Research report

Facilitation of preparatory behavior in an artificial prey paradigm by D1-subfamily dopamine receptor activation

Matthew R. Tinsley a,*, George V. Rebec b, William Timberlake a

a Center for the Integrative Study of Animal Behavior and Department of Psychology, Indiana University at Bloomington, 1101 E. Tenth St., Bloomington, IN 47405-7007, USA
b Program in Neural Science and Department of Psychology, Indiana University at Bloomington, 1101 E. Tenth St., Bloomington, IN 47405-7007, USA

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Abstract

Dopamine agonists facilitate, and antagonists inhibit, conditioned preparatory behaviors in rats. Similar effects are demonstrated on an unconditioned preparatory behavior: predatory search and contact of a moving artificial prey stimulus. Apomorphine (0.1, 0.2 mg/kg), a direct agonist, had no effect relative to a within-subject injection of saline vehicle but d-amphetamine (0.1 mg/kg), an indirect agonist, increased contact frequency without altering overall motor activation. To determine the relative importance of the D1 and D2 subfamilies of receptors in the amphetamine effect, separate groups of animals received amphetamine co-injected with either SCH23390 (0.01 and 0.005 mg/kg) or eticlopride (0.01 mg/kg), D1 and D2 antagonists, respectively. Whereas the eticlopride–amphetamine group showed no change in contact frequency from baseline, co-injections of either dose of SCH23390 and amphetamine led to near total suppression of contact, as did treatment with SCH23390 (0.005 mg/kg) alone. Treatment with 0.01 mg/kg eticlopride alone increased contact frequency while treatment with a higher dose (0.1 mg/kg) had no effect. Treatment with the D1-subfamily agonist SKF81297 (0.1 mg/kg) increased contact frequency. Collectively, these results support the hypothesis that dopamine mediates unconditioned preparatory behavior and suggest differing roles for the D1 and D2 receptor subfamilies. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Preparatory behavior; Amphetamine; Apomorphine; Eticlopride; SCH23390; SKF81297

1. Introduction

Although the distinction between preparatory and consummatory behaviors is an old one in ethology and psychology [7,33,46], it has only recently been applied to the effects of dopaminergic manipulations on motivated behavior [3,31]. Preparatory behaviors, such as locomotor search, are a class of diverse behaviors that precede and increase the probability of consummatory acts, such as feeding or drinking. Dopaminergic manipulations have supported the preparatory/consummatory distinction by affecting preparatory behaviors more than simple consummatory acts. For example, behavior related to food hoarding is disrupted by a dopamine receptor antagonist, pimozide [5], and is severely reduced following lesions of the ventral tegmental area (VTA), the source of the mesolimbic dopaminergic system. Neither of these treatments affected the consummatory behavior of eating [21,23,24]. Similarly, neurochemical and electrophysiological recordings have shown greater increases in dopaminergic activity during preparatory than during consummatory behavior [3,26,30]. Further, performance of conditioned preparatory behaviors, such as lever pressing on a food reward schedule or approach to a food magazine, is also reduced by central dopamine lesions [11] or dopamine antagonists [45] while consummatory behavior is unaffected.
The generality of these results is supported by research showing the effects of dopamine agonists and antagonists on preparatory behavior related to reinforcers other than food. For example, systemic amphetamine treatment increases instrumental responding for access to a female [10] without affecting copulatory behavior and antagonists such as haloperidol have been shown to suppress active avoidance, a preparatory behavior motivated by aversive reinforcement, without affecting escape from shock, which could be viewed as consummatory since it occurs after contact with the reinforcer [32,44].

An added specificity of analysis has resulted from the classification of dopamine receptors as D1-like (D1 and D5) or D2-like (D2, D3 and D4) [17,20] making possible a more precise delineation of dopamine-mediated effects on preparatory behaviors. Use of receptor-specific agonists and antagonists has established that both D1 and D2 activation elicit behavioral activation [9,25,27]. D2 receptor activation appears to acts as a positive stimulus in place preference learning and self-administration studies [36], while D1 receptors may be critical for reinforcement processes that underlie conditioned locomotion, the classical conditioning of locomotor drug effects to the situational context [6,28]. A role for D1 receptors has been also demonstrated in reward-mediated incentive learning [18,19,29]. One current hypothesis is that both D1 and D2 receptor activation are required for the full expression of dopamine mediated behaviors [28,42].

Despite the considerable amount of evidence that dopaminergic manipulations affect preparatory rather than consummatory behavior, it is worth noting that this evidence is restricted to conditioned behaviors such as instrumental responding and functionally ambiguous behaviors like conditioned locomotion. To establish that dopamine agonists facilitate unconditioned preparatory behaviors, it must be shown that these drugs facilitate an explicitly untrained behavior that occurs before the end of a functional behavioral sequence. Artificial prey search, chase and capture satisfy these requirements because predatory behavior comprises a sequence of spontaneous preparatory behaviors, including search, target orientation, approach and attack, that is followed by the stereotyped consummatory behaviors of killing and eating the prey [8]. In the present experiments the possibility is explored that dopamine manipulations modify unconditioned predatory behavior in the laboratory by testing the reactions of rats to moving artificial prey that can be chased and captured, but not killed or consumed.

The artificial prey paradigm was originally developed [40] to investigate the interaction between species-specific foraging behavior and classical conditioning procedures, (see also [4,34,37–39]). Presentation of the artificial prey stimulus, a rolling ball bearing, to food deprived rats has been shown to reliably evoke spontaneous, species-typical predatory behaviors, related in form to that displayed toward live prey [37]. Similar results have been shown in a variety of other rodent species [41]. These behaviors include species-characteristic orientation, approach, chase and capture as well as subsequent carrying and handling of the ball bearing. Preparatory behavior, as a class, includes all the behaviors up to the point the animal makes contact with the artificial prey stimulus. However, contact with the ball bearing was used as an index of preparatory behavior because it has been shown to be highly correlated with orienting and approach toward the moving stimulus [40], and is easier to score reliably.

The investigation began by determining if the general dopamine agonists, amphetamine and apomorphine, increased unconditioned preparatory behavior displayed toward an moving artificial prey stimulus relative to that occurring in a within-subjects saline control condition. Then a variety of drug treatments were used to distinguish the relative importance of the D1 and D2 receptor subfamilies in facilitating the unconditioned preparatory behavior. Finally we attempted to determine if a D1-selective agonist could support the same facilitation of preparatory behavior as a general agonist.

2. Materials and methods

A total of 144 experimentally naive female Sprague–Dawley rats, aged between 90 and 120 days, in 12 groups of 12 were used. All subjects were bred in the departmental animal colony (source animals supplied by Harlan Industries, Indianapolis, IN) and kept under a 12:12 h light:dark cycle with the lights off at 18:00 h. Subjects were given water ad libitum and animals were maintained at 85% of their free-feeding weight by varying the amount of their single daily meal during the study. Animal-use protocols for this and all subsequent experiments were approved by the Bloomington Institutional Animal Care and Use Committee.

The subjects were trained in custom-built aluminum chambers (50 cm × 35 cm × 30 cm), each with a clear, colorless plastic front, hinged at the bottom. The chamber contained a food hopper, water dispenser, and two platforms (one in front of the food dispenser and one in the middle of the back wall), as well as two stimulus lights and two speakers and a sunken trackway parallel to the front wall and 12.5 cm from it. A 1.6-cm diameter stainless steel ball bearing was sent down this trackway, entering the chamber through the side wall opposite the feeder and leaving...
through the side wall where the feeder is placed. The ball bearings took approximately 4 s to move down the trackway and exit the chamber, if undisturbed. The floors of the chamber and the trackway itself are tilted to ensure that a ball bearing will roll out of the apparatus no matter where it is left. The experimenter scored the frequency of contact elicited. Contact with the ball bearing has been shown to be highly correlated with the preparatory behaviors of orientation and approach and is easier to score. Microswitches placed under the platforms and light sensors on the food hopper were used to assess the animal’s general activity.

Before the study, subjects were weighed daily, allowing them to become used to handling and transportation. In addition, each subject received a total of 40 min exposure to the apparatus during the 2 days prior to the baseline recording.

Following the pre-treatment handling, baseline bearing contact was assessed during 6 days of testing. Each animal was placed in the training chamber and randomly presented with either a rolling ball bearing (12 trials) or the opening of the moving panel on the rear wall (12 trials) every 48 s. Moving panel trials were intended to be used to establish a measure of the animal’s response to simple movement. The order of presentation was pseudo-randomized with up to three consecutive presentations of each stimulus. Unfortunately, the automated recording of responding to the moving panel was not sufficiently reliable to allow analysis but there was little indication of systematic differences between treatments. Each time a deliberate contact with the ball bearing was made (i.e. a contact initiated by the animal rather than contacts in which the ball bearing rolled into the subject) the trial was scored as a contact trial. The proportion of contact trials to all trials, reported as a percentage, was used in later comparisons as a measure of baseline ball bearing contact frequency.

During the final 4 days of baseline recording, the animals were habituated to the injection protocol by daily injection of 1 ml/kg of physiological saline solution subcutaneously (sc) into the skin at the scruff of the neck 25 min before the session.

During the final, test, phase of the study, the animals were injected with daily alternations of either drug or vehicle control 25 min before testing. Then the animals were placed in the chamber and presented with ball bearings in the same manner as during the pre-treatment baseline.

For the amphetamine, amphetamine–eticlopride and amphetamine–SCH23390 groups, testing occurred across 6 days of alternating vehicle and drug treatments. Because no difference emerged between the first and later pairs of test days in these groups, all other groups were tested across 2 days of alternating treatment.

2.1. Drugs

d-Amphetamine sulfate (Sigma), apomorphine hydrochloride (Sigma), eticlopride hydrochloride (Research Biochemicals), SCH23390 hydrochloride (Research Biochemicals) and SKF81297 hydrobromide (Research Biochemicals) were mixed in 0.9% saline solution and administered as the salt. Co-injected drugs were mixed separately and combined by volume immediately before injection. Drugs were given using the same procedure used in pre-training habituation: sc injection into the skin at the scruff of the neck. The treatment groups included: 0.1 mg/kg d-amphetamine; 0.1, 0.2 and 0.5 mg/kg apomorphine; 0.1 mg/kg d-amphetamine with 0.01 mg/kg eticlopride; 0.1 mg/kg d-amphetamine with 0.01 mg/kg SCH23390; 0.1 mg/kg d-amphetamine with 0.005 mg/kg SCH23390; 0.005 mg/kg SCH23390; 0.01 mg/kg eticlopride; 0.1 mg/kg eticlopride; 0.005 mg/kg SCH23390 with 0.01 mg/kg eticlopride and 0.1 mg/kg SKF81297.

At 0.1 mg/kg amphetamine has been shown to facilitate conditioned responding without causing general motor activation [43]. We chose to use this dose to avoid the possibility that any treatment effects that were found on contact behavior could be due to motor effects of the drug. The apomorphine doses were chosen to give a broad enough dosage range to establish a dose-response curve. The doses of eticlopride and SCH23390 are known to be effective in blocking performance on a conditioning task sensitive to dopaminergic input [44].

2.2. Data analysis

Two-tailed within-subjects t-tests were used to establish the effect of each drug condition by comparing contact frequencies on drug treatment days with contact frequencies during saline treatment days. For the amphetamine, amphetamine–eticlopride and amphetamine–SCH23390 groups, the median vehicle treatment contact frequency was compared with the median drug treatment contact frequency after 6 days of alternating vehicle and drug treatments. For all other groups, mean vehicle and drug contact frequency were compared after 2 days of alternating treatment.

3. Results

Table 1 summarizes the effects of drug treatment for each condition. Average baseline contact frequencies, obtained following saline injection, varied from 8 to 40% across groups, reflecting a high degree of individual variation in this unconditioned behavior. The overall average baseline contact frequency was 19%, which is low compared with previous research [34], and may...
Table 1
Contact differences among treatment groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean contact differenceb (Drug—vehicle) (± S.E.M.)</th>
<th>t-value (tcrit = 2.20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>0.1</td>
<td>17.42 ± 6.92</td>
<td>2.52</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>0.1</td>
<td>-4.17 ± 2.18</td>
<td>-1.91</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>-4.08 ± 2.62</td>
<td>-1.56</td>
<td>n.s</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>-11.17 ± 3.13</td>
<td>-3.57</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>0.1</td>
<td>-20.83 ± 5.25</td>
<td>-5.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>with SCH23390</td>
<td>0.01</td>
<td>-16.75 ± 4.14</td>
<td>-3.19</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>0.1</td>
<td>-16.58 ± 5.33</td>
<td>-3.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SCH23390</td>
<td>0.005</td>
<td>20.17 ± 7.11</td>
<td>2.83</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>0.1</td>
<td>0.08 ± 2.34</td>
<td>0.04</td>
<td>n.s.</td>
</tr>
<tr>
<td>with eticlopride</td>
<td>0.01</td>
<td>-6.17 ± 3.11</td>
<td>-1.98</td>
<td>n.s.</td>
</tr>
<tr>
<td>SCH23390</td>
<td>0.05</td>
<td>11.08 ± 3.70</td>
<td>2.99</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>with Eticlopride</td>
<td>0.01</td>
<td>2.25 ± 6.73</td>
<td>0.33</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* n.s. = not significant.

b See text for statistical details.

indicate a suppression of predatory behavior due to the aversive nature of the injection protocol.

Fig. 1 displays the treatment effects for amphetamine and apomorphine groups. Amphetamine (0.1 mg/kg) significantly increased ball bearing contact frequency ($t(11) = 2.52, P < 0.05$). This effect was not accompanied by an increase in overall activity, as measured by duration of contact with the rear wall platform ($t(11) = -1.45$). Apomorphine treatment led to non-significant decreases in contact at the 0.1 and 0.2 mg/kg doses ($t(11) = -1.91$ and $-1.56$, respectively) but a significant decrease at 0.5 mg/kg ($t(11) = -3.57, P < 0.05$). The latter was accompanied by marked behavioral stereotypy, manifested as head tilting and floor licking.

Fig. 2 summarizes the effects of the D1-selective manipulations. A combination of amphetamine and the D1 antagonist SCH23390 (0.01 mg/kg) led to clear suppression of movement as well as a significant reduction in contact frequency ($t(11) = -5.04, P < 0.05$). A combination of amphetamine and a lower dose of SCH23390 (0.005 mg/kg) also led to reduced contact frequency ($t(11) = -3.19, P < 0.05$) but without the overt general behavioral suppression noted at the
higher dose. Treatment with SCH23390 (0.005 mg/kg) alone led to a significant reduction in contact frequency ($t(11) = -3.11, P < 0.05$). There was a significant increase in contact with the D1 agonist SKF81297 ($t(11) = 2.83, P < 0.05$) compared to vehicle injection.

Fig. 3 shows the effects of treatments with the D2-selective antagonist, eticlopride. No significant difference emerged when vehicle control treatments were compared with a combination of amphetamine and eticlopride (0.01 mg/kg) ($t(11) = 0.04$). Combining eticlopride and SCH23390 in the same injection did not lead to a significant reduction in contact frequency compared with vehicle control ($t(11) = -1.98$), whereas injections of eticlopride (0.01 mg/kg) alone led to a significant increase ($t(11) = 2.99, P < 0.05$). Treatment with a higher dose of eticlopride (0.1 mg/kg) had no effect ($t(11) = 0.33$).
4. Discussion

Treatment with general dopamine agonists demonstrated facilitation of unconditioned preparatory behavior by the indirect agonist amphetamine and suppression by the direct agonist apomorphine. Although the lack of facilitation with apomorphine suggests the possibility that a non-dopaminergic action of amphetamine was responsible for the increase in the amphetamine treated group, differences between these drugs on ball bearing contact can be explained by their differing mechanisms of action [1,12,14,35]. Treatment with amphetamine, because it results in increased dopamine release, facilitates those behaviors the animal would produce in an untreated state. In contrast, apomorphine, which activates postsynaptic dopamine receptors, tends to result in restricted and perseverative behaviors [14], an effect demonstrated at the highest apomorphine dose. It might seem possible that amphetamine treatment resulted in general behavioral activation that the locomotor activity measure was not sensitive enough to detect and that this activation was responsible for the increased preparatory behavior. However, this seems unlikely both because previous research has shown the measure of locomotor activity to be adequate [34] and because ball bearing contact behavior is a complex and co-ordinated motor response which is likely to be disrupted, rather than enhanced, by any drug-based motor effect.

The results from the selective agonist and antagonist treatment groups showed that D1-receptor antagonists differentially suppress ball bearing contact (the SCH23390 alone group and the amphetamine/SCH23390 (0.005) co-injection group), whereas D1 agonism facilitated contact (the SKF81297 group). The results from the eticlopride treatment groups suggest both an important independent role for D2 receptors and a modulatory effect on D1-based activation. Thus, both an important independent role for D2 receptors, tends to result in restricted and perseverative behaviors [43]. In partial contrast, the data suggest that both D1- and D2-selective compounds facilitate unconditioned preparatory behavior and that D2 receptor activation is necessary for D1-based activation to occur.

The mechanism by which D1 receptor activation modulates unconditioned preparatory behavior may be explained by research on the role of D1 receptors in incentive mediated learning. Beninger and Miller [2] hypothesized that activation of D1 receptors encodes the rewarding properties of behavior. Thus, the frequency of a behavior will increase if D1 activation occurs in close temporal proximity to some stimulus, as with rewarded lever pressing, or if the behavioral paradigm used only requires the association of increased dopamine activation with the test environment, as in place conditioning. However, a direct D1 agonist can inhibit a behavior dependent on close temporal contiguity with reward by acting on postsynaptic D1 receptors continuously. Because ball bearing contact is a preparatory behavior, one would expect contact to be related to contextual excitation, and so to be facilitated by a direct D1 agonist. However, one would also expect contact with the ball bearing to have rewarding properties and to be facilitated by an indirect agonist. The results with SKF81297 and amphetamine suggest that unconditioned ball bearing contact behavior has both these reward components.

The results from the eticlopride groups are more difficult to explain because they vary across the co-injection groups, but studies using dopamine iontophoresis following systemic treatment with dopamine antagonists in behaving rats [22] suggest a possible mechanism. Whereas the D1 antagonist SCH23390 elevates cell firing rate in striatum and attenuates dopamine-induced inhibition relative to control conditions, eticlopride has a weak depressing effect on cell firing and is ineffective in blocking dopamine-induced inhibition. This suggests that, at low doses, eticlopride produces a preferential blockade of dopamine autoreceptors, leading to the subsequent increase in striatal dopamine release [13,16] and suppression of cell firing [22]. This increased release could lead to greater activation of postsynaptic D1 receptors and, in the context of the current study, an increase in ball bearing contact via the same mechanism as amphetamine and SKF81297. At higher doses, this presynaptic specificity would be lost, resulting in the lack of an effect seen with the higher eticlopride dose.

The opposing effects of eticlopride and SCH23390 on contact frequency were mirrored by the opposing effects of these drugs on striatal neurons [22]. Additionally, the finding that co-injected eticlopride and SCH23390 causes no change in contact frequency, compared with baseline, is similar to evidence [22] finding that co-injected eticlopride and SCH23390 have almost no effect on those striatal cells that respond to iontophoretic glutamate [22]. It is tempting to speculate that such evidence implicates dopaminergic modulation of glutamate in the behavioral effects. However, the lack of an effect on contact behavior for the am-
phendrine-eticlopride group is problematic given that this analysis suggests that the preferential blockade of pre-synaptic D2 receptors should augment the amphetamine effect. Further assessment of the amphetamine-eticlopride interaction is warranted.

Finally, the findings with respect to the effects of D1 and D2 receptor activation on unconditioned ball bearing contact frequency do not support a simple relationship between dopamine receptors and the behavior of the animal. For example, D1 receptor activation is not related to the activity of a simple motivational state underlying preparatory behavior, if only because this is incompatible with the findings on the effects of D2-selective drugs. The results do support the conclusion that the effects of dopaminergic drugs on preparatory behavior are similar for both behavior that is explicitly conditioned and those that are part of the animal’s pre-existing behavioral repertoire, though these effects may occur at noticeably lower doses for unconditioned behaviors.

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