Tactile Stimulation in Adult Rats Modulates Dopaminergic Molecular Parameters in the *Nucleus Accumbens* Preventing Amphetamine Relapse.

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Research Article

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Abstract

Amphetamine (AMPH) is a psychostimulant drug frequently related to addiction, which is characterized by functional and molecular changes in the brain reward system, favoring relapse development and pharmacotherapies have shown low effectiveness. Considering the beneficial influences of tactile stimulation (TS) in different diseases that affect the central nervous system (CNS), here we evaluated if TS applied in adult rats could prevent or minimize the AMPH-relapse behavior also accessing molecular neuroadaptations in the *Nucleus accumbens* (NAc). Following AMPH conditioning in the conditioned place preference (CPP) paradigm, male rats were submitted to TS (15-min session, 3 times a day, for 8 days) during the drug abstinence period, which were re-exposed to the drug in the CPP paradigm for additional 3 days for relapse observation and molecular assessment. Our findings showed that besides AMPH relapse; TS prevented the dopamine transporter (DAT), dopamine 1 receptor (D1R), tyrosine hydroxylase (TH), mu opioid receptor (MOR) increase and AMPH-induced delta FosB (ΔFosB). Based on these outcomes, we propose TS as a useful tool to treat psychostimulant addiction, which subsequent to clinical studies**;** it could be included in detoxification programs together with pharmacotherapies and psychological treatments already conventionally established.

Highlights

- AMPH-exposed rats developed drug-preference and -relapse behavior;
- AMPH increased TH, D1R, DAT, ΔFosB and MOR in the *Nucleus accumbens*;
- Tactile stimulation (TS) in adult rats prevented these molecular changes;
- TS prevented AMPH relapse;

Introduction

The covid-19 pandemic has generated periods of social isolation worldwide. It is known that even a short period of social isolation can cause psychological distress, leading people to seek alcohol and psychoactive substances, thus aggravating the existing public health problem, increasing damages related to drug addiction [1]. Addiction is related to brain pathological neuroadaptations, which persist even after the drug withdrawal. In this sense, psychostimulant drugs such as amphetamine (AMPH) are strongly addictive, since this drug is known to stimulate the reward system, increasing the well-being sensation, pleasure and causing euphoria, which together promote dependence, tolerance and withdrawal syndrome [2]. Addiction also involves relapse, which is defined as a behavior of returning to the search / consumption of the drug after a period of self-imposed or enforced abstinence [3].

In this sense, drug users are often susceptible to drug relapse following days, months or even years of abstinence. [4]. Therefore, preventing the recurrence of drug abuse behavior is considered the most difficult aspect when treating addiction. Behavioral and pharmacological evidences indicate the important role of the dopaminergic pathway in the *Nucleus accumbens* (NAc) in drug rewarding, being

the dopamine (DA) the most important neurotransmitter in the initial drug action and addiction development [5].

It is well known that AMPH increases DA levels in the synaptic cleft, since it decreases monoamines degradation by partial inhibition of the monoamino oxidase (MAO), while preventing vesicular reuptake, consequently inverting the dopamine transporter (DAT) activity [6]. This DA levels increase was proposed as a sufficient condition to produce reward symptoms [7]. Besides, Tyrosine hydroxylase (TH) is the key enzyme in the DA synthesis process, whereas the dopamine receptor 1 (D1R) is responsible for its stimulus propagation [8]. Repeated exposure or chronic use of addictive drugs promotes a ΔFosB accumulation, which is a transcription factor closely involved in the reward and reinforcement symptoms [9, 10]. In addition, there is evidence that an interaction between the opioid and dopaminergic system mediates the reward system, since the activation of this μ- opioid receptor (MOR) in the mesolimbic brain area is responsible for DA release changes [11].

Despite advances in discoveries concerning neuroadaptative changes underlying addiction, there is no approved drug therapy to treat AMPH- and other psychostimulant-addicted individuals. Indeed, current pharmacotherapies only decrease withdrawal symptoms, being unable to prevent frequent relapse episodes [12]. As relapse is a growing concern, our research group has made efforts to search new non-pharmacological adjuvant therapies for drug addiction, especially to prevent relapse. Among them, we can highlight the physical exercise practice [13, 14], neonatal tactile stimulation (TS) [15, 16], omega-3 supplementation [17] and AMPH-isotherapic administration [18] and others.

Considering the different possibilities involving alternative therapies that can be applied together with drug addiction conventional treatments, neonatal TS caused beneficial changes in both AMPH-induced behavioral- and neurological- sensitization [19]. In fact, TS is a well-described procedure in the literature [20], which constitutes a craniosacral handling already demonstrated in preclinical studies involving neonates, since TS was able to prevent the preference for psychostimulant drugs [15, 16]. In this context, preclinical studies have shown that even when applied in the adulthood, TS improves neuropsychiatric disorders like depression [21]. In view of this, become of great importance to evaluate the possible beneficial influences of the adulthood TS on AMPH relapse, considering the AMPH-induced neurobiological and behavioral modifications.

Materials And Methods

Animals

Forty male *Wistar* rats (50 days old) from the breeding facility of the Federal University of Santa Maria (UFSM), RS, Brazil were used. This experimental protocol was approved by the Animal Ethics Committee of the Federal University of Santa Maria under de number 3991221118, associated to the National Council for the Control of Animal Experimentations (CONCEA), following international norms of care and animal maintenance. The animals were distributed in groups of five animals per Plexiglas cages, with

food and water *ad libitum*. They were kept in a room with controlled temperature (22 - 23°C) on a 12 h-light/dark cycle with lights on at 7:00 a.m.

Drugs

For this study, d-l-AMPH (Merck®) (4.0 mg/kg/mL) was applied according to previous studies [13, 22].

Experimental protocol

Two experimental groups were conditioned with saline (NaCl 0.9%; n=20, i.p.) or AMPH (4.0 mg/Kg; n=20, i.p., for eight consecutive days) in the conditioned place preference (CPP) paradigm. After 24h, animals were observed in the same apparatus without drug administration (CPP 1st test). Sequentially, each experimental group were re-designated in either tactile stimulation (TS) (15-min sessions, 3 times a day for 8 consecutive days) or not (unhandled; UH), resulting in four experimental groups: i) saline/UH (n=10); ii) saline/TS (n=10); iii) AMPH/UH (n=10); iv) AMPH/TS (n=10). One day after TS protocol, animals were re-expose to AMPH or saline in the CPP paradigm, using 4.0 mg/Kg; i.p., for three consecutive days, [23] for relapse evaluation (2nd CPP test). Additional behavioral test performed in the Y-maze apparatus. Sequentially, animals were anesthetized, euthanized and the *Nucleus accumbens* (NAc) dissected for further molecular analysis as shown in the figure 1.

CPP protocol

CPP is an animal learning paradigm that mediates the types of learning and memory, and it is a well-described behavioral model to assess hedonic reinforcement effects of drugs [24]. The apparatus consists of two compartments of equal size (45 cm × 45 cm × 50 cm) but with different visual cues and a third neutral compartment with equivalent intensity of light in both. The apparatus was cleaned with alcohol 20% using a wet sponge and paper towel before the introduction of each animal. On the first day the animal is initially exposed without medication in an equal way to the chambers (habituation), on the following day without the door, the animal is allowed to freely pass and have a round trip between the two compartments to evaluate a possible natural preference of the animal (pre-test). The next day, the door is inserted, blocking the passage between the compartments. The animal receives the drug (d,l-amphetamine, 4,0 mg/kg, i.p.) or saline (physiological solution 0,9%, i.p.) and sufficient time is given to allow a relapse until the peak of the drug's effect to be reached, in this case 25 min, with an interval of 4 h between each administration. This conditioning continues for 8 days. After this conditioning phase, on the testing day, rats were placed in the common compartment with free access to both compartments. The time spent in the drug-paired environment was interpreted as preference, while the time spent away from that environment was interpreted as aversion. After the tactile stimulation protocol (described below), the animals were submitted to more three days of AMPH re-exposition in CPP, which was followed by an additional test of drug preference as described above. Relapse symptoms were quantified by the highest time spent in the drug-paired environment in this second drug exposure [23, 25].

Tactile Stimulation

Tactile stimulation in adulthood consisted of removing the animals from home cage and petting them individually on the experimenter's lap with one hand for 15 min. The TS was applied 3 times per day between 09:00 a.m. and 05:00 p.m., for 8 days. After the procedures, the animals returned to their home cages. The procedure was based on TS protocols that were performed in adult rats [21].

Y-maze test

This test is used to assess working memory in rodents, using the assessment of spontaneous alternation, where the rat explores the three arms alternately driven by innate curiosity to explore new area, alternation is achieved when an animal visits all three arms clockwise or counterclockwise sequentially [26].

The Y-maze apparatus consists of three arms 32cm x 10cm x 26cm (length, width and height, respectively). The animal is placed in the center of the Y-labyrinth apparatus and allowed to freely explore it for 5 min. The sequence (alternation) and the total number of entries in the arms were quantified. The alternation percentage is calculate using the following formula: (Total alternation number/Total entries number-2)*100 [27].

Tissue preparations

Following 24 h of the last behavioral assessment, animals were anesthetized (isoflurane, the dose to the effect) and euthanized by exsanguination. Half of the animals in each group (n=5) had the brains removed and the regions of the NAc were dissected and stored in a freezer at − 80°C for subsequent molecular analysis.

Molecular Assessments: Western Blotting

NAc tissue was homogenized in a lysis buffer [21] for determination of total protein concentration, according to Bicinchoninic Acid (BCA) Protein Assay Kit (Pierce, IL) using bovine serum albumin as standard. After, protein samples were separated by electrophoresis on a 10% polyacrylamide gel and electrotransferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA). Non-specific binding sites were blocked and membranes were rinsed in buffer and incubated with primary antibodies: anti-β-actin (1:50000; Sigma-Aldrich, St. Louis, USA), anti-DAT (1:500; Santa Cruz Biotechnology), anti-D1R (1:500, Santa Cruz Biotechnology), anti-TH (1:500, Santa Cruz Biotechnology), anti-ΔfosB (1:1000, Santa Cruz Biotechnology), anti-MOR (1:500, Santa Cruz Biotechnology) followed by anti-goat (1:1000; Santa Cruz Biotechnology, CA, USA) or anti-rabbit (1:20000; Santa Cruz Biotechnology, CA, USA) IgG horseradish peroxidase conjugate. Immunocomplexes were visualized using Luminata (Millipore, USA) according to the manufacturer's instructions. Film signals were digitally scanned (Chemidoc™ Imaging Systems) and then quantified using ImageJ software. β-actin was used as an internal control, so that data were standardized according to actin values.

Statistical analysis

CPP data before TS protocol were analyzed by Student's T-test. For other outcomes that were obtained from behavioral and molecular assays, they were analyzed by two-way analysis of variance (ANOVA)

followed by Tukey's *post hoc* test (software package Statistica 10.0), and expressed as the mean ± standard error (S.E.M.). A value of $p<0.05$ was considered statistically significant for all comparisons made.

Results

Conditioned place preference (CPP) paradigm: AMPH-preference development and AMPH relapse after tactile stimulation (TS) is show in Figure 2:

Student's T-test showed that, as expected, animals injected with the drug showed AMPH-conditioned place preference (AMPH-CPP) in relation to saline injected group ($p<0.000$) (Fig. 2A).

Two-way ANOVA revealed a significant main effect of the drug and handling [$F(1,36)=23.35$, $p<0.000$ and 10.37, $p<0.003$, respectively] on AMPH-CPP.

After AMPH withdrawal and re-exposure, Tukey's test showed that TS was able to prevent drug relapse in comparison to UH group ($p<0.001$). In fact, CPP behavior observed in the AMPH/TS group was comparable to those observed in both saline-UH and -TS groups (Fig. 2B).

Y-maze task: Working memory and locomotor performance in animals exposed to AMPH and submitted to TS in adulthood is show in Figure 3:

No differences between the experimental groups in both total entries (Figure 3A) and % alternation (Figure 3B) in the Y-maze arms was observed, indicating that the AMPH-CPP observed in the UH group (Fig. 2B) was not consequent of an artifact, that is, the place preference was not related to memory disorder or motor damage.

Influence of TS on dopaminergic molecular targets (D1R and DAT) and TH immunoreactivity in the Nucleus accumbens **(NAc) of adult rats after AMPH relapse is show in Figure 4:**

Two-way ANOVA revealed a significant main effect of handling [$F(1,16)= 23.18$, $p<0.000$] on DAT and a significant main effect of the drug on D1R [$F(1,16)=4.56$, $p<0.05$] and TH [$F(1,16)=12.604$, $p<0.005$] immunoreactivity.

Tukey's test showed that the AMPH re-exposure increased D1R, DAT and TH levels in relation to saline injected group (*$p<0.05$; $p<0.000$* and *$p<0.01$*, respectively). In addition, TS was able to prevent these AMPH-induced increase in D1R (Fig. 4A), DAT (Fig. 4B) and TH (Fig. 4C), in comparison to AMPH/UH group, making such levels comparable to saline/TS group.

Interestingly, D1R, DAT and TH levels showed positive correlation with AMPH-relapse behavior ($r^2=0.32$, $p<0.0007$; $r^2 = 0.37$, $p = 0.0002$; and $r^2= 0.57$, $p=0.0001$, respectively) (Fig. 4D, E and F, respectively).

Influence of TS on ΔFosB and μ-opioid receptor (MOR) immunoreactivity in the NAc of rats after AMPH relapse is show in Figure 5:

Two-way ANOVA revealed a significant main effect of handling [F (1, 16) = 16,341, p<0,001] on ΔFosB levels and a significant main effect of the drug and handling [F (1,16)=31.5, p<0.000 and 14.88, p<0.05, respectively] on MOR immunoreactivity. *Post hoc* test showed that while AMPH re-exposure increased both ΔFosB (p<0.001) and MOR (p<0.05) levels in relation to saline group, TS attenuated this AMPH-induced increase whose levels remaining higher than those observed in TS/saline group (p<0.05 for both).

Discussion

Drug addiction causes irreparable damages to the individuals, their families and to the whole society, besides being related to a high cost for the health system. It is know that current pharmacological treatments are only palliative ones and unable to prevent dreaded relapse episodes [28]. This deficiency has stimulated studies in our laboratory, especially the current study, since the tactile stimulation (TS) is a non-invasive approach, which can be used together with conventional pharmacotherapy. To the best of our knowledge, this is the first study to show beneficial TS-induced neuroadaptations in adult rats following amphetamine (AMPH) addiction. We evaluated the TS influence when applied in adult rats on AMPH relapse, occurring together with the evaluation of molecular parameters that are closely related to drug addiction, which were quantified in the *Nucleus accumbens* (NAc). The evaluation happened as follows: i) animals showed relapse behavior subsequently to preference, withdrawal and re-exposure to drug; ii) during AMPH withdrawal, the TS protocol applied in adult animals was able to prevent AMPH relapse following drug re-exposure; iii) experimental groups did not present memory- or locomotion-impairment in the Y-maze task; and, iv) while AMPH increased D1R, DAT, TH, ΔFosB and MOR immunocontent in the NAc, TS was able to decrease these molecular markers levels.

Previous studies from our laboratory have shown CPP paradigm in experimental protocols related to AMPH addiction [14, 29, 30], besides other addictive substances such as cocaine [16] and morphine [31–33]. Thus, following drug extinction, events such as stress or drug re-exposure can reestablish the addiction, what may be experimentally observed by restoring the drug preference in the CPP paradigm [34]. Our current findings show that subsequently to drug abstinence, AMPH-conditioned animals presented drug-relapse behavior, which was observed through AMPH-CPP, confirming that there is a relation between AMPH-induced hedonic effects and environmental cues, such as those present in the CPP paradigm. Inversely, animals that were submitted to adulthood TS protocol, during the abstinence period, did not show relapse after AMPH re-exposure. In fact, our previous studies have already shown beneficial influences of neonatal TS in the prevention of both AMPH- and cocaine-CPP [15, 16]. In addition, neonatal TS modifies juvenile behavior, attenuates drug-induced behavioral sensitization and reorganizes the brain regions involved in drug addiction [35]. However, our current outcomes show the TS procedure in the treatment form, that is, it was applied in adult animals that previously showed AMPH-CPP, whose behavior indicates addiction. During the abstinence period, these animals were submitted to

adulthood TS, which prevented AMPH-relapse behavior after drug re-exposure. As far as we observed, this finding is innovative, since only neonatal TS has been experimentally studied [36] and clinically applied [37]; however, literature data are scarce when TS is applied in adult animals.

In this context, memory impairments could lead to false outcomes, since animals do not keep information connecting environmental clues and hedonic effects [38, 39], making the memory evaluation essential. Similarly, Y-maze task is also able to show locomotor performance in rodents, whose disturbances could cause interpretation mistakes. In this sense, our outcomes did not show any significant difference in the memory and locomotion among the experimental groups, as already described in the literature [40, 41]. These data allow us to propose that the place preference observed in the animals following AMPH re-exposure in the CPP paradigm was directly related to the AMPH-induced hedonic effects, but not to locomotor or memory impairments.

Although AMPH exerts behavioral and cognitive effects by modulating the activity of monoaminergic neurotransmitters, it is known that its reinforcing and euphoric effects are mediate mainly by the dopaminergic system [42]. AMPH are substrates for monoamine vesicular transporters and are absorbed by the neuron cells to increase the non-vesicular dopamine release (DA), this is enhanced by the inhibitory AMPH effect on monoamine oxidase [43]. This DA increase, which ends up reversing the DA transport in presynaptic neurons by the DA transporter (DAT) [44]. DA synthesis and availability are dependent on the enzyme tyrosine hydroxylase activity (TH) [45]. It is known that behavioral changes induced by psychostimulants are mediate by D1 receptor [46].

Connecting these data with our current findings, animals that were conditioned and re-exposure to AMPH, showed increased D1R, DAT and TH immunoreactivity in the NAc, while these events in cascade were inhibited by TS exposure during drug abstinence. Indeed, the increase DA release in the synaptic cleft [43], recruits an increase of the DAT [47], allowing us to propose that the increased level of this transporter in the NAc, as also observed in our findings, was due to increased levels of DA induced by AMPH [48]. Furthermore, DAT exerts influences on the expression and phosphorylation of TH in terminals of the NAc, where decreased levels of DAT were related to a smaller content of TH-protein and DA in the synaptic cleft, indicating that DAT participates in the regulation of DA homeostasis in dopaminergic terminals [49]. Of particular importance for our molecular findings, increased levels of DA in the synaptic cleft has been recognized to increase the D1R density [50] especially in the NAc, as demonstrated in methamphetamine (METH) chronic users [51]. These outcomes allow us propose that adulthood TS modulated DA release in the synaptic cleft, so preventing the activation of the dopaminergic cascade through increasing D1R, DAT and TH immunocontent in the NAc, as observed here.

In fact, TS exposure has promoted an increased DA release in both NAc and tegmental ventral area (VTA) [52]. Until now, the action mechanism involved in the increased DA levels in the synaptic cleft is unknown, but is possible that this increasing evokes the adaptive response preventing AMPH effects on the dopaminergic signaling because TS was able to modulate such releasing. A possible hypothesis would be an increased DAT pre-synaptic activity, thus decreasing DA levels and consequently minimizing reward

behaviors linked to AMPH-CPP, as observed here. Unfortunately, at this time, it was not possible to quantify the functionality of the transporter, and it was possible to quantify its levels, allowing us to only give hypothesis, which deserves to be clarified. In fact, we can claim that environmental or behavioral changes could mediate information for the central nervous system neurons (CNS). Besides, the environmental enrichment can be considered a stimulus comparable to TS, demonstrating that environmental manipulations are able to modulate mechanisms underlying the brain plasticity, thus regulating TH amount and increasing neurons generation [53, 54].

In the sequence of these molecular events, AMPH abstinence, following its chronic exposure, has been described as to favor the return of DA basal levels in the NAc [55], while the TS showed itself to able to increase DA levels in the same brain circuit [52]. These evidences allow us to propose that the exposure of adult rats to TS modulated the mesolimbic dopaminergic system. Supposedly, to keep the baseline DA levels moderately increased, while there was an abrupt drop in these levels in abstinent animals that were not re-exposed to TS, since TS was able to kept both D1R and DAT of this pathway in the same levels that were observed in animals that were not exposed to AMPH. These outcomes may be interpreted as an adaptive response to drug abstinence with consequent AMPH relapse what supports our findings obtained in the behavioral evaluations.

The transcription factor ΔFosB (35-37 kDa) was investigated because it is involved in the effects of natural and drug rewards, whose effects supposedly contribute to neuroadaptations in DA-regulated signaling [56, 57]. Besides that consequent binding of dopamine to D1 receptors, signals the activation of the cell-signaling cascade to increase the phosphorylation of factors and transcription in response to cAMP, such as the expression of immediate and long-lasting genes, such as ΔFosB [58]. Here we observe that AMPH increased ΔFosB in the NAc, as already described [59, 60], while TS decreased this chronic transcriptional factor. Besides this, little is known about the specific molecular mechanisms that mediate the TS, especially how it acts on ΔFosB in the Nac. In addition, environmental enrichment has already been shown to reduce ΔFosB in other brain areas related to anxiety, reducing C-Fos expression in the Nac in response to cocaine [61]. This shows that different alternatives related to both environmental and body stimulation can reduce a transcription factor closely linked to drug addiction.

Studies have related the dopaminergic- to opiod-system, suggesting that reward and motivation are closely modulated by these pathways [62–64]. In this sense, μ-opioid receptor (MOR) is an important mediator of rewarding properties and additive behavior [65, 66] and they are located in the GABAergic interneurons [67]. NAc is a brain area with considerable MOR density [68]. Increased MOR resulting from psychostimulant drug administration is related to increased vulnerability to relapse [69], what was also observed in our AMPH-exposed animals. Based on this, our findings show that TS is able to reduce MOR immunoreactivity in AMPH-exposed animals, indicating a possible decreased in impulsivity and vulnerability to AMPH relapse, which was confirmed in our behavioral outcomes. Similar opioid regulation response is shared by physical exercise and it has been seen that it can prevent relapse in rats addicted to amphetamine [14]. This way, we propose that the two treatment methods produce a similar regulation response. It seems that massage treatment induces the release of endogenous opioid peptides, given that

results in humans indicate that a massage session is followed by an increase in plasma β-endorphins [70].

In addition, human studies have also shown that an increase in dopamine, improved mood, reduced anxiety and reduced levels of cortisol (stress hormone) even after the first message [71, 72]. Considering that the hypothalamic-pituitary-adrenal (HPA) axis, responsible for controlling the stress response, has a substantial relation with the mesolimbic “reward” pathway, since stressors can activate this pathway [73], and environmental stress is considered a fundamental factor for relapse in addictive drugs [74, 75]. Glucocorticoids increase the DA biosynthesis by increasing the tyrosine hydroxylase activity (TH), the rate-limiting enzyme in DA synthesis [76], and they can regulate DAT expression [77]. TS applied to adult rats has already shown a beneficial influence on both stress and depression-like behaviors, decreased plasma ACTH and corticosterone levels, as well as the ability to decrease the HPA axis activity, minimizing the cascade of corticosteroids, inciting the GR expression [21] whose effect size was equivalent to those observed after conventional treatment with antidepressants [78, 79]. Also according to [16], TS is a form of prevention in face of stressful stimuli throughout life and, considering this, we may propose that it exerts prolonged beneficial influences. Based on this, we may infer that as an initial mechanism, the TS beneficial effects in adult rats may be related to the decrease in stress degree during the withdrawal period, which consequently caused a dopaminergic axis modulation, being sufficient to prevent AMPH relapse.

Considering the TS protocol effectiveness, which was applied in adult animals, and that the behavioral changes in the CPP and the molecular markers that involve DA generation and signaling, we may infer that TS could be stimulating the release of DA regular basis, while the drastic changes caused by the AMPH withdrawal happened. The neural modifications on the AMPH-induced mesolimbic dopaminergic pathway in the NAc may be modulated by TS, as observed in this study.

Conclusion

From these findings, we showed that adulthood TS was able to prevent drug-seeking behavior after the abstinence period, modulating molecular parameters in the brain. In the sense, it is of great importance to point out that our study differs from other previous ones related to TS. To the best of our knowledge, this is the first study to show that this non-invasive and safe procedure applied in adulthood may favorably contribute to addiction situations. We suggest its use after clinical studies, in the treatment associated with already used conventional medicines to prevent the relapse to psychostimulant drugs, such as AMPH.

Declarations

Consent to participate

Not applicable.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution / Consent to publish

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Domênika R. Rossato]; [Higor Z Rosa], [Jéssica Leandra O. Rosa], [Laura H. Milanesi], [Lívia D'Avila] and [Vinicia G. Metz]. The first draft of the manuscript was written by [Domênika R.Rossato] and [Marilise E Burger] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability statement

The datasets generated during and/or analyzed during the current study are not publicly available due to security of the no published findings already, but they will be available from the corresponding author on reasonable request.

Research Involving Animals

All procedures were approved by the Animal Ethics Committee of Federal University of Santa Maria and were carried out according to the Guidelines for Animal Experiments.

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Figures

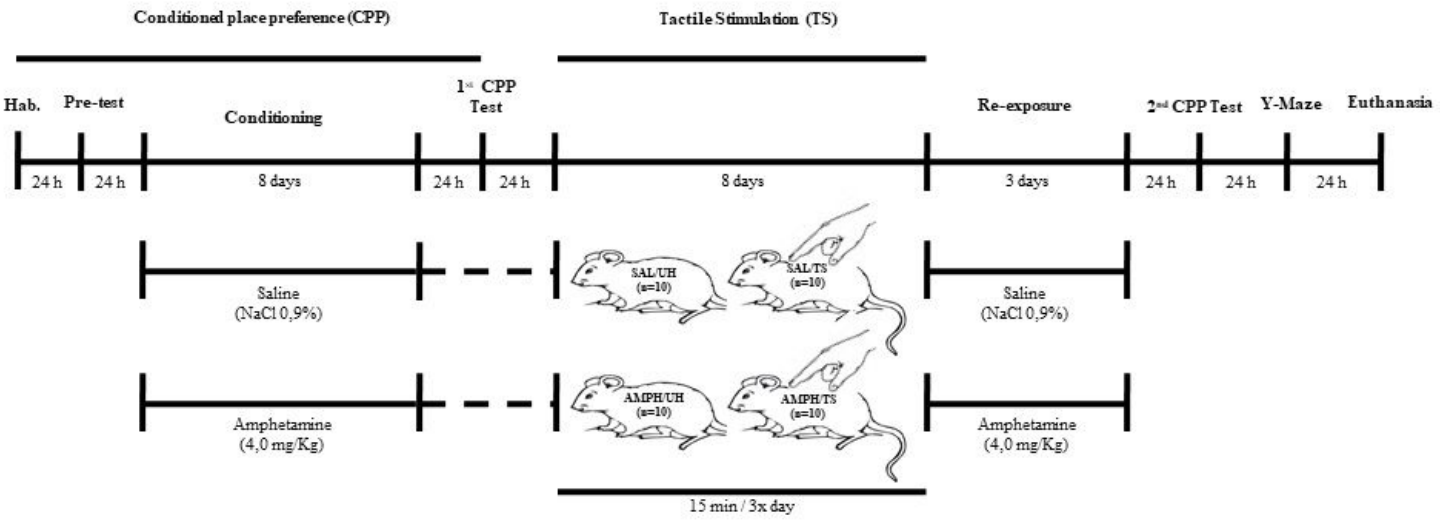

Figure 1

Experimental design: The animals were submitted to basal behavioral evaluation (CPP pre-test) 24h after the animals were conditioned with AMPH (4 mg/kg, i.p.) for 8 days and evaluated in the CPP paradigm

(1st CPP test). Subsequently, the animals were submitted to TS protocol (15 min, 3 times a day, for 8 days), which was followed by three additional days of AMPH-CPP paradigm. Twenty-four hours after the last AMPH re-exposure (4 mg/kg, i.p.) for 3 days, animals were evaluated again in the CPP (2nd CPP test). At the sequence, locomotion and work-memory parameters were performed in Y-maze test. All animals were euthanized for molecular assessments in the *Nucleus accumbens*.

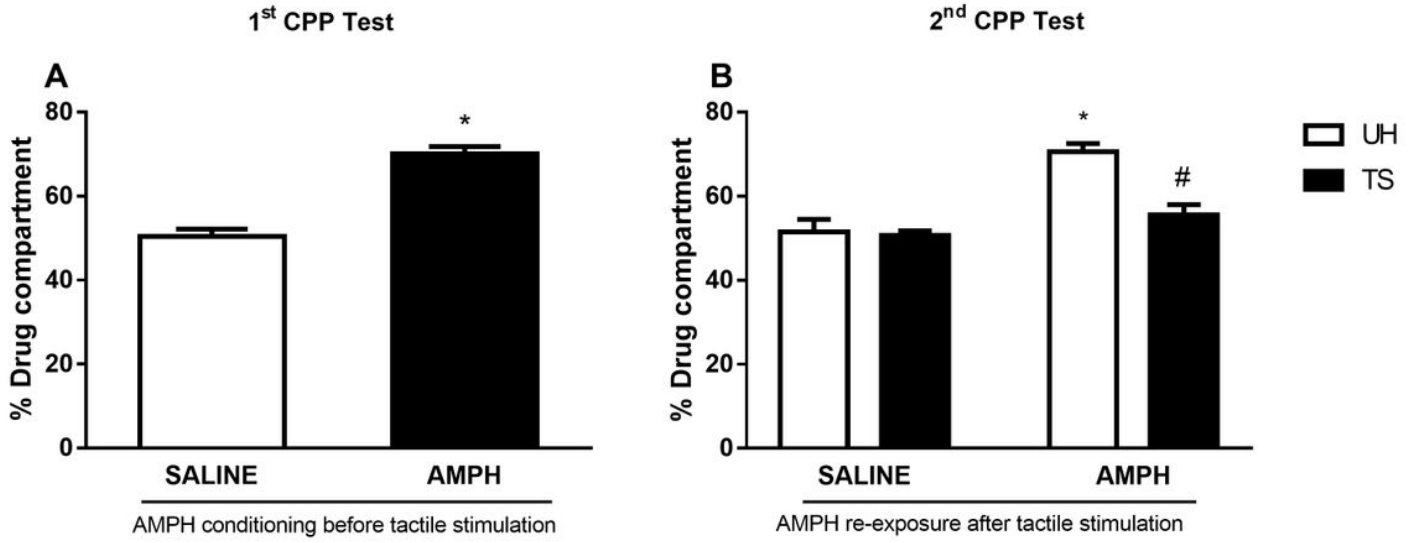

Figure 2

Amphetamine conditioned place preference (AMPH-CPP) (AMPH, 4 mg/kg, i.p. for 8 days) observed in CPP paradigm before 8 days of TS, (CPP 1st test) (2A); AMPH-CPP after AMPH withdrawal, TS exposure (15 min, 3 times a day for 8 days) and AMPH re-exposure (4 mg/kg; i.p. for 3 days) (CPP 2nd test) (2B). (Data are expressed as mean ± S.E.M. *indicates significant difference from AMPH injection for saline (*p<0.05*). # indicates significant difference from AMPH/UH group (*p<0.05*).

Abbreviations: Compartment place preference (CPP); amphetamine (AMPH); unhandled (UH) and tactile stimulation (TS).

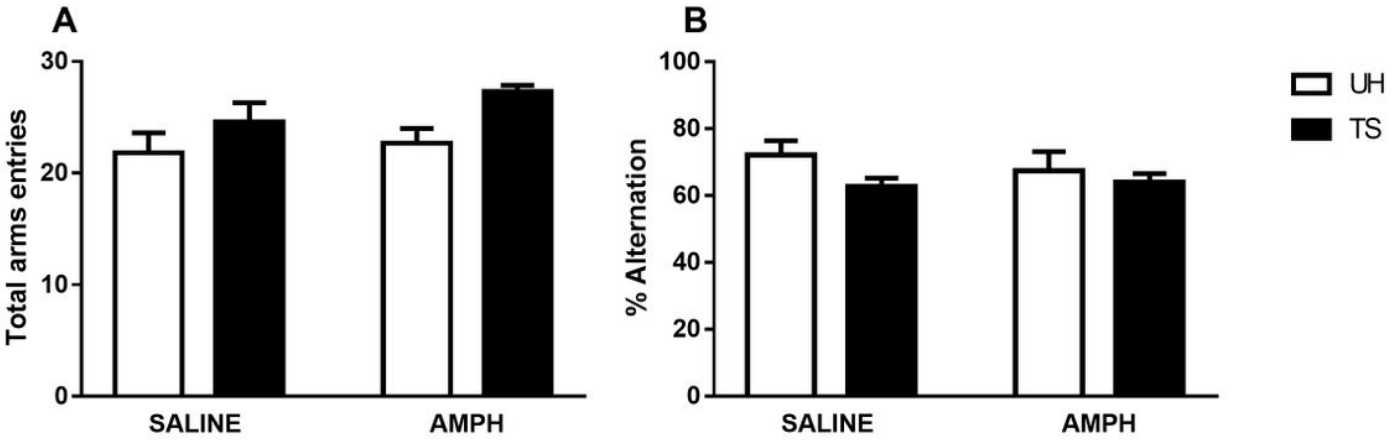

Figure 3

Quantification of both locomotor performance (A) and working memory (B) of adult animals exposed to AMPH and submitted to TS. Memory was assessed by correct alternation percentage. Total arm entries (Fig. 3a) and % alternation (Fig. 3b) were quantified. Data are expressed as mean ± S.E.M.

Abbreviations: Compartment place preference (CPP); amphetamine (AMPH); unhandled (UH) and tactile stimulation (TS).

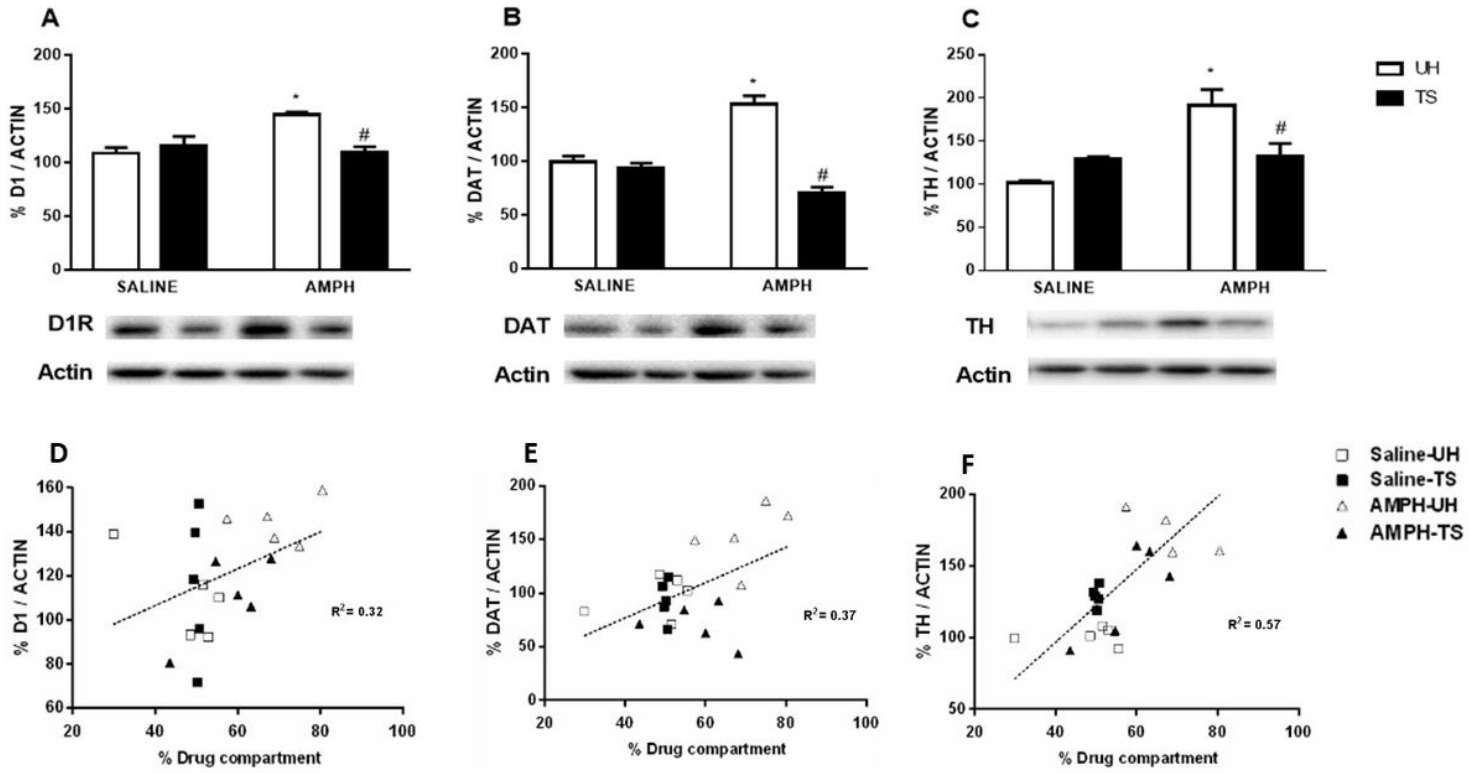

Figure 4

D1R (A), DAT (B) and TH (C) levels were quantified in the NAc of rats difference from AMPH injection for saline (*p<0.05*). # indicates significant difference from AMPH/UH group (*p<0.05*). Correlations between the percentage of the time spent in the AMPH-associated compartment in the CPP paradigm and D1R (D), DAT (E) and TH (F) in the animals that were exposed to tactile stimulation (TS) or unhandled (UH) during drug withdrawal.

Abbreviations: Amphetamine (AMPH); dopamine 1 receptor (D1R); dopamine transporter (DAT), amphetamine (AMPH), tyrosine hydroxylase (TH), unhandled (UH), tactile stimulation (TS) and compartment place preference (CPP).

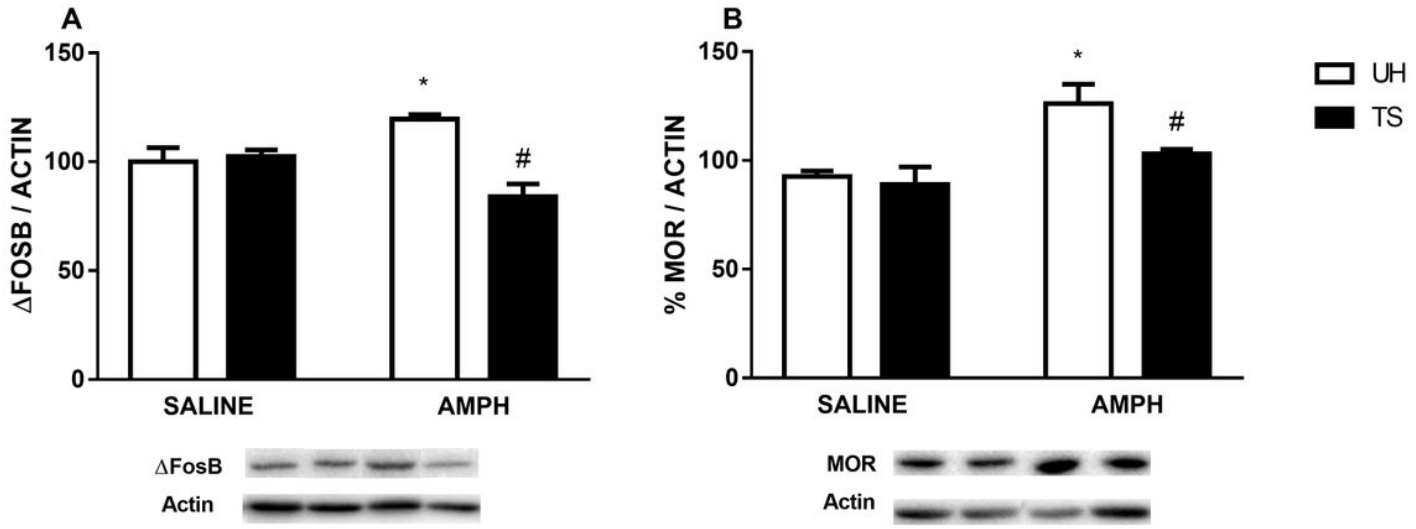

Figure 5

ΔFosB (A) and MOR (B) levels were quantified in the NAc of rats submitted to TS and re-exposure to AMPH. Each band in the sequence correspond to one bar in the figure. Data are expressed as mean ± S.E.M. *indicates significant difference from AMPH injection for saline (*p<0.05*). # indicates significant difference from AMPH/UH group (*p<0.05*).

Abbreviations: DeltaFosB (ΔFosB); Mu opioid receptor (MOR); Amphetamine (AMPH); amphetamine (AMPH); unhandled (UH) and tactile stimulation (TS).