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EFFECTS OF SPIPERONE ON SELF-STIMULATION AND OTHER ACTIVITIES OF THE MONGOLIAN GERBIL

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1 Self-stimulation to lever pressing and capacitance probe touching was obtained in Mongolian gerbils (*Meriones unguiculatus*) from electrode placements within the medial forebrain bundle.

2 Lever pressing was more sensitive to the decremental effects of a central depressant, pentobarbitone, than capacitance probe touching, suggesting its greater responsiveness to disturbances of motor function.

3 Spiperone (0.005 to 0.05 mg/kg) attenuated capacitance probe touching and lever pressing equally, a finding explained by action on either reward pathways or on the ability to initiate responding.

4 This same dose range of spiperone (0.005 to 0.05 mg/kg) attenuated locomotor activity, whether spontaneous or evoked by non-contingent electrical stimulation, and produced catalepsy.

5 The spiperone-induced attentuation of self-stimulation was not necessarily a result of its action on dopaminergic reward pathways since the effects could equally well be explained by a failure to initiate responding.

Introduction

Intraperitoneal injections of the neuroleptics, pimozide and spiperone, attenuate self-stimulation obtained from electrode placements in various mesencephalic and prosencephalic sites (Rolls, 1974; Mora, Rolls, Burton & Shaw, 1976). Since these compounds, at least in small doses, are thought to be selective antagonists at dopamine receptors (Andén, Butcher, Corrodi, Fuxe & Ungerstedt, 1970), the existence of an ascending dopaminergic reward pathway was inferred. However, in self-stimulation studies, electrical stimuli are delivered in response to a motor task, usually lever-pressing and therefore a reduction in responding after neuroleptics could be secondary to a performance deficit rather than an action on a reward pathway. Thus it has been argued that attenuation after neuroleptics is due to impairment of motor abilities essential to lever pressing (for references see Rolls, 1974).

Attempts have been made to minimize the motor components of self-stimulation, for example, in the rate-free procedure in which lever pressing is replaced by a less complex motor task (Valenstein & Meyers, 1964). The present paper compares the effects of spiperone on self-stimulation obtained with conventional lever pressing to its effects on self-stimulation obtained with a capacitance probe requiring only touch, it being reasoned that the more complex lever pressing task would be more sensitive to disruption of motor function; thus these two systems were affected differently by pentobarbitone. We also describe the effects of spiperone on a simple test for catalepsy and on locomotor activity.

Methods

Female gerbils (*Meriones unguiculatus*, 60–70 g), under halothane anaesthesia, were implanted with bipolar electrodes, constructed from 0.076 mm diameter insulated platinum wire; resistance of implanted electrodes varied between 20 and 70 kohms. The stereotactic co-ordinates, for the medial forebrain bundle were P, 1.1; L, 1.4 and D, 6.25 according to the atlas of Loskota, Lomax & Verity (1974).

At least one week was allowed for recovery after which time gerbils were trained to touch a capacitance probe ('Omron' type TLB KSR: Tateisi Electronics, Japan) or to press a conventional lever (depression weight 13 g) to obtain brain stimulation. Each stimulus consisted of a 500 ms train of 100 monophasic rectangular pulses/s (pulse width 1 ms) from a constant current source. The current required

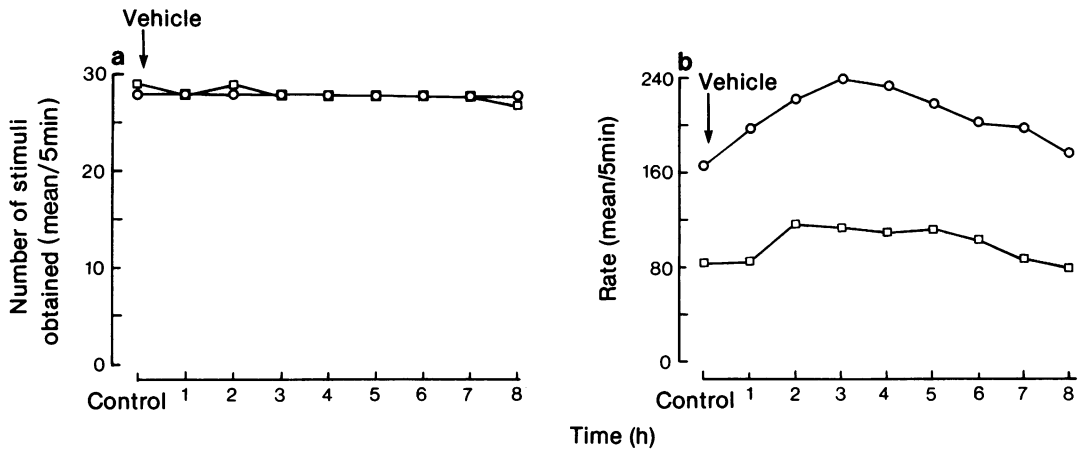

Figure 1 Mean self-stimulation rates of 5 gerbils receiving spiperone vehicle (0.25 ml/100 g s.c.) at 50 minutes. The mean rates are calculated from the average of the twelve 5 min counts obtained from each animal every hour; the control values are derived from ten 5 min counts: (a) shows the mean number of stimuli obtained on the 10 s fixed interval schedule and (b) shows the rate of responding. (○): Capacitance probe; (□): lever. The two experiments with the different manipulanda were separated by an interval of 1 week.

to maintain self-stimulation was determined for each animal and ranged from 50–300 μA. The stimulation parameters were monitored throughout experiments on an oscilloscope. To facilitate a steady rate of pressing for at least 9 h, a fixed interval schedule of 10s was implemented.

Gerbils were given two training sessions, the first to determine the optimum current for evoking consistent responding and the second to give a 9 h control for the subsequent drug experiments. During the experimental sessions, spiperone was administered subcutaneously after an initial 50 min control period. Five different gerbils were tested with each dose of spiperone (0.005, 0.0075, 0.01, 0.025 and 0.05 mg/kg s.c.); the tests for capacitance probe touching and lever pressing were made in the same gerbils at an interval of 1 week.

In the test for catalepsy, 54 gerbils were divided into 6 groups each of 9 gerbils. The groups were each prescribed a different dose of spiperone (5 groups) or control vehicle (1 group). The gerbils were then placed singly with their forepaws across a horizontal bar and their hindpaws across a second parallel bar placed 6.5 cm in the same horizontal plane. The time the gerbils remained in position was determined 10 times, each determination being made immediately after the other. The gerbils then received the appropriate drug treatment and the procedure was repeated after 2.5 hours.

To measure spontaneous locomotor activity, 30 gerbils were divided into 6 equal groups. The gerbils in one group received control vehicle, the remaining groups each receiving a different dose of spiperone. Immediately afterwards each group was placed in a cage on an Animex activity meter, the activity of each group was expressed as a percentage of the activity of the control group.

To determine the effects of spiperone on locomotor activity evoked by non-contingent electrical stimulation of the medial forebrain bundle, gerbils were placed singly into the experimental chamber ($n = 4$). Locomotor activity, detected by Animex activity meters, was evoked by electrical stimulation parameters similar to those used for self-stimulation, except that the stimuli were presented automatically every 10 s for 2 periods each of 1 h, before and 2.5 h after spiperone.

Drugs used were spiperone (Janssen Pharmaceuticals) and pentobarbitone sodium (Sagatal, May & Baker, Ltd.). Spiperone was dissolved in 0.01 N acetic acid (1 mg/1 ml) and then diluted with 0.9% w/v NaCl solution (saline) so that the required dose was contained in 0.25 ml/100 g body weight.

Results

The mean response rates and number of stimuli obtained on lever pressing (5 animals) with those obtained on capacitance probe touching (5 animals), over an 8 h experimental period can be compared in Figure 1. The 'spiperone vehicle' (0.25 ml/100 g s.c.) was given at 50 min after the start of the experiment. While response rates in both situations increased by between 35 and 45% over the first 3 h (Figure 1b) declining to approximately the control

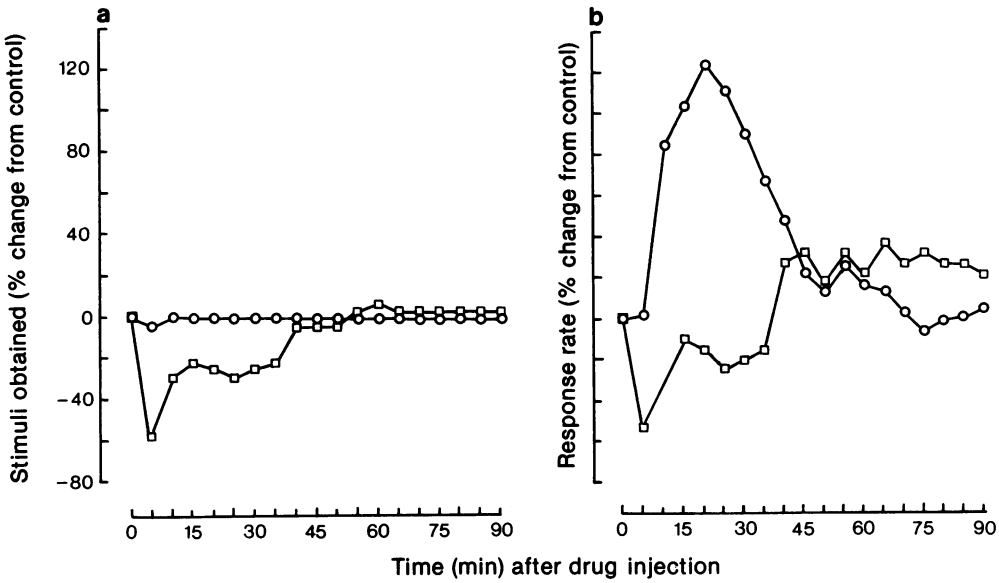

Figure 2 Mean self-stimulation rates of 5 gerbils receiving pentobarbitone sodium (22 mg/kg s.c.) at zero time. Control rates are calculated as described in Figure 1. (a) Shows the stimuli obtained and (b) the rate of responding as percentage changes from the control rates. (○): Capacitance probe; (□): lever. The two experiments with the different manipulanda were separated by an interval of 1 week.

rate at 8 h, the number of stimuli obtained showed no significant change throughout the entire period (Figure 1a).

Since lever pressing is a more complex task than capacitance probe touching, it was expected that a drug which reduced self-stimulation by impairing the ability to perform operant tasks rather than by a specific action on reward pathways would more readily affect lever pressing. Evidence that this was so was first obtained with sodium pentobarbitone (Figure 2). Thus 20 min after pentobarbitone (22 mg/kg s.c.) the number of lever presses and number of stimuli obtained were reduced by 15% and 25% respectively, from control rates of 63/5 min and 28/5 minutes. In contrast the number of capacitance probe touches increased by 125%, from 47/5 min to 106/5 minutes; the number of stimuli obtained remained constant at 28/5 minutes. These results indicate that lever pressing is more sensitive than probe touching to an impairment of motor ability. Hence a gross decrement in motor performance would be manifest as a difference between the dose-response curves obtained with the two manipulanda. Larger doses of pentobarbitone attenuated both level pressing and capacitance probe touching.

After spiperone (0.005 to 0.05 mg/kg s.c.) only a dose-related decrease in self-stimulation was seen whether elicited by lever pressing or by capacitance probe touching (Figure 3); the animals were alert and did not appear sedated. A smaller dose of spiperone (0.001 mg/kg) lacked effect. In the capacitance probe experiments, small doses of spiperone (0.005 and 0.0075 mg/kg) produced a gradual attenuation of self-stimulation, the effect increasing over the ensuing 9 h, whereas larger doses (0.01, 0.025 and 0.05 mg/kg) produced rapid abolition of self-stimulation with paradoxically speedier recovery. Indeed, self-stimulation recommenced sooner after 0.05 mg/kg than after 0.01 mg/kg. viz 3 h compared with 4.5 h (cf. Figure 3d and 3f). In experiments with the lever, the decline in self-stimulation after spiperone was similar to that seen with the probe. This is seen in Figure 4 in which the mean effects of spiperone over the first 3 h after injection, on self-stimulation in response to capacitance probe touching and to lever pressing are compared. However, recovery of lever pressing was slower than recovery of capacitance probe touching. For example, in a gerbil in which the effects of spiperone (0.05 mg/kg s.c.) were studied on both lever pressing and, one week later, on capacitance probe touching, self-stimulation to capacitance probe touching recommenced after 4 h compared with 16 h for lever pressing.

Behaviour of self-stimulating gerbils during onset of, and recovery from effects of spiperone suggested that catalepsy could have contributed to the reduc-

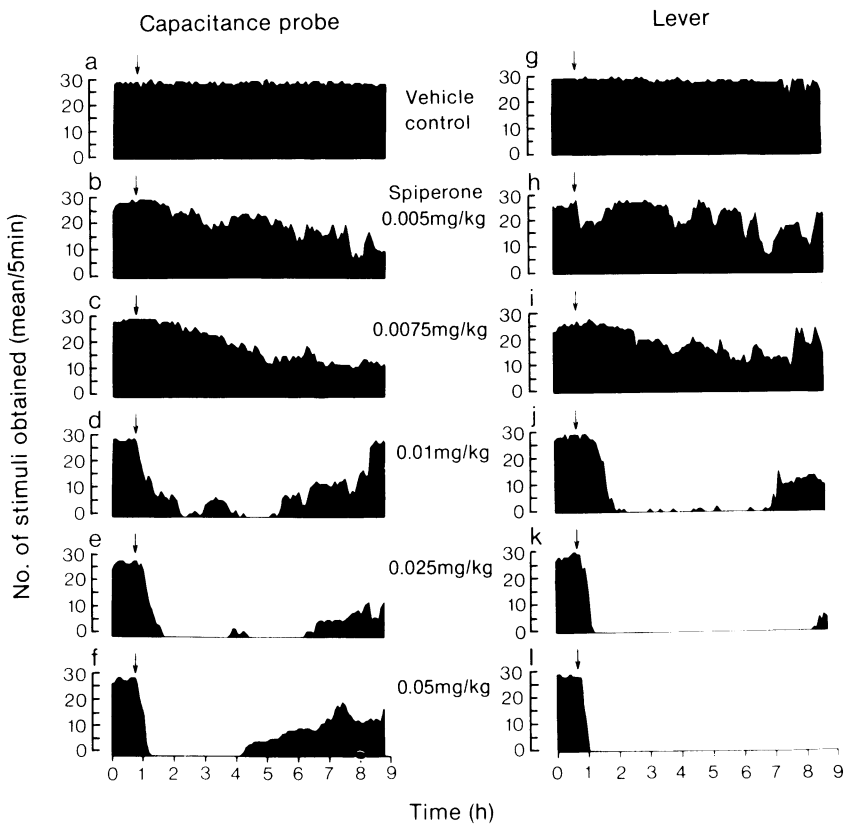

Figure 3 Mean effects of spiperone and control vehicle on capacitance probe touching and on lever pressing. Control vehicle or different doses of spiperone were given to 6 groups of gerbils (each group contained 5 gerbils, $n = 30$) at 50 minutes. The mean number of stimuli obtained in consecutive 5 min periods from each of the groups is plotted. Each gerbil was tested for both capacitance probe touching and lever pressing, these experiments being separated by an interval of 1 week.

tion in responding. Thus, immediately after delivery of each electrical stimulus, all animals remained immobile for a few seconds before resuming any activity. In about half the gerbils tested this restoration of activity was preceded by a single jump, the gerbils hitting the roof of the box with their snouts (the roof was lined with latex to prevent injury). Since the gerbils were not sedated after spiperone, the immobility may have been due to catalepsy. Therefore, the ability of spiperone to induce catalepsy was determined from the time that gerbils remained across two horizontal bars, measured on 10 occasions. On the first three trials, approximately 2.5 h after spiperone, treated animals responded similarly to controls but on the 4th and subsequent trials gerbils exhibited a marked dose-dependent catalepsy (Figure 5); the difference between control and spiperone (0.05 mg/kg) was significant ($P < 0.05$) on the 10th trial.

Catalepsy would also explain the highly significant reductions in both spontaneous motor activity ($P < 0.005$; Figure 6) and in motor activity evoked by non-contingent electrical stimulation of self-stimulation sites ($P < 0.001$; Figure 7). Non-contingent electrical stimulation produced a characteristic behavioural response in gerbils tested 2.5 h after spiperone (0.025 mg/kg). Thus after delivery of approximately 10 stimuli, gerbils made a single jump landing on both fore and hind paws. With subsequent stimuli, they gradually assumed a rearing posture and again jumped, to land once more on both fore and hind paws. This behavioural cycle was repeated throughout the experimental period.

Discussion

The results confirm that self-stimulation may be obtained from electrode placements within the medial forebrain bundle of gerbils (Routtenberg & Kramis, 1967). With a fixed interval schedule (10 s), the reinforcement rate was constant over at least 8 h per-

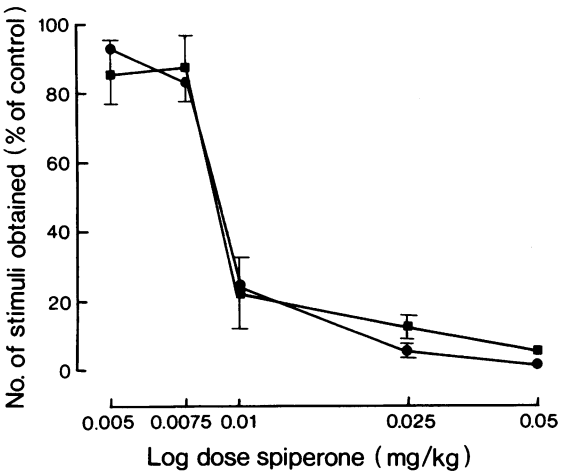

Figure 4 Log dose-response curves to the effects of spiperone on self-stimulation in gerbils. (●): Capacitance probe; (■): lever. Each point represents the mean effect observed in 5 animals over the first 3 h after drug injection. The data were obtained from the individual 5 min means plotted in Figure 3 and expressed as a percentage of the stimuli obtained in the control group (n = 30). The vertical bars represent s.e. means, those for the highest dose of spiperone being smaller than the symbols.

mitting the course of action of the drug under test to be studied. By using a conventional lever and a capacitance probe in separate experiments it was hoped to be able to identify decrements of self-stimulation performance due to a selective action on reward pathways rather than on motor performance. Recently Huston & Ornstein (1976) have demonstrated that the lever press response is more complex than previously realized, since the apparent attenuation of reward produced by various pharmacological or surgical manipulations merely reflected an inability to lever press; simpler responses were unaffected. In this study, pentobarbitone (22 mg/kg) increased self-stimulation to capacitance probe touching but decreased self-stimulation to lever pressing. In contrast to pentobarbitone, spiperone attenuated self-stimulation equally in the two experimental situations. It therefore appeared unlikely that spiperone impaired the ability of gerbils to lever press. Unexpectedly, the recovery phases differed considerably, recovery occurring much sooner with the capacitance probe than with the lever; the reason for the difference is not known but presumably relates to the differing complexities of the two motor tasks.

However, the finding that spiperone produced catatonia suggests an alternative explanation to an action on a reward pathway since a spiperone-induced difficulty in initiating motor activity would also attenuate

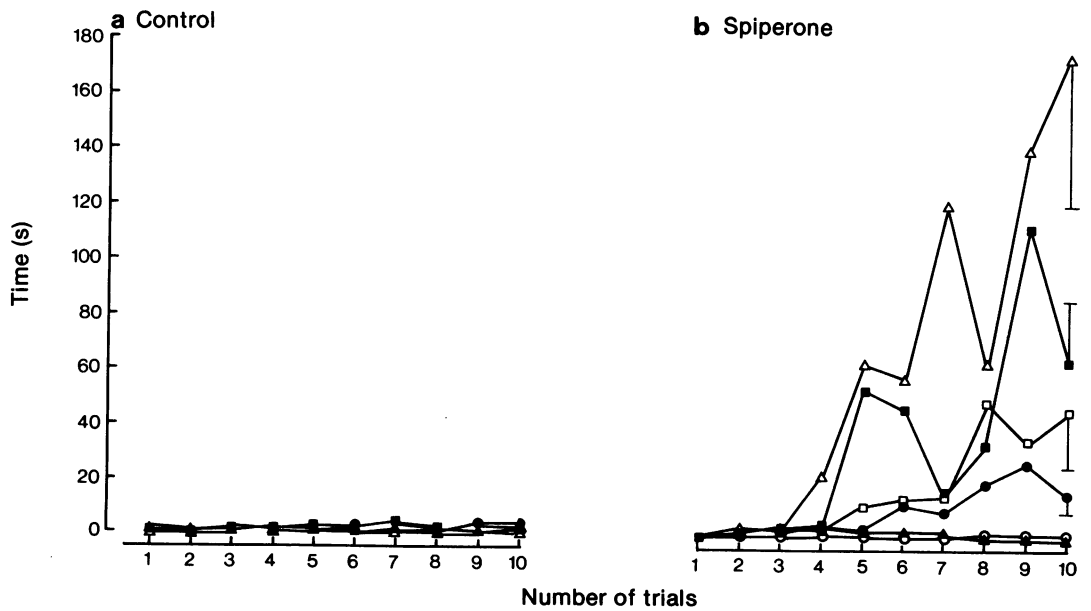

Figure 5 Effects of number of trials on spiperone-induced catalepsy in gerbils. The symbols represent the mean time that gerbils remained across horizontal bars. The vertical bars at the 10th trial indicate s.e. of means; those for the lowest dose of spiperone and vehicle control were smaller than the symbol. Animals were tested 2.5 h after spiperone vehicle (▲) or spiperone 0.005 mg/kg (○), 0.0075 mg/kg (●), 0.01 mg/kg (□), 0.025 mg/kg (■) and 0.05 mg/kg (△). Nine animals were tested at each dose level (n = 54).

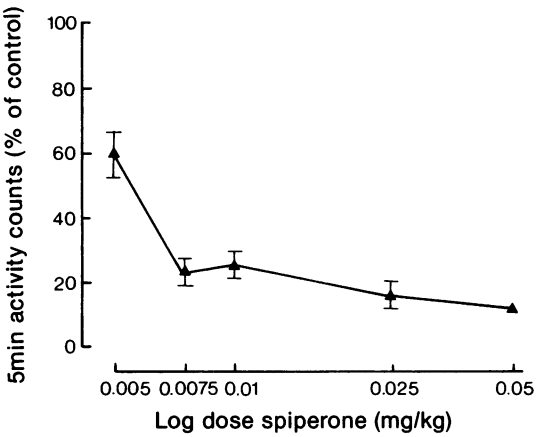

Figure 6 Log dose-response curve to the effects of spiperone on spontaneous locomotor activity in gerbils. As in Figure 4, each point represents the mean effect observed in 5 animals over the first 3 h after drug injection and is expressed as a percentage of the activity in the vehicle control group ($n = 30$). The reductions in activity after the different doses of spiperone are all significant ($P < 0.005$). The vertical bars represent s.e. means, that for the highest dose of spiperone being smaller than the symbol.

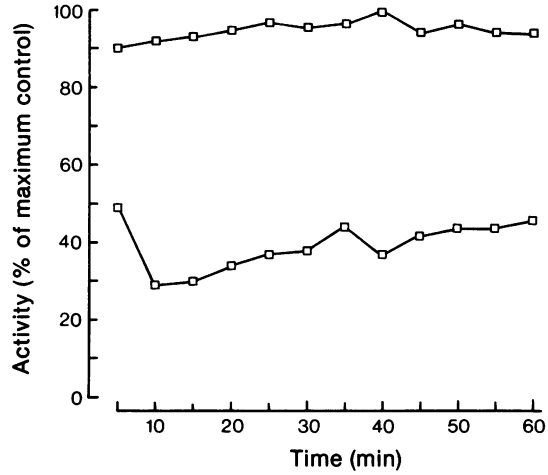

Figure 7 Spiperone-induced attenuation of locomotor activity evoked by non-contingent electrical stimulation of the median forebrain bundle in the gerbil. The mean 5 min activity counts are expressed as percentages of the maximum 5 min activity count obtained in the control period ($n = 4$). The lower trace starts 2.5 h after spiperone 0.025 mg/kg s.c.) and is significantly different from the control trace ($P < 0.001$).

self-stimulation and, more importantly, would affect lever pressing and capacitance probe touching equally. Difficulty in initiating a response was the explanation given for a selective action of haloperidol on conditioned avoidance as opposed to escape responding (Fibiger, Zis & Phillips, 1975). Interestingly, failure to initiate a response would affect operant-controlled feeding and drinking more than *ad libitum* feeding and drinking (Rolls, Rolls, Kelly, Shaw, Wood & Dale, 1974; Rolls, 1974).

While a cataleptic posture was a prominent behavioural feature following self-stimulation in spiperone-treated gerbils, in a non-operant test, catalepsy was only demonstrable on the 4th and subsequent trials that treated gerbils were tested. Recently Stanley & Glick (1976) have shown repeated testing to enhance dramatically haloperidol-induced catalepsy in rats. Perhaps in the self-stimulation experiments, the electrical stimuli subserved a similar function to the stimuli from repeated testing. The cataleptic posture induced by self-stimulation in spiperone-treated gerbils was frequently terminated by a 'jump'. Jumping was also seen in spiperone-treated gerbils receiving non-contingent electrical stimulation. This was unlikely to be related to the gerbils' propensity to exhibit epileptiform seizures (Thiessen, Lindzey & Friend, 1968; Kaplan & Miezejeski, 1972) since a similar exaggerated movement (a backward jump) was frequently seen to terminate spiperone-induced catalepsy in rats, the forepaws of which were placed against an elevated hoizontal bar (P. Jenner, personal communication). In gerbils, this jump, whether a reflex act or an exaggerated voluntary movement, restored normal activity suggesting a parallel with the clinical finding that in Parkinsonian patients, a strong environmental stimulus may temporarily overcome akinesia (Selby, 1968).

The results demonstrate that in gerbils, the selective dopamine antagonist, spiperone, attenuates self-stimulation evoked from sites within the medial forebrain bundle. From its similar effects on lever pressing and capacitance probe touching it was concluded that spiperone was attenuating self-stimulation either by reducing reward (by action on dopaminergic reward pathways) or by impairing motor initiation (by interfering with the dopaminergic-cholinergic balance within the extrapyramidal system). The latter explanation is favoured by findings that similar doses of spiperone produced catalepsy and reduced motor activity whether spontaneous or evoked by non-contingent electrical stimulation.

Separation of the effects of dopamine on reward pathways from effects on motor pathways, in self-stimulation experiments in which reward is contingent upon a motor task, can only be achieved if the receptors in the two pathways can be shown to be

different and selective antagonists become available or, if the reward component of self-stimulation mediated by another system does not involve dopamine. This last possibility is being investigated with self-stimulation evoked from the ascending coeruleocortical system.

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