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Median Raphe Injections of 8-OH-DPAT Lower Frequency Thresholds for Lateral Hypothalamic Self-Stimulation

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FLETCHER, P. J., M. TAMPAKERAS AND J. S. YEOMANS. *Median raphe injections of 8-OH-DPAT lower frequency thresholds for lateral hypothalamic self-stimulation.* PHARMACOL BIOCHEM BEHAV **52**(1) 65–71, 1995.—The selective 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) reduces the activity of brain 5-HT neurons via somatodendritic autoreceptors located in the midbrain raphe nuclei. This action of 8-OH-DPAT results in reduced 5-HT synthesis and release of 5-HT in terminal regions. Previous studies have shown that injecting 8-OH-DPAT into the raphe nuclei stimulates feeding, sexual behaviour, and locomotor activity, and serves as an unconditioned stimulus for inducing a conditioned place preference. This behavioural profile suggests that raphe injections of 8-OH-DPAT facilitate reward-related behaviour. The present study tested this hypothesis by investigating the effects of median raphe injections of 8-OH-DPAT on sensitivity to lateral hypothalamic (LH) self-stimulation. Frequencies required to sustain half-maximal rates of responding were determined following injection of saline or various doses of 8-OH-DPAT (0.2–5 μg) into the median raphe. In four rats with accurate injection sites 8-OH-DPAT dose-dependently lowered frequency thresholds by up to 40%. In the remaining rats injection sites were located outside the median raphe, and 8-OH-DPAT either slightly raised or failed to lower frequency thresholds. These results show that 8-OH-DPAT injected into the median raphe facilitates brain stimulation reward, and suggest that acute reductions in 5-HT neurotransmission may enhance sensitivity to rewarding stimuli. The possible interactions between 5-HT neurons and efferent systems utilizing dopamine and acetylcholine as neurotransmitters in mediating this effect are discussed.

8-OH-DPAT Median raphe Brain stimulation reward Frequency threshold Serotonin

ACTIVITY within the mesolimbic dopamine system is involved in controlling reward-related behaviours such as drug self-administration, intracranial electrical self-stimulation, feeding, and sexual behaviour [(5,46) for reviews]. Manipulations of brain 5-hydroxytryptamine (5-HT) function also alter such behaviours. Self-administration of cocaine or amphetamine is enhanced by treatments that reduce 5-HT activity, such as 5,7-dihydroxytryptamine (5,7-DHT) (25,27) and the nonselective 5-HT receptor antagonist metergoline (28), and is reduced by indirect 5-HT agonists such as fluoxetine (6,37). These results imply that increased 5-HT activity reduces, and decreased 5-HT activity enhances, the rewarding effects of amphetamine and cocaine. However, this simple conclusion is complicated by the observations that amphetamine-induced conditioned place preference is attenuated by the 5-$HT_{2/1C}$ receptor antagonist ritanserin (32), but not by 5,7-DHT (42). The involvement of 5-HT in reward processes is complex. This complexity derives in part from the diffuse 5-HT innervation of the forebrain (4) and the existence of multiple 5-HT receptor subtypes. The availability of selective 5-HT receptor ligands and advances in the understanding of the functional roles of the various 5-HT receptor subtypes may help in clarifying the role that 5-HT systems play in controlling behaviour.

The compound 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) is a selective 5-HT_{1A} agonist (1). Receptors of the 5-HT_{1A} subtype are found in the somatodendritic region of 5-HT-containing neurons in the dorsal and median raphe nuclei (45). Injections of 8-OH-DPAT into the dorsal or median raphe nucleus inhibit the activity of raphe 5-HT neurons (40), leading to a reduction in 5-HT synthesis and release in termi-

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nal areas (17,19). This relatively selective action makes 8-OH-DPAT, when injected into the raphe nuclei, a useful tool for investigating the role of brain 5-HT systems in controlling behaviour.

Several studies have suggested that 8-OH-DPAT may engage brain systems involved in reward. Rats prefer an environment paired with raphe injections of 8-OH-DPAT over one paired with saline injections (13). Low doses of peripherally injected 8-OH-DPAT, which preferentially activate raphe 5-HT_{1A} (18) receptors, also induce a conditioned place preference and increase responding for rewarding lateral hypothalamic (LH) self-stimulation (30). Raphe injections of 8-OH-DPAT elicit feeding (12), male sexual behaviour (16), ethanol consumption (44), and locomotor activity (15). This profile of behaviour is consistent with an action on the neural substrates involved in controlling sensitivity to rewards.

The present experiment was conducted to test this hypothesis by examining the effects of 8-OH-DPAT on LH self-stimulation in a frequency threshold paradigm that has been shown previously to be sensitive for detecting both increases (47) and decreases (48) in frequency thresholds following intracranial injection of drugs. With this method the effects of drugs on performance (i.e., bar pressing) vs. reward (i.e., frequency threshold) can be distinguished (10) and the duration of drug action can be assessed by tracking the frequency threshold over time (47). It was predicted that if 8-OH-DPAT enhances activity in brain reward systems, animals should require a lower frequency of stimulation to maintain responding following 8-OH-DPAT treatment.

METHOD

Subjects

Adult male Wistar rats weighing 250–350 g at the time of surgery were used. They were housed in transparent Nalgene cages with the colony room maintained at approximately 22°C, with a 12L : 12D cycle (lights on 0830 h). Food and water were freely available at all times.

Surgery and Histology

Rats were anaesthetized with 60 mg/kg sodium pentobarbital (Somnotol) IP, and injected with 0.6 mg/kg atropine sulphate (SC). Stainless steel electrodes, 200–250 μm diameter, constructed from insect pins and insulated with Epoxylite except at the tips, were implanted bilaterally in the medial forebrain bundle at the level of the hypothalamus. Flat skull coordinates for electrode implantation were AP − 2.8, L ± 1.8, and V − 8.8 mm, relative to bregma. An electrode wrapped around four jeweler's screws anchored in the skull served as a ground. In each rat a stainless steel guide cannula (22 g, Plastic Products, Roanoke, VA) was implanted with the tip terminating 4 mm dorsal to the median raphe. The cannula was implanted at an angle of 20° to the vertical using the following coordinates, relative to interaural zero; AP + 1.2, L − 1.2, and V + 5.5 mm. Stylets (15 mm long) were used to keep each cannula patent. Training and testing of animals began 7 days after surgery.

Following completion of the experiments rats were deeply anaesthetized with Somnotol and a volume of 0.2 μl fast green dye was injected via the guide cannula, to aid in visualization of the injection site. The rats were then perfused transcardially with 10% formaldehyde solution, and the brains removed and stored in formaldehyde for 2–7 days. Frozen brains were then cut at 40-μm intervals in a cryostat and the sections stained using cresyl violet.

Behavioural Testing

Rats were trained to press a bar for 0.5-s trains of 0.1-ms duration square wave cathodal pulses; stimulation was delivered via a constant-current stimulator, and the current monitored by the voltage drop across a 100 Ohm resistor on an oscilloscope. For each rat a fixed current intensity (300 μA for five rats and 400 μA for two rats) was determined that yielded high rates of responding (40–60 responses per min) at a frequency of 25–40 Hz. Animals were tested in 1-min trials followed by a period of 15–30-s extinction with frequencies varied to yield response rates above and below half-maximal rates of responding on successive 1-min trials. They were overtrained on this procedure for at least 1 week until bar-pressing rates and frequencies yielding half-maximal rates of responding were stable. On test days, approximately 20 min prior to drug injection a baseline rate–frequency function was established by measuring response rates over a range of 4–5 frequencies administered in a random order. Two more replications of this over the next 15 min assessed the stability of this curve. These baseline curves also yielded measures of the half-maximal response rate and the frequency threshold required to sustain that level of responding. For drug injections rats were removed from the test box and lightly wrapped in a towel. After the injection they were placed back in the box and testing began immediately. Response rates were measured during 1-min trials separated by a 15–30-s extinction period, with frequency varied between trials. If on the first trial response rate fell below the criterion half-maximum rate (determined in baseline testing) the next higher frequency was tested, and so on in ascending order, until the criterion rate was exceeded. Conversely, if on the first trial response rate was above criterion the next lower frequency was chosen until responding fell below criterion. When on two trials recorded response rates were above and below the half maximal level of responding, frequency thresholds were calculated by plotting bar presses/trial vs. frequency, drawing a line between the two frequencies, and measuring the frequency from the intersection of the line with the half-maximal rate. One frequency threshold was determined on average every 3 min. Percentage changes from baseline threshold were then determined. No complete rate–frequency curves were obtained after drug injection.

Drugs and Drug Delivery

8-Hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT; Research Biochemicals, Inc.) was dissolved in 0.9% saline and injected through a fine glass needle, housed inside stainless steel tubing, attached to a Hamilton syringe via a length of plastic tubing. A volume of 0.5 μl was injected manually over a period of 1 min with the needle left in place for a further 30 s to allow the drug to diffuse away from the needle tip. At least 44 h intervened between successive injections, and the order of injections was randomized for individual animals. The animals received several mock injections during the week prior to drug injections to accustom them to the handling procedures necessary for drug injections.

Statistical Analysis

The mean percentage change from baseline frequency over the first 20 min after injection was determined for each drug treatment in each animal. This 20-min period was chosen because the drug effects appeared maximal and stable during this time. These values were based on four to eight observa-

tions per condition. These data were then subject to one-way analysis of variance (ANOVA) for independent measures because in practice there were slight differences in the number of observations per treatment. Following a significant F-value post hoc comparisons were made using Tukey's test.

RESULTS

Figure 1 shows a reconstruction of the injection sites in the animals used in these studies. Four rats (A5, B7, C5 and D1) had injection sites located in the median raphe; three rats (B5, B6 and C2) had injection sites located up to 1 mm lateral and dorsal to the median raphe. In all rats the stimulating electrode was located in the lateral hypothalamus within 1 mm lateral or dorsolateral to the fornix.

Figure 2 illustrates representative rate-frequency curves for rats B7 (tested at 1 and 5 μg 8-OH-DPAT) and D1 (tested with saline and 5 μg 8-OH-DPAT) at various time points postinjection. The rate-frequency curve determined prior to drug injection was a characteristic S-shape, with response rate increasing as a function of stimulation frequency. In both rats injection of 5 μg 8-OH-DPAT induced a parallel shift to the left of the rate-frequency function. This dose of 8-OH-DPAT did not increase the rate of responding above the maximal response rate observed under baseline conditions in any of the animals. Neither 1 μg 8-OH-DPAT in rat B7 nor saline in rat D1 shifted the rate-frequency function. Thus, lower levels of frequency were required to sustain half-maximal rates of responding in rats treated with 5 μg 8-OH-DPAT. Similar results were seen for the remaining two rats.

The changes in frequency thresholds for each of the four rats with median raphe injection sites are shown in Fig. 3. Prior to drug injection the frequency thresholds were stable. In rats C5 and D1 saline injected into the median raphe did not alter thresholds except for a small transient reduction in the first 5 min of testing. In rat A5 saline treatment slightly increased thresholds. Following injection of 8-OH-DPAT frequency thresholds were reduced. For the most part these reductions were dose dependent, with 5 μg 8-OH-DPAT inducing the largest shifts in frequency thresholds. However, 2.5 μg 8-OH-DPAT lowered thresholds in rats B7, C5, and D1; 1 μg 8-OH-DPAT lowered thresholds in rat C5, but not in the other three rats. The effect of 8-OH-DPAT had an immediate onset and appeared to peak between 5 and 30 min after injection. In all cases frequency thresholds returned to baseline levels approximately 50–60 min after injection.

Figure 4 shows the mean percentage shift in baseline frequency calculated over the first 20 min postinjection for these rats. Significant effects of dose were found for rats A5, $F(3, 20) = 42.06$, $p < 0.001$, B7, $F(2, 20) = 25.84$, $p < 0.001$, C5, $F(3, 25) = 14.03$, $p < 0.001$, and D1, $F(3, 23) = 78.09$, $p < 0.001$. In rats A5, C5, and D1 8-OH-DPAT dose-dependently lowered the frequency threshold compared to saline treatment. In rat A5 saline treatment appeared to elevate the frequency threshold above baseline. However, t-tests showed that the threshold was lowered, compared to baseline level, by injection of 1 μg, $t(8) = 3.78$, $p < 0.01$, and 5 μg, $t(9) = 19.5$, $p < 0.001$, 8-OH-DPAT. The 0.2-μg dose did not lower the threshold when compared to baseline. In rat B7 saline was not injected, but 1 μg 8-OH-DPAT did not shift the frequency threshold away from the baseline level as assessed by a t-test, $t(7) = 0.21$, $p > 0.4$. The two higher doses of 8-OH-DPAT significantly reduced thresholds when compared to this dose.

In contrast to the effects of 8-OH-DPAT in these animals, frequency thresholds were not reduced by 8-OH-DPAT in the three rats with injection sites located outside the median raphe (Fig. 5). In fact, 2.5 μg 8-OH-DPAT elevated thresholds by approximately 20%, but this effect was not seen at a higher dose.

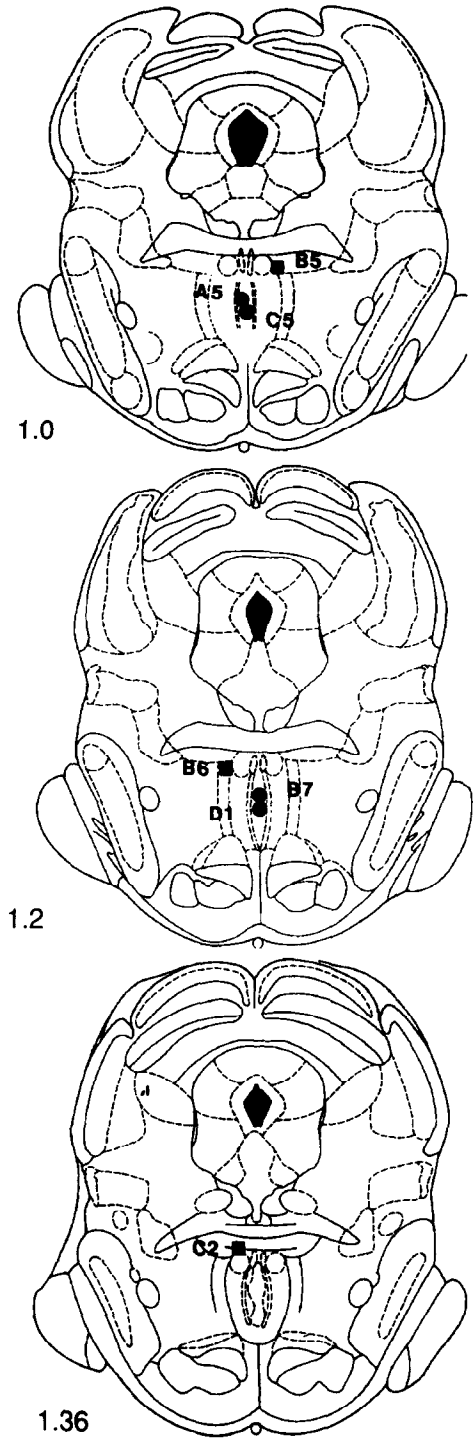

FIG. 1. Reconstruction of injection sites located in (circles) or near (squares) the median raphe nucleus. Sections are redrawn from the atlas of Paxinos and Watson (34).

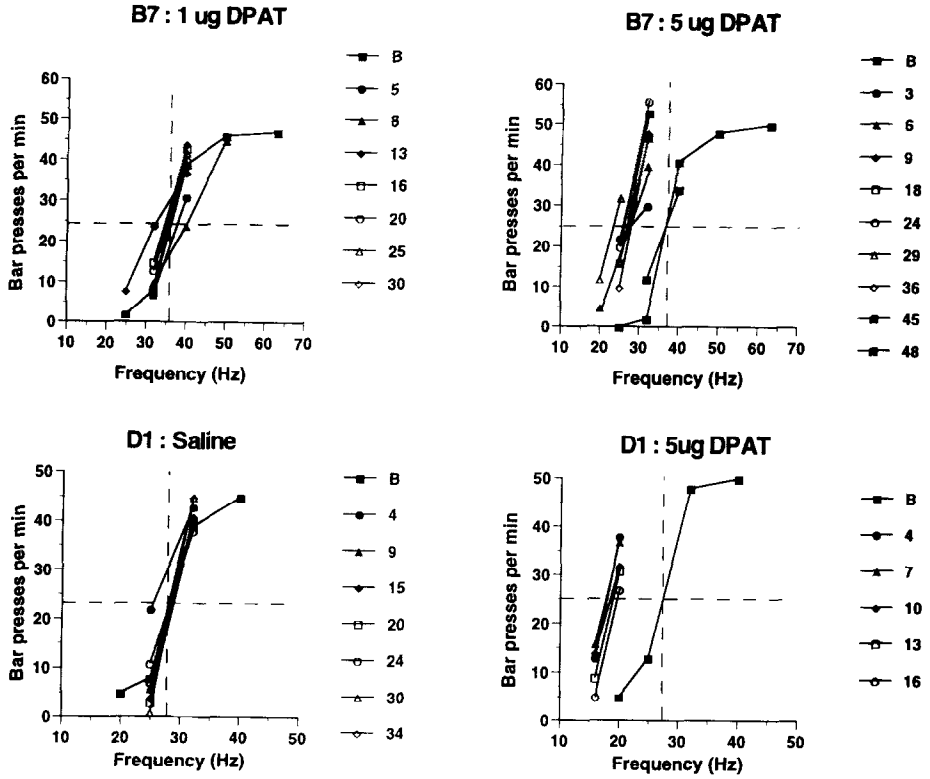

FIG. 2. Rate-frequency curves for rat B7 tested with i and 5 μg 8-OH-DPAT, and rat D1 tested with saline and 5 μg 8-OH-DPAT, at various times following injection. Numbers in the legend refer to minutes postinjection. Baseline (B) curves represent the means of three separate tests each conducted at four to five frequencies; SEMs for all points on these curves are within the symbol. All other lines were constructed from two tests with frequencies yielding above and below criterion levels of responding. The dotted lines intercepting the ordinate and abscissa represent half-maximal rates of responding, and the frequency required to maintain this level of responding, respectively.

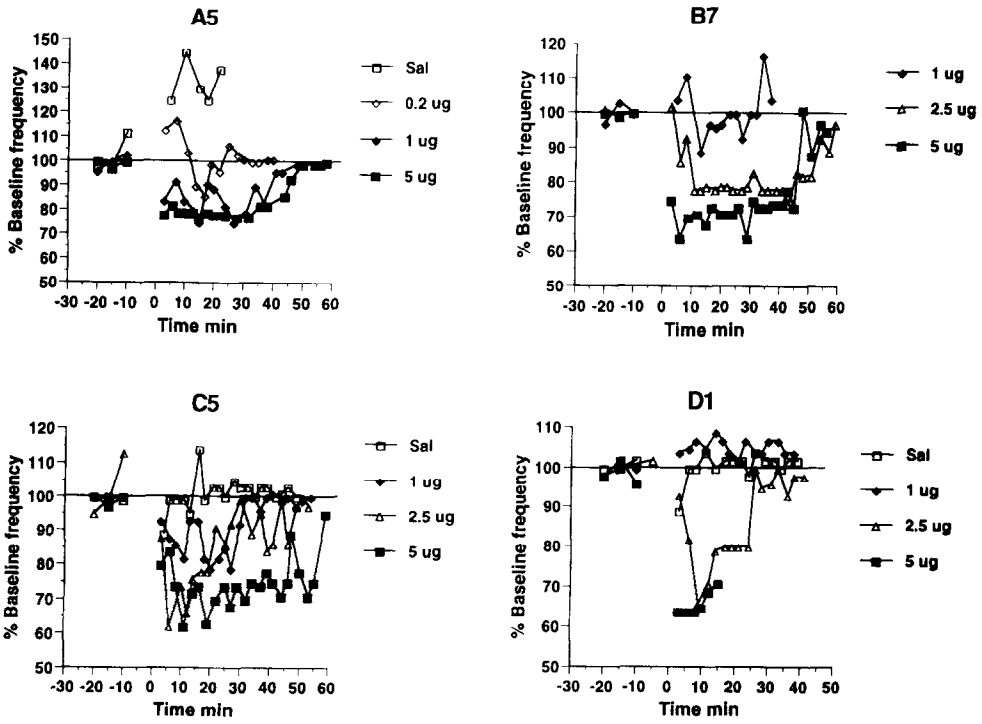

FIG. 3. Changes in frequency threshold for four rats with accurate median raphe injection sites following treatment with 8-OH-DPAT. The ordinate represents percent change in threshold from baseline levels. The abscissa represents time after injection (0 min); points to the left of 0 min show threshold changes determined prior to injection.

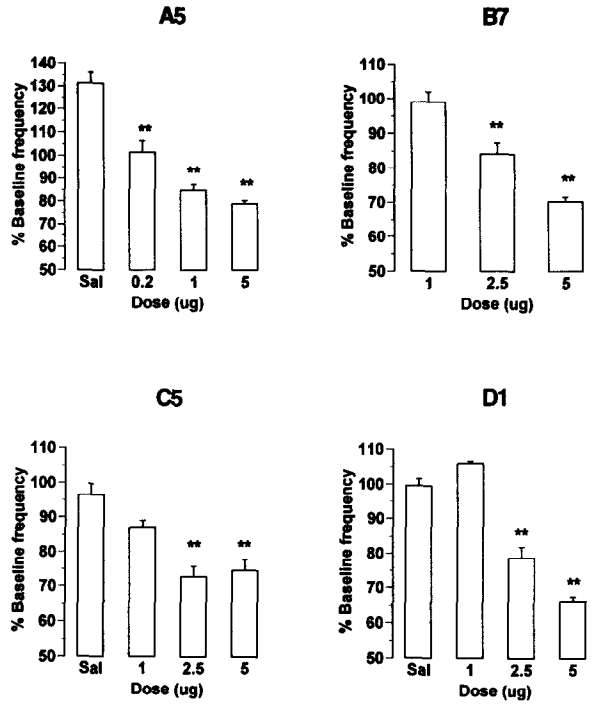

FIG. 4. Mean percentage shifts in frequency threshold following injection of 8-OH-DPAT in four rats with injection sites located in the median raphe. Values are the mean ± SEM percentage shift calculated over the first 20 min postinjection and are based on four to eight determinations per condition. $^{**}p < 0.01$ compared to Sal, or compared to 1 μg for rat B7.

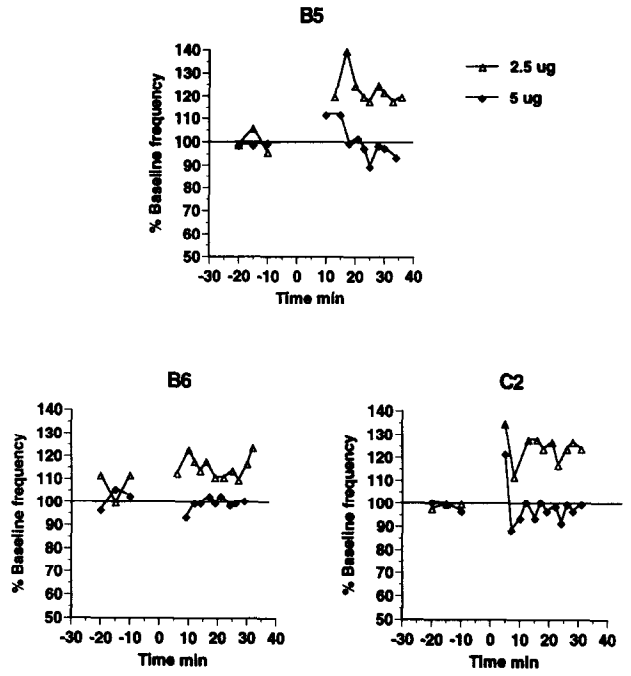

FIG. 5. Changes in frequency thresholds following injection of 2.5 and 5 μg 8-OH-DPAT in three rats with injection sites located outside the median raphe. The ordinate represents percent change in threshold from baseline levels. The abscissa represents time after injection (0 min); points to the left of 0 min show threshold changes determined prior to injection.

DISCUSSION

In rats lever pressing for LH stimulation, injections of 8-OH-DPAT directly into the median raphe nucleus lowered the frequency of pulses required to sustain half-maximal levels of responding. When injected into sites located outside the median raphe nucleus, 8-OH-DPAT did not lower frequency thresholds. The anatomical specificity of the effect of 8-OH-DPAT, together with the rapid onset of action, suggests that 8-OH-DPAT induces its effects via a localized action within the median raphe. The maximal reduction in frequency threshold observed was approximately 40% of baseline levels. This change in threshold is not as large as that observed after injection of amphetamine (14), but is similar to that reported following morphine injection into the ventral tegmental area (21).

The lowered frequency thresholds following 8-OH-DPAT injected into the median raphe nucleus shows that this treatment rendered animals more sensitive to the rewarding effects of lateral hypothalamic stimulation. This finding supports the hypothesis that median raphe injections of 8-OH-DPAT enhance responsivity to rewarding stimuli.

The lowering of frequency thresholds following median raphe injection of 8-OH-DPAT is consistent with the previously reported finding that a low dose of peripherally injected 8-OH-DPAT increased responding for a fixed frequency of LH stimulation on a VI 20 s schedule (30). Because 8-OH-DPAT is a selective 5-HT_{1A} receptor agonist and median raphe injections of this compound, over the dose range used in the present study, reduce 5-HT synthesis and/or release in terminal regions (19), it is likely that a reduction in 5-HT neurotransmission underlies these effects. Previous studies have shown also that reductions in 5-HT activity following treatment with the tryptophan hydroxylase inhibitor, para-chlorophenylalanine (35,36), or the nonselective 5-HT antagonist, methysergide (22), increased response rates for medial forebrain bundle stimulation. Thus, the results of studies using several different pharmacological manipulations provide consistent evidence that lowering brain 5-HT activity facilitates responding for rewarding brain stimulation, at least when the stimulating electrode is located within the medial forebrain bundle.

These results imply that brain 5-HT neurons may play an important role in modulating the activity of the brain's reward circuitry. Dopamine neurons, especially those projecting from the ventral tegmental area to the nucleus accumbens, are particularly important for reward-related behaviour [e.g., (46)]. Although the present study does not address directly the involvement of dopamine in mediating the effects of 8-OH-DPAT, other studies show that the activity of these dopamine neurons can be modified by alterations in 5-HT activity, with reductions in 5-HT activity apparently facilitating dopamine function (7–9). Thus, the facilitatory effect of 8-OH-DPAT on brain stimulation reward may arise from a removal of the inhibitory influence that 5-HT neurons exert over dopamine activity (7,29).

Behavioural studies provide several lines of evidence for this. Feeding induced by raphe injections of 8-OH-DPAT can be reversed by injections of flupenthixol into the nucleus accumbens (12), and the establishment of a conditioned place preference induced by 8-OH-DPAT can be reversed by dopa-

mine antagonists (33,39). These results point to an involvement of increased dopamine activity in the expression of these behaviours. Microinjections of 5-HT or LSD into the median raphe suppress the activity of 5-HT neurons and potentiate hyperactivity induced by the dopamine receptor agonist apomorphine (11). Conversely, the cataleptic effects of the dopamine receptor antagonist haloperidol are attenuated by 8-OH-DPAT injected into the dorsal or median raphe nucleus (20). Electrophysiological studies have shown that low doses of peripherally administered 8-OH-DPAT increase the firing rate of dopaminergic neurons in the VTA (2). Because this effect was not reproduced by local infusion of 8-OH-DPAT into the VTA, these authors suggest that the effect of 8-OH-DPAT is probably indirect, perhaps involving a suppression of 5-HT input to the VTA from the raphe nuclei.

Although these behavioural and electrophysiological studies support the hypothesis that 8-OH-DPAT facilitates dopamine function, results from neurochemical studies are less convincing. Although low doses of peripherally injected 8-OH-DPAT appear to increase dopamine release (3), median raphe injections of 8-OH-DPAT reduced 5-HT synthesis in various forebrain regions, but failed to significantly enhance dopamine synthesis, as measured indirectly by DOPA accumulation following decarboxylase inhibition, in the striatum and nucleus accumbens (17). However, these results are difficult to interpret because the injection procedure itself significantly enhanced DOPA accumulation. This shift in baseline was not apparent in another study (19), but these authors failed to observe any effect of 8-OH-DPAT on DOPA accumulation. Thus, the hypothesis that 8-OH-DPAT facilitates dopamine function by inhibiting 5-HT input to dopaminergic neurons is supported by behavioural results, but definitive neurochemical evidence is lacking. Facilitatory effects of lowered 5-HT activity, particularly following 5,7-DHT treatment, on dopamine-dependent behaviours are most obvious when dopamine systems have been engaged (41). For example, 5,7-DHT potentiated apomorphine-induced open field activity without altering baseline levels of activity alone (8). Given that the behaviours induced by 8-OH-DPAT, such as feeding, male sexual behaviour, locomotor activity, and lateral hypothalamic self-stimulation, involve significant activation of mesolimbic dopamine systems [e.g., (5)], it may be more appropriate to examine effects of 8-OH-DPAT on dopamine activity when dopamine systems have already been activated. In this regard it is interesting to note that injections of LSD into the median raphe increased K^+-stimulated release of dopamine from the nucleus accumbens (9).

Cholinergic neurons projecting from the pedunculopontine tegmental nuclei (PPT) to dopaminergic cell bodies in the VTA have been implicated in the rewarding effect of medial forebrain bundle self-stimulation (47,48). Disinhibition of this cholinergic system following scopolamine injections into the PPT lowers frequency thresholds by 20–80%. In contrast, inhibition of this system following carbachol injection into the PPT, or cholinergic antagonists into the VTA, greatly increases frequency thresholds. Presumably these effects involve changes in the activity of mesolimbic dopamine neurons (23). Serotonin strongly inhibits the activity of cholinergic PPT cells (24,26) via serotonergic inputs from the raphe nuclei (38,43). Also, 5-HT is believed to induce release of both dopamine and acetylcholinesterase from dopamine dendrites (31), which would protect dopamine neurons from overactivation. It can be hypothesized that removal of these inhibitory inputs, via median raphe injections of 8-OH-DPAT, increases dopaminergic activity resulting in a reduction of self-stimulation thresholds.

In summary, 8-OH-DPAT injected into the median raphe lowered frequency thresholds for rewarding LH self-stimulation. This finding shows that 8-OH-DPAT increases the sensitivity of rewarding electrical brain stimulation. Because 8-OH-DPAT selectively impedes 5-HT neurotransmission, these results indicate an important modulatory role for brain 5-HT neurons arising from the median raphe in controlling responsivity to rewarding stimuli.

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