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BRIEF COMMUNICATION

Effects of Dopamine-Receptor Blockade on Self-Stimulation in the Monkey[1]

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MORA, F., E. T. ROLLS, M. J. BURTON AND S. G. SHAW. *Effects of dopamine-receptor blockade on self-stimulation in the monkey.* PHARMAC. BIOCHEM. BEHAV. 4(2) 211–216, 1976. – In a dose-response experiment it was shown that intraperitoneal injections of 0.062 mg/kg, and 0.1 mg/kg of the dopamine-receptor blocking agent and neuroleptic spiroperidol severely attenuate self-stimulation in the orbitofrontal cortex, hypothalamus, and in the region of the locus coeruleus, in the rhesus monkey and in the squirrel monkey. In the rhesus monkey intracranial injections of 6 μg of spiroperidol bilaterally into the nucleus accumbens or the hypothalamus attenuated self-stimulation of the amygdala, and injections into the orbitofrontal cortex attenuated self-stimulation of the amygdala and lateral hypothalamus. Self-stimulation at other sites tested (including the region of the locus coeruleus) was much less affected by the injections, and injections into the region of the locus coeruleus were ineffective. These results together with other control experiments suggest that spiroperidol can attenuate self-stimulation in the monkey independently of any motor impairment or sedation produced, and that dopamine receptors in particular brain regions are involved in self-stimulation of particular brain sites.

Self-stimulation Dopamine Spiroperidol Reward Monkey

THERE is evidence that dopamine receptors are involved in self-stimulation in the rat. Self-stimulation can be obtained in the region of dopamine-containing cell bodies [2,4] and is attenuated by the administration of agents which block dopamine receptors such as haloperidol, pimozide [16] and spiroperidol [6,12]. Recently it has been shown that intracranial and intraperitoneal injections of blocking agents for dopamine receptors attenuate self-stimulation of a number of sites in addition to the lateral hypothalamus [9,11].

In this study we describe an investigation into whether dopamine receptors are involved in self-stimulation in the monkey. First, a dose-response curve of the effect of intraperitoneal injections of the dopamine-receptor blocking agent spiroperidol [1] on self-stimulation of different brain sites is described. Then the effects of intracranial injections of spiroperidol into different brain sites are described. The aims of this second experiment were to determine whether there are particular areas of the brain which are particularly sensitive to the effects of spiroperidol on self-stimulation, and to determine whether all self-stimulation sites are equally affected by dopamine-receptor blockade. The plan of the experiment was therefore to inject a given dose of spiroperidol into different brain areas, and to measure the effects on self-stimulation at different sites. This experimental design also indicates whether a side effect such as motor impairment or sedation accounts for any attenuation of self-stimulation observed (see below). The sites chosen for self-stimulation and for injections of spiroperidol were the orbitofrontal cortex, amygdala, nucleus accumbens, lateral hypothalamus and region of the locus coeruleus. Those areas were chosen because there is evidence that they are involved in self-stimulation in the monkey, in that self-stimulation occurs in those areas and in that single units in each of these areas are activated (usually trans-synaptically) in self-stimulation of each of the other areas [3, 9, 11].

METHOD

Animals

Three rhesus monkeys (*Macaca mulatta*) and two squirrel monkeys (*Saimiri sciureus*) weighing 2.8–3.25 kg and 0.50–0.70 kg were implanted under Nembutal anesthesia (0.6 mg/kg) with bilateral stainless-steel cannulae (0.6 mm o.d.). The cannulae could be used for local intracranial injection by inserting 0.3 mm o.d. cannulae connected to flexible polythene tubes into them, or for brain stimulation

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by passing stainless-steel wires insulated except for 0.2 mm at the tip through the cannulae. Using the atlas of Snider and Lee [14], the cannulae were aimed in the rhesus monkeys at the orbitofrontal cortex, nucleus accumbens, lateral hypothalamus, amygdala, and locus coeruleus. In the squirrel monkeys comparable sites were used [5]. One week after surgery the monkeys were tested for self-stimulation. The monkeys sat in a primate chair. A 0.3 sec train of 0.5 msec capacitatively-coupled negative pulses at a frequency of 100 Hz was applied to the electrode under test when the monkeys contacted a bar in front of them. When self-stimulation was found at a particular site the threshold for self-stimulation was determined by repeatedly decreasing and increasing the current, and the stability of the self-stimulation threshold and rate were confirmed over several days. The current was set to be just above the threshold current (i.e. the lowest current which would maintain continuous self-stimulation). Under these conditions the baseline self-stimulation rates for each structure in every monkey were determined.

Procedure

A dose-response curve of the effect of the dopamine-receptor blocking agent spiroperidol [1] on self-stimulation was made as follows. The spiroperidol (generously supplied by Janssen Pharmaceutica, Beerse, Belgium) was injected intraperitoneally in doses of 0.016, 0.062, and 0.1 mg/kg. Control injections were of the solvent only, 0.01 M tartaric acid. The monkeys were tested every 3 days. On a test day first the baseline rate of self-stimulation at every site was tested. Then the IP injections were given, and one hour later the self-stimulation rate was measured for each electrode for 10 one min periods, with 5 min periods allowed for changing the stimulation site and stabilization of the rate. The sites were tested in the following order: orbitofrontal cortex, amygdala, lateral hypothalamus and locus coeruleus. The self-stimulation rate over a period of 10 min for each electrode was calculated as a percentage of the corresponding mean baseline rate for that electrode to facilitate comparison between the electrodes. The plan of the experiments with intracranial injections was to compare the effects of injections of spiroperidol into different brain areas on self-stimulation of many different brain sites. In this particular experiment we used two rhesus monkeys and one of the squirrel monkeys. For the rhesus monkeys 2 μl of 3 μg/μl spiroperidol were injected bilaterally into a given site. Control injections of the solvent (0.01 M tartaric acid) were also given. The procedure was as for the IP injection tests. The self-stimulation rate over a period of 10 min for each electrode was calculated as a percentage of the corresponding mean control rate. In the squirrel monkey the procedure was as for the rhesus monkeys except that the spiroperidol (1 μg/μl in 1 μl volumes) was injected bilaterally until an effect was obtained. Haloperidol in a concentration of 2.5 μg/μl was also tested. Additional experiments with different doses were also performed (see Table 1).

Histology

At the conclusion of the experiments every site was verified histologically. The animals were anesthetized with Nembutal and a lesion (0.1 mA, 60 sec) was made to assist identification of the region in which stimulation and injections were given. The monkeys were perfused with isotonic saline followed by 10 percent formal-saline. The brains were frozen and sections were cut at 50 μ and stained with thionin. As shown in the sections from one of the rhesus monkeys illustrated in Fig. 3, the cannulae aimed at orbitofrontal cortex, lateral hypothalamus and amygdala were accurately placed, and the cannulae aimed at the nucleus accumbens ended in this particular monkey just below the nucleus accumbens close to the dura, so that the injections probably reached the nucleus accumbens, but self-stimulation did not occur probably because the stimulation electrode in protruding from the cannula touched the dura. The cannulae aimed at the locus coeruleus appeared to end within 2 mm of the locus coeruleus.

RESULTS

Intraperitoneal Injection

Dose-response curves for the effect of spiroperidol on self-stimulation in the rhesus monkeys and the squirrel monkeys are shown in Fig. 1. In this figure we represent the mean and standard error of the rate per min measured over a period of 10 min for each electrode 1 hr after injection in both groups of rhesus and squirrel monkeys. The results for each electrode were expressed as a percentage of the mean control (i.e. placebo) rate for that electrode. The mean rates for each electrode under the control condition, and on each testing day before injection, remained constant throughout the experiments. The experiments were repeated twice in rhesus monkey 1 at each drug dose and the same results were obtained in the two separate experimental sessions (see Fig. 2).

It is shown in Fig. 1 that in both the rhesus monkeys and the squirrel monkeys self-stimulation at all the sites tested was severely attenuated by a dose of spiroperidol of 0.062 or 0.1 mg/kg. At the lower dose of 0.016 mg/kg self-stimulation at most sites was unaffected, but self-stimulation of the orbitofrontal cortex was attenuated in two of the rhesus monkeys and one of the squirrel monkeys. The typical time course of the attenuation of self-stimulation by spiroperidol, and the repeatability of the results are illustrated in the two separate runs of the experiment in rhesus monkey 1 shown in Fig. 2. The results shown in Figs. 1 and 2 suggest that with a dose of 0.016 mg/kg of spiroperidol the effect on self-stimulation depends on which self-stimulation site is tested, and suggest that orbitofrontal cortex self-stimulation is particularly sensitive to dopamine-receptor blockade.

Intracranial Injection

The self-stimulation rate (expressed as a percentage of the preinjection control rate) for different sites one hr after injection of solutions of spiroperidol bilaterally into different brain areas of the monkeys is shown in Table 1. The error figures represent an estimate of the standard errors calculated from 10 one-minute observations of self-stimulation rate; and the significance of the difference of these observations from the preinjection control rates using a t-test and confirmed with a U test is shown by $*p<0.02$ or $\dagger p<0.001$.

The largest effect found with a 6 μg dose was attenuation of the rate of self-stimulation of the amygdala by 70 percent by injection into the nucleus accumbens (see Table

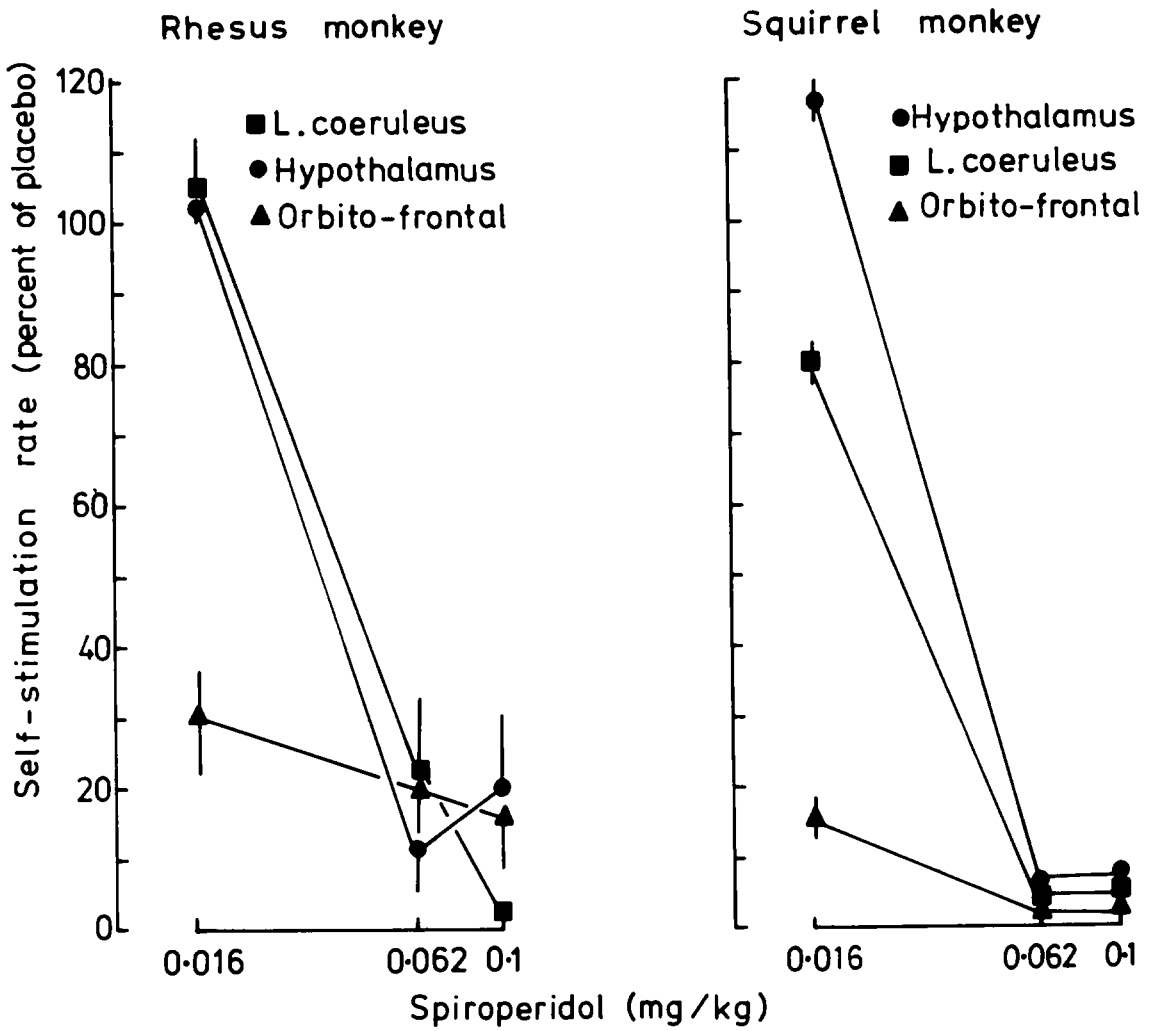

FIG. 1. Dose-response curves for the effect of IP injections of spiroperidol on self-stimulation rate at different sites in 3 rhesus monkeys and 2 squirrel monkeys. The self-stimulation rate for a particular electrode is shown as a percentage of the self-stimulation rate after a placebo injection, and for the rhesus monkey is the mean of two separate experimental determinations of every point. The vertical lines indicate an estimate of the standard error of the ten one-minute observations of self-stimulation rate. The self-stimulation electrodes were in the hypothalamus, the orbitofrontal cortex, and near the locus coeruleus.

1). Similar results, and dose-related effects, were seen with 9 μg of spiroperidol in which the attenuation of self-stimulation of the amygdala was 99 percent. This effect was confirmed with the squirrel monkey in which 5 μg of haloperidol injected into the same structure (nucleus accumbens) totally attenuated self-stimulation of the amygdala. Observations in other structures were that injections into the orbitofrontal cortex (O. F. Cortex) partially attenuated self-stimulation of the hypothalamus (by 46 percent) and of the amygdala (by 32 percent), and injections into the hypothalamus attenuated self-stimulation of the amygdala (by 35 percent). There was never any large effect of injections of spiroperidol into the region of the locus coeruleus or of injections into other areas on self-stimulation of the region of the locus coeruleus. These results were confirmed in additional experiments (see Table 1). The latency for a measurable effect was 10–20 min, and the effect reached its maximum at 20–30 min and remained at this level for more than 4 hr.

DISCUSSION

Intraperitoneal Injections

The experiment with intraperitoneal injections shows that in the monkey spiroperidol attenuates intracranial self-stimulation at the different sites tested at doses of 0.062 and 0.1 mg/kg. At the lower dose of 0.016 mg/kg some evidence was obtained that orbitofrontal cortex self-stimulation is particularly sensitive to dopamine-receptor blockade. Although the animals could still work for glucose at a dose of 0.062 mg/kg of spiroperidol, a general effect of the spiroperidol on behavior (such as a motor impairment or sedation) was suspected, since at the higher dose of 0.1 mg/kg the monkeys looked drowsy. To determine whether spiroperidol attenuated self-stimulation by this type of general effect on behavior or by a specific effect on self-stimulation, in Experiment 2 spiroperidol was injected directly into different brain sites. An attenuation of self-stimulation at only some self-stimulation sites would

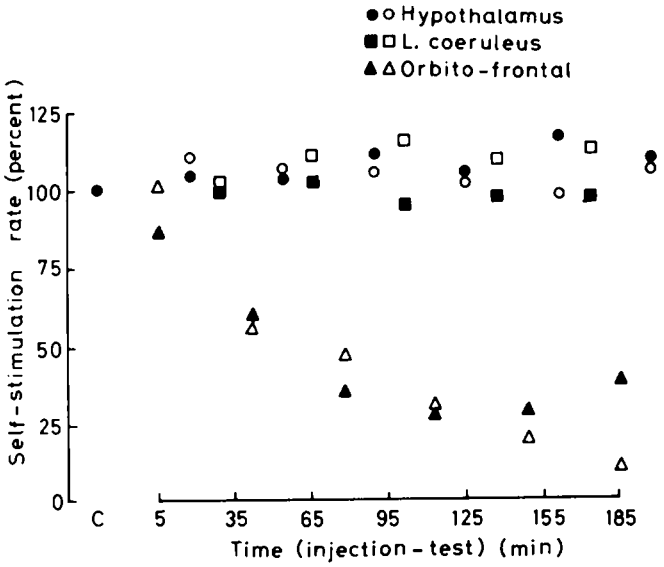

FIG. 2. The effect of 0.016 mg/kg of spiroperidol IP on self-stimulation with electrodes in the hypothalamus, orbitofrontal cortex and in the region of locus coeruleus, in rhesus monkey 1. The solid and open symbols represent the two separate experimental determinations of every point. The self-stimulation rate for a particular electrode is shown as a percentage of the self-stimulation rate after a placebo injection. C represents the control rate.

show that the effect on behavior was specific, and would provide evidence on the neural system which was involved in self-stimulation of these particular self-stimulation sites.

Intracranial Injections

The finding with intracranial injections of spiroperidol that self-stimulation in the region of the locus coeruleus was almost unaffected even when self-stimulation at some other sites was severely attenuated suggests that the attenuation of self-stimulation found at the affected site was not due to a general effect on behavior such as motor impairment or sedation. In addition, after the tests for self-stimulation the monkeys were able to perform the motor response task to obtain 20 percent glucose solution (see Table 1). Thus it appears that the attenuation of self-stimulation found at some sites can not be explained by a single effect such as motor impairment [8]. The finding that injections of spiroperidol into the orbitofrontal cortex attenuated self-stimulation of the amygdala and lateral hypothalamus suggests that the orbitofrontal cortex is involved in self-stimulation of some limbic structures. This suggestion is consistent with first, the electrophysiological findings that single units in the orbitofrontal cortex are activated in self-stimulation of the lateral hypothalamus and amygdala and vice versa [3], second, that anesthetization of the sulcal prefrontal cortex in the rat (which may

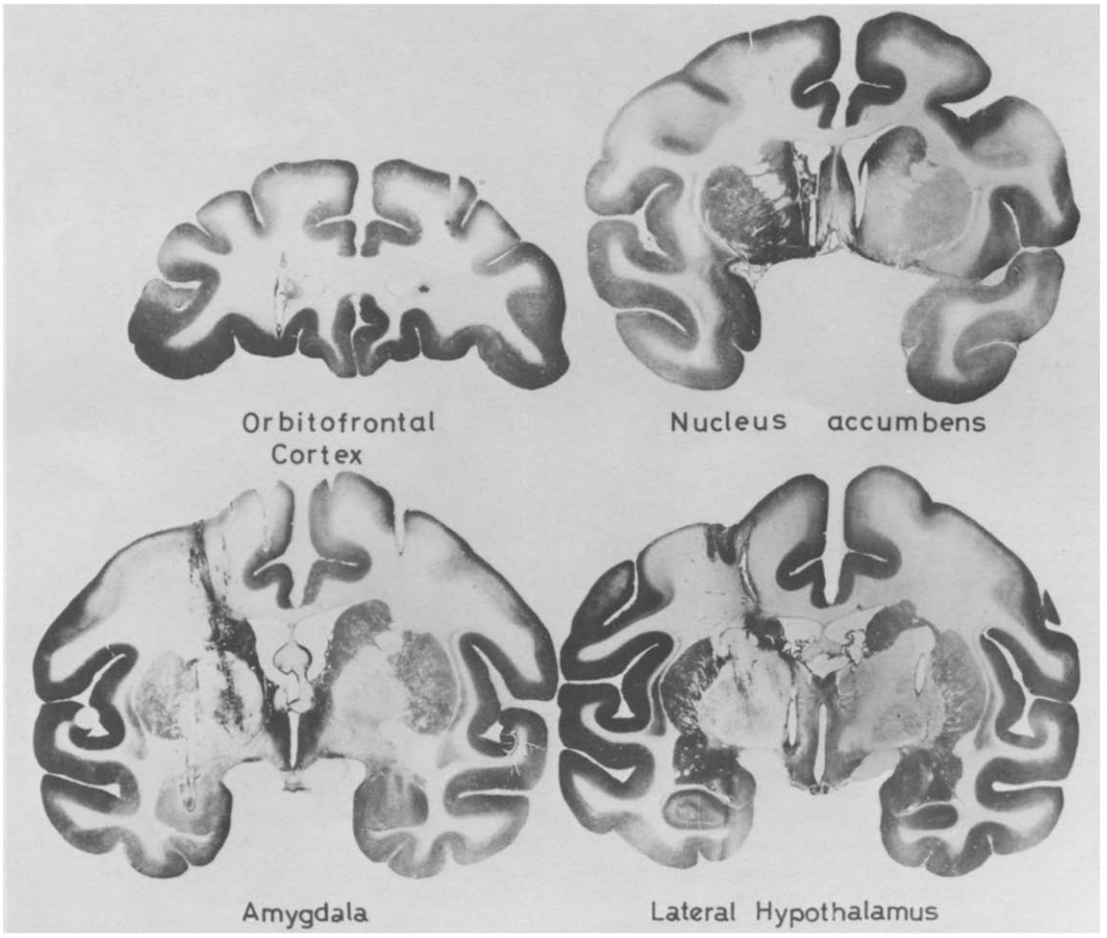

FIG. 3. Sites of cannulae used for injection and for stimulation in the rhesus monkey. No self-stimulation occurred at the nucleus accumbens site in this monkey, because the stimulation site was close to the base of the brain.

TABLE 1

EFFECTS OF INTRACRANIAL INJECTIONS OF SPIROPERIDOL ON SELF-STIMULATION

Monkeys	Injection Site Spiroperidol	Self-Stimulation Rate (Percent of Control Rate) O.F. Cortex	Amygdala	Hypothalamus	L. Coeruleus	N. Accumbens	Rate for Glucose as Percent of Control Rate	Volume Injected Bilaterally	Concentration
R1 and R2	O.F. Cortex	–	68 ± 10*	54 ± 13.2*	82 ± 9.2	–	87 ± 4.6	2 μl	3 μg/μl
R1 and R2	N. Accumbens	74 ± 4.3	30 ± 10†	96 ± 6.3	92 ± 4.6	–	160 ± 3.0	2 μl	3 μg/μl
R1 and R2	Amygdala	87 ± 10.0	–	82 ± 2.6	81 ± 3.3	–	92 ± 0.6	2 μl	3 μg/μl
R1 and R2	Hypothalamus	90 ± 4.3	65 ± 3*	–	82 ± 1.3	–	100 ± 5.0	2 μl	3 μg/μl
R1 and R2	L. Coeruleus	103 ± 4.3	112 ± 6	120 ± 4.0	–	–	–	2 μl	3 μg/μl
				Additional Experiments					
R1	Amygdala	32	–	64	85	–	–	3 μl	4 μg/μl
R1	N. Accumbens	67	1	71	50	–	–	3 μg	3 μg/μl
R1	O.F. Cortex	–	–	47	83	–	–	2 μl	3 μg/μl
R2	O.F. Cortex	12		–	–	18		2 μl	3 μg/μl
R2	N. Accumbens	25	–		–	4	–	3 μl	4 μg/μl
R2	Amygdala	84	–	–	–	36	–	2 μl	3 μg/μl
R2	Hypothalamus	98	33	–	–	117	–	2 μl	3 μg/μl
								(Haloperidol)	
SQ5	N. Accumbens	–	0			0		2 μl	2.5 μg/μl

*$p<0.02$ †$p<0.001$

correspond to the orbitofrontal cortex of the monkey) [7] attenuated self-stimulation of the lateral hypothalamus [10], third, that there are dopaminergic terminals in the region of the prefrontal cortex in the rat [15], and fourth, that single cells in this prefrontal region which are activated during self-stimulation of the lateral hypothalamus of the rat alter their firing rate when a dopaminergic agonist (apomorphine) or antagonist (spiroperidol) is administered systemically in low doses (personal observations). Thus, dopaminergic connections to the orbitofrontal cortex may be involved in self-stimulation of a number of different sites.

Although it is difficult to interpret at this stage, it is of interest that injections into the nucleus accumbens and lateral hypothalamus attenuated self-stimulation of the amygdala. It is known that single units in these two areas are activated during self-stimulation [3].

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