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# SNOUT CONTACT FIXATION, CLIMBING AND GNAWING DURING APOMORPHINE STEREOTYPY IN RATS FROM TWO SUBSTRAINS

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Apomorphine, at doses ⩾10 mg/kg (intraperitoneally), produced two patterns of stereotypy. In rats from one supplier it induced predominantly gnawing while in those from another predominantly climbing, suggesting that the response to the drug is influenced by genetic and/or experiential factors. At lower doses, apomorphine induced climbing in both groups ($ED_{50}$ = 1.4 mg/kg in each group) but oral behavior in only one of them ($ED_{50}$ = 1.3 mg/kg in one, and 8 mg/kg in the second group). Thus, at a given dose of apomorphine, different patterns of stereotypy may result from an interaction between two phenomena: the relative setting of the thresholds to mouth and to climb, and an inverse relation between oral activity and climbing. Analysis of climbing suggests that this response is comprised of two (previously unidentified) fundamental effects of apomorphine: snout contact fixation and bodywise forward progression.

Stereotyped behavior  Verticalization  Climbing behavior  Sniffing  Locomotion  Apomorphine

## 1. Introduction

It is generally considered that the stereotypy of rodents treated with apomorphine, a stimulant of dopamine receptors (Ernst, 1967), consists of continuous sniffing, licking, or gnawing (Ernst, 1967; Costall and Naylor, 1973; McKenzie, 1972). However, there are recent descriptions of another equally stereotyped behavior which does not apparently consist of sniffing or mouthing. Mice and rats treated with comparable doses of apomorphine are reported to rear persistently, climb, or cling to cage walls, presumably without any significant sniffing, licking, or gnawing, since these are not mentioned (Baldessarini et al., 1977; Decsi et al., 1979; Hershkowitz and Szechtman, 1979; Protais et al., 1976; Wilcox et al., 1979). It is not clear why this stereotyped behavior, which is called stereotyped rearing, climbing behavior, or verticalization, is observed at times instead of stereotyped mouthing. During our work on apomorphine stereotypy, we noticed pronounced climbing and little mouthing in Wistar rats from one supplier but the reverse in those from another. Therefore, one purpose of the present study was to document these differences. A second purpose was to document that even though the stereotyped behaviors of rats from the two suppliers may appear different, they nevertheless possess at least one invariant feature: snout contact maintenance.

## 2. Materials and methods

### 2.1. Animals

Male Wistar albino rats (3–4 months of age, 250–350 g) were obtained from two independent breeding colonies at the Weizmann Institute of Science: those from the Department of Hormone

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Research are referred to as rats from Supplier A; those from the Department of Animal Breeding are referred to as rats from Supplier B. All were housed 4 per cage with free food and water in a colony room maintained at 22°C with lights on from 05:00 to 19:00 h. Tests were conducted at same time each day during the light portion of the day-night cycle.

### 2.2. Drugs

Apomorphine hydrochloride was dissolved in saline (2.5 mg/ml) plus 0.1% ascorbic acid and was injected intraperitoneally (i.p.).

### 2.3. Apparatus

Climbing was measured in a $10 \times 13 \times 35$ cm Plexiglas enclosure which rested on a raised metal floor. To provide an opportunity for gnawing, the floor was perforated throughout its extent by 0.8 cm diameter holes spaced 0.3 cm apart. In addition, rats were able to gnaw at Plexiglas blocks ($0.5 \times 0.5 \times 2.5$ cm) glued to corners at the bottom, middle, and top of the cage (see fig. 1).

Behavior in the open field was assessed on a flat table ($140 \times 60$ cm and 92 cm high), placed in the middle of the room, away from any walls and without any objects on it.

### 2.4. Behavioral tests

After 10 min of adaptation to the apparatus, rats from each supplier were injected with saline, or 1.25, 2.5, 5, 10 or 20 mg/kg of apomorphine ($n = 7$ at each dose); each animal was tested once only. On any one test day, 6 rats (3 from each supplier) were assigned randomly as to dosage and tested simultaneously. A rat was considered to initiate an episode of climbing whenever it was not grooming and its paws were above a mark on the wall 8 cm high, a distance that is halfway to the small Plexiglas block (fig. 1) and less than half the height of a rearing rat. The length of time the rat remained thus elevated was recorded on an event recorder. In addition the presence or absence of mouthing, licking, or biting, was noted at 5 min intervals; a rat was scored 2 when this activity appeared to be very intense (that is, uninterrupted for 15 s or longer), 1 when it was mild (intermittent, lasting approximately 5–10 s), and 0 when it was absent. Recording ended at 60 min but animals remained under observation until the occurrence of body grooming, which in our experience signalled the end of apomorphine's behavioral activation.

Observation of activity in the open field was carried out on a different group of 7 rats from Supplier B (pilot observations of rats from Supplier A indicated that their performance on the variable of interest, namely, snout contact, did not differ from rats of Supplier B).

## 3. Results

### 3.1. Description of behavior

In the small Plexiglas enclosure the typical climbing response appeared as follows: within 2 min of injection of apomorphine, the rat stood up on its hindlegs, and with all four legs engaged in incessant climbing movements along the wall (fig. 1A left). After about 5 min, the climbing attempts became less dramatic, but not less incessant, involving sporadic use of a hindleg in climbing (fig. 1B left). Later, the hindlegs became rooted to the ground as the rat remained upright supporting some of its weight with its forelimbs on the walls (fig. 1C left). Still later, it scanned lower and lower portions of the vertical surface (fig. 1D left); some rats even came to rest on all fours while only the snout made small up and down movements against the wall. Finally, after about 30–120 min depending on the dose (table 1), the rat groomed its face and body, and settled down quietly on all fours, suggesting that the appearance of grooming signals the end of the drug's behavioral activation. Thus, during the course of drug action, climbing on all fours turns into rearing on hindlegs, then into rearing with flexed hindlegs, and in some rats into raising of head only (see also Szechtman et al., 1980).

Apomorphine-induced climbing was clearly different from the rearing of normal rats. First of all, normal rats maintained an upright rearing posture

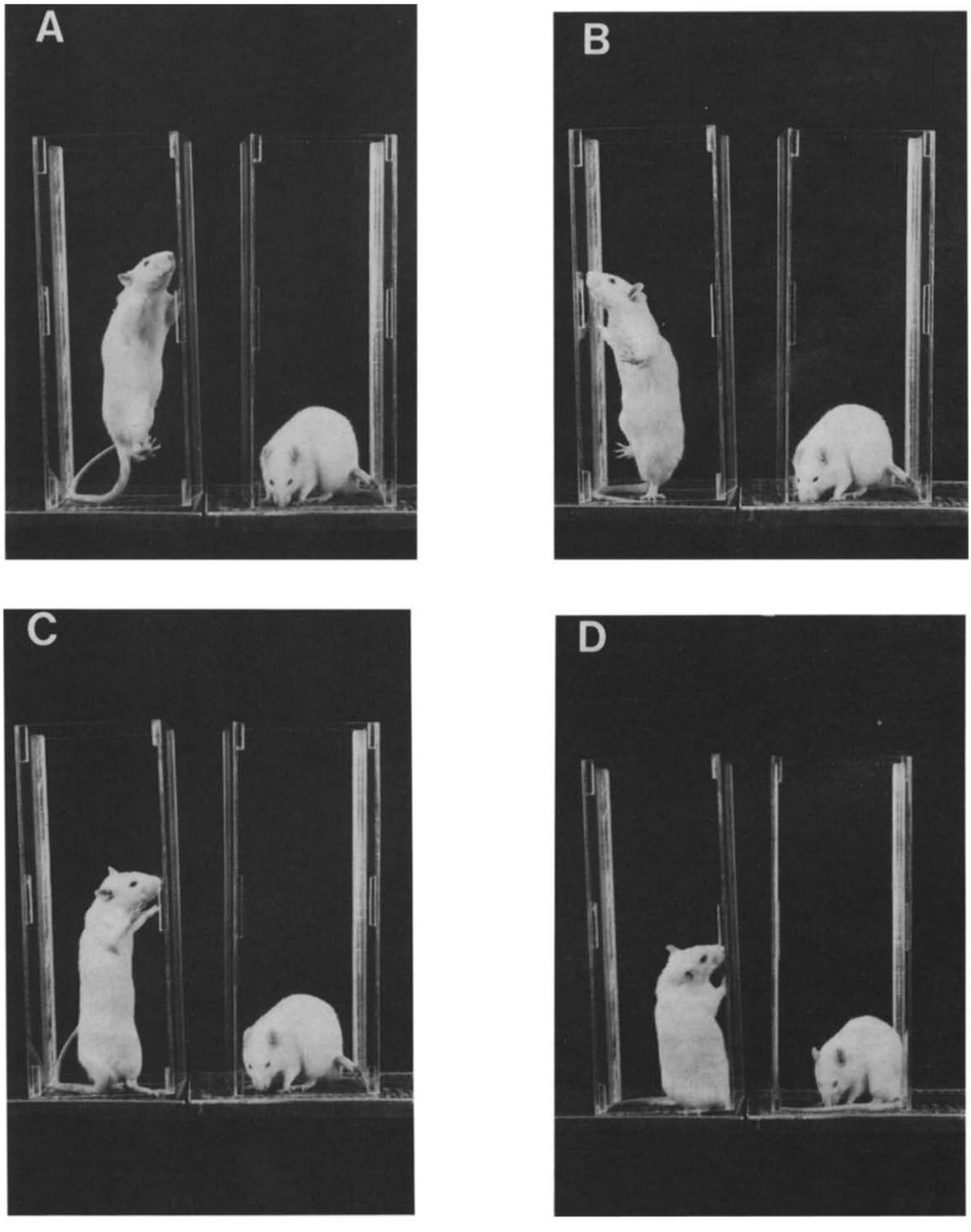


Fig. 1. Two responses to apomorphine: climbing and gnawing. As time after injection increases from A to D, the rat climbing on the left side of the photographs (from Supplier B) scans lower and lower portions of the wall, while the animal on the right side of the photographs (from Supplier A) continuously gnaws. For purposes of this illustration, both rats were injected (i.p.) with 10 mg/kg of apomorphine. However, at every dose, most rats which climb exhibit similar changes in the appearance of climbing.

TABLE 1

Duration of apomorphine's action as measured by three behavioral end points.

| Dose (mg/kg) | Release snout contact [1] | | Groom face [1] | | Groom body [1] | |
|---|---|---|---|---|---|---|
| | Supplier A [2] | Supplier B [2] | Supplier A | Supplier B | Supplier A | Supplier B |
| 0 | 5± 0 | 5± 0 [3] | 5±0 | 5± 0 | 12±3 | 10± 3 |
| 1.25 | 15± 6 | 28± 8 | 26±7 | 39± 8 | 33±7 | 44± 7 |
| 2.5 | 38± 7 | 51± 1 | 45±5 | 52± 2 | 54±6 | 63± 6 |
| 5.0 | 61± 9 | 60± 5 | 68±4 | 64± 5 | 71±4 | 67± 5 |
| 10.0 | 73± 2 | 72± 3 | 81±4 | 76± 3 | 87±4 | 88± 3 |
| 20.0 | 76±10 | 94±13 | 114±4 | 94±13 | 116±4 | 100±12 |

[1] Values are times in min ($\bar{X} \pm$ S.E.M.). Times were recorded to the last 5 min.

[2] n = 7 at each dose.

[3] 2×6 ANOVAs for each variable indicated that the effects of drug dosage were significant, but that the groups were not different from each other.

for only a few seconds at a time before lowering their forelimbs to the ground ($\bar{X} \pm$ S.E.M. = 4.6 ± 0.4 s, n = 14). In contrast, apomorphine-treated rats remained upright much longer ($P < 0.01$, t-test), in extreme cases as long as 40 min (123.8 ± 37.6 s, n = 70). Second, undrugged rats sometimes kept their forelimbs free in the air during rearing. In contrast, apomorphine-treated rats always rested them against the wall (fig. 1). Third, normal rats rooted their hindlegs to the ground to maintain stable support during rearing, and thus did not climb, whereas apomorphine-treated animals in the first minutes after injection made stepping movements with their hindlegs (figs. 1A and 1B, left).

Mouthing, licking, or gnawing, were sometimes combined with climbing, particularly in rats from Supplier A. In those instances, rats licked or gnawed the walls, or licked or gnawed the small Plexiglas block glued to corners halfway up the cage (see fig. 1). Often, however, rats from Supplier A did not climb (especially at higher doses of apomorphine, see below), but directed their oral behavior to the cage floor, as in figs. 1A–D, right.

### *3.2. Dose-response characteristics*

Fig. 2 indicates that in rats from Supplier A oral behavior was induced more readily than climbing ($P < 0.007$); in rats from Supplier B the reverse was true ($P < 0.005$; test for difference between two correlated proportions). Consequently, the incidence of oral behavior was greater in rats from Supplier A than Supplier B ($P < 0.001$, test for difference between proportions). However, the expected difference between the two groups in the incidence of climbing was not quite statistically significant ($P < 0.07$): it appeared to be present only at higher, but not lower, doses of apomorphine (fig. 2). Indeed, table 2 indicates that compared to rats from Supplier B, the duration of climbing in rats from Supplier A was significantly shorter only at higher ($\geq 10$ mg/kg) doses of the drug; in contrast, the rats differed in the extent of oral activity at doses $\geq 2.5$ mg/kg. As expected, the $ED_{50}$ for induction of oral behavior were different in rats from Supplier A and Supplier B ($P < 0.05$), but those for climbing were not (fig. 2). In general, then, the sensitivity to apomorphine did not differ when measured by climbing, but did when measured by induction of mouthing, licking, or gnawing.

The duration of action of apomorphine, as measured by the appearance of face or body grooming, was dose-dependent and was not influenced by the source of supply of the animals (table 1). This suggests that in the two populations of rats, pharmacokinetics of apomorphine are not different and consequently their distinct profiles of motor behavior are a reflection of some other factor(s).

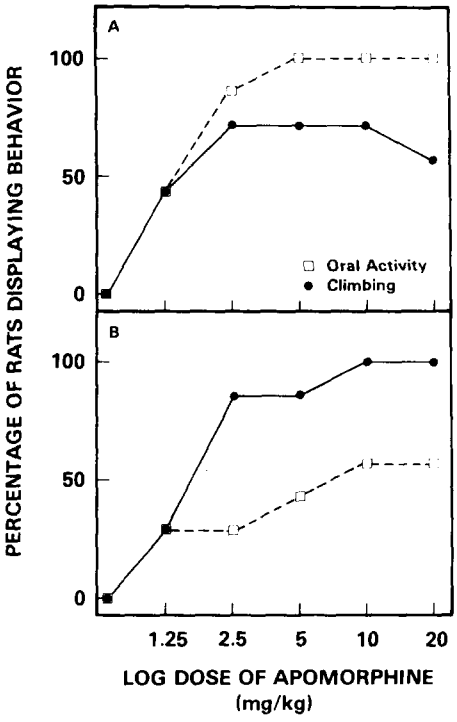


Fig. 2. Percentage of rats from Supplier A (panel A) and Supplier B (panel B) exhibiting climbing and oral behavior (mouthing, licking, or biting) at different doses of apomorphine. A rat was considered to exhibit climbing if its total time upright in the 60 min test was at least 500 s, that is, thrice the highest control value. A rat was said to show oral behavior if it either mouthed, licked, or gnawed, for at least 5 s during five or more of the twelve 5 min intervals. N = 7 at each dose. The $ED_{50}$ (and 95% confidence limits) for oral behavior in Supplier A and Supplier B rats were 1.3 (0.7–2.4) and 8 (2.6–24.5) mg/kg, respectively; those for climbing were 1.4 (0.4–5.2) and 1.4 (0.8–2.4) mg/kg calculated according to the method of Litchfield and Wilcoxon (1949).

### *3.3. Snout contact fixation*

Despite its varied appearance, the behavior of all drug-injected rats possessed the following invariant characteristic: no matter what movement the apomorphine-treated rats made, their snout was actually touching or was at most a few millimeters away from a surface (fig. 1). Thus, whether climbing or mouthing, rats from either supplier maintained uninterrupted snout-to-surface contact, from about 2 min after injection to shortly before grooming (table 1).

Others (e.g. Ernst, 1967; Costall and Naylor, 1973) appear to have labelled snout-to-surface contact 'sniffing'. Whether this behavior pattern is in fact sniffing needs to be examined, i.e., it should be shown that in apomorphine-treated rats the patterns of vibrissae movements and air inspiration resemble those of controls during exploratory sniffing. More importantly, however, categorizing the response as 'sniffing' obscures the fact that when apomorphine-treated animals sniff, lick, bite, or gnaw, they still keep their snout in contact with a surface. Viewed this way, it became very clear that all apomorphine-treated rats, regardless of supplier, maintained snout-to-surface contact, without interruption, until the effects of the drug began to wear off (table 1). This suggests that maintenance of snout-to-surface contact may be a fundamental aspect of the behavioral action of apomorphine (see also Szechtman et al., 1980, 1981).

If the foregoing is valid, then apomorphine-

TABLE 2

Effect of apomorphine (i.p.) on climbing and oral behavior in rats from different suppliers.

| Dose (mg/kg) | Duration of climbing (s) | | | Oral activity score | | |
|---|---|---|---|---|---|---|
| | Supplier A [1] | Supplier B [1] | P [2] | Supplier A | Supplier B | P [2] |
| 0 | 59 ± 15 | 115 ± 51 | n.s. | 0 | 0 | n.s. |
| 1.25 | 520 ± 226 | 578 ± 315 | n.s. | 6.6 ± 2.7 | 4.4 ± 2.5 | n.s. |
| 2.5 | 1909 ± 566 | 2519 ± 426 | n.s. | 14.0 ± 2.9 | 4.3 ± 2.3 | <0.002 |
| 5.0 | 1712 ± 550 | 2198 ± 535 | n.s. | 18.7 ± 1.5 | 4.9 ± 1.8 | <0.001 |
| 10.0 | 1280 ± 301 | 2474 ± 470 | <0.01 | 21.3 ± 0.8 | 9.0 ± 2.7 | <0.001 |
| 20.0 | 455 ± 164 | 2244 ± 253 | <0.001 | 18.9 ± 1.6 | 7.4 ± 2.4 | <0.001 |

[1] n = 7 at each dose. Each value is mean ± S.E.M.
[2] Two-tailed t-test using error variance from the 2×6 ANOVA.

treated rats should never assume the upright posture in the absence of walls. Indeed, they do not: rats from Supplier B were injected with apomorphine (10 mg/kg i.p.) and placed on a smooth table away from any walls or objects. Within 2 min of injection, the snout was brought into close contact with the table surface and not released until the drug began to wear off ($\overline{X} \pm$S.E.M. to release contact = 62 ± 3 min, n = 7). Consequently, there was no spontaneous rearing during this period, supporting the notion that snout contact is a fundamental sign of apomorphine's action (the behavior of rats in the open field is analyzed in detail in a separate publication; Szechtman et al., 1980; Szechtman et al., in preparation).

The maintenance of snout contact with a surface is an active process. Otherwise, it would not have been preserved, unbroken, through the diversity of movements which the animals made in the different environments. Therefore, we label the close snout-to-surface contact shown by apomorphine-treated rats, snout contact fixation (see Golani et al., 1979 for a discussion of snout contact fixation as a separate behavioral subsystem).

## 4. Discussion

### *4.1. Climbing and mouthing*

At high doses apomorphine induces predominantly stereotyped mouthing in rats from one supplier (Supplier A) but stereotyped climbing in rats of the same breed from a different supplier (Supplier B). Thus genetic and/or experiential factors influence the stereotyped response to apomorphine.

The two profiles of stereotypy do not reflect simply the disposition of one population to mouth and of the other to climb. Dose-response characteristics of apomorphine-induced oral behavior and of apomorphine-induced climbing indicate that in one of the groups, the $ED_{50}$ to induce mouthing is several times greater than in the other group (1.3 vs. 8 mg/kg) but the $ED_{50}$ to induce climbing are equal (1.4 mg/kg). Thus the threshold to mouth is close to the threshold to climb in one population but relatively higher in the other. This result is expressed in the observation that at lower doses of apomorphine one group climbs and mouths while the other mostly climbs. Interestingly, the fact that at higher doses rats from Supplier A mouth vigorously but climb very little (table 2) suggests that intense mouthing may antagonize climbing. (It should be noted that in our test situation it was possible to exhibit mouthing and climbing simultaneously: rats could gnaw at the small Plexiglas blocks glued halfway up the wall corners or lick the cage walls, which, in fact, many did.)

Overall, then, the variety of patterns of stereotypy observed at different doses of apomorphine in the two populations of rats may reflect the interaction between two phenomena: the relative setting of the thresholds to mouth and to climb, and a reciprocal relation between mouthing and climbing.

### *4.2. Snout contact fixation*

In one respect, every apomorphine-treated rat behaves similarly: regardless of supplier or of the particular environment in which it is tested, every rat maintains its snout in close proximity to surfaces. Snout contact is maintained no matter what stereotyped behavior the animal shows, be it climbing, walking, sniffing, licking, or gnawing. Since all the different stereotypies contain snout contact, snout contact fixation may be a fundamental behavioral effect of apomorphine (see also Szechtman et al., 1980, 1981).

In previous work (Szechtman et al., 1980), we have shown that several of the diverse forms of locomotion seen under apomorphine (forward locomotion, circling and pivoting) represent the interaction of two relatively independent variables that emerge successively during the drug's action: bodywise forward progression and shift of front or turning (in the Eshkol-Wachman movement notation terminology, shift of front refers to a change in horizontal orientation of the midline longitudinal axis of the body; see Golani et al., 1979). In the present paper, we document more fully that superimposed on these two variables there is a third one, snout contact fixation. The interaction of all three appear to account for most of the

phenomena seen in exploratory locomotion in the open field and in the small Plexiglas enclosure. For instance, the interaction between snout contact fixation and bodywise forward locomotion can account for such seemingly qualitative variations in the response to apomorphine as locomotion without rearing in an open field, climbing in a small enclosure, and repetitive falling from the top of a small cylinder ('cliff jumping', Weismann, 1971). The absence of rearing during locomotion in an open field reflects the fact that rearing there would entail breaking snout contact. In contrast, in a small enclosure, being upright does not involve breaking snout contact as the rat elevates itself with its snout against the wall. In fact, the climbing response can be considered as bodywise forward locomotion which, because of the structure of the immediate physical environment, is directed vertically along the wall. Similarly, 'cliff-jumping' is an instance of locomotion downwards in the environment, but forward bodywise—jumping, or falling, results when the progressing animal can no longer hold onto the wall. Thus, the same behavioral subsystems, snout contact and forward progression, are molded into seemingly qualitatively different behaviors by the different surfaces that the rat encounters.

The phenomenon which we regard as snout contact fixation is interpreted differently by others. Thus, the fact that rats keep their snout in close proximity to surfaces is generally labelled 'sniffing' (e.g. Ernst, 1967; Costall and Naylor, 1973), or 'S-behavior', where 'S-behavior' consists of sniffing and 'repetitive head and limb movements' (Ljungberg and Ungerstedt, 1977a). This behavioral response, whether viewed as quantitatively (Costall and Naylor, 1973) or qualitatively (Ljungberg and Ungerstedt, 1977b) different from gnawing, is thought to be absent during oral behavior. In contrast, this study demonstrates that snout contact is present for almost as long as apomorphine exerts its behavioral effects. Therefore, we consider snout contact to be a fundamental behavioral effect of apomorphine. Snout contact fixation should be viewed as an expression of a unique independent behavioral subsystem (Golani et al., 1979)—sniffing, mouthing, licking, or biting, may or may not be superimposed on it, depending on environmental, pharmacological, and genetic and/or experiential factors.

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