

UC Santa Barbara

UC Santa Barbara Previously Published Works

Title

The effect of MK-801 on stress-ethanol cross-sensitization is dissociable from its effects on nNOS activity

Permalink

<https://escholarship.org/uc/item/04k378pb>

Journal

Alcohol, 112

ISSN

0741-8329

Authors

Borges Dos Santos, Jaqueline Rocha

Rae, Mariana

Teixeira, Simone Aparecida

et al.

Publication Date

2023-11-01

DOI

10.1016/j.alcohol.2023.06.004

Copyright Information

This work is made available under the terms of a Creative Commons Attribution-NoDerivatives License, available at <https://creativecommons.org/licenses/by-nd/4.0/>

Peer reviewed



The effect of MK-801 on stress-ethanol cross-sensitization is dissociable from its effects on nNOS activity

Jaqueline Rocha Borges dos Santos ^{a, b}, Mariana Rae ^a, Simone Aparecida Teixeira ^a,
Marcelo Nicolás Muscará ^a, Karen K. Szumlinski ^c, Rosana Camarini ^{a, *}

^a Department of Pharmacology, Institute of Biomedical Sciences, Universidade de São Paulo, SP, Brazil

^b Department of Pharmaceutical Sciences, Institute of Biological and Health Sciences, Universidade Federal Rural Do Rio de Janeiro, RJ, Brazil

^c Department of Psychological and Brain Sciences, Department of Molecular, Cellular and Developmental Biology and the Neuroscience Research Institute, University of California Santa Barbara, Santa Barbara, CA, United States

ARTICLE INFO

Article history:

Received 28 March 2023

Received in revised form

12 June 2023

Accepted 26 June 2023

Keywords:

Cross-sensitization

Dizocilpine

Glutamate

Hippocampus

N-methyl-D-aspartate

Nitric oxide

Prefrontal cortex

ABSTRACT

Locomotor behavioral sensitization represents an animal model for understanding neuroadaptive processes related to repeated drug exposure. Repeated stress can elicit a cross-sensitization to the stimulant response of ethanol, which involves neuronal nitric oxide synthase (nNOS). Activation of N-methyl D-aspartate (NMDA) glutamate receptors triggers nNOS and the synthesis of nitric oxide (NO). In this study, we investigated the effects of blocking NMDA receptors using the NMDA receptor antagonist MK-801 on the cross-sensitization between restraint stress and ethanol. We also evaluated the nNOS activity in the prefrontal cortex (PFC) and hippocampus. Mice were pretreated with saline or MK-801 30 min before an injection of saline or stress exposure for 14 days. On the following day, they were challenged with either saline or 1.8 g/kg ethanol. Swiss male mice pretreated with 0.25 mg/kg MK-801 exhibited a sensitized response to ethanol. Moreover, MK-801 potentiated the cross-sensitization between stress and ethanol. However, MK-801 prevented the enhanced nNOS activity in stress-exposed groups (challenged with saline or ethanol) in the PFC; the antagonist also prevented the ethanol-induced increase in nNOS activity and reduced this enzyme activity in mice exposed to stress in the hippocampus. These data indicate that systemic treatment with the NMDA antagonist potentiated, rather than blocked, ethanol-induced behavioral sensitization and that this effect is dissociable from the capacity of NMDA antagonists to reduce ethanol/stress-induced NOS stimulation in the PFC and hippocampus.

© 2023 Elsevier Inc. All rights reserved.

Introduction

Behavioral sensitization is defined as progressive increase in behavioral outcomes after repeated drug administration and it is considered to reflect neuroadaptations (Robinson & Berridge, 2008). The excitatory neurotransmitter glutamate has long been implicated in the mechanisms underlying behavioral sensitization to many drugs of abuse (Vanderschuren & Kalivas, 2000; Wolf, 1998). For examples, both competitive and non-competitive N-methyl-D-aspartate (NMDA) antagonists prevented behavioral sensitization to psychomotor stimulants (Kalivas & Alesdatter, 1993; Wolf & Jeziorski, 1993). With respect to ethanol, the

co-administration of glutamate receptor antagonists with ethanol prevented the development (Broadbent & Weitemier, 1999; Camarini, Frussa-Filho, Monteiro, & Calil, 2000) and expression of ethanol-induced behavioral sensitization in mice (Nona & Nobrega, 2018). On the other hand, the local administration of the non-competitive NMDA receptor antagonist MK-801 into the nucleus accumbens induced a cross-sensitization to ethanol in ethanol-sensitized mice, without impacting the locomotor response to ethanol in non-sensitized animals (Abrahao & Souza-Formigoni, 2012). Taken together, these prior studies indicate that the activation of NMDA receptors gates ethanol-induced behavioral sensitization.

It is known that the activation of NMDA receptors triggers the influx of calcium ions into the postsynaptic neuron, initiating various intracellular signaling pathways. One of these signaling pathways involves the activation of neuronal nitric oxide synthase (nNOS), which is an enzyme responsible for synthesizing nitric

* Corresponding author. Departamento de Farmacologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Lineu Prestes, 1524, SP, 05508-900, Brazil.
E-mail address: camarini@icb.usp.br (R. Camarini).

oxide (NO) (Blanke & VanDongen, 2009, chap. 13). Notably, NO generated by the activity of nNOS modulates the extracellular levels of various neurotransmitters (e.g., glutamate) in the central nervous system (Wegener, Volke, Harvey, & Rosenberg, 2003) and plays a critical role in the behavioral sensitization to various drugs of abuse, including ethanol (Itzhak, Roger-Sánchez, & Anderson, 2009; Santos-Rocha et al., 2018). nNOS is an enzyme that plays a crucial role in various brain regions associated with stress (Gadek-Michalska, Tedez, Bugajski, & Bugajski, 2019) and drug craving (Itzhak & Anderson, 2008). Furthermore, NO can control different neurosecretory processes, particularly the release of the stress hormone corticotropin releasing factor (CRF) (Riedel, 2000). In the context of stress interactions with drugs of abuse, MK-801 increases plasma corticosterone, induces cross-sensitization to amphetamine (Wedzony & Czyrak, 1994), and potentiates stress-induced cross-sensitization with amphetamine (Cruz, Marin, Leão, & Planeta, 2012). Conversely, the disruption of NOS activity, either through pharmacological means or genetic manipulation, reduces the development of psychostimulant or ethanol sensitization (Itzhak, 1997; Itzhak, Ali, Martin, Black, & Huang, 1998; Itzhak & Anderson, 2008; Santos-Rocha et al., 2018).

Both the NMDA antagonist (Adams & Moghaddam, 2001) and NMDA itself (Lorrain, Baccei, Bristow, Anderson, & Varney, 2003) have been shown to impact glutamatergic neurotransmission in the rat PFC, indicating the intricate involvement of NMDA in this system. Additionally, the effects of MK-801 on the glutamate system in the PFC are blocked by L-NAME, an inhibitor of NOS (Roemaker, Gudelsky, Ahlbrand, & Richtand, 2012).

Stress exposure also plays an important role in ethanol-induced behaviors (Favoretto, Nunes, Macedo, Lopes, & Quadros, 2020). Both acute and chronic stress increase ethanol intake and potentiate the effects of alcohol on the brain's motivational systems (Delis et al., 2013; Doremus-Fitzwater, Paniccia, Gano, Vore, & Deak, 2018; Heilig & Koob, 2007; Koob, 2013; Richardson, Lee, O'Dell, Koob, & Rivier, 2008). Moreover, we have demonstrated that repeated stress can cross-sensitize with ethanol in both adolescent and adult mice (Carrara-Nascimento, Griffin, Pastrello, Olive, & Camarini, 2011; Quadir et al., 2016; Santos-Rocha et al., 2018). In support of a permissive role for NMDA receptor-mediated activation of neuronal nNOS in stress-drug cross-sensitization, we have demonstrated that chronic unpredictable stress increases nNOS activity in the frontal cortex and hippocampus in adult mice, and that nNOS inhibition prevented stress-ethanol cross-sensitization (Santos-Rocha et al., 2018). Moreover, the nNOS inhibitor 7-nitroindazole prevents ethanol sensitization and cross-sensitization between ethanol and chronic unpredictable stress (Santos-Rocha et al., 2018), raising a potential role for nNOS activation upon NMDA receptor stimulation in both ethanol-induced behavioral sensitization and its cross-sensitization with stress.

The search for mechanisms that influence drug-induced plasticity, such as sensitization, is highly relevant for understanding the underlying processes involved in addiction. However, it is important to carefully evaluate the general assumption that glutamate antagonists can universally prevent or inhibit the development or expression of behavioral sensitization. This notion has been a topic of debate, as highlighted in a seminal review on MK-801 (Tzschentke & Schmidt, 2000). Our hypothesis is that repeated stress can induce cross-sensitization to ethanol in a manner dependent upon the activation of the NMDA-NOS signaling pathway. Although cross-sensitization between stress and ethanol has been demonstrated, the role of NMDA receptors in this phenomenon has not been established. Therefore, we aimed to study whether MK-801, an NMDA antagonist, would influence the cross-sensitization between stress and ethanol. As NMDA receptor

stimulation can activate nNOS, we also examined for changes in nNOS activity – as a read-out of changes in NMDA-dependent nNOS activation/receptor function – within the PFC and hippocampus – both regions highly affected by stressors (McEwen, Nasca, & Gray, 2016).

Material and methods

Subjects

Adult Swiss male mice (total $n = 126$) were provided by the Instituto de Ciências Biomédicas (Institute of Biomedical Sciences) of the Universidade de São Paulo. Groups of 4 animals were housed in polypropylene cages (27.5 cm length $\times$ 16.5 cm width $\times$ 13 cm height). They were individually housed at $21 \pm 1^\circ\text{C}$ with food and water *ad libitum* on a 12-h light/dark cycle with the lights turned on at 7:00 AM for at least a 3-day acclimatization period. Each animal was used in only one experimental procedure. The experiments were conducted according to the guidelines of the Brazilian National Council for Control of Animal Experimentation (CONCEA), in accordance with the Brazilian National Law number 11,794 of October 8, 2008, Decree 6899 of July 15, 2009, after approval from the Ethics Committee of Animal Use (Protocol #134/09) of the Institute of Biomedical Sciences of the University of São Paulo.

Drugs

Ethanol (95% v/v; Merck do Brasil, Rio de Janeiro, RJ, Brazil) was diluted in a saline solution (0.9% NaCl in distilled water) to produce a 20% v/v solution that was administered intraperitoneally (i.p.) at a dose of 1.8 g/kg. Control animals received isovolumetric saline injections (i.p.).

MK-801 (dizocilpine maleate, Merck do Brasil, Rio de Janeiro, Brazil) was prepared in 0.9% physiological saline and administered i.p. at a dose of 0.25 mg/kg in a volume of 10 mL/kg (Camarini et al., 2000; Meyer & Phillips, 2007).

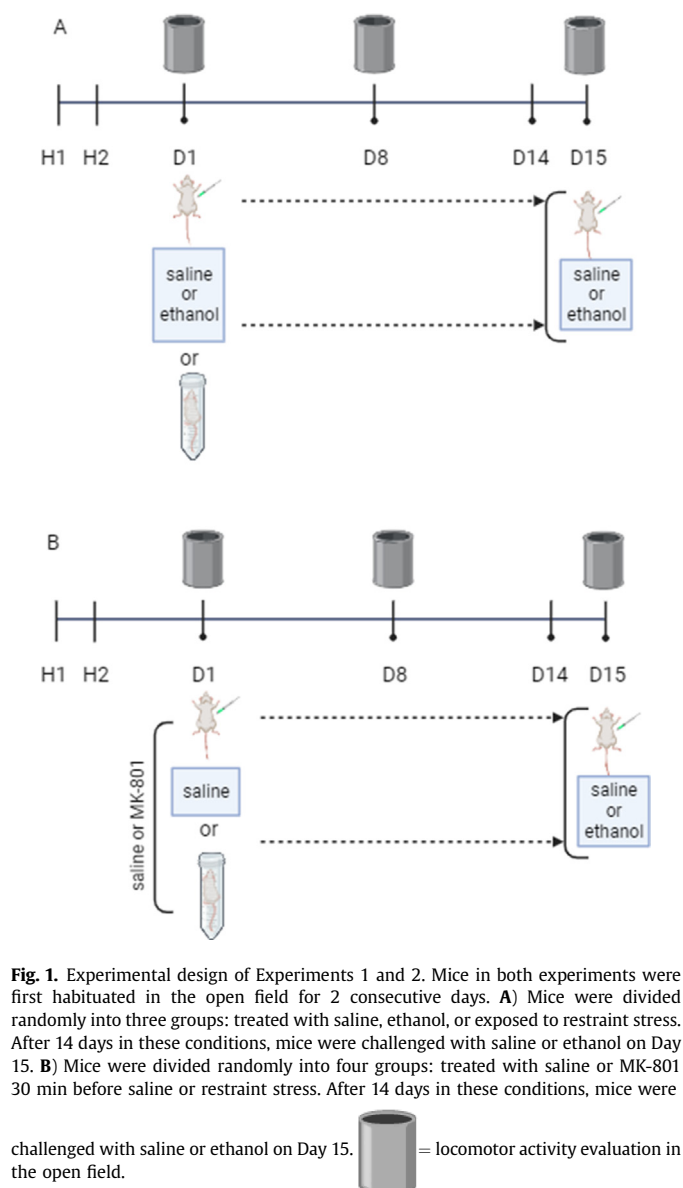
Apparatus

All behavioral procedures were performed during the light phase of the light/dark cycle. Total locomotor activity was evaluated in the open-field (40-cm diameter arena, surrounded by a 50-cm wall for 10 min) and recorded with a digital camera. EthoVision 3.1 software (Noldus, Wageningen, the Netherlands) was used to track and quantify the distance traveled by each animal.

Stress paradigm and experimental procedures

The experimental design is depicted in Fig. 1.

Experiment 1 (Fig. 1A). We first tested whether repeated restraint stress would elicit a sensitized locomotor response to acute ethanol. Prior to any repeated treatment, all animals were acclimated to the open field for 10 min, following an i.p. saline injection over the course of 2 habituation days (data not shown). On the following day, three groups of mice were repeatedly injected with saline, ethanol, or exposed to restraint stress for 14 days ($n = 14$ per group). Mice in the stress group were exposed to 14 consecutive days of restraint stress. The restraint stress consisted of restraining the animal in a plastic cylinder for 1 h/day, between 10:00 and 11:00 AM. On day 15, mice from each group were randomly divided into two subgroups and the locomotor activity was recorded for 10 min after a challenge injection with either saline or ethanol ($n = 7$ per group). Mice were tested in the open field after saline



injections on habituation days (H1 and H2) and after either saline, ethanol, or restraint stress on days 1 (D1), 8 (D8), and 15 (D15).

Having established a cross-sensitization between ethanol and stress, we next evaluated the effects of MK-801 on this cross-sensitization.

Experiment 2 (Fig. 1B). As in the first experiment, mice were injected i.p. with saline and habituated to the open field for 10 min over the course of 2 days (data not shown). Then, over the next 14 days, mice were pretreated with saline or 0.25 mg/kg MK-801, 30 min before restraint stress or saline i.p., as a control for the injection stress ($n = 20$ – 24 /group). To examine for the development of sensitization, locomotor activity was assessed for 10 min in the open field on days 1 and 8 (respectively, D1 and D8) of repeated restraint stress (or saline control procedures), immediately after the stress/saline procedure. Following the 14-day stress/saline phase of the experiment, the groups were then further subdivided to receive a challenge injection of either saline or 1.8 g/kg ethanol and were immediately tested in the open field for 10 min on day 15 (D15; $n = 10$ – 12 /group).

Mice were euthanized by decapitation 3 h after the assessment of locomotor activity on D15 for determination of nNOS activity.

Ca^{2+} -dependent nNOS assay

We conducted an assay to measure the conversion of [3H] L-arginine into [3H] L-citrulline in homogenates from PFC and hippocampus, as previously described (Teixeira et al., 2002). This assay enables the assessment of Ca^{2+} -dependent activation of NOS (which reflects mainly nNOS and endothelial NOS or eNOS) in our regions of interest. Three hours after the experiments, the animals were euthanized, and the hippocampus (including the dentate gyrus, CA1, CA2, CA3, and CA4 regions) and PFC (including pre-limbic, infralimbic, and anterior cingulate cortex) were dissected as previously described in Contó, Pautassi, and Camarini (2022) and Sultan (2013). Specifically, the brain was sectioned using a chilled razor blade and the PFC was dissected using brain punches (1.2-mm, Harris Micro-Punch, Ted Pella), according to the mouse brain atlas by Franklin and Paxinos (1997). This assay enabled us to assess the Ca^{2+} -dependent activation of NOS (which reflects mainly nNOS and endothelial NOS or eNOS) in both the hippocampus and PFC.

In order to obtain sufficient tissue for the assay, samples from the hippocampus and prefrontal cortex were pooled between two mice and then were homogenized in five volumes of cold incubation buffer (50-mM Tris HCl buffer, pH 7.4) containing 1-mM phenylmethylsulfonyl fluoride (PMSF) and 1-mM L-citrulline. The homogenates were incubated for 30 min with 1-mM NADPH, 2-mM $CaCl_2$, and 10- μ M L-arginine containing 100 000 disintegrations per minute (dpm) of [2,3,4,5- 3H] L-arginine monohydrochloride at room temperature (25–27 °C). Pharmacological controls of enzymatic activity were also carried out. For each sample, the specific NOS-mediated conversion of L-arginine to L-citrulline was obtained by subtraction of the conversion in the presence of 1-mM L-NAME (a non-selective NOS inhibitor), and the Ca^{2+} -dependent enzyme activity was confirmed by the null L-arginine to L-citrulline conversion in the absence of $CaCl_2$ and in the presence of 5-mM EGTA in the incubation medium (Teixeira et al., 2002).

The protein content of the samples was determined by Bradford assay, and the activity of nNOS was expressed as picomoles L-citrulline produced/minute per mg of protein. The Beckman Coulter 5500B Gamma Counter (California, United States) was used as the nNOS activity scintillation counter.

Statistical analysis

The variables were checked for normality (Shapiro–Wilk test) and homogeneity (Levene test). Data from the cross-sensitization between ethanol and stress were analyzed by a factorial analysis of variance (ANOVA) with pre-exposure (saline, ethanol, or stress) and challenge injection (saline or ethanol) as between-factors. The experimental design to analyze the effects of MK-801 on the cross-sensitization between repeated stress and ethanol consisted of eight groups (four groups – Saline/Saline, Saline/Stress, MK/Saline, and MK/Stress – that were subsequently challenged with saline or ethanol). First, we conducted *t* test analysis to determine whether the populations within the same treatment group before the challenge injection were different from each other. Since no differences were found (data not shown), the groups with the same repeated pretreatment were combined for analysis of the locomotor data from D1 and D8 of repeated stress/saline. The effects of MK-801 on cross-sensitization between restraint stress and ethanol were analyzed using a 3-way repeated-measure ANOVA, with pretreatment (MK-801 or saline) and stress (saline injection or restraint stress) as between-subjects factors, and days (D1 and D8)

as repeated-measures. Differences in the expression of locomotor activity, and nNOS activity to a challenge dose of ethanol on D15 were all analyzed using 3-way ANOVAs (pretreatment $\times$ stress $\times$ challenge injection). Tukey *post hoc* test was used when appropriate. All data are expressed as mean $\pm$ standard error of the mean (SEM). Significance was defined at the 0.05 level.

Results

Cross-sensitization between stress and ethanol

Consistent with our prior studies employing chronic unpredictable stress procedures (Quadir et al., 2016; Santos-Rocha et al., 2018), we confirmed that repeated restraint stress cross-sensitized to an ethanol challenge injection, and the magnitude of this cross-sensitization was of a similar magnitude as that exhibited by mice sensitized to repeated ethanol (Fig. 2). A two-way ANOVA revealed a significant pre-exposure $\times$ challenge interaction ($F_{(2,36)} = 5.45$, $p < 0.05$, $\eta^2 p = 0.23$), and significant main effects of stress and challenge injection (see Supplemental material). Compared to repeated saline control mice, both repeated ethanol injections and chronic restraint stress increased the acute locomotor response to the ethanol challenge injection (Tukey *post hoc* tests: Saline-Ethanol vs. Stress-Ethanol, $p < 0.05$; Saline-Ethanol vs. Ethanol-Ethanol, $p < 0.05$), revealing a sensitization to ethanol and cross-sensitization between stress and ethanol.

Acute stress suppresses MK-801 hyperlocomotion while MK-801 potentiates cross-sensitization between stress and ethanol

Next, we evaluated the effects of repeated MK-801 and stress on locomotor activity. Analysis of data from D1 and D8 (Fig. 3A) using a 3-way repeated-measures ANOVA showed a significant MK801 $\times$ Stress $\times$ Days interaction ($F_{(1,80)} = 20.99$, $p < 0.05$, $\eta^2 p = 0.21$). All other main effects, interactions, and effect sizes can be found in Supplemental material. Tukey *post hoc* tests indicated that MK-801 induced a hyper-locomotor activity in the unstressed control (MK/SAL) mice on D1, relative to the other groups ($p < 0.05$), indicating that repeated stress exposure blunts acute MK-801-induced locomotor stimulation (see Fig. 3A; D1, MK/Saline vs. MK/Stress).

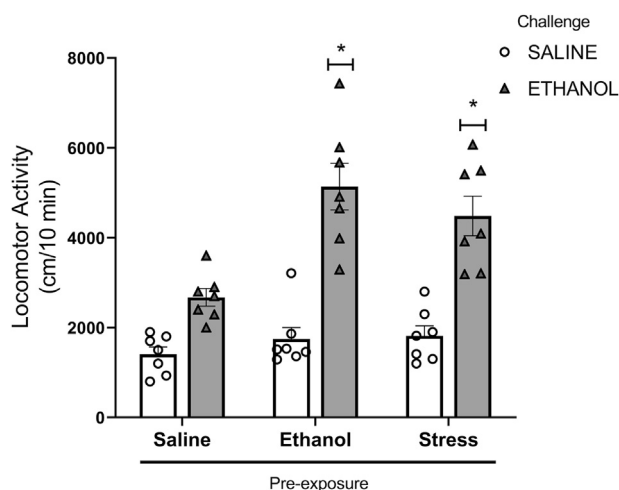


Fig. 2. Cross-sensitization between chronic restraint stress and 1.8 g/kg ethanol in male Swiss mice. Data are expressed as distance traveled (mean $\pm$ SE). Mice were exposed to saline i.p., 1.8 g/kg ethanol i.p., or chronic restraint stress over 14 days and then were challenged with saline or ethanol prior to locomotor testing. *differs from the group pre-treated with saline/challenged with ethanol, two-way ANOVA followed by Tukey post-tests ($n = 7$ /group).

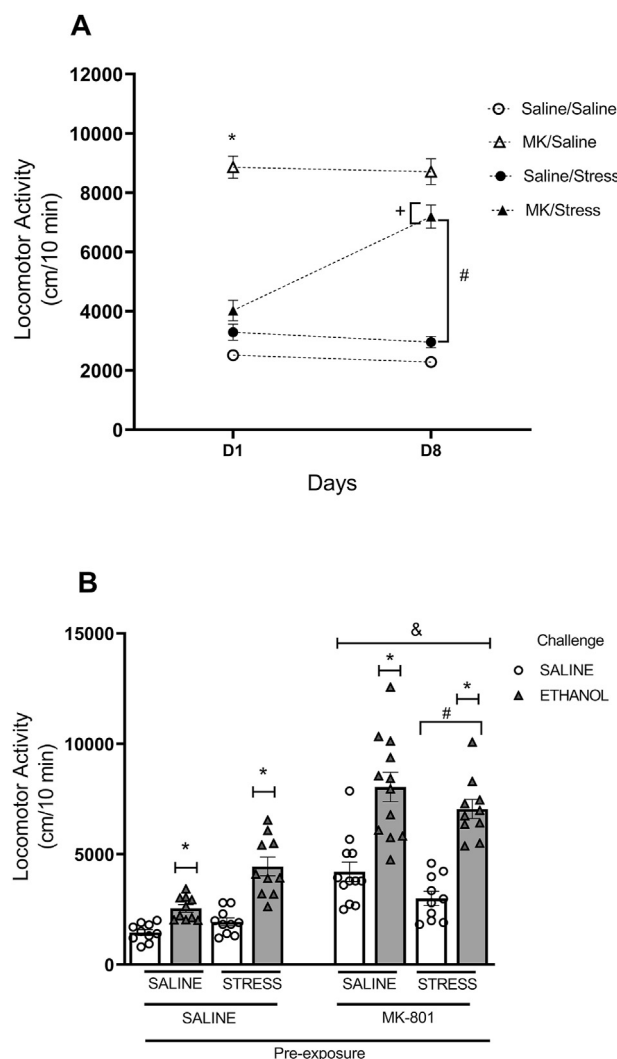


Fig. 3. A) Locomotor activity of mice pretreated with saline or MK-801 30 min before treatment with saline or restraint stress during 8 consecutive days (mean $\pm$ SE). The activity was quantified on Day 1 and Day 8. *differs from all groups, within the same day; +differs from D1, within the same group; #differs from Saline/Stress. Three-way repeated-measures ANOVA followed by Tukey test. B) Locomotor activity of mice challenged with saline (white bars) or 1.8 g/kg ethanol (gray bars) on Day 15, which were pretreated with saline or MK-801 30 min before an injection of saline or exposure to 1 h restraint stress for 14 consecutive days. *MK-801 pretreatment increases locomotor activity relative to saline (left vs. right graphs; three-way ANOVA), *ethanol-challenged groups show higher locomotor activity compared to the respective groups challenged with saline (left and right graphs, two-way ANOVA, followed by Tukey test), *stress yields a decrease in locomotor activity relative to the saline groups in MK-801-pretreated groups (right graph, two-way ANOVA, followed by Tukey test). $n = 10$ – 12 /group.

Although the high level of locomotor activity persisted on D8 in the no-stress mice pretreated with MK-801 (MK/Saline) mice, the locomotor activity of the MK/Stress group was now significantly higher than that of the Saline/Stress control on D8 ($p < 0.05$). To determine which group developed behavioral sensitization, the data were compared across days (D1 vs. D8), and analyses revealed an increase in locomotor activity across days only in MK/Stress mice (Fig. 3A), indicating that MK-801 co-treatment with restraint stress induces behavioral sensitization. In contrast, no significant change in locomotor activity was detected in repeated-stress (Saline/Stress) controls or in either of the no-stress control groups (Saline/Saline and MK/Saline). These data indicate that repeated (8 days) restraint stress, repeated saline, and repeated MK-801 treatment are insufficient to elicit behavioral sensitization in this study.

Subsequently, we evaluated the effects of MK-801 on cross-sensitization between restraint stress and ethanol in these mice. For this, the groups Saline/Saline, Saline/Stress, MK/Saline, and MK/Stress were challenged with either saline or 1.8 g/kg ethanol on D15 (Fig. 3B). A three-way ANOVA detected significant main effects of MK-801 ($F_{(1,76)} = 105.25, p < 0.0001; \eta^2p = 0.58$), challenge injection ($F_{(1,76)} = 98.13, p < 0.0001; \eta^2p = 0.56$), a significant MK-801 $\times$ challenge interaction ($F_{(1,76)} = 13.65, p < 0.01; \eta^2p = 0.15$), and MK-801 $\times$ stress interaction ($F_{(1,76)} = 15.57, p < 0.01; \eta^2p = 0.17$). All other main effects and effect sizes can be found in the Supplemental material. Tukey test revealed that mice pretreated repeatedly with MK-801 exhibited higher levels of locomotor activity than those pretreated repeatedly with saline (Fig. 3B, right vs. left). Further, the 3-way analysis was divided into 2-way analysis at each level of pretreatment. A two-way ANOVA conducted to analyze the groups pretreated with saline revealed a significant stress $\times$ challenge interaction ($F_{(1,36)} = 7.83, p < 0.01; \eta^2p = 0.18$; all other main effects and effect sizes can be found in the Supplemental material). *Post hoc* analyses revealed that acute ethanol increased locomotor activity as compared to saline control groups (Fig. 3B, left; Saline/Saline/Ethanol vs. Saline/Saline/Saline; Saline/Stress/Ethanol vs. Saline/Stress/Saline), replicating a cross-sensitization between repeated restraint stress and ethanol. Ethanol increased the locomotor activity of mice pre-exposed to stress (Saline/Saline/Ethanol vs. Saline/Stress/Ethanol), confirming a cross-sensitization effect. A two-way ANOVA conducted to analyze the groups pretreated with MK-801 revealed significant main effects of stress and challenge injection ($F_{(1,40)} = 4.8, p < 0.05; \eta^2p = 0.11$ and $F_{(1,40)} = 61.54, p < 0.05; \eta^2p = 0.61$). No statistically significant interaction between stress and challenge injection was found (see Supplemental material). As illustrated in Fig. 3B (right), mice challenged with ethanol exhibited higher levels of locomotor activity than those challenged with saline. Those mice exposed to stress showed blunted locomotor activity relative to those exposed to saline, confirming the interaction between MK-801 and stress.

Taking together, mice treated repeatedly with MK-801 exhibited a cross-sensitization to the acute ethanol injection as well as potentiated cross-sensitization between stress and ethanol (Fig. 3B, right vs. left).

MK-801 blunts nNOS activity in the PFC and hippocampus

Fig. 4 shows nNOS activity in the PFC (A) and hippocampus (B) homogenates.

Analyses of the data from the PFC by a 3-way ANOVA revealed significant main effects of MK-801 ($F_{(1,34)} = 6.80, p < 0.05; \eta^2p = 0.17$), challenge injection ($F_{(1,34)} = 12.25, p < 0.01; \eta^2p = 0.26$), and a significant MK-801 $\times$ stress interaction and MK-801 $\times$ challenge interaction ($F_{(1,34)} = 10.16, p < 0.05, \eta^2p = 0.23$; $F_{(1,34)} = 4.66, p < 0.05, \eta^2p = 0.12$, respectively). All other main effects and effect sizes can be found in the Supplemental material. MK-801 decreased nNOS activity in mice exposed to stress, as compared to those pretreated with saline. Further, the 3-way analysis was divided into 2-way analysis at each level of pretreatment. A two-way ANOVA conducted to analyze the groups pretreated with saline revealed significant main effects of stress and challenge injection ($F_{(1,16)} = 4.51, p < 0.05, \eta^2p = 0.22$; $F_{(1,16)} = 33.11, p < 0.05, \eta^2p = 0.67$, respectively). No statistically significant stress $\times$ challenge interaction was found ($F_{(1,16)} = 2.26, p > 0.05, \eta^2p = 0.12$). *Post hoc* analyses indicated that an acute challenge ethanol injection increased the nNOS activity in the PFC relative to saline controls, as well as stress-enhanced nNOS activity as compared to saline-pretreated groups (Fig. 3A). A two-way ANOVA

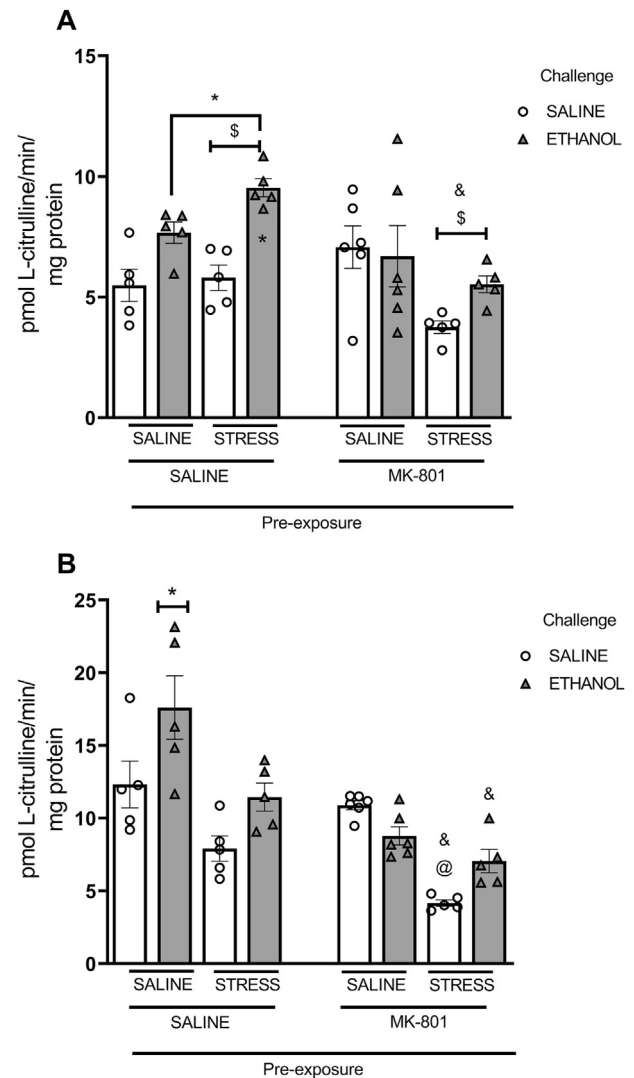


Fig. 4. A) Neuronal NO synthase (nNOS) activity in prefrontal cortex (PFC) homogenates and B) hippocampus homogenates prepared from mice challenged with saline (white bars) or 1.8 g/kg ethanol (gray bars) on Day 15 of treatment. Mice were pretreated with saline or MK-801 30 min prior to a saline injection or restraint stress exposure during 14 consecutive days. nNOS activity was measured 3 h after locomotor activity measurements. Data are represented as mean $\pm$ SEM. A) *MK-801 pretreatment decreases nNOS activity in mice exposed to stress relative to those pretreated with saline (left vs. right graphs; three-way ANOVA), *ethanol-challenged groups show higher nNOS activity compared to the respective groups challenged with saline (left graph, two-way ANOVA, followed by Tukey test); [§]stress yields an increase in nNOS activity relative to the saline groups in saline-pretreated groups (left graph), while this effect is inverted in MK-801-pretreated groups (right graph two-way ANOVA, followed by Tukey test). B) *differs from all groups; [@]MK-801 reduces nNOS activity in mice exposed to stress; [§]MK-801 decreases nNOS activity relative to control saline-saline-saline. Three-way ANOVA, followed by Tukey tests. n = 5–6/group (each sample represents a pool of 2 mice).

conducted to analyze the groups pretreated with MK-801 revealed a significant main effect of stress ($F_{(1,18)} = 6.50, p < 0.05, \eta^2p = 0.27$). No statistically significant main effects of challenge injection or stress $\times$ challenge injection interactions were found ($F_{(1,18)} = 0.64, p > 0.05, \eta^2p = 0.03$; $F_{(1,18)} = 1.50, p > 0.05, \eta^2p = 0.07$, respectively). In mice pretreated with MK-801, exposure to chronic stress yielded lower nNOS activity compared to saline controls. Thus, MK-801 pretreatment suppressed the elevated nNOS activity in the repeated-stress group (Fig. 4A).

A comparable analysis of nNOS activity in the hippocampus revealed a significant MK $\times$ Stress $\times$ challenge injection interaction ($F_{(1,34)} = 4.80$, $p < 0.05$, $\eta^2 p = 0.12$), allowing multiple comparisons. All other main effects and effect sizes can be found in the Supplemental material. *Post hoc* test showed that acute ethanol significantly increased hippocampal nNOS activity in the no-stress control mice relative to all groups, including those pretreated with MK-801 (Fig. 4B; Saline/Saline/Ethanol > other groups; all p 's < 0.05). Thus, this acute ethanol effect was prevented by repeated MK-801 pretreatment (MK/Saline/Ethanol < Saline/Saline/Ethanol; $p < 0.05$) and blunted also by repeated stress (Saline/Stress/Ethanol < Saline/Saline/Ethanol; $p < 0.05$). In the mice pretreated repeatedly with MK-801, exposure to repeated stress also decreased nNOS activity in the hippocampus (MK/Stress/Saline < MK/Saline/Saline; $p < 0.05$). Moreover, relative to control Saline/Saline/Saline, MK-801 also reduced nNOS activity in mice repeatedly exposed to stress.

Discussion

MK-801 effects on cross-sensitization between ethanol and stress

In this study, we conducted experiments to investigate stress-induced locomotor cross-sensitization to ethanol and the cross-sensitization between ethanol and MK-801, building upon prior literature (Meyer & Phillips, 2003; Phillips, Roberts, & Lessov, 1997; Quadir et al., 2016; Santos-Rocha et al., 2018). However, our findings differed from previous studies (Broadbent & Weitemier, 1999; Camarini et al., 2000; Meyer & Phillips, 2003), as repeated daily pretreatment with 0.25 mg/kg MK-801 did not prevent the development of ethanol-induced sensitization. Instead, mice treated repeatedly with MK-801 exhibited a cross-sensitization to the acute ethanol injection. Several factors may contribute to these discrepant results, including strain, dose, and context.

First, it is worth noting that most studies on ethanol sensitization utilize DBA/2 mice (Broadbent & Weitemier, 1999; Meyer & Phillips, 2003), while we have used Swiss mice. Regarding MK-801 dose, previous research has indicated that repeated administration of MK-801 at 0.1 mg/kg with ethanol potentiated ethanol sensitization in DBA/2J mice (Meyer & Phillips, 2003), while 0.25 mg/kg MK-801 did not interfere in development of sensitization in this strain (Nona & Nobrega, 2018). It should be noted that the DBA/2J mice employed in these two latter studies originated from different commercial vendors – Charles River (Quebec, Canada) in the case of Nona and Nobrega (2018), and Jackson Laboratory (Bar Harbor, Maine, United States) in Meyer and Phillips (2003) – and breeder-dependent differences in behavioral responses have been demonstrated with respect to vulnerability to stress (Theilmann et al., 2016) and ethanol intake (Palm, Roman, & Nylander, 2011). Furthermore, the context in which MK-801 is administered may influence its effects (Camarini et al., 2000; Carlezon, Mendrek, & Wise, 1995), as MK-801's ability to block drug-induced sensitization has been suggested to be state-dependent (Carlezon et al., 1995). In our study, ethanol was not administered in the test environment after each injection, which might have influenced the behavioral outcomes.

Another aspect to be considered in our study is the limited inter-individual variability observed in the response to ethanol-induced behavioral sensitization in Swiss mice when compared to the findings reported by Abrahao and Souza-Formigoni (2012). It is worth noting that different environmental conditions in various animal facilities can give rise to distinct characteristics at both molecular and behavioral levels. These characteristics may manifest as heightened or reduced variations in the response to repeated ethanol administration, as demonstrated by Abrahao and Souza-

Formigoni (2012) and Quoilin, Didone, Tirelli, and Quertemont (2012), respectively. Nevertheless, we observed higher locomotor variability in the groups treated with ethanol compared to those treated with saline, suggesting that ethanol elicits more diverse responses than saline.

MK-801 was used in this study as a pharmacological tool to investigate the involvement of NMDA receptors in the cross-sensitization between stress and ethanol, and MK-801 pretreatment potentiated the hyperlocomotion induced by both repeated stress and acute ethanol manipulations. In line with a prior neuropharmacological study targeting the nucleus accumbens (Abrahao & Souza-Formigoni, 2012), we also detected a cross-sensitization between systemic MK-801 and ethanol. A cross-sensitization between repeated MK-801 and ethanol is not surprising given that ethanol is posited to act as a non-competitive NMDA receptor antagonist, and thus, exhibits a similar mechanism of action as MK-801 to inhibit NMDA receptor function, albeit the complexity of changes in NMDA receptor induced by ethanol (den Hartog et al., 2017). As no locomotor sensitization was produced in the present study by repeated restraint stress alone, the potentiation of stress-induced locomotor sensitization by MK-801 suggests that repeated non-competitive blockade of NMDA receptors induces neuroplastic changes that facilitate the locomotor-sensitizing effects of repeated stress.

Cross-sensitization between stress and ethanol may involve the activational state of the hypothalamic-pituitary-adrenal (HPA) axis, as pretreatment with metyrapone, a corticosterone synthesis inhibitor, prevents the development of ethanol sensitization (Pastor et al., 2012), and a glucocorticoid receptor antagonist blocks the cross-sensitization between stress and ethanol (Roberts, Lessov, & Phillips, 1995), indicating that HPA axis activity has a role for ethanol-induced sensitization and its cross-sensitization to stress. Indeed, there is evidence that NMDA receptors gate neuroendocrine control over the HPA axis (Marty, Mulpuri, Munier, & Spigelman, 2020), with MK-801 increasing plasma corticosterone levels (Kim, Jung, Moon, & Song, 2009). Based on the findings of Roberts et al. (1995) and Pastor et al. (2012), the MK-801-induced corticosterone secretion might be predicted to potentiate stress-induced behavioral sensitization and cross-sensitization. Further, chronic stress decreases NMDA receptor expression in the PFC (Lee & Goto, 2011), which could tune NMDA signaling and shift behavioral sensitivity of stressed mice to both the locomotor-activating effects of repeated MK-801 pretreatment and to acute ethanol. This could explain the larger effect in stressed animals. For instance, NMDA hypofunction in the PFC increases the rewarding effects of opioids (Bishop, Lauzon, Bechard, Gholizadeh, & Laviolette, 2011).

The present study confirms previous reports that acute administration of MK-801 induces hyperlocomotion in different mouse strains (Camarini et al., 2000; Szumlinski et al., 2005). In fact, there is evidence that hyperlocomotion induced by NMDA antagonists is mainly regulated by the PFC, by mechanisms that involve increases in glutamate efflux, and by stimulation of non-NMDA receptors glutamatergic neurotransmission (Moghaddam, Adams, Verma, & Daly, 1997). However, in contrast to other reports demonstrating MK-801-induced locomotor sensitization with repeated treatment (Wolf & Khansa, 1991), 8 injections of 0.25 mg/kg MK-801 failed to elicit a sensitization of locomotor hyperactivity in the present study, which likely reflects a ceiling effect upon behavior. Repeated MK-801 pretreatment also did not result in tolerance to its locomotor activating effects, as previously demonstrated after 21 days of treatment (Camarini et al., 2000), corroborating evidence that the effects of repeated MK-801 upon locomotor activity (sensitization, tolerance, or no change) vary according to not only dose, but also the number of injections (Broadbent & Weitemier, 1999; Camarini et al., 2000; Ida, Asami, & Kuribara, 1995).

Curiously, the acute locomotor stimulant effect of MK-801 was blunted by concomitant stress exposure. While the precise mechanism accounting for the stress-induced blunting of MK-801 hyperactivity is not entirely clear, acute restraint stress is reported to increase glutamate and aspartate levels in the PFC and hippocampus (Moghaddam, 1993). Although acute stress initially reduced the hyperlocomotion caused by MK-801, this effect was gradually reversed with repeated stressor exposure. Previous studies have shown that chronic stress disrupts glutamate homeostasis by altering the expression of pre- and postsynaptic glutamate receptors, leading to glutamatergic hyperactivity (Nasca et al., 2015; Quadir et al., 2016), as well as increased glutamate uptake and basal release (Fontella et al., 2004). Based on these findings, it can be hypothesized that the effects of stress on the glutamatergic system may compete with the action of MK-801 on glutamate receptors. In our study, the MK-801/stress group showed lower NOS activity as compared to MK/saline after repeated exposure, suggesting an interaction between stress and the NO system. By examining NOS levels between acute MK/saline and acute MK/stress, we may gain a better understanding of the interplay between stress, NMDA receptor activity, and NOS function. This knowledge could contribute to unraveling the complex dynamics of the stages of the sensitization process.

MK-801 effects on nNOS activity of mice exposed to stress and ethanol

It is well known that calcium influx through NMDA receptors activates NOS, stimulating the synthesis of NO (Garthwaite, Garthwaite, Palmer, & Moncada, 1989). NO is a gaseous molecule that diffuses in the brain and plays a major role in both inter- and intracellular communication (Picón-Pagès, García-Buendía, & Muñoz, 2019). NO participates in several effects of ethanol (Davis & Syapin, 2005), including in ethanol-induced stimulant locomotor effects, conditioned place preference (Itzhak & Martin, 2000), and ethanol behavioral sensitization (Itzhak & Anderson, 2008; Santos-Rocha et al., 2018). Several studies have shown a role for NO in behavioral sensitization to cocaine (Itzhak, 1997), methamphetamine (Inoue, Arai, Shibata, & Watanabe, 1996), and morphine (Zarrindast, Askari, Khalilzadeh, & Nouraei, 2006), as well as the cross-sensitization between cocaine and methamphetamine (Itzhak, 1997). In a previous study from our laboratory, nNOS inhibition by 7-nitroindazole prevented both ethanol sensitization and cross-sensitization between ethanol and stress (Santos-Rocha et al., 2018). To better understand the participation of NMDA-NO signaling in cross-sensitization between stress and ethanol, we evaluated the nNOS activity in the PFC and hippocampus of mice pretreated with MK-801.

MK-801 prevents stress-induced increases in PFC nNOS activity

We know from prior work that the anxiogenic-like phenotype induced by acute restraint stress is associated with increased nNOS expression in the prelimbic ventromedial PFC, 24 h after the stress (Vila-Verde, Marinho, Lisboa, & Guimarães, 2016). Further, chronic unpredictable stress increased nNOS activity in the PFC (Santos-Rocha et al., 2018). However, we failed to detect an increase in PFC nNOS activity following chronic restraint stress, which may reflect a habituation to repeated exposure to the same type of stressor. Acute ethanol increased nNOS activity in both unstressed and stressed mice relative to their saline-challenged controls, and the magnitude of this ethanol effect appeared to be larger in the mice that underwent repeated stress versus those that did not, indicating a cross-sensitization between the effects of repeated stress and ethanol on nNOS activity within the PFC. Consistent with

a role for NMDA receptors in gating nNOS activity, MK-801 pretreatment prevented the increase in PFC nNOS activity exhibited by mice in the cross-sensitized state. Thus, a double-dissociation exists between the effects of MK-801 on stress-ethanol behavioral versus nNOS cross-sensitization.

MK-801 prevents ethanol-induced increases in hippocampal nNOS activity and decreases nNOS activity in stressed animals.

As observed in the PFC, the acute ethanol challenge injection increased nNOS activity within the hippocampus of our mice, and this effect was blunted in mice with prior MK-801 pretreatment. Although MK-801 did not change hippocampal nNOS activity (MK/Saline/Saline vs. Saline/Saline/Saline), Liu et al. (2009) demonstrated that repeated MK-801 inhibited the NOS activity in hippocampus of rats.

Increases in NO levels in the hippocampus, but not in the PFC, have a major influence on the HPA axis, stimulating the production and release of adrenocorticotrophic hormone (ACTH) (Seo & Rivier, 2001). In the present study, a history of repeated restraint stress also attenuated the ethanol-induced increase in nNOS activity in the hippocampus.

We also demonstrated that MK-801 suppressed nNOS activity in the stressed group. Chronic mild stress is reported to induce hippocampal nNOS activation and HPA axis hyperactivity (Zhou et al., 2011), and variable stress-restress exposure also increases hippocampal iNOS, followed by downregulation of NMDA receptors (Harvey, Oosthuizen, Brand, Wegener, & Stein, 2004). Although nNOS activity did not change in our stressed mice, MK-801 mitigated the nNOS activation in this group, suggesting that hippocampal NO following repeated stress can be mediated by NMDA receptors.

Previous studies have implicated NMDA receptors and NOS in the neural plasticity associated with ethanol sensitization. For instance, the nNOS inhibitor 7-nitroindazole has been shown to prevent ethanol sensitization and cross-sensitization between ethanol and chronic unpredictable stress (Itzhak & Martin, 2000; Santos-Rocha et al., 2018). Additionally, the nNOS gene has been identified as crucial for the development of behavioral sensitization to ethanol in both adult and adolescent male mice (Itzhak & Anderson, 2008). It is noteworthy that prolonged ethanol exposure can lead to the upregulation or increased expression of NMDA receptors in certain brain regions such as the PFC and hippocampus (Nagy, 2008, as review). However, intriguingly, previous research has demonstrated reduced NMDA receptor function and expression specifically in the accumbal region of ethanol-sensitized mice (Abraham et al., 2013). In our study, we observed a dissociation between the effects of MK-801 on stress/ethanol cross-sensitization and NOS activity. MK-801 potentiated cross-sensitization while decreasing NOS activity in this group. The underlying mechanisms that contribute to the intricate relationship between NMDA-NOS and stress/ethanol sensitization are complex and not yet fully understood.

Study limitations

We acknowledge that there are some limitations to our study. Firstly, there is a lack of a demonstrated causal relationship between the nNOS results and behavioral sensitization. Our previous studies indicated that nNOS inhibition effectively reduced both ethanol sensitization and the cross-sensitization between chronic unpredictable stress and ethanol (Santos-Rocha et al., 2018). Although such findings confirmed the involvement of the NMDA-NO pathway in ethanol-induced sensitization and ethanol/stress cross-sensitization, the present experimental designs were correlational in nature.

Secondly, another limitation of our study is the exclusion of female mice. It is well-documented that females exhibit heightened sensitivity to ethanol sensitization compared to males. Therefore, the generalizability of our findings may be limited to the male population, and further investigation involving female mice is warranted to fully understand the impact of sex on cross sensitization between stress and ethanol.

Conclusion

In summary, repeated pretreatment with the non-competitive NMDA antagonist MK-801 potentiates locomotor cross-sensitization between repeated restraint stress and ethanol. While prior studies indicate that NMDA antagonists, including MK-801, can block behavioral sensitization to a number of drugs of abuse (Sripada, Gaytan, Swann, & Dafny, 2001), including ethanol (Broadbent & Weitemier, 1999; Camarini et al., 2000), this study provides evidence that the capacity of MK-801 to inhibit behavioral sensitization is altered significantly by a history of repeated restraint stress. It will be important in future studies to determine whether these results generalize to other chronic stressor models and other NMDA antagonists. The precise mechanism(s) through which MK-801 pretreatment potentiates stress-ethanol cross-sensitization are still unclear, but appear to be dissociated from effects on nNOS in both the PFC and hippocampus. Such findings do not preclude a role for NMDA-NO signaling within basal ganglia or other mesocorticolimbic structures that govern locomotor behavior, which will be an important next step in our understanding of the role for this clinically relevant signaling system in stress and ethanol interactions.

Author contributions

Conception and design of the study: JRBS and RC; acquisition of the data: JRBS, MR, and SAT; Formal analysis: JRBS, MR, KKS, and RC; drafting and revising the article: JRBS, MR, KKS, MNM, and RC. The authors read and approved the final manuscript.

Acknowledgments

Funding for this study was provided by grants #2018/05038-0 and 2014/24518-1 from São Paulo Research Foundation (Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP, Brazil) and National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, Brazil, Universal 408228/2016-4 and 408153/2016-4). RC and MNM are Research Fellows of CNPq; Fig. 1 was created with BioRender.com.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alcohol.2023.06.004>.

References

- Abraham, K. P., Ariwodola, O. J., Butler, T. R., Rau, A. R., Skelly, M. J., Carter, E., et al. (2013). Locomotor sensitization to ethanol impairs NMDA receptor-dependent synaptic plasticity in the nucleus accumbens and increases ethanol self-administration. *Journal of Neuroscience*, 33(11), 4834–4842.
- Abraham, K. P., & Souza-Formigoni, M. L. O. (2012). Behavioral sensitization to ethanol results in cross-sensitization to MK-801 but not to NMDA administered intra-accumbens. *Behavioural Brain Research*, 235(2), 218–224.
- Adams, B. W., & Moghaddam, B. (2001). Effect of clozapine, haloperidol, or M100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. *Biological Psychiatry*, 50(10), 750–757.
- Bishop, S. F., Lauzon, N. M., Bechard, M., Gholizadeh, S., & Laviolette, S. R. (2011). NMDA receptor hypofunction in the prelimbic cortex increases sensitivity to the rewarding properties of opiates via dopaminergic and amygdalar substrates. *Cerebral Cortex*, 21(1), 68–80.
- Blanke, M. L., & VanDongen, A. M. (2009). Activation mechanisms of the NMDA receptor. In A. M. VanDongen (Ed.), *Biology of the NMDA receptor*. Boca Raton, Florida: CRC Press/Taylor & Francis.
- Broadbent, J., & Weitemier, A. Z. (1999). Dizocilpine (MK-801) prevents the development of sensitization to ethanol in DBA/2J mice. *Alcohol and Alcoholism*, 34(3), 283–288.
- Camarini, R., Frussa-Filho, R., Monteiro, M. G., & Calil, H. M. (2000). MK-801 blocks the development of behavioral sensitization to ethanol. *Alcoholism: Clinical and Experimental Research*, 24(3), 285–290.
- Carlezon, W. A., Mendrek, A., & Wise, R. A. (1995). MK-801 disrupts the expression but not the development of bromocriptine sensitization: A state-dependency interpretation. *Synapse*, 20(1), 1–9.
- Carrara-Nascimento, P. F., Griffin, W. C., 3rd, Pastrello, D. M., Olive, M. F., & Camarini, R. (2011). Changes in extracellular levels of glutamate in the nucleus accumbens after ethanol-induced behavioral sensitization in adolescent and adult mice. *Alcohol*, 45(5), 451–460.
- Contó, M. B., Pautassi, R. M., & Camarini, R. (2022). Rewarding and antidepressant properties of ketamine and ethanol: Effects on the brain-derived neurotrophic factor and TrkB and p75NTR receptors. *Neuroscience*, 493, 1–14.
- Cruz, F. C., Marin, M. T., Leão, R. M., & Planeta, C. S. (2012). Stress-induced cross-sensitization to amphetamine is related to changes in the dopaminergic system. *Journal of Neural Transmission*, 119(4), 415–424.
- Davis, R. L., & Syapin, P. J. (2005). Interactions of alcohol and nitric oxide synthase in the brain. *Brain Research. Brain Research Reviews*, 49(3), 494–504.
- Delis, F., Thanos, P. K., Rombola, C., Roski, L., Grandy, D., Wang, G. J., et al. (2013). Chronic mild stress increases alcohol intake in mice with low dopamine D2 receptor levels. *Behavioral Neuroscience*, 127(1), 95–105.
- Doremus-Fitzwater, T. L., Panaccia, J. E., Gano, A., Vore, A. S., & Deak, T. (2018). Differential effects of acute versus chronic stress on ethanol sensitivity: Evidence for interactions on both behavioral and neuroimmune outcomes. *Brain, Behavior, and Immunity*, 70, 141–156.
- Favoreto, C. A., Nunes, Y. C., Macedo, G. C., Lopes, J. S. R., & Quadros, I. M. H. (2020). Chronic social defeat stress: Impacts on ethanol-induced stimulation, corticosterone response, and brain monoamine levels. *Journal of Psychopharmacology*, 34(4), 412–419.
- Fontella, F. Y., Vendite, D. A., Tabajara, A. S., Porciúncula, L. O., Torres, I. L. S., Jardim, F. M., et al. (2004). Repeated restraint stress alters hippocampal glutamate uptake and release in the rat. *Neurochemical Research*, 29(9), 1703–1709.
- Franklin, K. B., & Paxinos, G. (1997). *The mouse brain in Stereotaxic coordinates* (p. 186). Academic Press.
- Gadek-Michalska, A., Tedeuz, J., Bugajski, A., & Bugajski, J. (2019). Chronic isolation stress affects subsequent crowding stress-induced brain nitric oxide synthase (NOS) isoforms and hypothalamic-pituitary-adrenal (HPA) axis responses. *Neurotoxicity Research*, 36(3), 523–539.
- Garthwaite, J., Garthwaite, G., Palmer, R. M., & Moncada, S. (1989). NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. *European Journal of Pharmacology*, 172(4–5), 413–416.
- den Hartog, C. R., Gilstrap, M., Eaton, B., Lench, D. H., Mulholland, P. J., Homanics, G. E., et al. (2017). Effects of repeated ethanol exposures on NMDA receptor expression and locomotor sensitization in mice expressing ethanol resistant NMDA receptors. *Frontiers in Neuroscience*, 11, 84.
- Harvey, B. H., Oosthuizen, F., Brand, L., Wegener, G., & Stein, D. J. (2004). Stress–restraint evokes sustained iNOS activity and altered GABA levels and NMDA receptors in rat hippocampus. *Psychopharmacology*, 175(4), 494–502.
- Heilig, M., & Koob, G. F. (2007). A key role for corticotropin-releasing factor in alcohol dependence. *Trends in Neurosciences*, 30(8), 399–406.
- Ida, I., Asami, T., & Kuribara, H. (1995). Inhibition of cocaine sensitization by MK-801, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist: Evaluation by ambulatory activity in mice. *The Japanese Journal of Pharmacology*, 69(2), 83–90.
- Inoue, H., Arai, I., Shibata, S., & Watanabe, S. (1996). NG-nitro-L-arginine methyl ester attenuates the maintenance and expression of methamphetamine-induced behavioral sensitization and enhancement of striatal dopamine release. *Journal of Pharmacology and Experimental Therapeutics*, 277(3), 1424–1430.
- Itzhak, Y. (1997). Modulation of cocaine- and methamphetamine-induced behavioral sensitization by inhibition of brain nitric oxide synthase. *Journal of Pharmacology and Experimental Therapeutics*, 282(2), 521–527.
- Itzhak, Y., Ali, S. F., Martin, J. L., Black, M. D., & Huang, P. L. (1998). Resistance of neuronal nitric oxide synthase-deficient mice to cocaine-induced locomotor sensitization. *Psychopharmacology*, 140(3), 378–386.
- Itzhak, Y., & Anderson, K. L. (2008). Ethanol-induced behavioral sensitization in adolescent and adult mice: Role of the nNOS gene. *Alcoholism: Clinical and Experimental Research*, 32(10), 1839–1848.
- Itzhak, Y., & Martin, J. L. (2000). Blockade of alcohol-induced locomotor sensitization and conditioned place preference in DBA mice by 7-nitroindazole. *Brain Research*, 858(2), 402–407.
- Itzhak, Y., Roger-Sánchez, C., & Anderson, K. L. (2009). Role of the nNOS gene in ethanol-induced conditioned place preference in mice. *Alcohol*, 43(4), 285–291.
- Kalivas, P. W., & Alesdatter, J. E. (1993). Involvement of N-methyl-D-aspartate receptor stimulation in the ventral tegmental area and amygdala in behavioral

- sensitization to cocaine. *Journal of Pharmacology and Experimental Therapeutics*, 267(1), 486–495.
- Kim, D. H., Jung, J. S., Moon, Y. S., & Song, D. K. (2009). Central or peripheral norepinephrine depletion enhances MK-801-induced plasma corticosterone level in mice. *Progress in Neuro-psychopharmacology & Biological Psychiatry*, 33(1), 45–48.
- Koob, G. F. (2013). Addiction is a reward deficit and stress surfeit disorder. *Frontiers in Psychiatry*, 4, 72.
- Lee, Y. A., & Goto, Y. (2011). Chronic stress modulation of prefrontal cortical NMDA receptor expression disrupts limbic structure-prefrontal cortex interaction. *European Journal of Neuroscience*, 34(3), 426–436.
- Liu, L. Y., Li, Q. J., Hu, Y. Y., Guo, X. H., Chu, S. C., & Li, W. B. (2009). [Intrathecal injection of MK-801 inhibited the NOS activity and NO content of hippocampus in rat during the process of formalin-induced inflammatory pain]. *Zhongguo ying yong sheng li xue za zhi [Chinese journal of applied physiology]*, 25(3), 423–427.
- Lorrain, D. S., Bacceti, C. S., Bristow, L. J., Anderson, J. J., & Varney, M. A. (2003). Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: Modulation by a group II selective metabotropic glutamate receptor agonist LY379268. *Neuroscience*, 117(3), 697–706.
- Marty, V. N., Mulpuri, Y., Munier, J. J., & Spigelman, I. (2020). Chronic alcohol disrupts hypothalamic responses to stress by modifying CRF and NMDA receptor function. *Neuropharmacology*, 167, Article 107991.
- McEwen, B. S., Nasca, C., & Gray, J. D. (2016). Stress effects on neuronal structure: Hippocampus, amygdala, and prefrontal cortex. *Neuropsychopharmacology*, 41(1), 3–23.
- Meyer, P. J., & Phillips, T. J. (2003). Bivalent effects of MK-801 on ethanol-induced sensitization do not parallel its effects on ethanol-induced tolerance. *Behavioral Neuroscience*, 117(3), 641–649.
- Meyer, P. J., & Phillips, T. J. (2007). Behavioral sensitization to ethanol does not result in cross-sensitization to NMDA receptor antagonists. *Psychopharmacology*, 195(1), 103–115.
- Moghaddam, B. (1993). Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: Comparison to hippocampus and basal ganglia. *Journal of Neurochemistry*, 60(5), 1650–1657.
- Moghaddam, B., Adams, B., Verma, A., & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 17, 2921–2927.
- Nagy, J. (2008). Alcohol related changes in regulation of NMDA receptor functions. *Current Neuropharmacology*, 6(1), 39–54.
- Nasca, C., Zelli, D., Bigio, B., Piccinin, S., Scaccianoce, S., Nisticò, R., et al. (2015). Stress dynamically regulates behavior and glutamatergic gene expression in hippocampus by opening a window of epigenetic plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, 112(48), 14960–14965.
- Nona, C. N., & Nobrega, J. N. (2018). A role for nucleus accumbens glutamate in the expression but not the induction of behavioural sensitization to ethanol. *Behavioural Brain Research*, 336, 269–281.
- Palm, S., Roman, E., & Nylander, I. (2011). Differences in voluntary ethanol consumption in Wistar rats from five different suppliers. *Alcohol*, 45(6), 607–614.
- Pastor, R., Reed, C., Meyer, P. J., McKinnon, C., Ryabinin, A. E., & Phillips, T. J. (2012). Role of corticotropin-releasing factor and corticosterone in behavioral sensitization to ethanol. *Journal of Pharmacology and Experimental Therapeutics*, 341(2), 455–463.
- Phillips, T. J., Roberts, A. J., & Lessov, C. N. (1997). Behavioral sensitization to ethanol: Genetics and the effects of stress. *Pharmacology, Biochemistry, and Behavior*, 57(3), 487–493.
- Picón-Pagès, P., García-Buendía, J., & Muñoz, F. J. (2019). Functions and dysfunctions of nitric oxide in brain. *Biochimica et Biophysica Acta. Molecular Basis of Disease*, 1865(8), 1949–1967.
- Quadir, S., Santos, J. R. B., Campbell, R. R., Wroten, M. G., Singh, N., Holloway, J. J., et al. (2016). Homer2 regulates alcohol and stress cross-sensitization. *Addiction Biology*, 21(3), 613–633.
- Quoilin, C., Didone, V., Tirelli, E., & Quertemont, E. (2012). Developmental differences in ethanol-induced sensitization using postweanling, adolescent, and adult Swiss mice. *Psychopharmacology*, 219(4), 1165–1177.
- Richardson, H. N., Lee, S. Y., O'Dell, L. E., Koob, G. F., & Rivier, C. L. (2008). Alcohol self-administration acutely stimulates the hypothalamic-pituitary-adrenal axis, but alcohol dependence leads to a dampened neuroendocrine state. *European Journal of Neuroscience*, 28(8), 1641–1653.
- Riedel, W. (2000). Role of nitric oxide in the control of the hypothalamic-pituitary-adrenocortical axis. *Zeitschrift für Rheumatologie*, 59(Suppl 2:II), 36–42.
- Roberts, A. J., Lessov, C. N., & Phillips, T. J. (1995). Critical Role for glucocorticoid receptors in stress- and ethanol-induced locomotor sensitization. *Journal of Pharmacology and Experimental Therapeutics*, 275(2), 790–797.
- Robinson, T. E., & Berridge, K. C. (2008). Review. The incentive sensitization theory of addiction: Some current issues. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 363(1507), 3137–3146.
- Roenker, N. L., Gudelsky, G. A., Ahlbrand, R., Horn, P. S., & Richtand, N. M. (2012). Evidence for involvement of nitric oxide and GABA(B) receptors in MK-801-stimulated release of glutamate in rat prefrontal cortex. *Neuropharmacology*, 63(4), 575–581.
- Santos-Rocha, J. B., Rae, M., Teixeira, A. M. A., Teixeira, S. A., Munhoz, C. D., Muscará, M. N., et al. (2018). Involvement of neuronal nitric oxide synthase in cross-sensitization between chronic unpredictable stress and ethanol in adolescent and adult mice. *Alcohol*, 68, 71–79.
- Seo, D. O., & Rivier, C. (2001). Microinfusion of a nitric oxide donor in discrete brain regions activates the hypothalamic-pituitary-adrenal axis. *Journal of Neuroendocrinology*, 13(11), 925–933.
- Sripada, S., Gaytan, O., Swann, A., & Dafny, N. (2001). The role of MK-801 in sensitization to stimulants. *Brain Research. Brain Research Reviews*, 35(2), 97–114.
- Sultan, F. A. (2013). Dissection of different areas from mouse Hippocampus. *Bio-Protocol*, 3(21), e955.
- Szumliński, K. K., Lominac, K. D., Kleschen, M. J., Oleson, E. B., Dehoff, M. H., Schwarz, M. K., et al. (2005). Behavioral and neurochemical phenotyping of Homer1 mutant mice: Possible relevance to schizophrenia. *Genes, Brain and Behavior*, 4(5), 273–288.
- Teixeira, S. A., Castro, G. M., Papes, F., Martins, M. L., Rogério, F., Langone, F., et al. (2002). Expression and activity of nitric oxide synthase isoforms in rat brain during the development of experimental allergic encephalomyelitis. *Brain Research. Molecular Brain Research*, 99(1), 17–25.
- Theilmann, W., Kleimann, A., Rhein, M., Bleich, S., Frieling, H., Löscher, W., et al. (2016). Behavioral differences of male Wistar rats from different vendors in vulnerability and resilience to chronic mild stress are reflected in epigenetic regulation and expression of p11. *Brain Research*, 1642, 505–515.
- Tzschentke, T. M., & Schmidt, W. J. (2000). Blockade of behavioral sensitization by MK-801: Fact or artifact? A review of preclinical data. *Psychopharmacology*, 151(2–3), 142–151.
- Vanderschuren, L. J., & Kalivas, P. W. (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: A critical review of preclinical studies. *Psychopharmacology*, 151(2–3), 99–120.
- Vila-Verde, C., Marinho, A. L., Lisboa, S. F., & Guimarães, F. S. (2016). Nitric oxide in the prelimbic medial prefrontal cortex is involved in the anxiogenic-like effect induced by acute restraint stress in rats. *Neuroscience*, 320, 30–42.
- Wedzony, K., & Czyrak, A. (1994). The role of corticosteroids in the acquisition of sensitization to locomotor stimulant effects of MK-801. *Brain Research*, 657(1–2), 351–356.
- Wegener, G., Volke, V., Harvey, B. H., & Rosenberg, R. (2003). Local, but not systemic, administration of serotonergic antidepressants decreases hippocampal nitric oxide synthase activity. *Brain Research*, 959(1), 128–134.
- Wolf, M. E. (1998). The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Progress in Neurobiology*, 54(6), 679–720.
- Wolf, M. E., & Jeziorski, M. (1993). Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. *Brain Research*, 613(2), 291–294.
- Wolf, M. E., & Khansa, M. R. (1991). Repeated administration of MK-801 produces sensitization to its own locomotor stimulant effects but blocks sensitization to amphetamine. *Brain Research*, 562(1), 164–168.
- Zarrindast, M.-R., Askari, E., Khalilzadeh, A., & Nouraei, N. (2006). Morphine state-dependent learning sensitization and interaction with nitric oxide. *Pharmacology*, 78(2), 66–71.
- Zhou, Q. G., Zhu, L. J., Chen, C., Wu, H. Y., Luo, C. X., Chang, L., et al. (2011). Hippocampal neuronal nitric oxide synthase mediates the stress-related depressive behaviors of glucocorticoids by downregulating glucocorticoid receptor. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 31(21), 7579–7590.