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A Quantitative Analysis of Stereotyped Gnawing Induced by Apomorphine

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REDGRAVE, P., P. DEAN AND G. LEWIS. *A quantitative analysis of stereotyped gnawing induced by apomorphine.* PHARMAC. BIOCHEM. BEHAV. 17(5)873–876, 1982.—An apparatus was designed and constructed to enable a quantitative analysis of the stereotyped gnawing produced by the dopamine agonist apomorphine. Using this apparatus it was discovered that increasing the subcutaneous dose of apomorphine increased (1) the number of animals that gnawed, and (2) the duration of gnawing in those animals that gnawed at all doses. Other aspects of apomorphine-induced gnawing, in particular the latency to respond and the frequency and duration of individual gnaws, were relatively unaffected. Likely properties of the system responsible for the organization of stereotyped gnawing are discussed.

Apomorphine Stereotyped gnawing Quantitative analysis

BEHAVIOURAL stereotypies caused by increases in central dopamine (DA) transmission are characterised by the apparently purposeless repetition of relatively small sequences of behaviour (for reviews see [9,11]). An analysis of the details of these individual behavioural sequences may provide important information concerning the mechanism whereby changes in DA transmission influence behaviour.

Various procedures have been adopted to describe the types of stereotypy exhibited by rodents. Perhaps the most commonly used system is that of rating scales [1,2] which provide a qualitative description of stereotypy associated with different doses of drugs such as amphetamine and apomorphine. An alternative approach in which the presence or absence of different response categories is defined was reported recently [3]. These procedures have not, however, been able to provide satisfactory descriptions of quantitative aspects of individual stereotypies.

When relatively large doses of DA-agonist are administered to rats the most common stereotyped behaviour is gnawing and biting. While there have been several attempts to automate the recording of this behaviour [5, 7, 8], so far none has provided a satisfactory quantitative description of it. We therefore designed and constructed an apparatus to meet the following requirements: (1) it should facilitate the expression of gnawing rather than some other form of stereotyped behaviour; (2) gnawing should be exclusively directed to the part of the apparatus where it could be measured; (3) it should be capable of measuring characteristics of individual gnaws; (4) the process of detecting gnaws should be unnoticed by the animals and not triggered by events other than gnawing. Our solution to these problems was to use a small, smooth sided arena containing no suitable gnawing surface apart from two conspicuous metal plates. These plates were brought together only by gnawing; this completed an electrical circuit, an event which the animals could not detect. The apparatus was used to investigate quantitative changes in the stereotyped gnawing produced by different doses of the DA-receptor agonist apomorphine.

METHOD

Apparatus

A small box (20×23×30 cm) was constructed out of 3 mm perspex sheet. A raised floor was placed approximately 7 cm from the bottom of the box. A slot 20×0.7 cm was cut in the floor 1 cm away from, and parallel to, one of the longest sides of the box. Through this slot a pair of parallel metal plates were admitted to which rats direct their stereotyped gnawing and biting (see Fig. 1).

Biting or gnawing the plates caused one to touch the other, an event which switched a modified relay (see below). Relay closure was monitored continually by an ACT-N computer program (Campden Instruments) which indicated the frequency and duration of relay closures during specified time periods throughout a test session.

Certain facilities were incorporated into the apparatus to ensure that (1) the animal was oblivious of the electrical events associated with plate closure, and (2) a high proportion of the bites and gnaws directed towards the plates resulted in detectable closure. These features of the apparatus are illustrated in Fig. 2.

(1) Two measures were taken to prevent the animal being shocked while biting the plates. First, in order to reduce the current switched by the plates the original Campden relay module was modified by interposing a simple single transistor drive circuit between the metal plates and the relay. This alteration reduced the current through the plates to about 85 μA. Secondly, the plates were constructed so that the live part of the equipment was normally inaccessible to the rat (see Fig. 2).

(2) Three forms of adjustment were provided (see Fig. 2) to ensure that detection of gnaws and bites directed to the

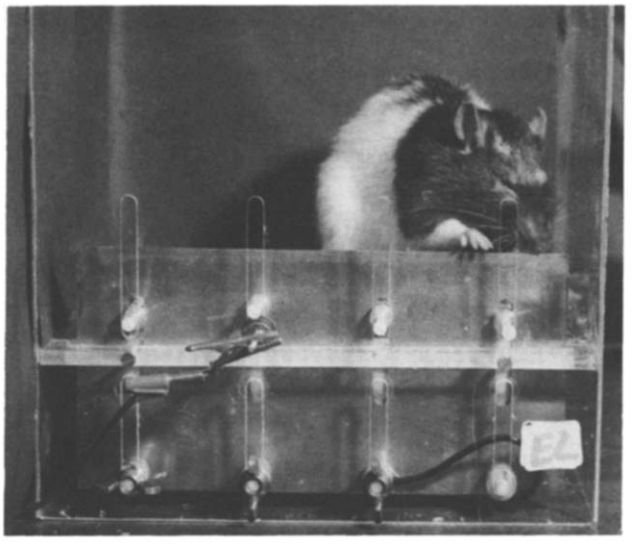

FIG. 1. A front view of the apparatus for the quantitative measurement of stereotyped gnawing.

plates was maximized: (a) the distance from the top of the plates to the floor of the box; (b) the distance between plates; (c) the force required to close the plates. We have found that a high proportion of the available gnaws and bites are detected when a=40 mm, b=2 mm and a metal disk (diameter 25 mm) weighing 40 g placed on the edge of the flexible plate just effects a closure.

Injection and Testing Procedures

Twelve male hooded rats weighing approximately 300 g were used as subjects. They were housed individually and were given free access to food and water except for the duration of experimental testing.

At the beginning of a test session subjects were habituated to the experimental apparatus for 60 min. Each animal then received either one of four doses of apomorphine HCl (McFarlan Smith), 2, 4, 6, 8, mg/kg, or a control injection of the ascorbate/saline vehicle (0.5 mg/ml). Injections were made sub-cutaneously into the flank. Each animal received each dose and the vehicle control in a counterbalanced order; successive injections into a single subject were separated by a minimum of two days.

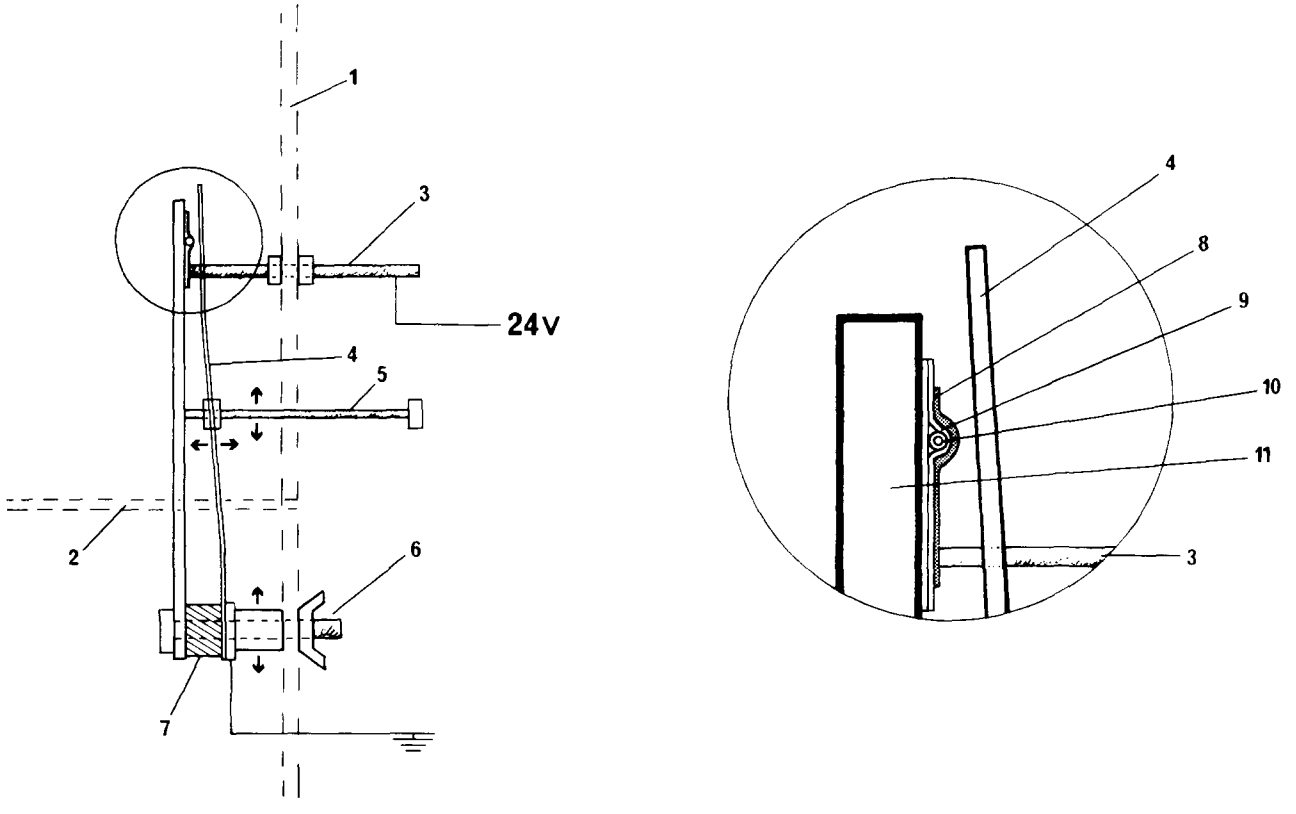

FIG. 2. Illustration of the components of the plates for the detection of stereotyped gnawing: (1) wall of box (3 mm perspex); (2) floor of box (3 mm perspex); (3) threaded brass rod (6BA) which provides the live connection of the single transistor drive circuit; (4) flexible spring steel plate (0.25 mm) which is connected to ground; (5) nylon screw (4BA) with lock nuts onto the flexible steel plate (slots in the wall of the box and flexible steel plate enable the following adjustments as indicated: distance between the plates, and the force required to close the plates); (6) nylon screw (0BA) with wing nut (slots in the wall of the box enables the distance between the top of the plates and the floor of the box to be varied); (7) insulating spacer (Klingerite); (8) strip of aluminium foil (which is in electrical contact with threaded brass rod (3)); (9) double sided insulating tape; (10) polyethylene tubing (pp20); (11) inflexible duraluminium plate (1 mm).

TABLE 1

QUANTITATIVE CHANGES IN STEREOTYPED GNAWING PRODUCED BY INCREASING DOSES OF APOMORPHINE

Gnawing Parameters (Means n=6 ± S.E.M.)	Apomorphine (mg/kg) 2	4	6	8
Total gnaws*	2150 ± 723	2263 ± 744	3820 ± 1120	5124 ± 409
Duration of gnawing (min)†	47 ± 7.7	75 ± 6.8	76 ± 15.1	107 ± 3.6
Gnawing latency (min)	20.8 ± 6.1	13.3 ± 1.7	19.1 ± 6.6	18.3 ± 3.8
Gnawing frequency (gnaws/min)	41.2 ± 15.0	28.2 ± 7.4	42.8 ± 9.1	47.9 ± 3.2
Peak gnawing frequency (gnaws/5 min)	387 ± 98	325 ± 72	376 ± 78	445 ± 40
Duration/gnaw (sec)	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.03	0.18 ± 0.06

*$p<0.02$; †$p<0.002$.

Plate closures from each apparatus were monitored automatically by the computer program (see above) for a 3 hr period following the administration of apomorphine. Every 5 min throughout the 3 hr session a record was taken of (1) the number of plate closures and (2) the length of time the plates were closed; from this information it was possible to derive (3) the mean duration/gnaw for each 5 min period.

RESULTS

Observation of apomorphine treated rats in the new apparatus revealed (1) that almost all the stereotyped gnawing and biting exhibited by animals was directed towards the metal plates, (2) a high proportion of the biting and gnawing directed towards the plates resulted in their closure, and (3) no other event other than gnawing or biting caused the plates to close. Taken as a whole these observations suggest that electronic detection of plate closure provides a very slightly conservative estimate of the total amount of stereotyped gnawing and biting produced by apomorphine.

One of the 12 rats tested with apomorphine failed to gnaw at any of the doses that were administered. The effect of apomorphine on gnawing for the remaining eleven animals is shown in Fig. 3. The increase in gnawing shown to follow the larger doses of apomorphine had two components. One was that more animals gnawed at the higher doses (2 mg/kg n=6; 4 mg/kg n=8; 6 mg/kg n=8; 8 mg/kg n=10). The second was that the rats which gnawed at low doses gnawed more at high doses.

A detailed analysis of the stereotyped gnawing of the six rats which gnawed at all doses of apomorphine is presented in Table 1. When the data of these animals were subjected to analyses of variance the main effects of increasing the dose of apomorphine were to increase the total number of gnaws, $F(3,15)=4.56$, $p=0.018$, and the length of time during which gnawing occurred, $F(3,15)=7.87$, $p=0.002$.

All other aspects of the gnawing response were relatively unaffected by increasing doses of apomorphine. Thus, there were no significant changes in the onset latency of gnawing, $F(3,15)=0.57$; the frequency of gnawing, i.e., total number of gnaws/total duration of gnawing, $F(3,15)=1.13$; or the peak frequency of gnawing, $F(3,15)=0.97$. Nor were the characteristics of individual gnaws affected, in that the mean duration/gnaw, i.e., the total time for which the plates were held closed/total number of gnaws, was not significantly altered by increasing doses of apomorphine, $F(3,15)=0.16$.

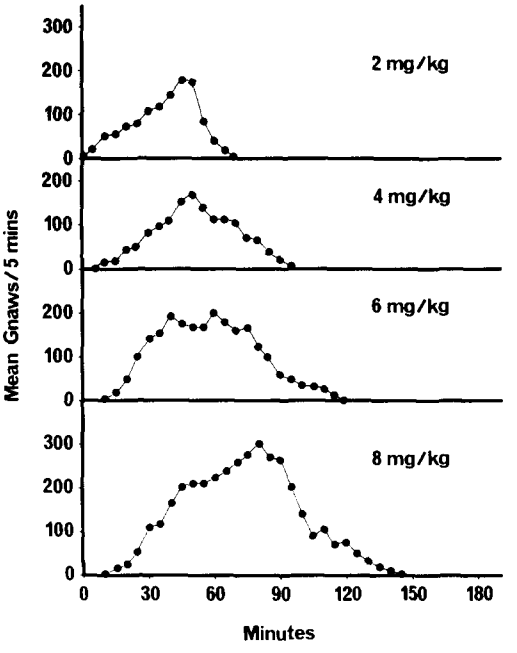

FIG. 3. Effects of different doses of apomorphine on mean numbers of gnaws/5 min throughout a period 3 hr after injection (n=11).

DISCUSSION

Two aspects of the results require comment. The first is how far the apparatus met the requirements listed in the introduction. As indicated in the results section animals gnawed on the metal plates rather than elsewhere and did not close the plates other than by gnawing. Detection of stereotyped gnawing was not perfect, however, because occasionally some animals exhibited weak gnawing which was insufficient to close the plates. Also, in subsequent experiments

we have observed on rare occasions that some animals will gnaw on parts of their own bodies, e.g., their tails or feet. Finally, although attributes of the apparatus such as its small size and smooth sides encouraged stereotyped gnawing, occasionally another form of oral stereotypy was observed, namely licking, which the present apparatus is not capable of measuring.

Secondly, using this apparatus it was discovered that increasing the subcutaneous dose of apomorphine had two main effects on stereotyped gnawing. One was to increase the number of animals that gnawed. The second, seen in those animals that gnawed at all doses, was to increase the length of time for which gnawing occurred. All other characteristics of apomorphine-induced gnawing revealed by the new method of recording (e.g., response latency, the mean and peak frequency of gnawing, and duration of individual gnaws) were relatively unaffected by changes in dose over the range studied.

Perhaps the simplest explanation of this pattern of results is that there is a system responsible for the organisation of gnawing movements which is normally under tonic inhibitory control. This system can be released in an all-or-none manner by apomorphine. Provided the environmental conditions are suitable [6,12] this release results in gnawing, the characteristics of which are determined more by the system released than by the extent of the release, i.e., the level of DA-receptor activation. In this respect, it is interesting that the acute effect of apomorphine on rotation in rats with unilateral 6-hydroxydopamine lesions is principally on the duration rather than the speed of turning [14,16].

This explanation is consistent with our previous suggestion that DA-related gnawing and biting may reflect a release of mechanisms in the superior colliculus following a reduction in nigrally mediated inhibitory control [10]. Recent data [4,15] suggest that the relevant collicular mechanism responsible for the production of some DA-related oral stereotypy is the sensitization of a perioral biting reflex. Certainly the present finding that increasing the dose of apomorphine had relatively little effect on the frequency and duration of individual gnaws is consistent with the proposal of an all-or-none release of a relatively simple reflex (see also [13]).

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