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Subchronic effects of fluoxetine on conditioned suppression produced by a hot air blast

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Abstract

Conditioned suppression is an animal model of anxiety disorders that has been broadly used to investigate the behavioral effects of different drugs. However, various methodological variables (e.g., the type of aversive stimulus) that supposedly interfere with the acquisition of conditioned suppression may also contribute to conflicting results among the studied drugs. Additionally, few studies have sought to investigate the effects of selective serotonin reuptake inhibitors (SSRIs). The present study investigated the effect of subchronic 5-day administration of 5 mg/kg fluoxetine in the retention of conditioned suppression produced by a hot air blast (HAB). The subjects were 12 albino Wistar rats distributed into an Experimental Group (EG) and Control Group (CG). After sessions were conducted to pair two stimuli, a sound and HAB, fluoxetine (EG) or saline (CG) was administered. Twenty-four hours after the last injection, a test session was conducted. The results showed no difference between groups. Fluoxetine (5 mg/kg) did not exert anxiolytic effects in this model of conditioned suppression produced by a HAB. **Keywords**: conditioned suppression; fluoxetine; subchronic; hot air blast.

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Introduction

Conditioned suppression is a procedure developed by Estes and Skinner (1941/1961) to study basic processes related to anxiety. In this procedure, regardless of any response emitted by the subject, a neutral stimulus is paired with an unconditional stimulus that is usually aversive. After several Pavlovian pairings, the neutral stimulus becomes a conditioned aversive stimulus that is able to elicit responses and indirectly suppress the operant response. This effect was termed *state of anxiety* by Estes and Skinner and later called *conditioned suppression*.

Generally, in this procedure, the effect of a drug is measured by the frequency of operant responding in the presence of the conditioned aversive stimulus (McNaughton & Zangrossi, 2008). The operation that indicates the rate of suppression of operant responding in the presence of the conditioned aversive stimulus is termed the *suppression ratio*. Few studies have used selective serotonin reuptake inhibitors (SSRIs) in conditioned suppression. A review of the literature published between 1961 and 1993 by Griebel (1995) found no experiments of conditioned suppression that used SSRIs. However, studies by Jakob (1995) and Guerra (2000) investigated the action of the SSRI fluoxetine in latent inhibition using a procedure similar to Estes and Skinner (1941/1961). This allowed us to evaluate the effect of this drug in the conditioned suppression model by analyzing the control groups that did not have a history of pre-exposure to neutral stimuli. The analysis of these groups in the studies by Jakob (1995) and Guerra (2000) indicated that fluoxetine did not exert an anxiolytic effect. Given these data, it is necessary to conduct studies that directly investigate the effect of fluoxetine in conditioned suppression and the parameters in which it may function as an anxiolytic.

In conditioned suppression, different parametric issues (e.g., schedules of reinforcement, conditioned suppression stability level, nature of the responses studied, and intensity of the aversive stimulus) may contribute to the occurrence of conflicting data among pharmacological agents (Appel, 1963; Hunt, 1961; Millenson & Leslie 1974). The type of unconditioned aversive stimulus is also one of the variables that play an important role in the Estes and Skinner (1941/1961) model. Few studies have been able to successfully reproduce the phenomenon of conditioned suppression with an unconditioned aversive stimulus that is different from electric shock, such as reprimand in humans

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(Reiter & DeVellis, 1975), a time-out period in rats (Leitenberg, Bertsch, & Coughlin, 1968), and a hot air blast (HAB) in rats (Nascimento & Carvalho Neto, 2011). Additionally, we are unaware of any studies that have investigated effects of drugs on conditioned suppression using aversive stimuli of different natures.

Given the necessity of studying the sensitivity of responses to SSRIs in conditioned suppression procedures using different aversive stimuli, the present study tested the effect of subchronic fluoxetine treatment in the retention of conditioned suppression produced by a HAB. The HAB is an aversive stimulus that has been used in conditioned suppression (Nascimento & Carvalho Neto, 2011), punishment (Carvalho Neto et al., 2005; Carvalho Neto, Maestri, & Menezes, 2007; Rodrigues, Nascimento, Cavalcante, & Carvalho Neto, 2008), and learned helplessness (Maestri, 2008) procedures.

Notably, in the procedure adopted in the present study, the subjects were pre-exposed to the neutral stimulus. This procedure was used to habituate possible responses elicited by the neutral stimulus and isolate the suppressive effects of the HAB. This design is common in the conditioned suppression literature (e.g., Ayres, 1968; Davis & McIntire, 1969; Hoffman & Fleshler, 1961; Hoffman & Barret, 1971). However, unlike latent inhibition, the time of pre-exposure to the neutral stimulus was reduced.

Methods

Subjects

A total of 12 male albino Wistar rats that were experimentally naive and approximately 3 months old were used. All of the animals were reared at the Biology Vivarium, Universidade Federal do Pará. During the experiment they were kept in pairs in polypropylene home cages lined with rice straw hay and deprived of water for 24 h before each session, with a continuous supply of food and access to water for 10 min after each experimental session. The subjects were randomly divided into two groups ($n = 6$ per group): Experimental Group (EG; fluoxetine) and Control Group (CG; saline). All of the recommendations of ethical animal care established by the Conselho Nacional de Controle de Experimentação Animal (CONCEA) were followed.

Apparatus

We used the same equipment as Nascimento and Carvalho Neto (2011). One operant conditioning chamber (Mod 3, Insight equipment) was adapted to the use of the HAB. The floor and lever that triggered the drinker were constructed of acrylic, and the ceiling was constructed of a wire net. On the ceiling were two cardboard supports–one on each side–that held hair dryers (model RV429AB, REVLON) that emitted the HABs. The hair dryers were turned on simultaneously by manual control and remained on for 5 s to produce the HABs. The dimensions of each HAB were the following: 216.5 dyn/cm² pressure, 85 dB noise intensity, and a 4 ± 1ºC increase in temperature relative to the 24 ± 1ºC environmental temperature inside the chamber. A thermo-hygrometer clock (Minipa MT-241) recorded the changes in temperature and air humidity inside the experimental chamber.

The neutral stimulus that preceded the HAB was a sound (60 s, frequency of 1000 cycles per second) emitted by a loudspeaker coupled to the chamber. The speaker was located above the lever. The sound and water manipulations occurred through a control box connected to the operant conditioning chamber, which also had a digital panel that recorded the lever press responses.

Drug treatments

Fluoxetine (5 mg/kg) was dissolved in saline (.9% NaCl, alone serving as a vehicle control) and administered intraperitoneally in a 1.0 ml/kg volume. The injections were administered at a fixed schedule in a different environment from where the experiment was conducted.

Procedure

Both groups were exposed to seven phases. (1) The rats were trained with the drinker, and lever press responding was shaped (one to three sessions). (2) The lever press response was strengthened on a continuous reinforcement schedule (CRF; two sessions). (3) The lever press response was further strengthened on a variable interval 60 s (VI 60 s) schedule. This phase was first performed with a gradual adaptation to the schedule using VI 5, 15, 25, 30, 40, 50 and 60 s and ended after five consecutive sessions in which the rates of lever press responding (responses/minute) on a VI 60 s schedule had a maximum variation of 20%, with no ascending or descending tendency. (4) The rats were then habituated to the sound (three sessions). The sound was presented twice during the session with a mean interval of 17 min between each period. The sound presentation followed a different order for each session (1st session: 4th and 18th minutes; 2nd session: 9th and 27th minutes; 3rd session: 5th and 24th minutes). (5) The stimuli were then paired (three sessions). Independent of any response emitted by the subject, the first stimulus that was presented was the sound followed by the HAB. The minutes of presentation of these stimuli were the same as those used in the 1st, 2nd, and 3rd session of the habituation-to-sound phase; therefore, two pairings of stimuli occurred per session. (6) The rats then received subchronic administration for five consecutive days. Each subject received a daily injection of fluoxetine or saline and then was returned to its home cage. In this phase, the subjects were kept under water deprivation conditions (i.e., daily access to water for 10 min) with access for at least 30 min before receiving the injection. (7) The conditioned suppression retention test was then conducted (one session) 24 h after the last drug administration exactly as in the 1st session in habituation-to-sound phase.

All sessions lasted 30 min and were conducted daily between 9:00 AM and 12:00 PM. The number of sessions used to habituate to the sound and to pair the stimuli were based on unpublished data (Nascimento & Carvalho Neto, 2008) and the results of Nascimento and Carvalho Neto (2011).

Statistical analysis

Data were analyzed using multivariate analysis of variance (MANOVA) followed by paired-samples *t*-tests to analyze the same group during the three sessions of habituation to the sound and pairing of the stimuli. Paired-samples *t*-tests were used to analyze the data from the conditioned suppression retention test and each habituation-to-sound and pairing-of-stimuli session between groups (EG, CG). In all cases, $p \leq .05$ was considered statistically significant. The suppression of lever press responding during the sound was analyzed using the suppression ratio: response rate (responses/minute) in the presence of sound divided by the response rate in the absence of stimuli (sound or sound + HAB). A score of 0 indicated total suppression of the lever press response. A score of 1.0 indicated no suppression. A score greater than 1.0 indicated an increase in lever press responding (Stein, Sidman, & Brady, 1958). The suppression ratio was calculated for the habituation to the sound, pairing of the stimuli, and conditioned suppression retention test.

Results

As illustrated in Figure 1, suppression of the lever press response remained after the 5 days of acquisition in both groups according to the conditioned suppression retention test. No significant difference in the mean suppression ratio in the conditioned suppression retention test was found between groups ($t[5] = 1.2, p = .3$), although the ratio in the CG was higher (.7) than in the EG (.4). Regarding the comparison of the mean suppression ratios between the last pairing of the stimuli and conditioned suppression retention test, the value was .6 and increased to .7 in the CG, and the value was .5 and decreased to .4 in the EG. Therefore, no significant difference was found between these sessions in these groups (CG: $t[5] = 3$, $p = .8$; EG: $t[5] = 1.2$, $p = .3$).

Figure 1 shows that in the first session of habituation to the sound, the mean suppression ratio values were .9 and .7 for the CG and EG, respectively ($t[5] = .7, p = .51$). These values increased to 1.1 in the 2nd session ($t[5] = .5$, $p = .64$) and remained at this value in the 3rd session ($t[5] = .1$, $p = .92$) in both groups. In the pairing-of-stimuli phase, the mean suppression ratios were 1.2 and 1.3 in the 1st session in the CG and EG, respectively ($t[5] = .3, p = .78$), gradually decreasing to .9 in both groups in the 2nd session ($t[5] = .2, p = .85$) and decreasing further to .6 and .5 in the 3rd session in the CG and EG, respectively ($t[5] = .3, p = .78$). Therefore, no significant difference was found between the mean suppression ratio values between the CG and EG for each habituation-to-sound and pairing-of-stimuli session. The MANOVA showed no difference in the mean suppression ratio over the habituation-to-sound and pairing-of-stimuli sessions between the CG and EG ($F[1, 33] = .003$, $p = .96$). However, a significant difference in the mean suppression ratio during the course of the habituation-to-sound and pairing-of-stimuli sessions was found between groups ($F[1, 33] = 13.6$, $p = .001$).

With regard to the comparison of the mean suppression ratio values between the sessions of each phase, Figure 1 shows that a significant difference occurred only between the 1st and 3rd pairing-of-stimuli sessions in the EG ($t[5] = 3.8$, $p = .01$) in which the

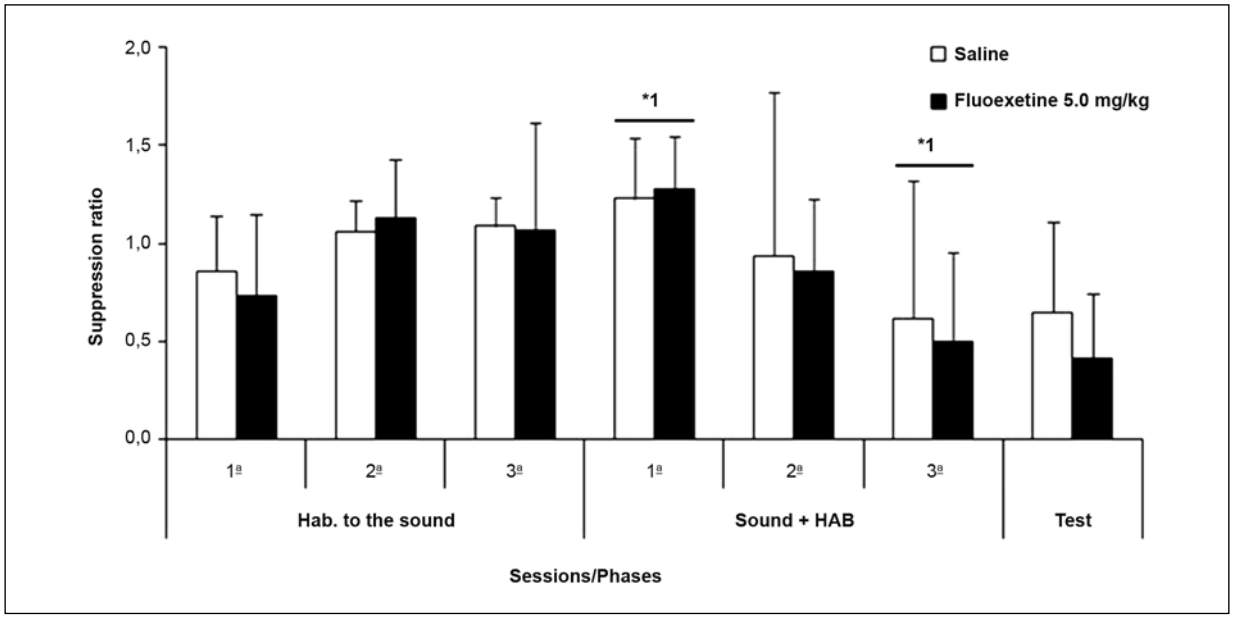

Figure 1. Overall mean suppression ratio of lever press responding during the habituation-to-sound, pairing-of-stimuli (sound + HAB) and test phases. *$p \leq .05$, significant difference between 1st and 3rd pairing-of-stimuli sessions in the experimental group (EG).

suppression ratio decreased from 1.3 to .5. In the present study, the CG and EG were methodologically similar until the pairing-of-stimuli phase because they received the drug only after this phase. Therefore, by combining the data from both groups from the 1st pairing-of-stimuli session and comparing these data with combined data from the 3rd pairing-of-stimuli session, a significant decrease in the mean suppression ratio values was found from the 1st to the 3rd session ($t[11] = 4.0$, $p = .002$; paired-samples t-test).

Figure 1 also shows that the mean suppression ratio values were higher in the last habituation-to-sound session than in the 3rd pairing-of-stimuli session in both groups, but these differences were not significant (CG: $t[5] = .9$, $p = .41$; EG: $t[5] = 1$, $p = .36$). No significant increase in the mean suppression ratio values was found between the last habituation-to-sound session and 1st pairing-of-stimuli session in either group (CG: $t[5] = .9$, $p = .41$; EG: $t[5] = 2.0$, $p = .10$).

Figure 2 shows the overall mean of the absolute frequency of lever press responding in the absence of the conditioned stimulus during the course of the phases. The mean absolute frequency of lever press responding in the conditioned suppression retention test was greater in the EG (14.8) than in the CG (10.2), but this difference was not significant ($t[5] = 1.1$, $p = .32$). These data suggest that the mean absolute frequency of lever press responding in the absence of the conditioned stimulus during the test was not affected by fluoxetine. Both groups exhibited an increase in the mean absolute frequency of lever press responding in the absence of the conditioned stimulus in the conditioned suppression retention test compared with the last pairing-of-stimuli session. This increase was significant (CG: $t[5] = 2.6$, $p = .05$; EG: $t[5] = 3.1$, $p = .03$).

Discussion

The suppression ratio values indicated that fluoxetine at a dose of 5 mg/kg did not exert anxiolytic or anxiogenic effects when administered subchronically considering the retention of conditioned suppression produced by the HAB. Additionally, the mean absolute frequency of lever press responding in the absence of stimuli was not affected by fluoxetine, suggesting no change in motor responses.

We are unaware of any studies that have investigated the effects of fluoxetine on the retention of conditioned suppression. Jakob (1995) and Guerra (2000) administered fluoxetine (10 and 5 mg/kg, respectively) before or during the acquisition of conditioned suppression produced by electric shock and measured lever press responding (Jakob, 1995) and licks on a lickometer (Guerra, 2000). In these studies, fluoxetine maintained the suppression ratio values at less than 0.3 with both chronic and acute treatment and did not exert anxiolytic effects. Moreover, Jakob (1995) also observed no change in lever press responding during the period in which the sound and shock were not presented.

Notably, the clinical use of fluoxetine is indicated for the treatment of obsessive compulsive disorder (OCD), and its anxiolytic effects are achieved after long-

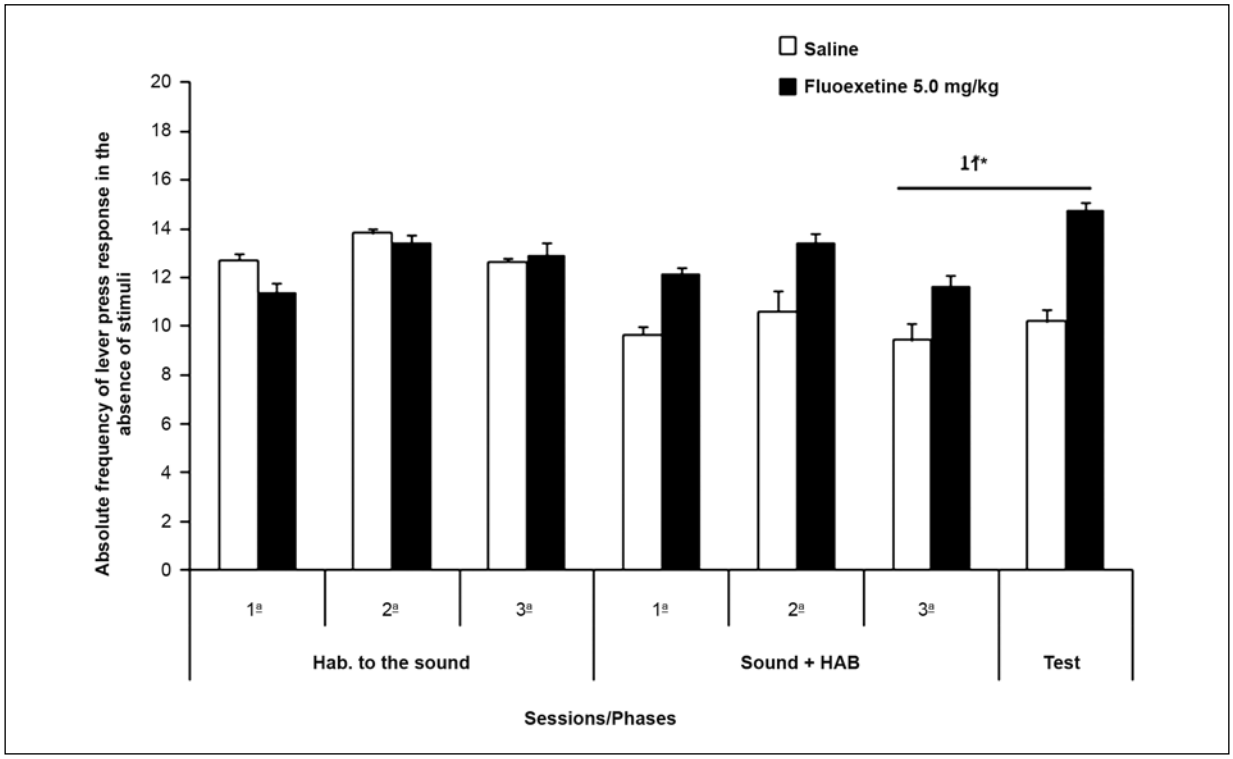

Figure 2. Overall mean of absolute frequency of lever press responding in the absence of the conditioned stimulus in the habituation-to-sound, pairing-of-stimuli (sound + HAB), and test phases. *$p \leq .05$, significant difference between the last pairing-of-stimuli session and test phase in both groups.

term use (Nutt, 2005; Sinclair & Nutt, 2007). Shuhama, Del-Bem, Loureiro, and Graeff (2007) indicated that the symptoms in an animal model related to OCD that are sensitive to drug treatment are stereotyped grooming, checking and hoarding routines, and veterinary pathology, such as paw-licking dermatitis in dogs.

Shuhama et al. (2007) suggested that conditioned suppression is a model related to generalized anxiety disorder. The measure is freezing behavior in response to a sound or environment in which the electric shock is presented, supposedly becoming a conditioned aversive stimulus. In contrast to Shuhama et al. (2007), conditioned suppression produced by a HAB may be related to another type of anxiety disorder. Nascimento and Carvalho Neto (2011) found that the frequency of freezing elicited by HAB-induced conditioned suppression was almost zero during the pairing of stimuli and presentation of a conditioned aversive stimulus (i.e., sound). However, stationary responses (i.e., remaining in the same location of the apparatus but possibly moving to other body parts) and exploratory responses (e.g., sniff responses) occurred at a high frequency (Nascimento & Carvalho Neto, 2011).

Further studies should evaluate parametric issues associated with conditioned suppression with a HAB to clarify the possible relationships with specific anxiety disorders. Although data obtained in the present study and data produced by Jakob (1995) and Guerra (2000) did not show a recovery of operant responding during the presentation of the conditioned aversive stimulus after different treatments with fluoxetine, we cannot conclude whether such procedures with a HAB and shock are sensitive to fluoxetine. Methodological variables such as the level of suppression of operant responding; drug administration before, during, or after the pairing-of-stimuli phase; nature of the aversive stimulus; and intensity of aversive stimulation (e.g., Castejón & Cubeddu, 1998; Appel, 1963) may directly influence the effects exerted by a drug.

With regard to the habituation-to-sound sessions, this phase is commonly used in conditioned suppression experiments so that the stimulus ceases to have any possible aversive function (i.e., novelty effect) and permanently becomes a neutral stimulus that does not interfere with the effects of aversive unconditioning (e.g., Ayres, 1968; Davis & McIntire, 1969; Hoffman & Fleshler, 1961; Hoffman & Barret, 1971). Figure 1 shows that the suppression ratio in the last habituation-to-sound session was greater than in the last pairing-of-stimuli session in both groups, suggesting no influence of the habituation-to-sound phase on the pairing-of-stimuli phase. However, this fact is not supported by the statistical analysis in which the difference between these phases was not significant in either group.

The use of the habituation-to-sound phase may have hampered the pairing of the sound stimulus and HAB because an increase was found in the suppression ratio between the last habituation-to-sound session and 1st pairing-of-stimuli session, although the increase was not significant. However, the number of pairing-of-stimuli sessions and type of aversive stimulation may have influenced these results. If the number of pairing sessions or number of pairings within a session was greater than that used in the present study, then the suppression ratio values could have been less than .5 in the last pairing-of-stimuli session. With regard to the nature of the aversive stimulus, some studies of conditioned suppression produced by shock (e.g., Hoffman & Barret, 1971; Carvalho Neto & Nascimento, 2011) found a sudden drop in operant responding in the first pairing session that reached complete suppression and consequently generated suppression ratio values of 0, in contrast to the habituation-to-sound session that had high suppression ratio values. These results differ from data obtained with conditioned suppression produced by a HAB (Carvalho Neto & Nascimento, 2011) in which the suppression of operant responding occurred gradually. Therefore, we cannot conclude that the habituation-to-sound phase directly influenced the data obtained during the pairing-of-stimuli phase. Data are needed from studies that investigate the influence of pre-exposure to the sound before the pairing-of-stimuli phase with different types of aversive stimuli and analyze different numbers of pairing sessions to further elucidate this issue.

Comparisons of the mean suppression ratios in the 1st and 3rd pairing-of-stimuli sessions indicated that although a decrease in the mean suppression ratio was found between the two sessions (see Figure 1), this difference was significant only for the EG. However, no significant differences in the mean suppression ratios were observed in the conditioned suppression retention tests in the CG and EG. Moreover, no differences in the mean suppression ratios were found in the 3rd pairing-of-stimuli sessions between the CG and EG. Therefore, we may assume that the different results of the comparisons between the 1st and 3rd sessions in the CG and EG did not interfere with the effect of fluoxetine. The differences found between the CG and EG with regard to the 1st and 3rd pairing-of-stimuli sessions may have resulted from individual differences between subjects in the CG. Both groups were subjected to the same experimental design before receiving the drug. We combined the data from both groups and analyzed the mean suppression ratio data from the 1st and 3rd pairing-of-stimuli sessions. The analysis revealed significant differences between sessions. Therefore, other studies that use a greater number of subjects in each group may be needed.

The joint analysis of the mean suppression ratios in the CG and EG in the 1st and 3rd pairing-of-stimuli sessions also indicated that the HAB effectively produced conditioned suppression. The sound acquired similar aversive properties as the HAB, thereby producing a decrease in the suppression ratio. Nascimento and Carvalho Neto (2011) used two rats as subjects, but only one exhibited conditioned suppression. The authors emphasized the need to expand the number of subjects to confirm the effectiveness of HAB as an aversive

stimulus in conditioned suppression. In contrast to Nascimento and Carvalho Neto (2011), the present study used 12 subjects, with six in each group.

With regard to comparing the last pairing-of-stimuli session with the conditioned suppression retention test, a significant difference was found in the mean absolute frequency of lever press responding in the absence of stimuli in both groups. Given this result, the HAB likely affected operant responding not only during the sound but also during the period in which no stimulus was presented. Thus, absence of the HAB in the conditioned suppression retention test may have facilitated the increase in the frequency of lever press responses. Valenstein (1959) studied guinea pigs, and Willis (1969) studied rats. They showed that lever press responding was suppressed not only during aversive conditioning but also during the period in which the conditioned and unconditioned stimuli were absent when a high-intensity electric shock was used. Valenstein (1959) and Willis (1969) reported data obtained only from pairing-of-stimuli sessions. Further studies should investigate these variables associated with not only a HAB but also other aversive stimuli.

In summary, although data from the present study showed that 5 mg/kg fluoxetine did not alter the suppression of lever press responding when administered subchronically, we cannot confirm that the conditioned suppression produced by the HAB was insensitive to this drug. Many issues need to be clarified such as the development of a dose-effect curve, analysis of different routes of administration and treatments, effects of the drug in other response categories (e.g., licks on a lickometer and exploratory responses), and drug administration during the acquisition of conditioned suppression. Moreover, further investigations of conditioned suppression with a HAB should also use other SSRIs and different classes of antidepressants and anxiolytics to validate this model for drug testing.

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