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Subcutaneous Injections of Apomorphine, Stimulus Generalization and Conditioning: Serious Pitfalls for the Examiner Using Apomorphine as a Tool

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COOLS, A. R., C. L. E. BROEKKAMP AND J. M. VAN ROSSUM. *Subcutaneous injections of apomorphine, stimulus generalization and conditioning: serious pitfalls for the examiner using apomorphine as a tool.* PHARMAC. BIOCHEM. BEHAV. 6(6) 705–708, 1977. – This report shows that stimulus generalization occurs in rats conditioned by a single injection of apomorphine. The data suggest that apomorphine initially acts as an unconditioned stimulus (UCS) of an unconditioned response (UCR) that, in turn, produces stimuli which become conditioned stimuli (CS) of a conditioned response (CR) having a nature identical to that of the UCR. The study also shows that behaviour elicited by a subcutaneous injection of apomorphine depends on the part of the body selected for administration. The mentioned properties should be taken into account when apomorphine is used as a tool in studies on brain and behaviour.

Apomorphine Site of SC injection Stimulus generalization Conditioning

APOMORPHINE BEING a potent agonist of dopamine receptors is believed to be a valid tool for elucidating biochemical, neurophysiological, psychopharmacological and behavioural aspects of central dopaminergic systems [1,12]. Despite this, the use of this drug may lead to several pitfalls. Firstly, there is no simple log dose-effect relationship as revealed by the results of a quantitative analysis of apomorphine-induced behavioural effects [8]. Secondly, the behaviour elicited by a subcutaneous injection depends on the part of the body selected for the injection. And, thirdly, apomorphine has such a strong conditioning effect that apomorphine-treated animals cannot be used twice within a certain period unless one wants to study the conditioning effects of apomorphine. This report presenting hard evidence in favour of the two last-mentioned pitfalls shows that apomorphine can only be used as a valid tool on condition that the properties described below are seriously taken into account.

Apomorphine given to rats induces the continuous display of sniffing, licking and gnawing depending on the route of administration and dosage given [16,22]. It has been suggested that the apomorphine-induced effects might be considered as the result of a conditioning process in which the initially induced response is conditioned by apomorphine [5]. As it turned out to be extremely difficult to change the nature of the initially induced response in rats by environmental cues [6], the procedure normally used in studies on conditioning could not be used to investigate this hypothesis. We, therefore, decided to use the approach that is described below in order to investigate whether or not apomorphine triggers a conditioning process in rats.

In studies involving stimulus generalization, an animal is trained to respond in the presence of two or more stimuli. A generalization test is then given during extinction; several stimuli along the same dimension as the training stimulus (S^d) are presented, and the animal's tendency to respond in the presence of each test stimulus (S^Δ) is recorded. The extent to which the animal's behaviour is controlled by the S^d is related to the amount of responding in the presence of S^Δ [24]. It is evident that generalization only occurs on the condition that conditioning has been established. Accordingly, we investigated whether or not stimulus generalization occurs in rats having received a single injection of apomorphine.

METHOD

Male, Wistar rats (200 ± 20 g) were used. The behaviour following systemic injections of apomorphine was measured in semi-circular, wooden cages (radius 40 cm; height 50 cm) having a clear Plexiglas front and standing in a room with dimmed light. The display of licking and gnawing of each individually placed rat was recorded by means of the time-sample method: at the beginning of each 20 sec the cages (3 per session) were scanned and a note made of the behaviour of each rat at that time; droppings were removed during the experimental session to prevent the display of mouth-filling. The observation period lasting 10 min started

10 min after the injection; all experiments were performed between 09:00 and 17:00 hr. The behaviour was also qualitatively analysed during the first five min after the injection on the generalization test day (see Results)).

Apomorphine dosages served as S^d. According to the hypothesis, the continuous display of a certain group of behavioural elements elicited by a single injection of apomorphine is due to a learning process; therefore, no additional training for pairing the stimulus with the response was given. We used the following S^d's: S^{d1} = a dose of 0.9 mg/kg SC given into the neck (Test 1); S^{d2} = a dose of 1.3 mg/kg SC given into the neck (Test 2); S^{d3} = a dose of 1.3 mg/kg SC given into the flank (Test 3).

In order to test the occurrence of stimulus generalization, four series of experiments were performed. (A, Test 4) Naive rats (n = 6) having received S^{d1} on Day 1 received S^{d2} as S^{Δ} on the generalization test day (Day 3). (B, Test 5) Naive rats (n = 6) having received S^{d2} on Day 1 received S^{d3} as S^{Δ} on the test day. (C, Test 6) Naive rats (n = 6) having received S^{d3} on Day 1 received S^{d2} on the test day. (D, Test 7) Naive rats (n = 6) having received S^{d2} on Day 1 received a saline injection SC given into the neck as S^{Δ} on the test day.

In order to establish whether possible generalization between environmental cues and apomorphine could contribute to the amount of generalization obtained, three additional series of experiments were performed. (E, Test 11) Naive rats (n = 6) having received S^{d2} on Day 1 in the earlier described cages received the same S^{d2} on Day 3 in somewhat larger cages (radius 45 cm; height 60 cm): on the test day, the cages were strongly illuminated by means of a bulb (200 W) hanging 30 cm above the floor of the cates. (F, Test 12) Naive rats (n = 6) having received S^{d2} on Day 1 in the larger, stronger illuminated cages received a saline injection SC given into the neck as S^{Δ} on the test day under the same circumstances. (G, Test 13). The last-mentioned experiment was repeated with a different group of naive rats (n = 6) with S^{d1} instead of S^{d2} on Day 1. The following sessions served as controls for the last series of experiments. (H, Test 8) Naive rats placed in the larger, stronger illuminated cages without any treatment (I, Test 9) naive rats placed in the larger, stronger illuminated cages received S^{d1}, and (J, Test 10) naive rats placed in the larger, stronger illuminated cages received S^{d2}.

RESULTS

Table 1 shows that S^{d1} given to naive rats on Day 1 produced significantly less gnawing and significantly more licking than S^{d2} (Table 1, Tests 1 and 2), whereas S^{d1} did produce effects identical to those elicited by S^{d3} (Table 1, Tests 1 and 3). In addition, S^{d3} also produced significantly less gnawing and significantly more licking than S^{d2} (Table 1, Tests 2 and 3). Accordingly, they could be used as different S^{Δ}'s.

As also shown in Table 1, the effects elicited by apomorphine on the generalization test day were completely determined by the effects elicited by the injection given on Day 1: in none of the tests (Table 1,

TABLE 1

STIMULUS GENERALIZATION AND DIFFERENT APOMORPHINE TREATMENTS

Test	Treatment Day 1	Treatment Day 3	Gnawing	p	Licking	p
1	S^{d1}		4.50 ± 2.19	† < 0.005	25.50 ± 2.19	† < 0.005
2	S^{d2}		25.17 ± 2.10	† < 0.005	4.82 ± 2.10	† < 0.005
3	S^{d3}		5.67 ± 2.71	† NS	21.83 ± 3.91	† NS
4	S^{d1}	S^{Δ} (=S^{d2})	7.33 ± 3.03	‡ NS	20.25 ± 2.76	‡ NS
				§ < 0.005		§ < 0.005
5	S^{d2}	S^{Δ} (=S^{d3})	23.33 ± 3.70	‡ NS	5.00 ± 3.72	‡ NS
				§ < 0.005		§ < 0.005
6	S^{d3}	S^{Δ} (=S^{d2})	1.50 ± 1.31	‡ NS	15.17 ± 4.33	‡ NS
				§ < 0.005		§ < 0.005
8	CS		0.00		0.00	
9	S^{d1}-C¶		0.00	† < 0.005	28.80 ± 1.17	† NS
10	S^{d2}-C		24.60 ± 2.13	† NS	5.10 ± 2.10	† NS
11	S^{d2}	S^{Δ} (=S^{d2}-C)	22.78 ± 3.19	‡ NS	4.53 ± 2.45	‡ NS
				§ NS		§ NS
12	S^{d2}-C	S^{Δ} (=CS)	0.00	‡ < 0.005	0.00	‡ < 0.005
				§ NS		§ NS
13	S^{d1}-C	S^{Δ} (=CS)	0.00	‡ NS	0.00	‡ < 0.005
				§ NS		§ NS

*S^{d1} = 0.9 mg/kg, SC, neck, S^{d2} = 1.3 mg/kg, SC, neck; S^{d3} = 1.3 mg/kg, SC, flank; S^{Δ} = treatment on generalization test day; C = environmental cue and CS =environmental cue combined with saline injection (SC, neck).

†Means ± SEM produced by S^{d1} (S^{d2}, S^{d3}, S^{d1}-C and S^{d2}-C) given to naive rats compared with those produced by S^{d2} (S^{d3}, S^{d1}, S^{d1} and S^{d2}) given to naive rats (Student's t-test).

‡Means ± SEM produced by S^{Δ} compared with those produced by the S^d given to the same rats (Student's t-test for paired observations).

§Means ± SEM produced by S^{Δ} given to rats pretreated with S^d on Day 1 compared with those produced by the same S^{Δ} given to naive rats.

¶Pairing of the environmental cue with apomorphine resulted in the suppression of gnawing.

Tests 4–6) the amount of gnawing and licking displayed on the generalization test day was significantly different from that displayed on the first day. The experiments in which saline was given as S^{Δ} on the test day revealed that saline remained ineffective with respect to the continuous display of the apomorphine-like effects; however, it did elicit an abortive response, i.e., the display of discontinuous gnawing, during the first minutes following the injection of saline in 66.7% of the rats tested (data not shown).

The final series of experiments analyzing the influence of environmental cues revealed that they did not behave as conditioning stimuli: (1) changing the environment on the generalization test day did not affect the effects (Table 1, Test 11) and (2) the environmental cues given on the generalization test day following their pairing with the apomorphine treatment on the first day did not elicit the continuous display of apomorphine-like effects (Table 1, Tests 12 and 13); however, some rats (47% of the animals tested) displayed an abortive response on that day: they showed a very short-lasting response of which the nature was identical to that of the response elicited by apomorphine on Day 1; this effect lasted only a few minutes.

DISCUSSION

Apart from the fact that the outcome of Test 1–3 revealed that the S^{D}'s could be used as different S^{Δ}'s, it also revealed that the effects produced by a subcutaneous injection depend on the part of the body selected for the injection; at the moment, we cannot give an explanation for the observation that apomorphine SC given into the neck is much more effective than apomorphine SC given into the flank. The results, however, stress that information about the precise site of injection is required for comparing data reported in the literature. Furthermore, the Tests 4–6 have clearly shown that the effects produced by apomorphine on the generalization test day were completely determined by the type of apomorphine treatment given on the first day. The possibility that the first injection might have sensitized the animals to apomorphine can be excluded, since the effects elicited by a relatively low dosage on Day 1 were not surmounted or replaced by effects elicited by a relatively more effective dosage given on the test day (Table 1, Test 4). As saline given on the generalization test day was unable to elicit the apomorphine syndrome, it is unlikely that a special case of stimulus generalization, i.e., state-dependent learning [18,25], underlies the phenomena observed in the remaining experiments. Anyhow, neither of them can occur without the establishment of conditioning. Thus, our data offer hard evidence that a single injection of apomorphine triggers a conditioning process. These results are consistent with the work with apomorphine on self-stimulation [5] as well as with the results of a study in which apomorphine is self-administered via jugular cannulas [2]. The results also point out that an animal treated once with apomorphine cannot be used again within a short period after the first injection unless one wants to study the conditioning effects of this drug. This is of utmost importance, since it has been reported that apomorphine can be repeatedly injected in rats without changing the animal's sensitivity to apomorphine [9].

Finally, the present studies have offered us an additional set of important data. Although environmental cues did not act as conditioning stimuli for the continuous display of the apomorphine-induced effects, it turned out that they were able to initiate the apomorphine-characteristic gnawing. On the basis of these observations, it can be suggested that apomorphine at best acted as an unconditioned stimulus for the single display of a certain set behavioural elements, whereas it did not act as such for the continuous display of this set of behavioural elements in these tests.

Taking into account the ability of apomorphine to trigger a conditioning process resulting in the continuous display of a certain set of behavioural elements as shown in the first series of experiments (Table 1, Tests 4–6), we suggest that apomorphine may act as follows. Apomorphine initially acts as an unconditioned stimulus (UCS) of an unconditioned response (UCR); the stimuli produced by the UCR become conditioned stimuli (CS) of a conditioned response (CR) of which the nature is not only determined by, but also identical to, the UCR. Thus, we propose that apomorphine triggers a process, in which both classical and operant conditioning are intermingled. In this context, it is of importance to stress that the nature of the UCR depends on (a) the genetic load as indicated by the fact that animals belonging to the same species display more or less the same type of effects [6,16], (b) the individual developmental history as indicated by the fact that socially deprived rats display more intense effects than rats grown up together [19], (c) the internal state as indicated by the fact that starvation changes the intensity of the elicited response [20], (d) the dosage, site and route of administration (this study), and (e) environmental cues as indicated by the fact that effects of aversive conditioning in apomorphine-treated rats are limited to the cage, in which the experiments are performed [13]. The influence of environmental cues in the aversive conditioning test is not in conflict with our data, since the rats in the first study display a response, of which the nature is directed by the experimentator, whereas the rats in our study display a response, of which the nature is directed by the animal itself; actually, these two sets of data imply that an artificially induced UCR is more sensitive to environmental cues than an UCR, of which the nature is determined by the animal itself.

The present study not only offers evidence in favour of the concept that apomorphine reinforces the behaviour displayed during the onset of the drug action [5], but is also compatible with the more general suggestion that stereotyped behaviour triggered by agents which increase the dopaminergic activity is directed by conditioning of the behaviour present at the initiation of the drug action [4, 5, 6, 11].

Our results also point out that one has to be very careful with using apomorphine as a valid tool unless attention is given to its conditioning properties and to the fact that the part of the body selected for subcutaneous injections directs the effects elicited by apomorphine.

As a final remark, it is intriguing to note that apomorphine being a dopamine receptor agonist not only indirectly triggers an increased activity within certain serotonergic fibres arising from the raphe nuclei [7, 15, 23], but also requires an intact serotonergic system to elicit the stereotyped behaviour [7,10]; as the serotonergic fibres arising from the raphe contain reinforcing properties [3, 14, 17, 21], serotonin has to be seriously considered as an important factor in the conditioning process triggered by apomorphine.

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