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ORIGINAL INVESTIGATION

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Intracranial self-stimulation under a progressive-ratio schedule in rats: effects of strength of stimulation, d-amphetamine, 7-OH-DPAT and haloperidol

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Abstract Progressive-ratio (PR) schedules, which have been widely used to study the reinforcing efficacy of various reinforcers (in particular IV psychostimulants), have been very seldom applied to the study of positively reinforcing electrical brain stimulation (EBS). In the present study, rats were required to emit a progressively increasing number of lever-presses (3,4,6,7,9,11,14,16, etc.) for access to successive reinforcers (periods of VTA self-stimulation). Each period of self-stimulation consisted of ten trains of square pulses of EBS; each train was available under a continuous reinforcement schedule. The number of periods of EBS earned during a session was deemed the breaking point (BP). After acquisition and stabilization of self-stimulation, a study was carried out to verify that changes in the strength of the EBS (i.e. changes in the frequency, the intensity or the pulse duration, one parameter at a time) induced changes in the BP. The effects of IP pretreatments with *d*-amphetamine, the dopamine D_3/D_2 receptor agonist 7-OH-DPAT and haloperidol were then assessed. Decreases in the strength of EBS decreased the BP. However, increasing the strength above training values resulted in minimal increases in the BP. *d*-Amphetamine (0.25–1 mg/kg) dose-dependently increased the BP; additionally, when the reinforcer was withheld (i.e. in conditions of extinction, with the stimulator turned off) *d*-amphetamine was also found to augment the BP. This might indicate that *d*-amphetamine preferentially potentiated the motivational (non-rewarded presses) aspects of VTA self-stimulation under this type of PR schedule. 7-OH-DPAT had biphasic effects: at low doses (0.01 and 0.03 mg/kg), it tended to decrease the BP while higher doses (1 and 3 mg/kg) robustly increased the BP. Under conditions of extinction, 7-OH-DPAT (1 mg/kg) had a tendency to increase the BP, but this effect was not statistically significant and did not approach the magnitude of effects observed with *d*-amphetamine. Haloperidol (0.08–0.48 mg/kg IP) dose-dependently reduced the BP, suggestive of a decrease in the reinforcing efficacy of the EBS. These results show that rats can be trained to self-administer EBS of the VTA under a PR schedule of reinforcement and that this behaviour is sensitive to disruption or potentiation of dopaminergic neurotransmission.

Key words 7-OH-DPAT · *d*-Amphetamine · Dopamine · Extinction · Haloperidol · Progressive-ratio · Rat · Self-stimulation · Ventral tegmental area

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Introduction

Progressive-ratio (PR) schedules of reinforcement, originally developed by Hodos (1961) to study the "relative reward strength of stimuli" have been employed in rats (Roberts et al. 1989), monkeys (Yanagita, 1973; Griffiths et al. 1978), dogs (Risner and Silcox, 1981), pigeons (Wanchisen et al. 1988), pigs (Dantzer 1976) and humans (McLeod and Griffiths, 1983; Paule et al. 1988) and with diverse reinforcers including: money (Hughes et al. 1985), IV psychostimulants (Winger and Woods, 1985; Depoortere et al. 1993; Li et al. 1994), food pellets (Poncelet et al. 1983; Depoortere et al. 1996), liquids (Hodos, 1961, Cheeta et al. 1995) and cigarette puffs (Willner et al. 1995). A PR schedule requires an increasing amount of work (number of operant responses, or ratio requirement) to be emitted within a time limit for delivery of each successive reinforcer. The "breaking point" (BP) is defined as the number of reinforcers earned during a session or, alternatively, as the highest ratio requirement completed

before the subject stops responding. The BP is considered to reflect the efficacy of the reinforcer, so that the higher the BP, the higher the efficacy.

To the best of our knowledge, the only studies that applied PR schedules of reinforcement to intracranial self-stimulation – a frequently studied reinforcer in rats – were published by Hodos (1965) and Keesey and Goldstein (1968). Furthermore, we are not aware of any published pharmacological exploration of self-stimulation behaviour maintained by such schedules. This absence of pharmacological data, in particular concerning the dopaminergic system, on this type of self-stimulation schedule is unfortunate. The dopaminergic system is considered to play a key role in positively reinforced behaviours and recent developments in molecular biology have brought the number of subtypes of DA receptors to five (the D_1/D_5 and $D_2/D_3/D_4$ families: see Jaber et al. 1996 for recent review). Studying the effects of ligands selective for these subtypes of receptor – and in particular the D_3 subtype shown to be restricted to areas documented to be of prime importance in positively reinforced behaviours (nucleus accumbens in particular; Fibiger and Phillips 1988) – is a key task for advancing comprehension of the function of central dopaminergic systems. The most widely used schedule of intracranial self-stimulation, the curve-shift procedure, shows in our opinion some limitations as a method for investigating the effects of direct DA receptor agonists. Specifically, it has been found (Leith 1983; Nakajima and O'Regan 1991; Depoortere et al. 1996) that these compounds mostly shift the frequency/response curve to the right, an effect similar to that observed with dopaminergic antagonists. This effect, therefore, seems to be in contradiction with a potentiation of the dopaminergic neurotransmission, although several explanations can account for this paradoxical effect (see for example Herberg et al. 1976 or Leith 1983). Notwithstanding the mechanism underlying these rightward shifts, the similarity between the effects of agonists and antagonists (rightward shifts in both cases) makes the interpretation of the results of agonist/antagonist interaction studies particularly difficult.

This limitation of the curve-shift procedure led us to assass if rats implanted with electrodes in the ventral tegmental area (VTA) could self-stimulate under a PR schedule of reinforcement. The objective was to assess whether such a procedure could represent a viable alternative to the curve-shift method for studying the effects of direct DA receptor agonists. The PR procedure was adapted from that used to train rats to earn food pellets in a previous experiment (Depoortere et al. 1996). We first undertook a limited parametric study to verify that, as is the case with changes in the dose of self-administered cocaine (Winger and Woods, 1985; Roberts et al. 1989; Depoortere et al. 1993; Rowlett et al. 1996), in the sucrose content (Cheeta et al. 1995) or in the volume of sweetened milk (Hodos and Kalman, 1963), changes in the parameters of the EBS would lead to consistent and orderly changes in the BP. We assessed changes of either the frequency, the intensity or the pulse duration of the EBS. In the second part of the study, in order to verify that this experimental procedure was sensitive to the effects of manipulations of the DA system, we investigated the effects of the DA releaser *d*-amphetamine, of the DA receptor antagonist haloperidol and of the DA D_3/D_2 receptor agonist 7-OH-DPAT (Levesque et al. 1992).

Materials and methods

Animals

Male Wistar rats (Charles River, France) were housed individually and their weights kept at 450 ± 20 g by restricting access to chow. All rats were kept on a 12-h light-dark cycle (light on between 7.00 a.m. and 7.00 p.m.) in a colony room at 21°C. Animals were housed and tested in accordance with current French legislation on animal experimentation.

Apparatus

Rats were trained and tested in six operant chambers (Med Associates, East Fairfield, VT., USA) fitted with one lever and a house-light. Each chamber was enclosed in a ventilated and sound-attenuated cubicle, and was connected to an IBM PC-compatible computer via an interface (LVB, Med Associates). All events were recorded and controlled by the "Med-PC" software (Med Associates). Electrical brain stimulation (EBS) was delivered by optically-isolated neurostimulators (model 215-II, Hugo Sachs, Hugstetten, Germany) through a 5-lead spring-shielded cable suspended from the ceiling of the chamber. The intensity of EBS was controlled on an oscilloscope via a 100 kOhms resistor put in series in the stimulation circuit.

Surgery

Rats were injected with atropine (1 mg/kg SC) followed by an ip injection of a mixture of ketamine (75 mg/kg) and diazepam (5 mg/kg). After induction of anaesthesia, they were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, Calif., USA) in the flat-skull position. Two electrodes were implanted in the VTA, one in each side of the brain. Coordinates (with respect to lambda) were: AP: 3.7 mm, ML: 1 mm, DV: 8.9 mm from skull surface (Paxinos and Watson 1986). Each electrode was made of two 175 μm stainless steel Teflon-coated threads (A-M Systems, Everett, Wash., USA), twisted and held together with cyanoacrylate glue, with a 0.2–0.4 mm dorso-ventral inter-tip distance. Each of the four threads was soldered on to one of the five pins of a miniature female connector; each rat had thus four stimulation sites: two threads on each side of the brain. The connector was then embedded in acrylate resin and anchored to the skull by means of four stainless steel screws, one of which was used as the common anode.

Self–stimulation shaping under a continuous reinforcement schedule

One week after surgery, rats were placed in the operant chamber for self–stimulation shaping. EBS consisted of square cathodal

pulses of 1 ms duration, delivered at a frequency of 100 Hz, with the intensity adjusted for each rat (see below); the duration of the train of cathodal pulses was 0.2 s. The stimulation site retained for shaping was that where stimulation sustained self-stimulation behaviour with the least deleterious motor side-effects (such as head rotation). During the following few days, rats were shaped once daily during 30-min sessions on a continuous reinforcement (CRF) schedule (each lever-press resulted in the delivery of a 0.2-s train of EBS). The intensity of EBS was adjusted (range: 60–110 μA) so as to obtain between 100 and 120 lever-presses/min. When stability of lever-pressing was observed during 3 consecutive days, rats were switched to a PR schedule.

Self–stimulation training under a progressive-ratio reinforcement schedule

Under the PR schedule (Fig. 1), rats were required to emit an increasing number of presses (ratio requirement, upward dotted lines in Fig. 1) to have access to each successive period of EBS (box in Fig. 1). The ratio requirement was increased as follows: 3, 4, 6, 7, 9, 11, 14, 16, 19, 22, 26, 29, 34, 38, 43, 49, 55, 62, 70, 78, 87, 98, 109, 122, 136, 151, 168, 187, 208, 231, 256, 284, 315, 349, 387, 429, 475, 526, 582 and 645). The pattern of progression (that does not follow a mathematical law) of the ratio requirement was inspired by a previously published progression (Depoortere et al. 1993) and was determined from a pilot study. Presses of the lever were not reinforced during these periods of ratio requirement, during which the house light was turned on. Rats had 10 min to complete the ratio requirement during each of these periods; failure to do so resulted in the end of the session. Completion of the ratio requirement resulted in access to the reinforcer which consisted of a period when self-stimulation was available under a CRF schedule: each lever-press was reinforced by the delivery of a train of electrical pulses. Each train (0.4 s duration) consisted of 1 ms cathodal pulses delivered at a frequency of 63 HZ (baseline parameters); the intensity was individually adjusted so that each rat would reach a BP between 18 and 22 during a baseline session. Two consecutive trains were separated by a 2 s time-out, during which lever-pressing had no effect; ten presses were reinforced. The house light was turned off during this period of EBS, and a maximum of 40 reinforced periods were available during each daily session. If, for example, as indicated by the cross along the foremost right dotted line in Fig. 1, a rat failed to complete the ratio requirement (87 lever-presses) to gain access to the 21st reinforcer during the 10 min time limit, the BP was 20 (number of reinforcers obtained).

Fig. 1 Schematic representation of the progressive-ratio schedule. See text for more details

Although this schedule could alternatively be described as a "PR/CRF chained schedule", we chose, for convenience, to retain the term "PR schedule".

Variation of the strength of the stimulation

In this part of the experiment, a single EBS parameter (either the frequency, the pulse duration or the intensity) was changed at a time. The levels tested for each parameter were as follows: frequency: 25, 33, 63, 100 and 167 Hz; pulse duration: 0.1, 0.5, 1, 2 and 5 ms; intensity: 50, 70, 90, 100, 110, 130 and 150% of the baseline value (individually adjusted for each rat). For each parameter, values were tested in a randomized order, with at least two baseline sessions between each test session.

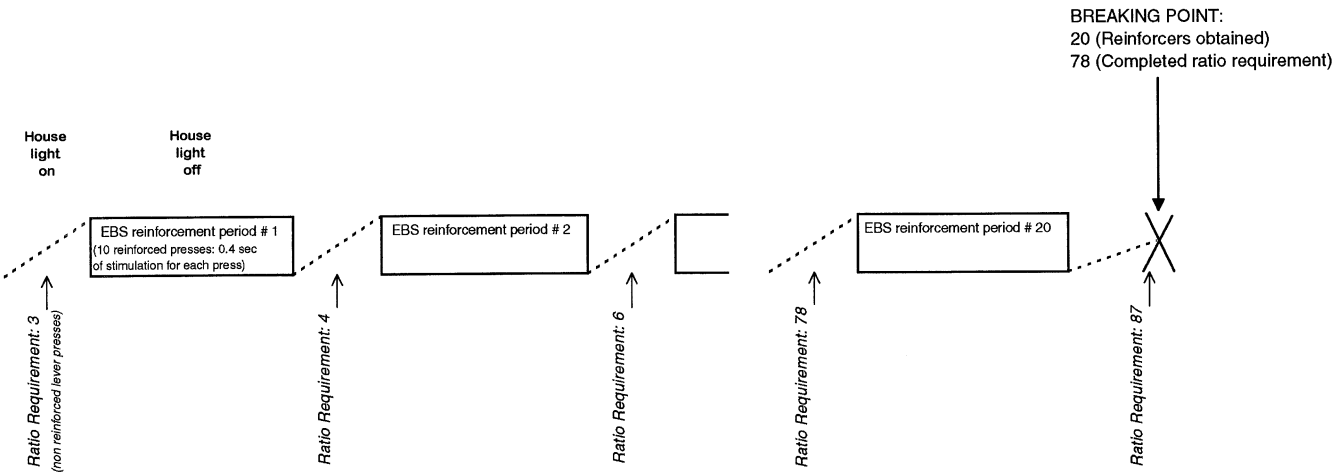
Pharmacological study

In this part of the experiment, we assessed the effects of pretreatment with *d*-amphetamine (0.25, 0.50 and 1 mg/kg), (±)7-OH-DPAT (0.01, 0.03, 0.1, 1 and 3 mg/kg) or haloperidol (0.08, 0.12, 0.24 and 0.48 mg/kg). *d*-Amphetamine (Boyer, Paris, France), 7-OH-DPAT [7-hydroxy-2-(di-*N*-propylamino)-tetralin; RBI, Natick, Mass., USA] and haloperidol (Sigma Chemical Co., St Louis, Ma., USA) were dissolved in saline and given ip in a volume of 2 ml/kg, 15 min (*d*-amphetamine, 7-OH-DPAT) or 60 min (haloperidol) pre-session. Doses are expressed as weights of the base. Each rat was injected with all doses of each drug, given in a randomized order. For each drug, control consisted of the averaged data obtained from baseline sessions immediately preceding each drug session, with vehicle given at the appropriate time. To accustom rats to the injection procedure, saline was administered 15 min before each baseline session, and at least 48 h separated two consecutive drug treatment sessions.

Rats were additionally tested with *d*-amphetamine (0.125, 0.25 and 1 mg/kg) and 7-OH-DPAT (0.1, 1 and 3 mg/kg) in conditions of extinction, i.e. with the neurostimulator turned off throughout the session. Control consisted of data collected during an extinction session with a saline pretreatment. Between these extinction tests (one saline and 3 dose of *d*-amphetamine or three doses of 7-OH-DPAT), rats were trained using the baseline self-stimulation parameters.

Data analysis

The parameter recorded during each PR self-stimulation session was the breaking point (BP), defined as the number of EBS



reinforcement periods earned during a session. BP's were analysed by means of a one-way ANOVA for repeated measures (within factor: parameter of stimulation for the parametric study, or dose of drug for the pharmacological study), followed by post-hoc tests (one-tailed Dunnett's for *d*-amphetamine and haloperidol, two-tailed Dunnett's for 7-OH-DPAT due to the biphasic nature of the curve). Statistical analyses were performed using the "GB-Stat" software (Dynamic Microsystems, Inc., Silver Spring, Md., USA).

Results

Effects of varying the pulse duration, intensity or frequency of electrical brain stimulation on the breaking point

Under control conditions (baseline parameters: 1 ms duration, 100% of individually adjusted intensity, 63 Hz frequency, represented by an open square in each panel of Fig. 2), rats gained access to an average of 20 periods of EBS (Breaking Point: left axis). To gain access to this 20th reinforcer, rats had to emit 78 lever-presses (Ratio Requirement: right axis).

Changes in each of the three parameters of the EBS significantly ($F4,32 = 27.15$, $P < 0.0001$; $F6,24 = 7.93$, $P < 0.0001$ and $F4,24 = 9.77$, $P < 0.0001$ for the pulse duration, the intensity and the frequency, respectively) modified the BP (Fig. 2). Lowering each of the EBS parameters below the baseline value decreased the BP; however, augmenting the values above the baseline value did not significantly modify the BP.

Effects of pretreatment with *d*-amphetamine on the breaking point

d-Amphetamine significantly increased the BP ($F3,21 = 63.36$, $P < 0.0001$; Fig. 3A, solid squares). At the highest dose tested (1 mg/kg), the average BP was about 35 (ratio requirement: 387) as compared to an average of 19 reinforcers (ratio requirement: 70) for vehicle (solid square in shaded area of Fig. 3A).

When tested in conditions of extinction, by switching off the neurostimulators [i.e. withholding delivery of EBS during the normally reinforced periods ("No Stimulation": open circles, Fig. 3A)], rats injected with vehicle had a very low BP (average: four, open circle in shaded area Fig. 3A). This corresponded to a ratio requirement of 7, which was 10 times lower than the ratio corresponding to the average BP (19) obtained with the stimulator turned on (solid square in shaded area of Fig. 3A). When treated with *d*-amphetamine, the BP increased substantially ($F3,21 = 54.60$, $P < 0.0001$), reaching values of 13 and 33, translating into ratio requirements of 34 and 315, for 0.25 and 1 mg/kg, respectively.

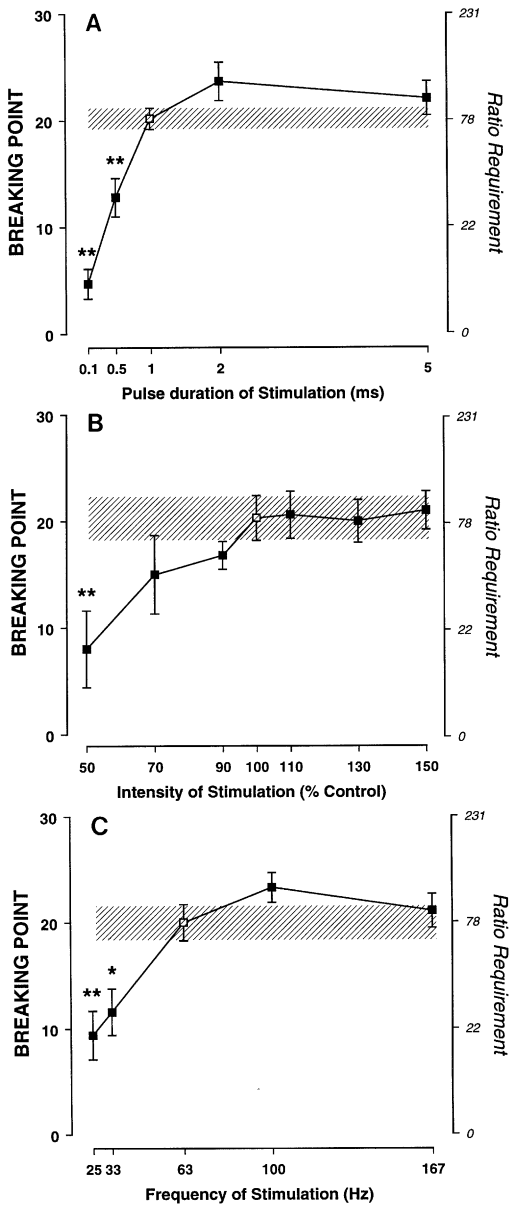


Fig. 2A–C Effects of changes in the pulse duration (**A**), intensity (**B**) or frequency (**C**) of the stimulation for rats self-stimulating in the ventral tegmental area under a progressive-ratio schedule. *Left axis*: breaking Point (number of periods of self-stimulation earned during a session); *right axis*: ratio requirement (number of presses to be emitted to obtain access to the corresponding reinforcer). Access to each successive period of self-stimulation was available after emission of an increasing number of lever-presses according to the sequence: 3, 4, 6, 7, 9, 11, 14, 16, 19, 22, 26, 29, 34, 38, 43, 49, 55, 62, 70 etc. For example, at the baseline frequency of 63 Hz (*lower panel*), rats obtained, on average, a breaking point of 20 i.e. had access to 20 self-stimulation periods, so that they emitted, on average 78 presses to earn access to this last period before quitting. Symbols are means; *vertical bars* are SEMs; the *open symbol* in each panel indicates the value used during baseline sessions; *shaded areas* indicate SEMs around these values. $*P < 0.05$, $**P < 0.01$ versus control (Dunnett's post-hoc test, two-tailed). $n = 9, 5, 7$ for the pulse duration, intensity and frequency experiments, respectively

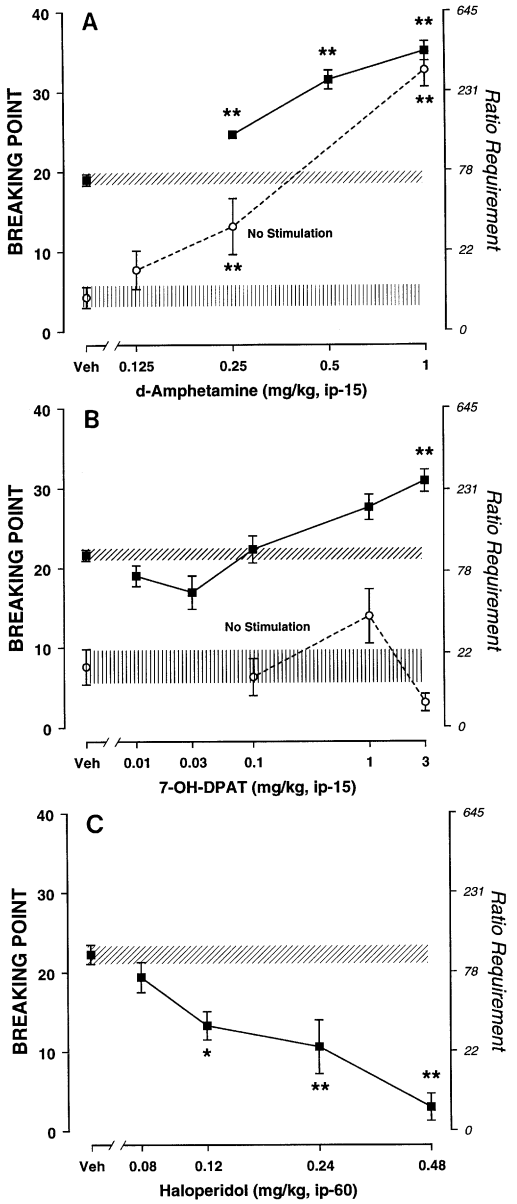


Fig. 3A–C Effects of *d*-amphetamine (**A**), 7-OH-DPAT (**B**) or haloperidol (**C**) on self–stimulation of the ventral tegmental area under a progressive-ratio schedule. For *d*-amphetamine and 7-OH-DPAT, the experiments were conducted under standard conditions of stimulation (63 Hz, 1 ms, intensity adjusted for each rat: *filled squares*, *solid line*) and in the absence of stimulation (*open circles*, *dashed line*). Control values (*Veh*) were obtained from baseline sessions (15 min pre-session vehicle injection) that preceded drug sessions during the course of the relevant drug treatment. See legend of Fig. 1 for other details. Symbols are means; *vertical bars* are SEMs; *shaded areas* indicate SEMs around the Veh value. $*P < 0.05$, $**P < 0.01$ versus vehicle (Dunnett's post-hoc test). $n = 7$ for the *d*-amphetamine (stimulation), 7-OH-DPAT (no stimulation) and haloperidol experiments; $n = 8$ for the 7-OH-DPAT (stimulation) and *d*-amphetamine (no stimulation) experiments

Effects of pretreatment with 7-OH-DPAT on the breaking point

7-OH-DPAT significantly modified the BP ($F5,35 = 9.92$, $P < 0.0001$; Fig. 3B, solid squares). While 0.03 mg/kg of 7-OH-DPAT slightly decreased the BP, the two higher doses increased the BP above baseline values.

Under conditions of extinction, following vehicle treatment, rats emitted few lever-presses with an average BP around 8. In these conditions, treatment with 7-OH-DPAT induced significant ($F3,21 = 6.04$, $P < 0.01$) changes in BP values. These changes differed from those observed following *d*-amphetamine in two respects: first, the curve had an inverted U-shape, and second the maximal BP reached at 1 mg/kg was about half that obtained under *d*-amphetamine (about 14 versus 33, reflecting a more than 8-fold difference in terms of ratio requirement).

Effects of pretreatment with haloperidol on the breaking point

Haloperidol significantly decreased the BP ($F4,24 = 16.0$, $P < 0.0001$; Fig. 3C). The lowest dose tested (0.08 mg/kg) was ineffective, while the highest dose (0.48 mg/kg) almost abolished self-stimulation: the average BP dropped from about 22 reinforcers (vehicle value: solid square in shaded area) to about 3 reinforcers, corresponding to a drop in the ratio requirement from 98 to 6.

In order to evaluate if the observed decrease in BP might have been due to a non specific motor incapacitating effect, we analyzed a complementary parameter, the time from the start of the session to the end of the delivery of the fifth reinforcer. It was found that this parameter was not affected by haloperidol (1888 s ± 79, 2083 s ± 241, 2857 s ± 783, 2005 s ± 215 for vehicle, 0.08, 0.16 and 0.24 mg/kg of haloperidol, respectively; ($F3,15 = 1.13$, $P = 0.37$). Times for 0.48 mg/kg of haloperidol could not be statistically analysed because of missing data (2 rats did not press at all).

Discussion

This study demonstrates that electrical stimulation of the VTA can serve as a reinforcer in a self-stimulation procedure under a PR schedule of reinforcement. The BP was shown to be sensitive to parametric manipulation of the EBS, as there was a positive relationship between a decrease of the pulse duration, intensity or frequency of the EBS and a decrease of the BP. Self-stimulation under this schedule of reinforcement was also shown to be sensitive to pharmacological manipulation of the dopaminergic system, as the DA receptor antagonist haloperidol reduced the BP,

whereas both the indirect DA receptor agonist *d*-amphetamine and the DA D_3/D_2 receptor selective agonist 7-OH-DPAT increased the BP. With *d*-amphetamine, responding was enhanced even under conditions of extinction, that is when the stimulator was turned off.

Lowering the strength of the EBS below baseline values, by manipulating either the intensity, pulse duration or frequency, resulted in a decrease in the BP. Likewise, a reduction of EBS strength has been shown to decrease self-stimulation behaviour with other schedules of reinforcement, such as the curve-shift methodology (Lynch and Wise, 1985; Nakajima and Patterson, 1997). The most likely explanation to account for these decreases in self-stimulation behaviour is that these milder EBSs possess lower intrinsic reinforcing efficacy. On the other hand, increases above baseline of the EBS strength – which might be expected to increase its reinforcing valence – did not augment the BP; testing of stimulation values higher than the ones reported in Fig. 2 was precluded because of the risks (marked motor side effect, head-mount loss, etc.) that can be associated with high strength EBS. Reasons for the failure to observe increases of BP are not clear: it is plausible that EBS of strength higher than that used in baseline sessions generated aversive effects that competed with the positively reinforcing effects and resulted in the BP reaching a ceiling. Alternatively, the parameters chosen for training may have produced maximal reinforcement. There are examples in the drug self-administration literature where increases of the dose of self-administered drugs above the training dose did not induce marked increases in BP (see Griffiths et al. 1978; Winger and Wood 1985; Depoortere et al. 1993). We have also found that in rats trained to press for one 45 mg food pellet under a PR schedule, increases in the number of pellets that could be earned (two or four pellets) did not result in a marked increase in the BP (unpublished results). Substantial increases in BP with increases in the magnitude of the reinforcer above baseline values have nonetheless been reported, using sweetened milk (Hodos and Kalman 1963), heroin (Roberts and Bennett 1993) or cocaine (Roberts et al. 1989). Hodos (1965) was also able to show that increasing the length of the train of EBS (from 0.15 to 0.5, 1, 5 and 10 s) increased the BP above baseline values (for a training length of 0.5 s). However, this was observed in only three out of four rats, each rat being implanted in a different brain area, so that it is difficult to draw any conclusion as to the generality of this finding. The magnitude of the reinforcer used for training, the particular nature of the PR schedule (steepness of the progression, etc..) or the type of reinforcer might be important factors in showing that an increase in the strength of the reinforcer above baseline conditions leads to an increase in the BP.

The indirect DA receptor agonist *d*-amphetamine dose-dependently increased the BP. Three explanations might account for this effect. First, *d*-amphetamine may have enhanced the reinforcing efficacy of the primary reinforcer, i.e. the EBS. There is considerable evidence in the literature that *d*-amphetamine potentiates primary reinforcers, and in particular positively reinforcing EBS (Schaefer and Holtzman 1979; Gallistel and Karras 1984; Schaefer and Michael 1988). The lack of increase of the BP with increases in EBS parameters above baseline values (see above), however, would seem to be in contradiction with this explanation. However, one could imagine that the neuronal substrate mediating the reinforcing effects of EBS was maximally activated by electrical pulses, but that its level of activity could be additionally increased by pharmacological means, such as potentiation of dopaminergic neurotransmission. Nevertheless, the finding that *d*-amphetamine increased lever-pressing regardless of whether the neurostimulator was switched on (conditions of extinction, see below) would suggest that its effects cannot be attributed to a selective enhancement of the rewarding value of the EBS.

A second explanation would be that *d*-amphetamine enhanced the incentive or motivation to lever-press to have access to the primary reinforcer. This alternative explanation finds strong experimental support in the observation that in the absence of the primary reinforcer (i.e. under extinction, when the stimulator is turned off), *d*-amphetamine also dramatically potentiated operant responding using this PR schedule of reinforcement. *d*-Amphetamine has been found, depending on the brain site, to either enhance (Olds, 1970; West and Michael 1990) or diminish (West and Michael 1990) self-stimulation under conditions of extinction. However it must be stressed that in these two studies, the period of extinction was immediately (within the same session) preceded by a period of self-stimulation during which the neurostimulator was active (which was not the case here), so that meaningful comparisons are difficult to establish. In the present study, with 1 mg/kg of *d*-amphetamine, rats reached a BP almost as high as that obtained when the stimulator was active: *d*-amphetamine is known to potentiate the efficacy of secondary (conditioned) reinforcers (Robbins et al. 1983; Beninger and Ranaldi 1992), so that it might have potentiated one or more of the various secondary reinforcers (contact with the lever, light extinction during periods of self-stimulation, etc.) that would be in effect in this type of operant task. Interestingly, McGregor and Roberts (1995), in a study where cocaine self-administration was available under a fixed-ratio schedule or a PR schedule, postulated that the PR schedule might be more sensitive to the "incentive value" of cocaine than the fixed-ratio schedule.

A third explanation would be that *d*-amphetamine – which has motor activating effects and disinhibitory properties – might have non-specifically increased responding through a rate-enhancing effect or by

generating a stereotyped behaviour directed towards the lever (Evenden and Robbins 1983). Our own unpublished data from rats trained under another schedule of self-stimulation ("frequency/rate curve-shift" method) would tend to contradict this third alternative. These rats when tested under extinction (with the stimulator turned off right from the beginning of the session, a condition that best corresponds to the condition of extinction used in this PR procedure) emitted hardly any lever-presses when injected with 1 mg/kg IP of *d*-amphetamine, a dose that robustly shifted the frequency-rate curve to the left with the stimulator on. This absence of effects would suggest that the tremendous increase in PR operant output seen with *d*-amphetamine in conditions of extinction is not due to a non specific (rate-enhancing/stereotypy-driven) effect of *d*-amphetamine on lever-pressing.

The DA D_3/D_2 receptor agonist 7-OH-DPAT (Levesque et al. 1992) showed a biphasic effect: low doses tended to lower the BP, while higher doses robustly shifted the BP towards higher values. This biphasic effect was observed in our previous study on responding under a PR schedule using food pellets as the reinforcer (Depoortere et al. 1996). A reduction of the BP at low doses can be attributed to action at presynaptic autoreceptors, giving rise to a lowering of DA outflow and mimicking the effects of DA receptor antagonists. The effects we saw in the present study are not as marked as those we observed in the food reinforced schedule; however, we saw these BP reductions with other DA receptor agonists used at low doses (manuscript in preparation) in this PR self-stimulation procedure. Higher doses of 7-OH-DPAT robustly increased the BP, which one might reasonably interpret as a potentiation of the reinforcing efficacy of the primary (and/or secondary) reinforcer(s), i.e. electrical stimulation of the VTA. This stands in stark contrast to the effects of direct DA receptor agonists in the curve-shift methodology: 7-OH-DPAT, another D_3/D_2 DA receptor agonist quinpirole and the non selective DA receptor agonist apomorphine shifted the curve to the right (Leith 1973; Nakajima and O'Regan 1991; Depoortere et al. 1996). Putative explanations for these paradoxical effects have been provided elsewhere (Herberg et al. 1976; Leith 1983; Depoortere et al. 1996).

One other notable difference between *d*-amphetamine and 7-OH-DPAT is that at 3 mg/kg, 7-OH-DPAT substantially increased the BP when self-stimulation was available, but did not modify the BP under conditions of extinction, whereas 1 mg/kg of *d*-amphetamine markedly augmented the BP under both conditions. At 3 mg/kg, 7-OH-DPAT induces stereotypies (predominantly sniffing: Daly and Waddington 1993; Depoortere et al. 1996,) which do not interfere with lever-pressing when the stimulator is turned on, but seem to compete with lever-pressing under extinction. This exemplifies the complexity of the interaction of stereotypies with operant responding.

An interesting aspect of self-stimulation under a PR schedule of reinforcement lies in the opposite effects of direct DA receptor agonists (which generally increase the BP) and of DA receptor antagonists (which decrease the BP). For that reason, this type of schedule might offer a decisive advantage over a curve-shift schedule, where the effects of these agonists cannot be readily distinguished from those of antagonists (both shift the curve to the right). These mirror image effects in the PR schedule are a definite plus in agonist-antagonist interaction studies, and should allow for the fine pharmacological dissection of the implication of the various DA receptor subtypes in self-stimulation behaviour. Choice of EBS as a reinforcer in a PR schedule also offers advantages over alternative reinforcers such as food/liquids or IV psychostimulants. First, the magnitude of the reinforcement provided by EBS can be very precisely titrated (by changes in any of the three parameters of square pulse stimuli), which facilitates the creation of groups of rats with comparable baseline operant output. Second, self-stimulation behaviour is a very stable behaviour and rats can be kept for long periods of time, which is not always the case for IV psychostimulants (due to catheter patency problems).

The DA receptor antagonist haloperidol dose-dependently decreased the BP: this decrease could be accounted for either by a reduction of the motivational/reinforcing efficacy of EBS of the VTA, or be due to motor incapacitation or sedation, or ultimately result from a subtle combination of the two effects. Effects of DA receptor antagonists on self-stimulation performance have been extensively explored in the past (examples: Carey 1983; Lynch and Wise 1985), and the exact nature of the phenomenon (anhedonia or motor deficit) subserving the observed decrease in self-stimulation behaviour has fuelled much debate in the literature (see, for example the review by Wise 1978). For the present study, analysis of an additional parameter (the time from the start of the session to the end of the delivery of the 5threinforcer) indicated that the capabilities of rats for operant responding were apparently not affected by haloperidol. In the light of this complementary analysis, it appears reasonable to conclude that the decrease in BP produced by haloperidol, at least at the 0.16 and 0.24 mg/kg doses, is more likely to stem primarily from a blunting of the motivational/reinforcing efficacy of the VTA stimulation than from an aspecific motoric effect.

In conclusion, this study demonstrated that self-stimulation of the VTA under a PR schedule of reinforcement is sensitive, to a certain extent, to manipulations of the parameters of the EBS, can be reduced by the DA receptor antagonist haloperidol and potentiated by augmentation of DA neurotransmission by *d*-amphetamine and by the DA D_3/D_2 receptor agonist 7-OH-DPAT. This suggests that this procedure provides a reasonable alternative to the curve-shift

methodology for the exploration of the effects of direct DA receptor agonists.

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