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ScienceDirect
Pharmacology, Biochemistry and Behavior 86 (2007) 441–448
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

7-OH-DPAT effects on latent inhibition: low dose facilitation but high dose blockade: Implications for dopamine receptor involvement in attentional processes

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Received 29 September 2006; received in revised form 23 December 2006; accepted 3 January 2007
Available online 11 January 2007

Abstract

7-OH-DPAT is a dopamine D2/D3 agonist, which at low doses acts preferentially on D3 receptors but at high doses it acts on D2 and D3 receptors. The present study investigated the contribution of D3 and D2 receptors on latent inhibition (LI) by using two dose levels of 7-OH-DPAT: a low dose, 0.1 mg/kg (D3 receptor activation) and a high dose, 1.0 mg/kg, (D2/D3 receptor activation) in a conditioned emotional response (CER) paradigm. The LI Protocols included CS pre-exposure (10 or 40 CS alone trials), CER induction and a non-drug CER test phase. Additionally, the drug effects upon CER acquisition without LI were assessed using the same treatments and test environment pre-exposure protocols but without the tone CS. The effects of 7-OH-DPAT on crossing, rearing and grooming were also measured in an open field 1 day after the CER test phase. The results showed that the low dose 7-OH-DPAT treatment potentiated LI at 10 but not at 40 CS pre-exposures. The high dose 7-OH-DPAT treatment blocked LI at both the 10 and 40 stimulus pre-exposures; and it also induced hyperactivity. Thus, D3 stimulation induced by a low dose of 7-OH-DPAT can facilitate LI but these effects are contingent upon and are specific to the number of stimulus presentations. Altogether, these findings indicate that D3 stimulation can enhance attentional processes, but D2 stimulation can impair attentional processes.

Keywords: Latent inhibition; 7-OH-DPAT; Grooming; Rearing; Crossing; Anti-psychotic drugs; D3 receptor; CER; ADHD

1. Introduction

Since it was cloned in 1990 (Sokoloff et al., 1990), numerous studies have investigated D3 dopamine (DA) receptor pharmacology and function. D3 receptor mRNA and protein are predominantly expressed in limbic brain areas (e.g., nucleus accumbens, island of Calleja, and hippocampus) known to be associated with motivated, appetitive, and emotional behaviours (Bouthenet et al., 1991; Diaz et al., 2000; Gurevich and Joyce, 1999; Landwehrmeyer et al., 1993a,b; Levant, 1998; Levesque et al., 1992; Richtand et al., 1995). D3 receptors are widely regarded as important targets for the development of therapeutic treatments for schizophrenia and drug abuse (Pilla et al., 1999; Shafer and Levant, 1998; Sokoloff et al., 1990, 1992).

In the past few years, several existing compounds were reported to display a greater selectivity for D3 receptors as compared to D2 receptors. For example, 7-Hydroxy-2(*N*,*N*-di-*n*-propylamino) tetralin (7-OH-DPAT) has been reported to be a selective ligand for D3 receptors (Hillefors-Berglund and Von Euler, 1994; Levesque et al., 1992). This DA agonist has been identified as having >100-, >1000- and >10,000-fold selectivity for D3 over D2, D4 and D1 receptors, respectively (Levesque et al., 1992). Several behavioural effects of this compound have been reported including effects pertinent to psychostimulant sensitization and conditionng (Mattingly et al., 2000, 2001) and psychostimulant drug-seeking (Neisewander et al., 2004; Fuchs et al., 2002).

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doi:10.1016/j.pbb.2007.01.001

However, an important factor concerning 7-OH-DPAT is that this drug produces a biphasic effect on behavioural activity in which locomotion is inhibited at lower doses and stimulated at higher doses (Ahlenius and Salmi, 1994; Daly and Waddington, 1993; Depoortere et al., 1996; Kagaya et al., 1996; Khroyan et al., 1995; McElroy et al., 1993; Svensson et al., 1994a,b). Behavioural characterizations of 7-OH-DPAT, using doses of up to 10 mg/kg, demonstrated U-shaped dose–response curves (Ahlenius and Salmi, 1994; Daly and Waddington, 1993; Pugsley et al., 1995), suggesting D3 receptor activation at low doses and increasing D2 receptor occupancy at higher doses. Estimates of in vivo D2 DA receptor occupancy across a range of 7-OH-DPAT doses, based upon D2 receptor protection from *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydro-quinilone (EEDQ) alkylation, suggest that 7-OH-DPAT doses below 0.3 mg/kg are devoid of significant D2 receptor occupancy (Levant et al., 1996).

The aim of the present study was to investigate the effect of 7-OH-DPAT on latent inhibition (LI). LI has been used to assess attentional deficits that are a putative central characteristic of certain forms of schizophrenia (Feldon and Weiner, 1991; Gray et al., 1991; Solomon et al., 1981; Weiner, 1990; Weiner et al., 1984). In the LI paradigm, repeated non-reinforced pre-exposure to a stimulus inhibits the formation of subsequent associations to that stimulus (Lubow and Moore, 1959). LI has been described as reflecting the process of learning to ignore irrelevant stimuli (Lubow, 1973, 1974; Mackintosh, 1975) and has been demonstrated behaviourally in both animals and humans (Lubow and Moore, 1959). LI is a robust phenomenon, readily demonstrable across a wide range of associative learning paradigms including Pavlovian and instrumental conditioning procedures (Moser et al., 2000).

The validity of the LI paradigm as a model for a schizophrenic attentional deficit is strengthened by the fact that overstimulation of the DA receptors generally by systemic administration of amphetamine impairs LI (De la Casa et al., 1993; Solomon et al., 1981; Weiner et al., 1981, 1988), whereas, anti-psychotics, including both typical and atypical compounds, reverse the amphetamine-induced impairment and can enhance LI when administered separately (Christison et al., 1988; Dunn et al., 1993; Feldon and Weiner, 1988, 1991; Moran et al., 1996; Solomon et al., 1981; Warburton et al., 1994; Weiner and Feldon, 1987). The neuroanatomical substrates of LI have been investigated and, from studies of lesions and microdialysis, there is substantial evidence pointing to the involvement of the limbic system and the dopaminergic system (Broersen et al., 1996; Ellenbroek et al., 1996; Feldon and Weiner, 1992; Gray et al., 1995).

The purpose of the present work in evaluating the effect 7-OH-DPAT on LI in rats is based on the hypothesis that activity in the dopaminergic pathways is exacerbated in schizophrenia. Interestingly, the DA affinity for the D3 receptor is up to 70 times greater than the affinity for the D2 receptor (Richtand et al., 2001). This means that any unbalance in the dopaminergic systems related to behavioral disorders will also be related the D3 receptor (Schwartz et al., 2000). In addition, the expression of D3 is higher than D2 in mesolimbic areas and lower in areas that compose motor circuits (Levant et al., 1992; Weiner and Brann, 1989; Mansour et al., 1990). Consequently, typical anti-psychotics, the action of which has been attributed to the blockage of D2 receptors (Seeman, 1987), should also act on D3 receptors, since for these receptor subtypes relative affinity partially overlaps (Schwartz et al., 2000; Joyce, 2001). The same comment applies to the atypical anti-psychotics, which have partial affinity for both D2 and D3 receptors (Schwartz et al., 2000; Joyce, 2001).

In the present study we undertook to assess the possible contribution of D3 receptors to the dopaminergic effects on LI by using a low dose of 7-OH-DPAT (0.1 mg/kg) in a conditioned emotional response (CER) paradigm. For a comparison we included a high dose 7-OH-DPAT treatment, which affects both D2 as well as D3 receptors. As an independent behavioural assessment of D2 stimulation by the high dose of 7-OH-DPAT, we measured behavioural activity in an open field since with increasing D2 agonism locomotor activation was expected. Additionally we assessed possible direct drug effects upon CER. This was important because a drug-induced impairment of CER would yield a false positive LI effect since LI effects are expressed in retardation of CER. Accordingly, we included a replicate 7-OH-DPAT CER protocol but without the tone stimulus. The additional protocol allowed determination as to whether the 7-OH-DPAT treatment by itself would enhance/retard CER.

2. Materials and methods

2.1. Subjects

Male Wistar albino rats provided by the State University of North Fluminense, initially weighing 250–300 g, were housed in individual plastic cages (25 × 18 × 17 cm). The vivarium was maintained at a constant temperature (22 ± 1 °C), with a 12/12 h light/dark cycle (lights on at 0700 h). For 7 days prior to all experimental procedures each animal was weighed and handled daily for 5 min. Food and water were available ad libitum during this period. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus

LI treatment Protocols was carried out in two operant conditioning boxes (24 × 23 × 21 cm) placed within a sound-attenuating enclosure (Insight Ltda, Brazil). The ventilation fan of the outside enclosure provided the background noise. A drinking bottle was located on one side of the box. Licks were detected by a lickometer circuit. The conditioned stimulus was an 80 dB, 2.8 kH, 10 s pure tone generated by a Sonalert module. The unconditioned stimulus was a 0.75 mA, 1 s scrambled foot shock applied to the stainless steel bars 0.25 cm in diameter spaced 1.5 cm apart. A 486 IBM personal computer controlled stimulus presentation and data recording.

Behavioural activity was measured in a separate open-field black arena chamber (60 × 60 × 45 cm). A closed-circuit video-camera (DISISEC, model IR575M), mounted 50 cm above the arena was used for recording and subsequent scoring of behavioural activity data. All behavioural testing was conducted

under dim red light to enhance the contrast between the white subject and dark background of the test chamber. Testing was conducted during the light phase (between 1400 and 1800 h). Masking noise was provided by a fan located in the experimental room and was turned on immediately prior to placing the animal in the experimental arena and turned off upon removal of the animal from the experimental arena. The complete test procedure was conducted automatically without the presence of the experimenter in the test room.

2.3. Drugs

7-OH-DPAT (Sigma, St. Louis, MO, USA) solutions were prepared by dissolving (0.1) and (1.0) mg/1 ml in a physiological saline solution (0.9%). All injections, including vehicle injections, were given in a volume of 1 ml/kg (s.c.) 25 min before pre-exposure and before conditioning. The selection of doses of 7-OH-DPAT was based on a previous study (Levant et al., 1996).

2.4. Procedure

2.4.1. LI procedure. Phase I

Before the beginning of the experiments, the rats were handled and weighted for 5 min during 7 consecutive days. During this period, there was a gradual increase of water restriction culminating with 1 h of free access to water per day. This 23 h water-deprivation schedule continued until the end of the LI procedures and food remained freely available during the entire experiment. After adaptation to their new housing conditions, rats were randomly assigned to experimental groups and each rat had experience with only one experimental chamber for the duration of the experiment. A CER Protocol was used modeled after Weiner et al. (1996) to investigate the effects of systemic 7-OH-DPAT treatment on LI. The stages of the LI procedure were as follows given 24 h apart, except for the baseline stage that was carried out during 5 consecutive days.

2.4.1.1. Baseline lick response assessment. Rats were handled for 5 min daily, and were trained to lick water in the experimental chamber for 20 min/day during 5 consecutive days. After the baseline session, each rat was returned to its home cage and allowed access to water for 40 min.

2.4.1.2. LI tone stimulus presentation. With the water bottle removed, each rat was placed into an experimental chamber. Tone pre-exposed rats received 10 or 40 tone stimulus presentations, 10-s each, with a fixed inter-stimulus interval of 50 s. The environment pre-exposed rats were placed into the experimental chamber without the tone stimulus for an identical period of time: 10 and 40 min.

2.4.1.3. CER induction. The CER Protocol was initiated 1 day after completion of LI tone stimulus presentation treatment. With the water bottle removed, each rat was placed in the experimental chamber. Five minutes later, each rat received the first of 3 tone–footshock pairings. Footshock was applied in the last 1 s of tone presentation. The second and third tone–shock pairings were given 5 and 10 min later, respectively. After the third pairing, each rat remained in its experimental chamber for an additional 5 min and was then removed.

2.4.1.4. Rebaseline. Following completion of the conditioning Protocols, the animals were retrained to drink until baseline levels were recovered.

2.4.1.5. CER test. Each rat was placed in the experimental chamber and allowed to drink. When the rat completed 75 licks, the tone was presented for 300 s, at which time, the animal was allowed to perform at least 10 more licks. Session was terminated if the subject failed to complete these additional 10 licks within 300 s. Lick suppression ratio was determined according to the formula $B/B+A$ where B is the period of the tone stimulus presentation (licks 76–100) and A is the equal temporal period immediately prior to the presentation of the tone stimulus (licks 51–75). In this index measure, a suppression ratio of 0.00 indicates complete lick suppression and no LI effect; while a ratio of 0.50 indicates no CER and, in the present study a maximal LI effect.

2.4.2. Locomotor behaviour assessment in open field. Phase II

Twenty-four hours after the CER test, Phase II was initiated to evaluate the behavioural activity effects of the low and high dose 7-OH-DPAT treatment. Rats were individually placed in the experimental open field for 20 min, removed and injected s.c. with 0.1 mg/kg 7-OH-DPAT, or 1.0 mg/kg 7-OH-DPAT or an equivalent volume of vehicle, and replaced into the open field for an additional 50 min. Behavioural activity was measured as crossings, rearings and grooming duration. For crossings, the open-field floor was divided into 8 equal-sized squares and the number of times that the rat passed from one square to another with its four paws was recorded. Rearing responses were scored as the number of the times that the animal reared up on its hindlimbs and raised its forelimbs off the floor onto the wall or into the air. Grooming was timed in seconds and included both facial and flank grooming behaviour. Behavioural activity was scored from video recordings by a trained observer who was unaware of the treatment.

2.5. Protocol 1: 10 vs. 40 tone stimulus presentations

Altogether, 72 rats were used in the experiment, 36 of which were randomly assigned to Protocol 1 and the other 36 to Protocol 2. The effects of vehicle, 0.1 and 1.0 mg/kg 7-OH-DPAT treatment on LI were tested using a low (10) and a high (40) number of tone stimulus presentations. A low number of stimulus presentations are used when an enhancement or facilitation of LI is expected. A high number of stimulus presentations are often used to insure that an LI effect occurs in the control animals. LI attenuation is then used as an index of a treatment induced impairment of LI in the experimental animals (Weiner et al., 1988). Thirty-six rats were randomly assigned to six experimental groups ($n=6$ per group) in a 2×3 factorial design of 10 and 40 presentation to the tone stimulus

factorially combined with vehicle, 7-OH-DPAT 0.1 or 7-OH-DPAT 1.0 mg/kg. Data from three animals (one from each group) was lost from the 10 trial phase due to apparatus failure. One day after completion of the LI protocols, the animals were tested in the open-field arena.

2.6. Protocol 2: 10 vs. 40 min test environment exposures without tone stimulus presentations

The CER design and procedures of Protocol 1 were followed except that there were no tone stimulus presentations. Protocol 2 was carried out to assess possible drug effects on CER acquisition without interacting LI effects to the tone CS. Thirty-six rats were randomly assigned to six experimental groups ($n=6$ per group) in a 2×3 factorial treatment design with 10 and 40 min exposures to the experimental chamber combined with vehicle, 0.1 and 1.0 mg/kg 7-OH-DPAT treatment. Data from three animals in the experimental groups with the 10 min exposure to the experimental chamber were lost due to apparatus failure. The CER induction and CER test procedures were the same as in Protocol 1. Also, 1 day after completion of the CER Protocols, all animals were tested for behavioural activity in the open-field arena.

2.7. Statistics

CER suppression scores were analyzed by a 2×2 ANOVA for the main factors of the number of CS presentation and CS pre-exposure treatment separately for each drug treatment. If the number of CS presentations versus CS Pre-exposure treatment interaction attained significance ($p<0.05$), Independent t tests were used to make specific group comparisons. For the behavioural activity data, the total time of the test (70 min) was divided into 7 intervals of 10 min duration in order to make within-treatment assessments. The results of behavioural activity (Phase II) from protocols 1 and 2 were pooled and the scores (crossing, rearing and grooming) were analyzed by repeated $2\times3\times7$ ANOVA, consisting of between-subject factors, the number of CS presentation and drug dose treatment and the interval factor (seven 10-min periods) as within-subject factor. If there was no statistical difference for the number of CS presentation in the behavioural activity measures across similar drug treatment groups (e.g., 0.1 mg/kg 7-OH-DPAT groups from protocols 1 and 2), the scores were combined for a group size $n=24$. When a significant effect of drug dose treatment versus interval interaction was recorded, data were further analyzed by one-way ANOVA followed by post-hoc Duncan's multiple range tests. $p<0.05$ was used as the criterion for statistical significance.

3. Results

3.1. Latent Inhibition

3.1.1. Protocol 1 vs. Protocol 2: 10 and 40 tone stimulus presentations vs. 10 and 40 min test environment exposures without the tone stimulus

The vehicle treatment effects shown in Fig. 1A indicate an interaction effect between the presence and absence of the CS during pre-exposure and the number of pre-exposures. The two-way ANOVA showed that the interaction was statistically significant ($F_{1,18}=4.6$; $p<0.05$) as well as the main effect of number of CS pre-exposures ($F_{1,18}=17.3$; $p<0.05$) but the effect of the number of pre-exposures was not significant

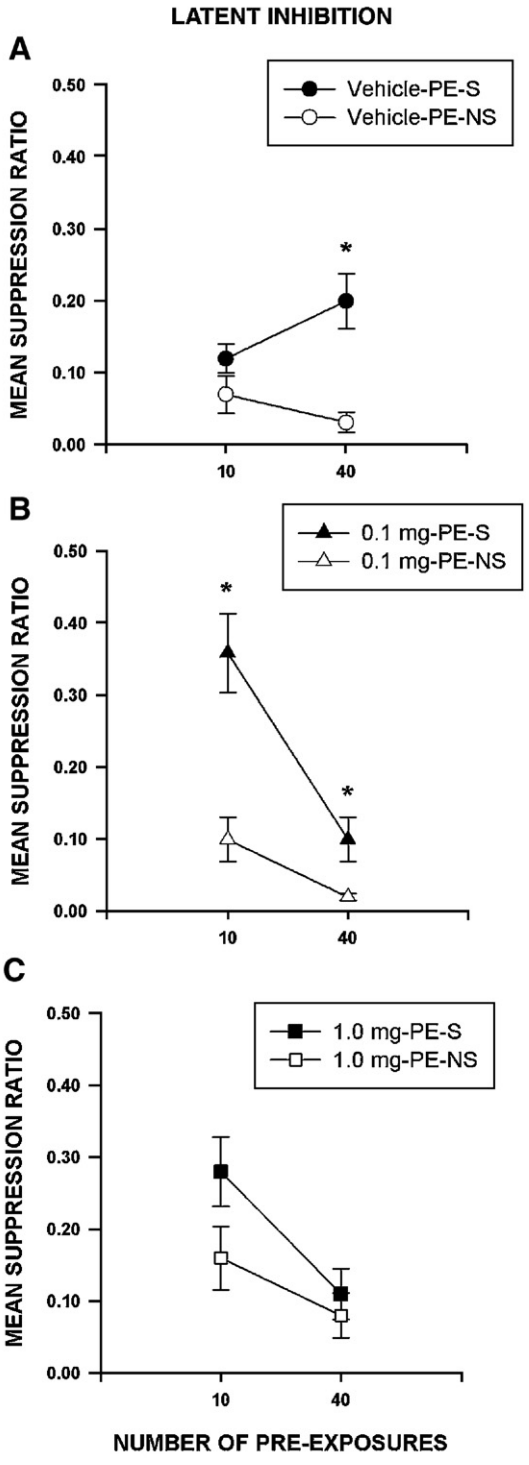

Fig. 1. Means and S.E.M. of suppression ratios for stimulus pre-exposure with the CS (PE-S) present and for pre-exposure without the CS (PE-NS) treatment conditions using 10 and 40 pre-exposure sessions (trials). * Denotes higher suppression ratio ($p<.05$) for the stimulus present group compared to the non-stimulus group.

($p>0.05$). To further evaluate this interaction, post-hoc independent *t*-tests were used to compare the stimulus pre-exposure (PE-S) and no stimulus pre-exposure (PE-NS) groups at 10 and 40 pre-exposure levels, respectively. At 10 pre-exposures, the mean group differences were not statistically significant ($p>0.05$) but at 40 pre-exposures, the mean difference between groups was statistically significant ($t_{10}=4.3$; $p<0.01$), clearly showing LI effects.

For the 7-OH-DPAT 0.1 mg/kg treatment (Fig. 1B), the two-way ANOVA again showed a significant interaction between the presence and absence of the stimulus during pre-exposure ($F_{1,18}=6.2$; $p<0.05$). In addition, the main effect of the number of pre-exposures ($F_{1,18}=26.25$; $p<0.01$) and the main effect of the number of pre-exposures ($F_{1,18}=24.12$; $p<0.01$). To further analyze the observed interaction, a post-hoc *t*-test was carried out and it showed a significant group mean difference between the PE-S and PE-NS groups for 10 pre-exposures ($t_8=4.04$; $p<0.01$) and for 40 pre-exposures ($t_{10}=2.8$; $p<0.05$). As can be seen in Fig. 1B the differences between groups were substantially reduced at 40 pre-exposures.

For the 7-OH-DPAT 1.0 mg/kg treatment (Fig. 1C), the two-way ANOVA revealed a significant main effect of the number of pre-exposures ($F_{1,18}=9.40$; $p<0.01$) but no main effect of pre-exposure to the stimulus ($p>0.05$) or to exposure to the stimulus vs. number of test environment pre-exposures interaction ($p>0.05$).

3.2. Phase II. Behavioural activity assessment

Fig. 2 presents the mean total activity over a 70 min period (first 20 min with no drug; second 50 min with drug) during the open-field test. For crossings (Fig. 2; top panel), the statistical analyses with a repeated three-way ANOVA indicated a drug treatment versus interval interaction ($F_{12,\ 390}=4.36$, $p<0.01$), a significant effect of interval ($F_{6,\ 390}=116.22$; $p<0.01$), and a significant effect of treatment ($F_{2,\ 65}=6.34$; $p<0.01$). However, there was no interval versus tone presentation versus treatment interaction ($p>0.05$), no interval versus tone presentation interaction ($p>0.05$) and no effect of CS tone presentation ($p>0.05$) To further analyze the treatment versus interval interaction, a one-way ANOVA followed by Duncan's multiple range test was performed. The treatment groups did not show statistical differences for interval 1 (no drug) and 2 (no drug) ($p>0.05$). However, the number of crossing in interval 1 was higher than the other intervals ($p<0.05$). For interval 3 (first 10 min post-drug), the 7-OH-DPAT 0.1 mg/kg group showed a lower number of crossing than the vehicle and 7-OH-DPAT 1.0 mg/kg groups ($p<0.05$). However, from interval 4 until the end of the test, the 7-OH-DPAT 1.0 mg/kg group showed a higher number of crossings than the vehicle and 7-OH-DPAT 0.1 mg/kg groups ($p<0.05$).

For rearing (Fig. 2; middle panel), the two-way ANOVA indicated treatment versus interval ($F_{12,\ 396}=4.52$; $p<0.01$) interaction, a significant effect of interval ($F_{6,\ 396}=60.87$; $p<0.01$), and a significant effect of treatment ($F_{2,\ 66}=4.84$; $p<0.05$). However, there was no interval versus tone presentation versus treatment interaction ($p>0.05$), no interval versus tone presentation interaction ($p>0.05$) and no effect of CS presentation ($p>0.05$). A one-way ANOVA followed by Duncan's multiple test showed that the experimental groups did not show statistical differences at interval 1 and interval 2

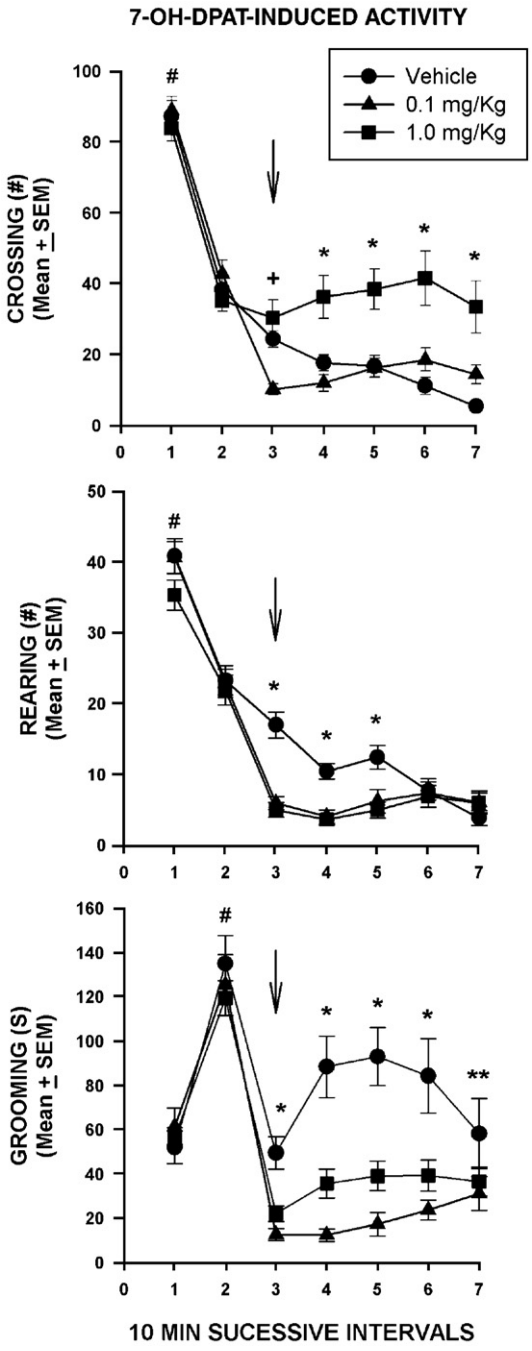

Fig. 2. Means and S.E.M. for crossing (top panel), rearing (middle panel) and grooming (bottom panel) in a 70 min session in the arena during 7-OH-DPAT-induced activity experiment. The results of behavioural activity (Phase II) from experiments 1 and 2 were pooled. Arrows denote the time points for treatment administrations. # Denotes a high activity time for all experimental groups than the other times. + Denotes low activity for the 7-OH-DPAT 0.1 mg/kg group that the vehicle and 7-OH-DPAT 1.0 mg/kg groups. * Denotes a higher activity than the other groups. ** Denotes high activity for the vehicle group when compared to the 7-OH-DPAT 1.0 mg/kg group ($p<0.05$; One-way ANOVA followed by Duncan's multiple range test).

(non-drug) ($p>0.05$). However, the number of rearings during interval 1 was higher than during other intervals ($p<0.05$). From interval 3 (first 10 min post-drug) until interval 5, the vehicle group showed a higher number of rearings than the 7-OH-DPAT 0.1 mg/kg and 7-OH-DPAT 1.0 mg/kg groups ($p<0.05$). There were no differences between the 7-OH-DPAT groups. However, from interval 6 until the end of the test, there were no statistical differences among the experimental groups ($p>0.05$).

For grooming (Fig. 2; bottom panel), the three-way ANOVA indicated treatment versus interval interaction ($F_{12,\ 396}=4.24$, $p<0.01$), a significant effect of interval ($F_{6,\ 396}=36.72$; $p<0.01$) and a significant effect of treatment ($F_{2,\ 66}=46.23$; $p<0.01$). However, there was no interval versus CS presentation versus treatment interaction ($p>0.05$), no interval versus CS presentation interaction ($p>0.05$) and no effect of CS presentation ($p>0.05$). To further analyze the treatment versus interval interaction, a one-way ANOVA followed by Duncan's multiple range test was performed. There were no statistical differences for the experimental groups for interval 1 and interval 2. However, grooming during interval 2 was higher than during the other intervals ($p<0.05$). From interval 3 (first 10 min post-drug) until interval 6, the vehicle group showed a higher grooming activity than the 7-OH-DPAT groups ($p<0.05$). There were no differences between the 7-OH-DPAT groups. However, at interval 7, only the 7-OH-DPAT 1.0 mg/kg group showed low grooming activity when compared to the vehicle group ($p<0.05$).

4. Discussion

The LI effect in the vehicle treated animals is consistent with the general literature. That is, the higher number of exposures to the test stimulus (40) induced a greater level of LI in the animals of the vehicle treatment group (pre-exposed vehicle group versus non-pre-exposed vehicle group) (Moser et al., 2000; Ruob et al., 1998; Weiner et al., 1996, 1997) than the small number of exposures to the test stimulus (10) which produced a negligible LI effect in the vehicle group (Dunn et al., 1993; Feldon and Weiner, 1991; Moran and Moser, 1992; Trimble et al., 1997; Weiner and Feldon, 1987). While the absolute increase of LI in the vehicle group appears to be modest in the present study, this increased exposure to the stimulus, however, was accompanied by increased exposure to the test environment context as well. Significantly, in our protocols the increased exposure to the test environment had a statistically reliable effect in the opposite direction to LI, namely, enhanced CER. Thus, the modest increase in LI in the vehicle group needs to be considered against a backdrop in which the CER suppression ratio baseline was decreasing.

In sharp contrast to the facilitation of LI with increased CS pre-exposure (10 to 40) in the vehicle group, LI in the low dose 7-OH-DPAT treatment decreased as test stimulus exposure increased. The low dose of 7-OH-DPAT, however, markedly potentiated LI at the low number of tone stimulus exposures (10). Interestingly, the facilitation of LI by the low dose of 7-OH-DPAT using a low number of pre-exposure stimuli is similar to results obtained with the typical anti-psychotic drug haloperidol (Trimble et al., 1997; Ruob et al., 1997) and atypical anti-psychotics such as clozapine (Trimble et al., 1998; Weiner et al., 1996) and risperidone (Alves and Silva, 2001).

In Protocol 2, without tone stimulus presentation, the low dose 7-OH-DPAT and vehicle treatment groups had essentially identical CER baseline suppression ratios, so that differences between vehicle and low dose 7-OH-DPAT on LI cannot be accounted for in terms of a differential change in baseline CER acquisition. The high dose 7-OH-DPAT treatment, however, had an overall effect of increasing the baseline CER suppression ratio; i.e., a drug-induced impairment in CER for the high dose 7-OH-DPAT group. In addition, there was an absence of an LI effect. This pattern of results suggests functionally the operation of two different drugs, one drug exerting its effect on attentional behaviour as expressed in potentiated LI with dopamine D3 receptors activation (low dose 7-OH-DPAT) and second drug exerting its influence primarily on the D2 receptors expressed in blockade of LI and CER effects with a primary D2 receptor activation (high dose 7-OH-DPAT) which was sufficient to overcame D3 receptor activation effects. Thus, a cautionary note is appropriate for dose/response drug studies.

In terms of behavioural activity effects, both the low and high doses of 7-OH-DPAT suppressed rearing and grooming (Daly and Waddington, 1993; Sobrian et al., 2003). The time course of the low dose 7-OH-DPAT effect upon locomotion indicated that at the time of LI testing, the low dose of 7-OH-DPAT had no effect upon locomotion, whereas, the high dose of 7-OH-DPAT generated a large increase in behavioural activity (Daly and Waddington, 1993; Depoortere et al., 1996; Gilbert et al., 1995; Kagaya et al., 1996; Khroyan et al., 1995). Although, both dose levels of 7-OH-DPAT decreased grooming, the effect was greatest for the low dose 7-OH-DPAT groups. In linking the effects of 7-OH-DPAT on motoric activity to LI, it needs to be noted that the LI protocols occurred 25 min after the drug treatment, whereas, the effects on motoric behaviour were measured immediately after the drug treatment. Thus, the 10 stimulus pre-exposure protocol occurred 25–38 min post-injection. At this post-injection time interval, activity level (Fig. 2) was similar in the vehicle and low dose 7-OH-DPAT groups but markedly elevated for the high dose 7-OH-DPAT group.

The present results highlight the importance of the interaction between number of CS pre-exposures of the LI Protocols with the 7-OH-DPAT drug effect. In addition to emphasizing this important methodological issue in drug effect assessment, the present results also raise the intriguing question regarding 7-OH-DPAT and LI; that is, the question of what drug-induced processes could be occurring over the time course of the drug effect to produce such strikingly different behavioural outcomes. The measurement of the spontaneous behavioural effects did not reveal any time course changes in the 7-OH-DPAT groups, which paralleled the changes in LI.

It is of interest that the 7-OH-DPAT low dose group during the 10 tones stimulus pre-exposure LI treatment had virtually identical locomotor activity levels to the vehicle groups. Nonetheless, rearing and grooming were reduced. Thus, this

treatment group could be considered to be relatively more quiescent and that this behavioural drug state is consistent with anti-psychotic drug treatment (Trimble et al., 1997), which improves LI. A question that needs further examination is the diminution of LI facilitation with increased stimulus presentations. An important follow-up experiment that is needed is to hold pre-exposure time constant (e.g., 40 min) and the numbers of stimulus presentations constant (e.g., 10) but vary the time when the stimulus presentation occurs (i.e., first 10 min/last 10 min) and compare LI effects. If LI enhancement only occurs with stimulus presentation in the first 10 min, then some type of biphasic function/interference drug-induced process is implicated.

The inclusion of a protocol without tone stimulus presentations pointed out a significant difference between the two drug dose levels of treatment upon CER performance. The high but not the low dose impaired CER acquisition as manifested in the increased suppression ratio. The high dose of 7-OH-DPAT increases D2 stimulation. At the same time, it may also increase D3 stimulation to a level so intense that a depolarization block is induced and stimulus salience becomes downgraded. Such a mechanism might explain the impaired CER acquisition with high dose of 7-OH-DPAT**.** Therefore, the possibility of D3 dysfunction also needs to be considered. In addition to these effects, the high dose 7-OH-DPAT induced pronounced hyperactivity. This effect is consistent with an increasing D2 agonist effect with 10-fold increase in 7-OH-DPAT dose level. These results are in accordance with data showing that high doses of 7-OH-DPAT, including doses above 0.3 mg/kg (Levant et al., 1996), increase behavioural activity (Daly and Waddington, 1993; Depoortere et al., 1996; Gilbert et al., 1995; Kagaya et al., 1996; Khroyan et al., 1995). The broader significance of hyperactivity by itself is uncertain. In the present study, however, it coincided with the higher suppression ratio observed in experiment 2. Taken together, these results point to a possible attentional impairment perhaps analogous to some behavioural disorders such as attentional deficit hyperactivity disorder (ADHD). Certainly, over stimulation of D2 receptors can be expressed by hyperactivity but it is also likely to disrupt complex behavioural processes that would impair acquisition of stimulus–response associations.

In summary, 7-OH-DPAT, at a low or D3 preferring dose level, can markedly enhance LI indicating a potent effect of dopaminergic activation upon attentional processes. This effect is critically linked to the number of stimulus pre-exposures but not to locomotor stimulant effects.

Acknowledgements

This research was supported by FAPERJ, VA Merit Review Grant and NIDA Grant R01 05366. L. C. M. is a recipient of a fellowship from UENF, Brazil. We thank Dr. Cristiane Salum for her helpful assistance with the latent inhibition procedures, Gina Nunes Teixeira for the technical assistance, Dr. Richard Ian Samuels for revision of the text. The experiments were performed in compliance with the recommendations of Brazilian Society of Neuroscience and Behavior (SBNeC), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

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