamber as the delimitation between the drug-paired and the drug-unpaired chambers, whether animals were inside these conditioning chambers or not. Thus, Reid et al. (1985) may have reported an artifact CPA for their short PC protocol without APE and an artifact CPP for the same protocol with APE. This possibility is accentuated when one considers that Reid et al. (1985) used 2 habituation sessions and only 4 conditioning trials for a dose of 1.0 g/kg in their experiments. Unless the weight of the animals (mean of 406 g) at the beginning of the PC protocol had effects on alcohol metabolism and distribution (Bloom et al., 1982), thus increasing the motivator magnitude, it is hard to see how this short protocol without APE has resulted in CPA and not in conditioning failure.

Regarding the 4 failures and 1 CPP for the dose range of 0.5 to 2.0 g/kg in group-housed Sprague Dawley rats (Alaux-Cantin et al., 2013; Barbier et al., 2008), except for the CPP report, the analysis done in this section has little impact on their comprehension. We can only say that the conditioning failure reports of Alaux-Cantin et al. (2013) and Barbier et al. (2008) do not seem to be associated with any of the cases analyzed in short PC protocols with APE. On the other hand, Pascual et al. (2012) reported 2 failures and 2 CPAs for the doses of 0.5 and 1.0 g/kg (ip), respectively, in group-housed Wistar rats subjected to short protocols

with APE. Those researchers reported the same pattern of results for the same doses in short protocols without APE. Considering this, while the failures reported are easily explained by latent inhibition (3 habituation sessions) and low alcohol dose (0.5 g/kg), the CPAs seem to be related to the high alcohol concentration (25%, v/v) used for ip administration.

Nonetheless, considering both long protocols without APE and PC protocols with APE, there is little evidence that such methods are associated with the determination of alcohol motivator valence by some sensitization/tolerance effect. Accordingly, when we compared single and group housing for the CPA-CPP distribution in long PC protocols and in PC protocols with APE, we found the same associations shown by short PC protocols (for long protocols: $F^2 = 10.632$, n = 15, p = 0.001, $\varphi_c = 0.873$; for PC protocols with APE: $F^2 = 7.935$, n = 30, p = 0.019, $\varphi_c = 0.561$). The evidence then indicates, as presented in **Appendix S8**, that long protocols and PC protocols with APE sensitize rats to alcohol mainly concerning variables that affect conditioned control installation and expression, with long PC protocols being more effective in this sensitization, at least for outbred strains of the albino rat. Then, for a sensitization capable of changing the valence of alcohol motivator valence from aversive to appetitive, a much longer PC protocol or extensive period of APE may be needed.

Appendix S10: Biases in PC apparatus and stimulus assignment criteria

Apparatus bias is characterized by the presence of an unconditioned preference for one of the conditioning chambers during the baseline test. The unconditioned preference is, by definition, an unconditioned behavioral relation and, thus, a populational feature (not an individual characteristic). From this consideration and those done in **Appendix S3** and **Appendix S5**, we have the ratio formula for measuring baseline preference:

$(t_0CC1/(t_0CC1+ t_0CC2))*100\%$,

where: t_0CC1 = baseline time in conditioning chamber 1; t_0CC2 = baseline time in conditioning chamber 2.

Whenever the baseline preference is strong and consistent among the animals composing a sample (i.e., if they spend, on average, significantly more than 50% of the time in a specific conditioning chamber in relation to the other), this is considered an unconditioned preference and the apparatus is considered biased. Whenever the result of this formula does not differ significantly from 50%, an unconditioned preference is not present, an unbiased apparatus is used, and the individual baseline preferences are a simple instance of biological and stimulatory variation. Cunningham et al. (2003) used a similar definition of apparatus bias and the same formula that we do here (the direct test of t_0CC1 and t_0CC2 difference may also be used for measuring apparatus bias. However, this procedure increases the probability that a researcher statistically detects bias in an apparatus where there is none: see **Appendix S3**).

In contrast, bias in conditioning protocol is defined by the selection criteria (also called “stimulus assignment criteria”) for pairing the drug with a chamber. There are at least 4 different criteria, 1 for the unbiased assignment and 3 for the biased assignment. If researchers use an unbiased (also called “counterbalanced” or “balanced”) stimulus assignment, they choose to pair the drug randomly with either conditioning chamber within the drug-treated group, regardless of using a biased or an unbiased apparatus. Thus, for some animals in this group, the drug is paired with confinement in one conditioning chamber and, for other animals in the same group, with confinement in the other conditioning chamber.

In the case of biased (or “unbalanced”) assignment, the fixed criterion happens when researchers choose a priori one and only one chamber to be paired with the drug, regardless of using a biased or unbiased apparatus. Thus, while in the unbiased assignment researchers try to dilute the possible influences of individual or unconditioned baseline preferences, in the biased fixed criterion researchers consider the baseline preferences irrelevant for stimulus

assignment[18]. In the other 2 biased criteria, the drug-paired chamber is either the preferred chamber or the non-preferred chamber during the baseline. Moreover, one must notice that, in these last two cases, basal preference may be determined in terms of individual preferences (whether the apparatus is biased or unbiased; this means that the drug-paired chamber is not necessarily the same for all drug-treated animals) or in terms of unconditioned preference (for biased apparatuses only).

The relations and use of biases constitute a controversial topic in the PC literature for various reasons. From the early reviews in the 1980s onward, one can find the recurring recommendation to avoid using biased apparatuses and assignments. This recommendation stemmed from the theoretical complexities involved in analyzing and interpreting the results of experiments conducted with them (these considerations can be found in Carr et al., 1989; Cunningham et al., 2011; Swerdlow et al., 1989). Moreover, the report of apparatus bias is quite problematic in the PC literature, as many researchers do not report the "raw" baseline times, the results of statistical tests used to access baseline preference, or report baseline times only after discarding "outlier animals" in the baseline test (Cunningham et al. 2003).

In contrast, some researchers believe that CPA instatement is easier when one uses the preferred-chamber criterion for stimulus assignment (preferably in combination with a biased apparatus), while others believe that instating CPP is easier when one uses the non-preferred-chamber criterion (again, preferably in combination with a biased apparatus). Although there is some evidence that could be interpreted as supporting these claims (see Bieńkowski et al., 1997a; 1997b; Le Foll and Goldberg, 2005; Mucha and Iversen, 1984; Schechter, 1992; Schechter and Krimmer, 1992) and there is indeed a predicted scenario in which these claims may be true (that of latent inhibition in biased apparatuses: unconditioned axiogenetic stimuli tend to decrease latent inhibition effects), the present data does not seem to support the generality of these claims.

Currently, from what we know, the most reliable data regarding the relations of biases in apparatus and stimulus assignment come from the meta-analysis of Bardo et al. (1995) and the elegant and insightful experimental study of Cunningham et al. (2003). Bardo et al. (1995)

18 While it is not clear whether this rationale is a valid one (for reasons exposed ahead in this appendix), the use of the fixed assignment criterion (whether in combination with an unbiased or a biased apparatus) is not recommended for another reason: the criterion has an implicit premise of conditioning equipotentiality for different conditioning chambers. However, even though animals may not present an unconditioned preference for one conditioning chamber in relation to the other, this does not imply that learning (i.e., conditioning) will occur with the same magnitude for different conditioning chambers. Thus, assuming that there is equipotentiality between different conditioning chambers is a probable source of interpretation error in drug-induced PC experiments, especially for drugs that have low pharmacological potency. For this main reason, the use of the balanced assignment criterion is preferred in respect to the use of the fixed assignment criterion.

reviewed the literature of morphine-, heroin-, amphetamine-, and cocaine-induced PC with rats. In their meta-analysis, none of the reviewed criteria for biased assignment (preferred- and non-preferred-chamber criteria) yielded unequivocally greater or smaller effect sizes in comparison with the unbiased one for CPP. The non-preferred-chamber criterion yielded significantly smaller effect sizes than the unbiased assignment for amphetamine, cocaine, and morphine, especially for the first 2 drugs. Meanwhile, although the preferred-chamber criterion only had data for amphetamine and heroin, merely for heroin it generated a significantly smaller effect size in comparison to both the non-preferred-chamber and the balanced criteria.

This could be interpreted as somewhat supporting the claim of easier CPA instatement with this criterion. However, for amphetamine, it generated an effect size that was as great as that of the balanced criterion and greater than that of the non-preferred-chamber criterion. Additionally, Bardo et al. (1995) found that, when researchers combined a presumably biased apparatus (i.e., an apparatus with a black chamber and a white chamber) with the presumably non-preferred-chamber criteria (i.e., the white chamber), this combination resulted in a significantly greater effect size only for amphetamine in respect to the balanced assignment for CPP. For morphine and cocaine, it resulted in significantly smaller effect sizes (heroin-induced PC seems to be less sensitive to bias effects. All criteria or bias combinations yielded similar effect sizes, except for the significantly lower effect produced by the preferred-chamber criterion).

Cunningham et al. (2003) manipulated apparatus bias by changing the combination of tactile cues available in a baseline preference test for alcohol-induced PC in mice. Accordingly, they used an unbiased and a biased apparatus in their experiments, both combined with an unbiased stimulus assignment. However, knowing the individual baseline scores, the tactile cue to which alcohol was paired for each animal, and using a large number of animals in each apparatus study, those researchers further segregated the animals into different groups. This segregation allowed the analysis of results as if each of these groups had been conditioned according to different criteria for biased stimulus assignment (i.e., according to the preferred- and non-preferred-chamber criteria) for each apparatus type. For the unbiased apparatus, significant place conditioning (CPP) was obtained despite the use of unbiased or biased stimulus assignment and, in this latter case, regardless of the assignment criteria. For the biased apparatus, place conditioning was again obtained for the unbiased stimulus assignment. However, in the case of the biased assignment, place conditioning was

detected only for the non-preferred-chamber criterion, while a conditioning failure was obtained with the preferred one.

These studies of Bardo et al. (1995) and Cunningham et al. (2003) do not support the idea that instatement of CPP or CPA is, in general, easier with a biased apparatus, biased stimulus assignment, or their combination. If anything, they point out that the use of biases causes much more variation in PC results than the use of unbiased counterparts, being as efficient as the use of unbiased counterparts in some cases and having some detrimental effects for PC in others. Moreover, the study of Cunningham et al. (2003) may indicate that the uneven outcomes detected for biased assignment criteria in a biased apparatus are likely due to a ceiling effect rather than to an interaction between the drug's effect and an unconditioned motivational response (e.g., "anxiety") to the initially non-preferred stimulus (i.e., some of the theoretical complexities said above). However, here one must consider that most researchers use a black conditioning chamber vs. a white conditioning chamber to establish a biased apparatus in PC studies. While we do know that this black-white contrast establishes preference bias by an unconditioned aversion to the white chamber, we currently do not know if this behavioral mechanism (i.e., unconditioned stimulatory aversion/anxiogenesis) is responsible for a biased preference for tactile stimuli such as those used by Cunningham et al. (2003).

On the other hand, this precarious scenario of the untrustworthy report of apparatus bias is also present in the alcohol-induced literature herein reviewed (**Figure S6**). As can be seen, most reports of apparatus bias are not reliable for short PC protocols. For 68.4% of these experiments, we do not have baseline reports to directly evaluate apparatus bias. Meanwhile, for 82.7% we do not know if baseline outliers were excluded or not from the study and, for the other 17.3%, we do not know the repercussions of outlier exclusion on the bias/baseline stated/reported as this statement/report only occurred (if it occurred) after the exclusion. These omission factors preclude the proper evaluation of the relationship between apparatus and stimulus assignment biases for results of short PC protocols.

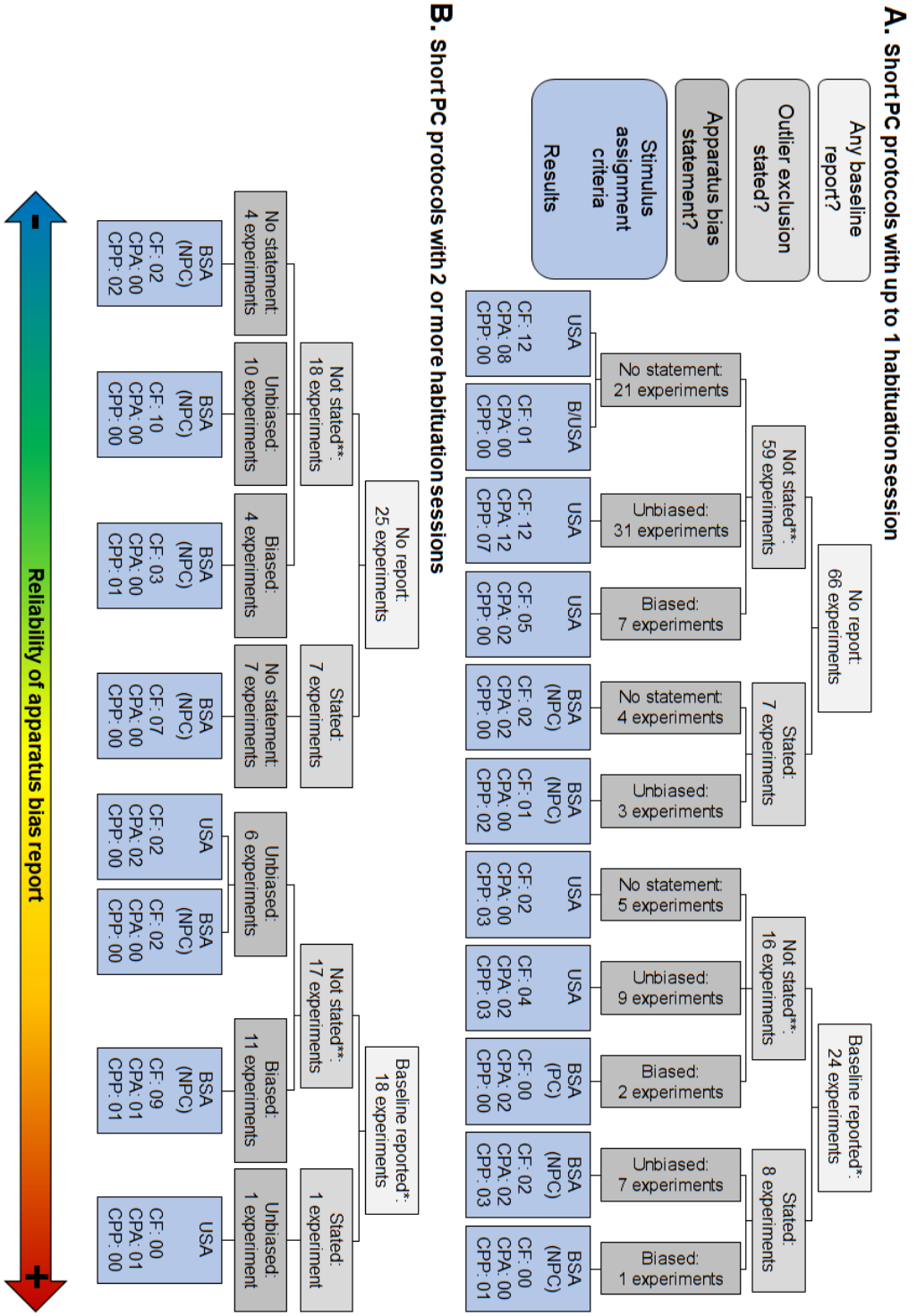


Figure S6 Results of short PC protocols organized according to reports of biases in apparatus and stimulus assignment criteria: 0 or 1 (a) and 2 or more (b) habituation sessions. BSA, biased stimulus assignment; USA, unbiased stimulus assignment; (PC), preferred-chamber criterion; (NPC), non-preferred-chamber criterion; B/USA, use of both biased (NPC) and unbiased criteria for different animals of the alcohol-treated group; CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference. * Baseline report refers to any report of baseline for the used apparatus as indicated in each selected article, even when such baseline is reported in the references of that article. ** Non-statement of outlier exclusion means that researchers may have excluded animals but did not state it or that researchers may not have excluded animals but did not state it, while the statement of exclusion always means that researchers excluded outliers. Note: Reliability of apparatus bias report refers to the report's trustworthiness, but it does not mean that the reported bias is reliable or not per se.

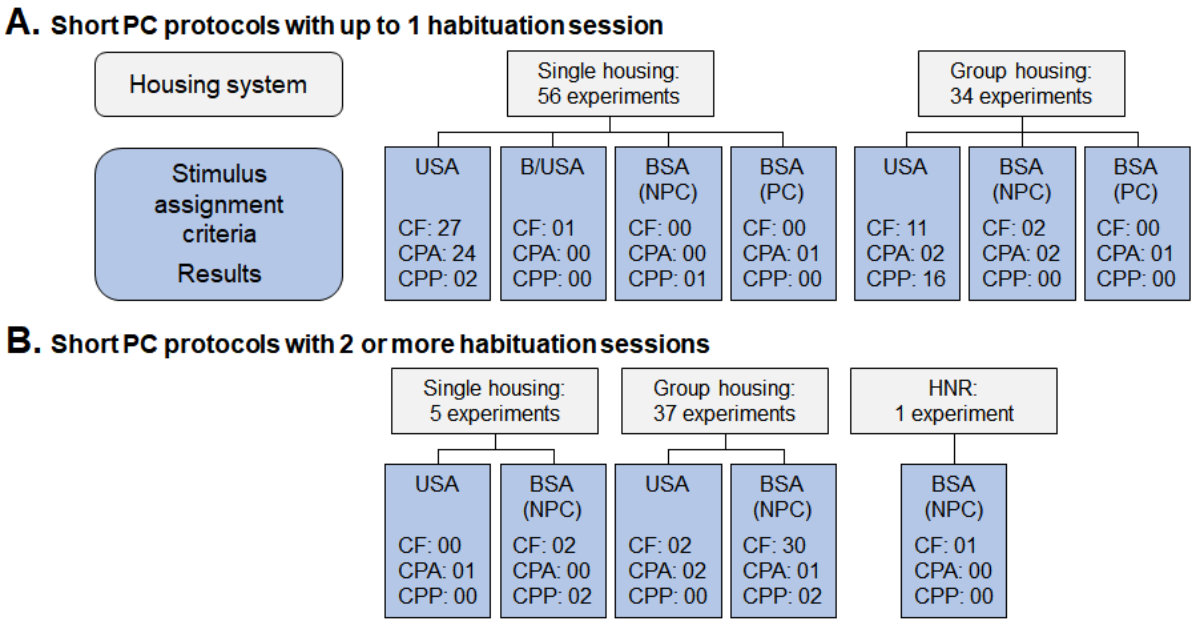


Figure S7 Results of short PC protocols organized according to housing systems and reports of stimulus assignment criteria: 0 or 1 (a) and 2 or more (b) habituation sessions. BSA, biased stimulus assignment; USA, unbiased stimulus assignment; (PC), preferred-chamber criterion; (NPC), non-preferred-chamber criterion; B/USA, use of both biased (NPC) and unbiased criteria for different animals of the alcohol-treated group; HNR, housing not reported; CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference.

Conversely, in the case of stimulus assignment, under the untrustworthy state of apparatus bias report, suitable evaluation of the aforesaid claims for CPP and CPA should occur in front of the other variable already identified as a predictor for these instances of successful conditioning, i.e., housing system (**Figure S7**). As one can see, the non-preferred-chamber criterion is not a better predictor for CPP than group-housing nor is the preferred-chamber criterion a better predictor for CPA than single-housing. While 21.74% of CPP reports occurred for the non-preferred-chamber criterion, 78.26% occurred for group housing (**Figure S7a** and **S7b**). For CPA prediction, the same can be said for the preferred-chamber criterion in respect to single housing: 4.76% CPA reports for the first one and 76.47% for the second. Thus, for the literature herein reviewed, the claims about the biased stimulus assignment criteria have little impact on the comprehension and prediction of different instances of conditioning success.

Appendix S11: Timetable of alcohol-induced PC and circadian modulation of alcohol effects

PC Timetable (i.e., PC experiments done in different time regimes of the light-dark cycle) is a variable that has received little experimental probation in drug-induced PC studies (Li et al., 2013; Webb et al., 2009). For alcohol, this variable is important for two reasons. First, the effects of alcohol on the body, including the brain, are largely determined by the rate at which it and its main metabolic product, acetaldehyde, are eliminated (Edenberg, 2007; Hurley and Edenberg, 2012). The main metabolic and elimination pathway for alcohol involves the hepatic isozymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) (reviews of other enzymatic and nonenzymatic elimination mechanisms in Kalant, 1971, 1996; Quertemont, 2004). The first enzyme oxidizes alcohol to acetaldehyde and ALDH oxidizes acetaldehyde to acetate. Indeed, in both humans and rats the orchestrated activities of these isozymes, in respect to acetaldehyde peripheric production, accumulation, and elimination, are the best predictors of susceptibility to alcohol use disorder (the case of humans: Edenberg, 2007; Hurley and Edenberg, 2012; Israel et al., 2011) and probability of greater alcohol consumption (the case of rats: Eriksson, 1973, 1980; Koivula et al., 1975; Koivisto and Eriksson, 1994; Nishiguchi et al., 2002; Quintanilla et al., 2005a, 2005b, 2006; Rivera-Meza et al., 2010; Sapag et al., 2003).

The functional variants of ADH that exhibit high alcohol oxidizing activity and the variants of ALDH that exhibit low acetaldehyde oxidizing activity display protective effects against alcohol consumption (Li, 2000). Moreover, as ADH and ALDH activities are positively proportional to total enzyme mass (Crabb et al., 1987; Lad et al., 1984), in outbred rats blood alcohol elimination rates (and probably acetaldehyde production, accumulation, and elimination rates) present an important circadian control related to variations in hepatic ADH activity (Sturtevant and Garber, 1980, 1981). ADH achieves its highest activity peak in the late period of the dark phase and its lowest activity peak in the late period of the light phase for rats ad libitum fed with a normal diet (Sturtevant and Garber, 1984). In agreement, rats display circadian variation in their behavioral (startle response and motor activity) and biological (plasma corticosterone, non-esterified fatty acids, blood alcohol levels, and body temperature) responses to alcohol (Brick et al., 1984).

Second, timetables are important because of brain circadian rhythms, especially those linked to the hypothalamic control of ADH activity (Crabb et al., 1985) and the activity of mesencephalic dopaminergic neurons projecting to the nucleus accumbens and the pre-frontal cortex, as the phasic release of dopamine by these projection systems have been implicated in

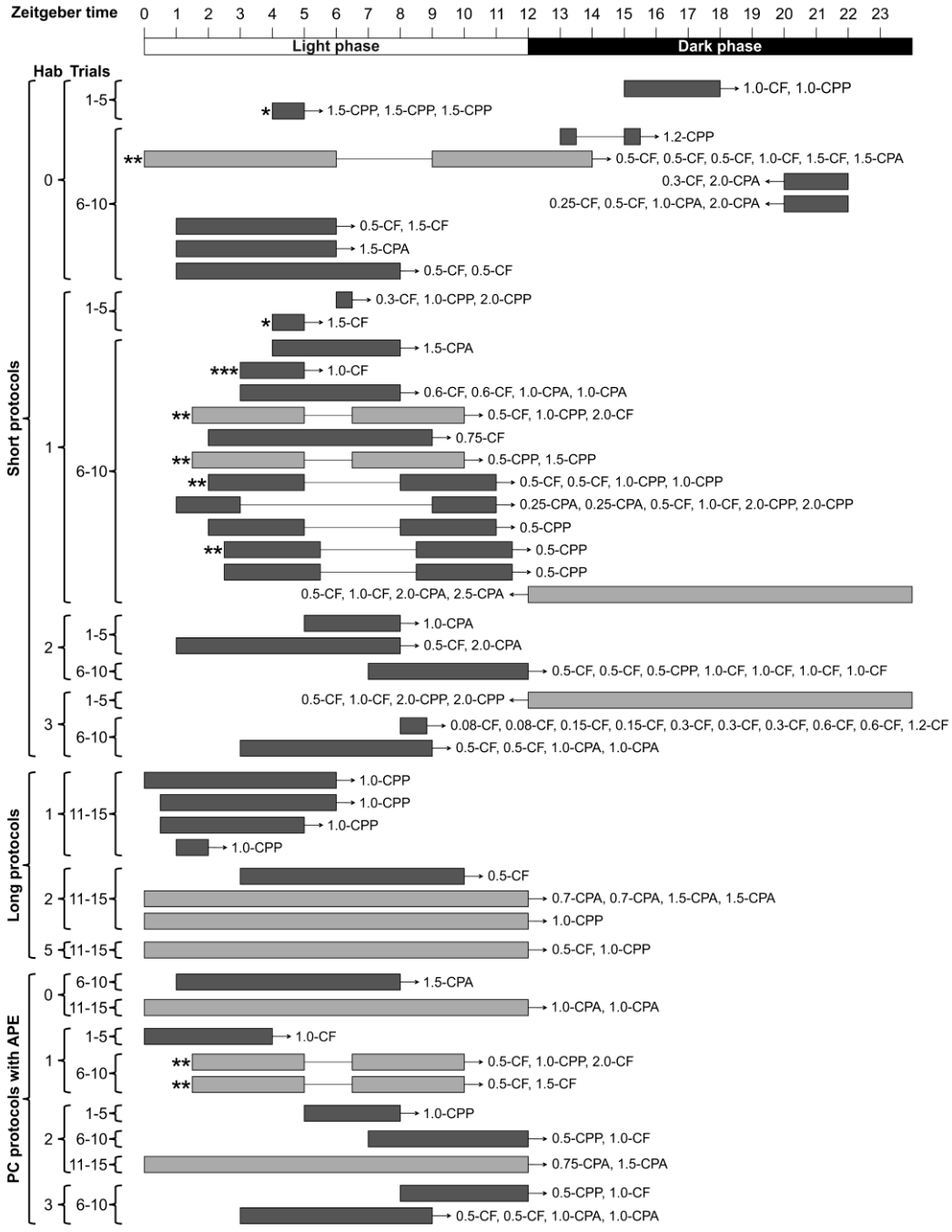


Figure S8 Timetables reported for alcohol-induced place conditioning protocols.
Hab, number of habituation sessions; Trials, number of conditioning trials; CF, conditioning failure; CPA, conditioned place aversion; CPP, conditioned place preference. Zeitgeber time 0 is the time when lights turned on and Zeitgeber time 12 is the time when lights turned off (12-12h light-dark cycles); Dark-gray bars indicate the timetable clearly reported in articles or kindly provided by authors via e-mail (Bahi, 2013; Bahi and Drier, 2013; Becker et al., 2006; Funk et al., 2004; Ise et al., 2013; Jones et al., 2010; Melis et al., 2007; Pascual et al., 2012; Zuo et al., 2017). Light-gray bars indicate timetables inconsistently reported in articles or partially provided by authors via e-mail (Alaux-Cantin et al., 2013; Barbier et al., 2008). Horizontal lines linking gray bars specify that 2 conditioning trials occurred on the same day for the same experiment, one at each time interval. Arrows indicate the alcohol dose (in g/kg) and the reported result of each experiment done in the experimental interval. * Morales et al. (2012) used a 14-10h light-dark cycle. ** van der Kooy et al. (1983), Barbier et al. (2008), Alaux-Cantin et al. (2013), Bahi (2013), and Bahi and Dreyer (2014) conducted counterbalance in the order of conditioning trials. *** Martijena et al. (2001) conducted the habituation session and post-test at ZT 07-09. **Note:** The expression "experimental interval" does not necessarily refer to the beginning and end of an experiment but mostly to the interval at which various experiments were conducted as reported in many articles.

the reinforcing effects of many drugs of abuse, including alcohol (Di Chiara and Imperato, 1986; Ding et al., 2009, 2015; Foddai et al., 2004; Koob, 1992; McBride et al., 1999; Pierce and Kumaresan, 2006; Yim and Gonzales, 2000). In the case of mesencephalic dopaminergic projections to the ventral and dorsal striatum, these present circadian variation in both tonic and phasic release of dopamine (Castañeda et al., 2004; Ferris et al., 2014). In a 12-12h light-dark cycle, the zenith (highest point) of the tonic release occurs approximately 6 hours after the start of the dark phase, while the nadir (lowest point) occurs approximately 6 hours after the start of the light phase. On the other hand, the phasic-release zenith occurs 6 hours after the start of the light phase and the nadir occurs 6 hours after the start of the dark phase (Ferris et al. 2014).

For these reasons, we extracted the timetables of alcohol-induced PC interventions (**Figure S8**). Quite unfortunately, of the 62 articles from which we extracted data for 192 experiments, we only know for certain the time interval in which experiments were carried out for 23 articles reporting short protocols (37.1% of total articles, 50.4% of short protocols), 5 articles reporting long protocols (8.1% of total articles, 18.5% of long protocols), and 9 articles reporting PC protocols with APE (14.5% of total articles, 43.8% of these PC protocols). This is shown by the dark-gray bars in **Figure S8**.

Additionally, we found these experiments did not distribute evenly for alcohol dose and number of conditioning trials or habituation sessions. Furthermore, the experimental time reported mostly refer to a set of 2 or more experimental groups, i.e., it does not refer to the beginning and end of an experiment but mostly to the interval at which various experiments were conducted, without specifying if there was or not a temporal order among experiments. This report variation prevents the proper evaluation of this possible modulation. Therefore, the only two assertions we can make are that, for articles reporting the time interval, most PC protocols were conducted during the light phase (**Figure S8**), and that the circadian modulation of alcohol-induced PC is a variable needing experimental probation.

Appendix S12: Report quality indicators, risk of report bias assessment, and theoretical limitation

Section 1. Report quality indicators and risk of report bias assessment

Throughout **Appendices S10** and **S11**, we have indicated report omissions in our sample of alcohol-induced PC in rats. These omissions compromise the reliability of any theoretical interpretation of this intervention based on animal model characteristics, known features of associative learning processes, PC-related variables, and alcohol effects. This is so because the omissions not only preclude researchers from evaluating the impact of relevant animal- and procedure-related variables on reported outcomes. They also indicate flaws in planning and conducting preclinical studies, which, in turn, are thought to reduce internal validity and predictive power of research (van der Worp et al., 2010; Landis et al., 2012). Since we have previously shown the omissions regarding the variables we considered relevant and provided supplementary material covering them, here we assess the existence of report biases in our sample with report quality indicators.

Table S10 shows the percentage of articles and experiments reporting 7 items (4 article-wise and 3 PC experiment-wise) of report quality and the mean (± SE) aggregate score of risk of bias. These percentages and scores are organized according to the type of PC experiment and the total number of articles and experiments. The mean aggregate score was calculated from the ratings of the 3 last items, with a range value of 0 to 1, where 0 means "total risk of bias" and 1 means "no risk of bias". Regarding article-wise items, 2 evaluated ethical issues related to animal welfare (compliance with regulatory requirements, pertinent to articles published after 1989) and conflicts of interests, one was general (compliance with ARRIVE guidelines, pertinent to articles published after June 2010), testing the adherence to a proposed improvement of reports, and one evaluated planning of the experimental design (sample size calculation method).

A temporal trend could be noticed for statements of compliance with regulatory requirements: 0 of 16 articles reporting in the 1990s, 16 of 20 in the 2000s, and 19 of 19 in the 2010s. This trend may indicate the standardization process of this report in the literature following the regulatory systems on laboratory animal research implemented in Canada, the European Union, and the USA in the late-1980s (Griffin and Locke, 2016; Olsson et al., 2016). It also seems to be the reason why attendance to this criterion is much lower in articles reporting short protocols in comparison to those reporting long and PC protocols with APE (**Table S10**). Of 16 articles failing to state regulatory requirements in the 1990s, 11 reported short PC protocols.

In the case of statement of conflict of interests, there is some indication that this ethical concern is rising in the literature, as all 9 articles reporting it were published from the late 2000s onwards. However, this was a timid trend, representing only 23.1% of the selected articles published in the 2000s and 2010s. On the other hand, all articles published after June 2010 failed the criterion on the statement of compliance with the ARRIVE guidelines (Kilkenny et al. 2010). Since these guidelines attempt to improve quality and transparency in reports of animal research, this failure indicates that such improvement is still necessary in the literature, as is the case in many other fields of in vivo research (Percie du Sert et al. 2020). This point is reinforced by the fact that none of the 62 articles stated methods for sample size calculation for any in vivo intervention therein reported (**Table S10**). This omission causes a lack of reassurance that all studies reported in all selected articles, including the PC experiments herein reviewed, were adequately planned in statistical power.

We evaluated the quality of experimental design and conduct of selected experiments by the PC experiment-wise items randomization of group allocation, exact descriptions of sample size, and blinded/automated assessment of outcomes. Report percentages of these items were lower in the subsets of short and PC protocols with APE than in the long protocol subset (**Table S10**). Of especial interest is the relation of the short protocol subset to the general sample as these experiments were reported in meaningful numbers in all 4 decades sampled (**Figure 1b**) and constitute 69.3% of our overall sample. Because of such a high percentage, the general sample also presents percentages of reports lower than 50% for the first 2 items. Then, together with the general failure in sample size calculation, these percentages may indicate a considerable risk of bias in the design and conduct of PC interventions, especially for short experiments. This is confirmed by the mean aggregate score of this subset and the general sample. These relations raise the issue of risk of bias dispersion over the sampled decades.

To facilitate the description of this dispersion, we used the mean aggregate score per decade. The overall sample had mean aggregate scores of 0.419 (± 0.079), 0.667 (± 0.042), 0.572 (± 0.043), 0.356 (± 0.029) for the 1980s, 1990s, 2000s, and 2010s, respectively. The short protocol subset had 0.405 (± 0.055), 0.742 (± 0.034), 0.494 (± 0.061), and 0.326 (± 0.027) for the 1980s, 1990s, 2000s, and 2010s, respectively. As expected, mean aggregate scores per decade in the general sample and the short protocol subset are related. The 1980s were also expected to present a greater risk of bias than the 1990s since stronger regulatory systems were adopted only in the late-1980s for in vivo research. What was not expected is the risk increase in the decades following the 1990s, especially the drastic increase in the

Table S 10 Report quality assessment in selected articles and experiments

Item type	**Article-wise items**				**PC experiment-wise items**			
Quality report item	**Statement of accordance with regulatory requirements**	**Statement of accordance with the ARRIVE guidelines**	**Statement on conflict of interest**	**Sample size calculation method**	**Randomization of PC groups allocation**	**Exact sample size description**	**Blinded or automated assessment**	**Mean aggregated score of PC-wise items (± SE)**
No. of reports (%) and aggregated score in SPCP	23/38 (60.5%)	0/14 (0%)	8/44 (18.2%)	0/44 (0%)	47/133 (35.34%)	64/133 (48.1%)	79/133 (59.4%)	0.476 (± 0.025)
No. of reports (%) and aggregated score in LPCP	8/11 (72.7%)	0/1 (0%)	1/13 (8%)	0/13 (0%)	16/27 (59.26%)	19/27 (70.4%)	22/27 (81.5%)	0.704 (± 0.060)
No. of reports (%) and aggregated score PC-APE	11/14 (78.6%)	0/5 (0%)	2/15 (13.3%)	0/15 (0%)	18/32 (56.3%)	8/32 (25%)	17/32 (53.1%)	0.448 (± 0.055)
Total No. of reports (%) and total aggregated score	35/55 (63.6%)	0/17 (0%)	9/62 (14.5%)	0/62 (0%)	81/192 (42.19%)	91/192 (47.4%)	118/192 (61.5%)	0.503 (± 0.022)

Percentages were calculated using all 62 articles and 192 experiments, according to the type of place conditioning experiment reported in each article. In the case of article-wise items, compliance with regulatory requirements and ARRIVE guidelines were applicable only for articles published from January 1990 and July 2010 onwards, respectively (55 articles in the first case and 17 in the second). SPCP, short place conditioning protocols without alcohol pre-exposure; LPCP, long place conditioning protocols without alcohol pre-exposure; PC-APE, place conditioning protocols with alcohol pre-exposure. **Note 1:** 1 article reported both SPCP and LPCP experiments, 8 reported both SPCP and PC-APE experiments, and 1 reported both LPCP and PC-APE experiments. For these articles, in the case of article-wise items, the (lack of) item report is counted 2 times, 1 in each pertinent type of PC protocol.

2010s.

This measure indicates that risk of bias is much less prevalent in short protocols published in the 1990s and 2000s (67.7% and 58.6% reporting randomization, respectively; 80.6% and 37.9% reporting exact sample size, respectively; 74.2% and 51.7% reporting blinded/automated assessment of outcome, respectively) than in those published in other decades. Meanwhile, short protocols published in the 1980s and the 2010s have a remarkably similar high risk of bias, differing only in the types of risk that were predominant in each decade (10.7% and 13.3% reporting randomization, respectively; 78.6% and 13.3% reporting exact sample size, respectively; 32.1% and 71.1% reporting blinded/automated assessment of outcome, respectively).

As we did not program other measurements in this topic, we do not know for certain the reasons for this trend of greater risk of bias following the 1990s. Even so, when reading the articles, we noticed 2 changes in temporal patterns of publication. The first is that the publications in the 1990s were dominated by research groups that had dealt with drug-induced PC in rats during the 1980s and even in the late 1970s. Then, the improvement in report quality during the 1990s could not only represent advances derived from the regulatory systems but also the expertise gains, protocol standardization, and automatization of outcome assessment taking place in these research groups. Meanwhile, the 2000s saw the rise of other research groups studying alcohol-induced PC with rats, many of which used locally made PC apparatuses.

The second one is the change in applications of alcohol-induced PC interventions. In the 1980s and 1990s, researchers were more interested in validating PC as a measure of alcohol “reward”, in understanding why alcohol-induced PC in rats mostly resulted in CPA and CF, and in finding ways to alter this pattern of results (i.e., in finding ways to produce CPP). This not only caused articles to be focused on alcohol-induced PC experiments during those decades but also propelled the use of long protocols and APE methods for rats and the adoption of mice as the preferred model organism for alcohol-induced PC in the following decades.

However, beginning around the mid-1990s, the use of the paradigm with rats was progressively instrumentalized. In this use, it was integrated into comparisons with other drug-induced PC interventions, in multi-behavioral designs to test the general effect of alcohol on behavior or the effect of other drugs on alcohol-induced PC interventions and to assess biological changes accompanying the behavioral results. This change in scope caused a greater load of information to be reported in articles, which could increase the chance of

information about the experimental designs of PC itself being less reported (the case of randomization) or reported with less accuracy (the case of sample size description). Thus, we suggest that the trend of greater risk of bias following the 1990s is related to these 2 changes in publication patterns.

Section 2. Theoretical limitation

Here we briefly discuss the limitation inherent to our theoretical analysis. When considering variables related to the conditioning failure-success dichotomy, we assumed that, beside variables disrupting installation or expression of conditioned control, only very low doses of alcohol have greater probability of producing conditioning failures. This assumption was done from a conservative experimental perspective: for this non-optimal effect on conditioned control, one may find compelling experimental evidence throughout the associative learning literature.

Nonetheless, in **Table S6** we pointed out that changes in preference may be interpreted according to the hypothesis of algebraic summation (in the terms of White et al., 1987) of the affective reinforcing and aversive properties of the primary motivator. Accordingly, given the conditions for alcohol to induce CPP in PC, increases in CPP magnitude would occur when the rewarding effects of alcohol are progressively greater than its aversive effects. However, as alcohol dose and/or concentration increase, so do its aversive effects. To the extent that these aversive effects increase, CPP magnitude begins to decrease to the point where no motivational property overcomes the other but "cancel" each other, turning the preference change insignificant (in the statistical sense). Beyond that point, the aversive properties would dominate and lead to CPA results.

Consequently, the transition point between CPP and CPA stipulated by the algebraic summation hypothesis would be a fourth effect class for conditioning failure. If this indeed is the case for PC, this transition point is a better explanation for the disparate results reported for the dose of 2.0 g/kg (ip) for group-housed rats: 2 CPP reported for a concentration of 12.5% (v/v) *vs.* 2 conditioning failures reported for a 20% (v/v) concentration (Barbier et al., 2008; Yu et al., 2016; Zuo et al. (2017). In addition to one of these failures, Barbier et al. (2008) also reported a CPP for a dose of 1.0 g/kg (ip, 20%, v/v), which is also in agreement with the prediction of the hypothesis. Similarly, considering the conditions for CPA instatement, this hypothesis also bears consequences for interpreting the conditioning failure report for a dose of 1.5 g/kg (ig, 10%, v/v) in relation to the CPA report for the same dose at a concentration of 20% (van der Kooy et al, 1983).

Referências aos apêndices S8 a S12

Alaux-Cantin, S., Warnault, V., Lgastelois, R., Botia, B., Pierrefiche, O., Vilpoux, C., Naassila, M. 2013. Alcohol intoxications during adolescence increase motivation for alcohol in adult rats and induce neuroadaptations in the nucleus accumbens. Neuropharmacol. 67, 521–531. https://doi.org/10.1016/j.neuropharm.2012.12.007

Bahi, A. 2013. Viral-mediated knockdown of mGLUR7 in the Nucleus Accumbens mediates excessive alcohol drinking and increased ethanol-elicited conditioned place preference in rats. Neuropsychopharmacol. 38, 2109–2119.

Bahi, A., Dreyer, J.-L. 2013. Striatal modulation of BDNF expression using microRNA124a-expressing lentiviral vectors impairs ethanol-induced conditioned-place preference and voluntary alcohol consumption. Eur. J. Neurosci. 38, 2328–2337.

Bahi. A., Dreyer, J.-L. 2014. Lentiviral vector-mediated dopamine D3 receptor modulation in the rat brain impairs alcohol intake and ethanol-induced conditioned place preference. Alcohol. Clin. Exp. Res. 38, 2369–2376.

Barbier, E., Pierrefiche, O., Vaudry, D., Vaudry, H., Daoust, M., Naassila, M. 2008. Long-term alterations in vulnerability to addiction to drugs of abuse and in brain gene expression after early life ethanol exposure. Neuropharmacol. 55, 1199–1211. https://doi.org/10.1016/j.neuropharm.2008.07.030

Bardo, M.T., Rowlett, J.K., Harris, M.J. 1995. Conditioned place preference using opiate and stimulant drugs: A meta-analysis. Neurosci. Biobehav. Rev. 19, 39–51. https://doi.org/10.1007/s002130000569

Becker, A., Schmitz, M., Grecksch, G. 2006. Kindling modifies morphine, cocaine and ethanol place preference. Exp. Brain Res. 168, 33–40. https://doi.org/10.1007/s00221-005-0081-3

Biała, G., Kotlińska, J. 1999. Blockade of acquisition of ethanol-induced conditioned place preference by N-methyl-D-aspartate receptor antagonists. Alcohol Alcohol. 34, 175–182. https://doi.org/10.1093/alcalc/34.2.175

Biała, G., Langwiński, R. 1996a. Effects of calcium channel antagonists on the reinforcing properties of morphine, ethanol and cocaine as measured by place conditioning. J. Physiol. Pharmacol. 47, 497–502.

Biała, G., Langwiński, R. 1996b. Rewarding properties of some drugs studied by place preference conditioning. Pol. J. Pharmacol. 48, 425–430.

Bieńkowski, P., Kuca, P., Kostowski, W. 1995. Conditioned place preference after prolonged pre-exposure to ethanol. Pol. J. Pharmacol. 47, 189–191.

Bieńkowski, P., Kuca, P., Piasecki, J., Kostowski, W. 1996. Low dose of ethanol induces conditioned place preference in rats after repeated exposures to ethanol or saline injections. Alcohol Alcohol. 31, 547–553. https://doi.org/10.1093/oxfordjournals.alcalc.a008190

Bieńkowski, P., Kuca, P., Piasecki, J., Kostowski, W. 1997a. 5-HT_3 receptor antagonist, tropisetron, does not influence ethanol-induced conditioned taste aversion and conditioned place aversion. Alcohol 14, 63–69. https://doi.org/10.1016/s0741-8329(96)00108-5

Bieńkowiski, P., Iwinska, K., Piasecki, J., Kostowski, W. 1997b. 5,7-dihydroxytryptamine lesion does not affect ethanol-induced conditioned taste and place aversion in rats. Alcohol 14, 439–443. https://doi.org/10.1016/s0741-8329(96)00191-7

Bloom, F., Lad, P., Pittiman, Q., Rogers, G. 1982. Blood alcohol levels in rats: Non-uniform yields from intraperitoneal doses based on body weight. Br. J. Pharmacol. 75, 251–254. https://doi.org/10.1111/j.1476-5381.1982.tb08780.x

Bozarth, M.A. 1990. Evidence for the rewarding effects of ethanol using the conditioned place preference method. Pharmacol. Biochem. Behav. 35, 485–487. https://doi.org/10.1016/0091-3057(90)90191-j

Brick, J., Pohorecky, L.A., Faulkner, W., Adams, M.N. 1984. Circadian variations in behavioral and biological sensitivity to ethanol. Alcohol. Clin. Exp. Res. 8, 204–211. https://doi.org/10.1111/j.1530-0277.1984.tb05840.x

Busse, G.D., Lawrence, E.T., Riley, A.L. 2005. The effects of alcohol preexposure on cocaine, alcohol and cocaine/alcohol place conditioning. Pharmacol. Biochem. Behav. 81, 459–465. https://doi.org/10.1016/j.pbb.2005.04.003

Carr, G.D., Fibiger, H.C., Phillips, A.G. 1989. Conditioned place preference as a measure of drug reward. In: Liebman, J.M., Cooper, S.J. (Eds.), Topics in experimental psychopharmacology (1): The neuropharmacological basis of reward. Clarendon Press/Oxford University Press, pp. 264–319.

Castañeda TR, Marquez de Prado B, Pietro D, Mora F (2004) Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: Modulation by light. J Pineal Res 36:177–185

Crabb DW, Morzorati SL, Simon JR, Li T-K (1985) Central nervous system control of alcohol dehydrogenase activity in rats. Life Sci 37:2381–2387.

Crabb DW, Bosron WF, Li T-K (1987) Ethanol metabolism. Pharmacol Ther 34:59–73. https://doi.org/10.1016/0163-7258(87)90092-1

Cunningham CL, Ferree NK, Howard MA (2003) Apparatus bias and place conditioning with ethanol in mice. Psychopharmacol 170:409–422.

Cunningham CL, Groblewski PA, Voorhees CM (2011) Place conditioning. In: Olmstead MC (ed) Neuromethods (53): Animal models of drug addiction. Springer, pp. 167–189.

Diana M, Peana AT, Sirca D, Lintas A, Melis M, Enrico P (2008) Crucial role of acetaldehyde in alcohol activation of the mesolimbic dopamine system. Ann NY Acad Sci 1139:307–317. https://doi.org/10.1196/annals.1432.009

Di Chiara G, Imperato A (1986) Preferential stimulation of dopamine release in the nucleus accumbens by opiates, alcohol, and barbiturates: Studies with transcerebral dialysis in freely moving rats. Ann NY Acad Sci 473:367–381.

Ding ZM, Rodd ZA, Engleman EA, McBride WJ (2009) Sensitization of ventral tegmental area dopamine neurons to the stimulating effects of ethanol. Alcohol Clin Exp Res 33:1571–1581.

Ding ZM, Ingraham CM, Rodd ZA, McBride WJ (2015) The reinforcing effects of ethanol within the posterior ventral tegmental area depend on dopamine neurotransmission to forebrain cortico-limbic systems. Addict Biol 20:458–468.

Edenberg HJ (2007) The genetics of alcohol metabolism: Role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health 30:5–13.

Eriksson CJP (1973) Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. Biochem Pharmacol 22:2283–2292.

Eriksson CJP (1980) The aversive effect of acetaldehyde on alcohol drinking behavior in the rat. Alcohol Clin Exp Res 4:107–111.

Ferris MJ, España RA, Locke JL, Konstantopoulos JK, Rose JH, Chen R, Jones SR (2014) Dopamine transporters govern diurnal variation in extracellular dopamine tone. PNAS 111:E2751-E2759.

Fidler TL, Bakner L, Cunningham CL (2004) Conditioned place aversion induced by intragastric administration of ethanol in rats. Pharmacol Biochem Behav 77:731–743. https://doi.org/10.1016/j.pbb.2004.01.010

Foddai M, Dosia G, Spiga S, Diana M (2004) Acetaldehyde increases dopaminergic neuronal activity in the VTA. Neuropsychopharmacol 29:530-536.

Funk D, Vohra S, Lê AD (2004) Influence of stressors on the rewarding effects of alcohol in Wistar rats: Studies with alcohol deprivation and place conditioning. Psychopharmacol (Berl) 176:82–87. https://doi.org/10.1007/s00213-004-1859-x

Gawel K, Labuz K, Gibula-Bruzda E, Jenda M, Marszalek-Grabska M, Silberring J, Kotlińska JH (2016) Acquisition and reinstatement of ethanol-induced conditioned place preference in rats: Effects of the cholinesterase inhibitors donepezil and rivastigmine. J Psychopharmacol 30:1–12. https://doi.org/10.1177/0269881116642539

Gibula-Bruzda E, Marszalek-Grabska M, Gawel K, Witkowska E, Izdebski J, Kotlińska JH (2015) The influence of the new enkephalin derivative, cyclo[N^{ε},N^{β}-carbonyl-D-Lys^2,Dap^5] enkephalinamide (cUENK6), on reinstatement of ethanol-induced conditioned place preference in rats. Physiol Behav 145:50–56. https://doi.org/10.1016/j.physbeh.2015.03.031

Hurley TD, Edenberg HJ (2012) Genes encoding enzymes involved in ethanol metabolism. Alcohol Res Curr Rev 34:339–344.

Ise Y, Mori T, Katayama S, Nagase H, Suzuki T (2013) Rewarding effects of ethanol combined with low doses of morphine through dopamine D_1 receptors. J Nippon Med Sch 80:34–41. https://doi.org/10.1272/jnms.80.34

Israel Y, Rivera-Meza M, Quintanilla ME, Spag A, Tampier L (2011) Acetaldehyde burst protection of ADH1B*2 against alcoholism: An additional hormesis protection against esophageal cancers following alcohol consumption? Alcohol. Clin Exp Res 35:806–810.

Jones BC, Ben-Hamida S, De Vasconcelos AP, Kelche C, Lazarus C, Jackisch R, Cassel JC (2010) Effects of ethanol and ecstasy on conditioned place preference in the rat. J Psychopharmacol 24:275–279.

Kalant H (1971) Absorption, diffusion, distribution, and elimination of ethanol: Effects on biological membranes. In: Kissin B, Begleiter H (eds) The Biology of Alcoholism (1): Biochemistry. Springer, pp. 1–62.

Kalant H (1996) Pharmacokinetics of ethanol: Absorption, distribution, and elimination. In: Begleiter H, Kissin B (eds) The Pharmacology of Alcohol and Alcohol Dependence. Oxford University Press, pp. 15–58.

Koivisto T, Eriksson CJP (1994) Hepatic aldehyde and alcohol dehydrogenase in alcohol-preferring and alcohol-avoiding rat lines. Biochem Pharmacol 48:1551–1558.

Koivula T, Lindros KO (1975) Effects of long-term ethanol treatment on aldehyde and alcohol dehydrogenase activities in rat liver. Biochem Pharmacol 24:1937–1942.

Koob GF (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 13:177–184.

Kotlińska J, Biała G, Rafalski P, Bochenski M, Danysz W (2004) Effect of neramexane on ethanol dependence and reinforcement. Eur J Pharmacol 503:95–98. https://doi.org/10.1016/j.ejphar.2004.09.036

Kotlińska J, Pachuta A, Dylag T, Silberring J (2007) Neuropeptide FF (NPFF) reduces the expression of morphine- but not of ethanol-induced conditioned place preference in rats. Pept 28:2235–2242. https://doi.org/10.1016/j.peptides.2007.08.006

Kotlinska JH, Bochenski M, Danysz W (2011) The role of group I mGlu receptors in the expression of ethanol-induced conditioned place preference and ethanol withdrawal seizures in rats. Eur J Pharmacol 670:154–161. https://doi.org/10.1016/j.ejphar.2011.09.025

Lad P, Shoemaker WJ, Leffert HL (1984) Developmental changes in rat liver alcohol dehydrogenase. Dev Biol 105:526–529. https://doi.org/10.1016/0012-1606(84)90310-5

Le Foll B, Goldberg SR (2005) Nicotine induces place preferences over a large range of doses in rats. Psychopharmacol 178:481–492.

Li S-X, Wei Y-M, Shi H-S, Luo Y-X, Ding Z-B, Xue Y-X, Lu L, Yu C-X (2013) Glycogen synthase kinase-3β in the ventral tegmental area mediates diurnal variations in cocaine-induced conditioned place preference in rats. Addict Biol 19: 996–1005.

Li T-K (2000) Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. J Stud Alcohol 61:5–12.

López M, Cantora R (2010) Opposite effects of ethanol on taste and place conditioning in rats. Rev. Iberoam Psicol Salud 1:207–221.

Marglin SH, MacKechnie DK, Mattie ME, Hui Y, Reid LD (1988) Ethanol with small doses of morphine establishes a conditioned place preference. Alcohol 5:309–313. https://doi.org/10.1016/0741-8329(88)90071-7

Martijena ID, Lacerra C, Bustos SG, Molina VA (2001) Chronic benzodiazepine administration facilitates the subsequent development of ethanol dependence. Brain Res 891:236–246.

McBride WJ, Murphy JM, Ikemoto S (1999) Localization of brain reinforcement mechanisms: Intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res 101:129–152.

Melis M, Enrico P, Peana AT, Diana M (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. Eur J Neurosci 26: 2824–2833. https://doi.org/10.1111/j.1460-9568.2007.05887.x

Morales M, Varlinskaya EI, Spear, LP (2012) Evidence for conditioned place preference to a moderate dose of ethanol in adult male Sprague-Dawley rats. Alcohol 46:643–648. https://doi.org/10.1016/j.alcohol.2012.06.001

Mucha RF, Iversen SD (1984) Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: A procedural examination. Psychopharmacol 82:241–247. https://doi.org/10.1007/BF00427782

Nishigushi M, Kinoshita H, Mostofa J, Tanigushi T, Ouchi H, Minami T, Hatake K et al. (2002). Differential blood acetaldehyde concentration following ethanol administration in newly developed high alcohol preference and low alcohol preference rat model system. Alcohol Acohol 37:9–12.

Pascual M, Do Couto BR, Alfonso-Loeches S, Aguilar MA, Rodriguez-Arias, Guerri C (2012) Changes in histone acetylation in the prefrontal cortex of ethanol-exposed adolescent rats are associated with ethanol-induced place conditioning. Neuropharmacol 62:2309–2319. https://doi.org/10.1016/j.neuropharm.2012.01.011

Peana AT, Assaretti AR, Muggironi G, Enrico P, Diana M (2009) Reduction of ethanol-derived acetaldehyde induced motivational properties by l-cysteine. Alcohol Clin Exp Res 33:43–48. https://doi.org/10.1111/j.1530-0277.2008.00809.x

Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? Neurosci Behav Rev 30:215–238.

Quertemont E (2004) Genetic polymorphism in ethanol metabolism: Acetaldehyde contribution to alcohol abuse and alcoholism. Mol Psychiat 9:570–581.

Quintanilla ME, Tampier L, Sapag A, Israel Y (2005a) Polymorphisms in the mitochondrial aldehyde dehydrogenase gene (*Aldh2*) determine peak blood acetaldehyde levels and voluntary ethanol consumption in rats. Pharmacogenet. Genom 15:1–5.

Quintanilla ME, Tampier L, Valle-Prieto A, Sapag A, Israel Y (2005b) Complex I regulates mutant mitochondrial aldehyde dehydrogenase activity and voluntary ethanol consumption in rats. FASEB J 19:36–42.

Quintanilla ME, Israel Y, Sapag A, Tampier L (2006) The UChA and UChB rat lines: Metabolic and genetic differences influencing ethanol intake. Addict Biol 11:310–323.

Reid LD, Hunter GA, Beaman CM, Hubbell CL (1985) Toward understanding ethanol's capacity to be reinforcing: A conditioned place preference following injections of ethanol. Pharmacol Biochem Behav 22:483–487. https://doi.org/10.1016/0091-3057(85)90051-6

Rivera-Meza M, Quintanilla ME, Tampier L, Mura CV, Sapag A, Israel Y (2010) Mechanisms of protection against alcoholism by an alcohol dehydrogenase polymorphism: Development of an animal model. FASEB J 24:266–274.

Sapag A, Tampier L, Valle-Prieto A, Quintanilla ME, Moncada C, Israel Y (2003) Mutations in mitochondrial aldehyde dehydrogenase (ALDH2) change cofactor affinity and segregate with voluntary alcohol consumption in rats. Pharmacogenet 13:509–515.

Sable HJK, White SL, Steinpreis RE (2004) Effects of chronic naltrexone treatment in rats on place preference and locomotor activation after acute administration of cocaethylene or ethanol plus cocaine. Alcohol 33:51–61. https://doi.org/10.1016/j.alcohol.2004.04.004

Schechter MD (1992) Locomotor activity but not conditioned place preference is differentially affected by moderate dose of ethanol administered to P and NP rats. Alcohol 9:185–188. https://doi.org/10.1016/0741-8329(92)90050-k

Schechter MD, Krimmer EC (1992) Differences in response to the aversive properties and activity effects of low dose ethanol in LAS and HAS selectively bred rats. Psychopharmacol 107:564–568. https://doi.org/10.1007/BF02245271

Spina L, Longoni R, Vinci S, Ibba F, Peana AT, Muggironi G, Spiga S, Acquas E (2010) Role of dopamine D_1 receptors and extracellular signal regulated kinase in the motivational properties of acetaldehyde as assessed by place preference conditioning. Alcohol Clin Exp Res 34:607–616. https://doi.org/10.1111/j.1530-https://doi.org/0277.2009.01129.x

Stewart RB, Grupp LA (1985) Some determinants of the motivational properties of ethanol in the rat: Concurrent administration of food and social stimuli. Psychopharmacol (Berl) 87:43–50. https://doi.org/10.1007/BF00431776

Sturtevant RP, Garber SL (1980) Light-dark and feeding regimens affect circadian phasing of blood-ethanol decay rates. Pharmacol Biochem Behav 13:637–642. https://doi.org/10.1016/0091-3057(80)90005-2

Sturtevant RP, Garber SL (1981) Meal timing dominates the lighting regimen as a synchronizer of the circadian blood ethanol clearance rate rhythm in rats. J Nutr 111:2000–2005. https://doi.org/10.1093/jn/111.11.2000

Sturtevant RP, Garber SL (1984) Circadian rhythms of alcohol dehydrogenase and MEOS in the rat. Proc Soc Exp Biol Med 175:299–303. https://doi.org/10.3181/00379727-175-3-rc2

Swerdlow NR, Gilbert D, Koob GF (1989) Conditioned drug effects on spatial preference: Critical evaluation. In: Boulton AA, Baker GB, Greenshaw AJ (eds) Neuromethods (13): Psychopharmacology. Humana Press, pp. 399–446.

Tzschentke TM (1998) Measuring reward with the Conditioned place preference paradigm: A comprehensive review of drug effects, recent progress, and new issues. Prog Neurobiol 56:613–672. https://doi.org/10.1016/s0301-0082(98)00060-4

van der Kooy D, O'shaughnessy M, Mucha RF, Kalant H (1983) Motivational properties of ethanol in naive rats as studied by place conditioning. Pharmacol Biochem Behav 19:441–445. https://doi.org/10.1016/0091-3057(83)90117-x

Webb IC, Baltazar RM, Wang X, Pitchers KK, Coolen LM, Lehman MN (2009) Diurnal variation in natural and drug reward, mesolimbic tyrosine hydroxilase, and clock gene expression in the male rat. J Biol Rhythms 24:465–476.

White NM, Messier C, Carr GD (1987) Operatinalizing and measuring the organization of drugs on behavior, in Methods of assessing the reinforcing properties of abused drugs (Bozarth MA ed), pp 591–617. Spring-Verlag, New York.

Yim HJ, Gonzales RA (2000) Ethanol-induced increases in dopamine extracellular concentration in rat nucleus accumbens are accounted for by increased release and not uptake inhibition. Alcohol 22:107–115.

Yu L, Wang L, Zhao X, Song M, Wang X (2016) Role of single prolonged stress in acquisition of alcohol conditioned place preference in rats. Life Sci. 151, 259-263. http://dx.doi.org/10.1016/j.lfs.2016.03.004

Zuo W, Fu R, Hopf FW, Xie G, Krnjevic K, Li J, Ye J-H (2017) Ethanol drives aversive conditioning through dopamine I receptor and glutamate receptor-mediated activation of lateral habenula neurons. Addict Biol 22:103–116.

SEÇÃO DE DADOS EXTRAÍDOS: APÊNDICES S13 E S14

Appendix S13

https://www.researchgate.net/publication/371124529_acer15092_sup_0004_Appendix_S13_Data_Extractedxlsx?_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uIiwicGFnZSI6InByb2ZpbGUiLCJwcmV2aW91c1BhZ2UiOiJwdWJsaWNhdGlvbiJ9fQ

Appendix S14

https://www.researchgate.net/publication/371124625_acer15092_sup_0005_Appendix_S14_Effect_size_estimates_95_CIxlsx?_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uIiwicGFnZSI6InByb2ZpbGUiLCJwcmV2aW91c1BhZ2UiOiJwdWJsaWNhdGlvbiJ9fQ

ANEXO A: CARTA DE APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 35315
Título: CONDICIONAMENTO AO LOCAL INDUZIDO POR ETANOL EM RATOS: INTERACOES ENTRE ISOLAMENTO SOCIAL, COMPRIMENTO DOS APARATOS, HORARIO DOS EXPERIMENTOS DE CONDICIONAMENTO E MODIFICACOES NEURONAIS NA VIA DOPAMIN

Vigência: 11/05/2018 à 30/08/2021

Pesquisadores:

Equipe UFRGS:

MIRNA BAINY LEAL - coordenador desde 11/05/2018
Douglas Marques da Silva - Aluno de Doutorado desde 11/05/2018

Equipe Externa:

José Inácio Lemos Carvalho Monteiro - pesquisador desde 11/05/2018
Eliane Dallegrave - pesquisador desde 11/05/2018

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 03/09/2018 - Sala 330 do Anexo I do Prédio da Reitoria - Campus Centro - Av. Paulo Gama-100/ Porto Alegre - RS, em seus aspectos éticos e metodológicos, para a utilização de 433 ratos Wistar machos (de 60 dias), provenientes do CREAL/UFRGS; de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Terça-Feira, 18 de Setembro de 2018

ALEXANDRE TAVARES DUARTE DE OLIVEIRA
Vice Coordenador da comissão de ética