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Behavioural Responses to Amphetamine and Apomorphine in Pigs

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TERLOUW, E. M. C., G. DE ROSA, A. B. LAWRENCE, A. W. ILLIUS AND J. LADEWIG. *Behavioural responses to amphetamine and apomorphine in pigs.* PHARMACOL BIOCHEM BEHAV 43(2) 329-340, 1992.—The effects of different doses of amphetamine (0-1.5 mg/kg) and apomorphine (0-1.0 mg/kg) on behaviour of pigs were compared. Amphetamine induced an increase in levels of nosing and rooting and of locomotion. These increases were, however, related to increased levels of standing. At higher doses (1.0-1.5 mg/kg), amphetamine specifically induced a rigid standing posture with jerking head and limb movements. Apomorphine at 0.1-1.0 mg/kg increased locomotion. In contrast to amphetamine, this effect was specific as it was not explained by increased levels of standing. At 1.0 mg/kg, apomorphine specifically induced "locomotion while the pigs maintained snout contact with the floor or trough." In addition, at this dose it induced drinking in one test, while licking in another. These differences may in part be due to differences in the test environment. Apomorphine exerted a strong conditioning effect, as indicated by the lack of behavioural variability in the postinjection period. This effect may explain the large interindividual variation in apomorphine response. Amphetamine and apomorphine elicit different behavioural syndromes in pigs, suggesting that they act on different neural systems. In addition, neither amphetamine nor apomorphine elicited behaviour that closely resembles environmentally induced stereotypies.

Amphetamine Apomorphine Stereotypies Compulsive behaviour Locomotion Oral activities Drinking Snout contact fixation Pigs

THE term "stereotypy" has been defined as "a repetitive activity without obvious goal or function" (18,23,34). Stereotypies often develop in zoo and farm animals, including pigs (1,8, 31,55). Sows kept under restrictive feeding and housing conditions can perform a variety of stereotypic behaviours including bar biting, chewing of the tether chain, vacuum chewing, and weaving (1,8,55). While these stereotypies are environmentally induced, the term stereotypy has also been used to describe certain behaviours induced by dopamine agonists, such as repetitive licking, gnawing, and limb movements in laboratory rodents (11,36,39,53). As behavioural syndromes fitting the definition of stereotypy can be induced both by dopamine agonists and long-term environmental stress, it has been speculated that dopaminergic systems are fundamental to the environmentally induced stereotypies (9,48,49). In support of this hypothesis, amphetamine potentiates the performance of environmentally induced stereotypies in chimpanzees (3). Furthermore, haloperidol, which is known to inhibit dopamine agonist-induced stereotypies (32), also inhibited environmentally induced stereotypies in voles and pigs (22,59).

The effect of amphetamine and apomorphine on behaviour has been investigated across a number of species, including laboratory rodents, pigeons, cats, dogs, cattle, sheep, monkeys, and humans (11,16,28,33,36,50,52,54). Although one recent study investigated the effect of apomorphine on the behaviour of suckling piglets (14), to our knowledge no study has systematically investigated dopamine agonist-induced behaviour in pigs. The present study, therefore, analysed in detail the behavioural effects of different doses of two dopamine agonists, amphetamine and apomorphine, in pigs. Similarities and differences between drug-induced and environmentally induced stereotypies are discussed.

EXPERIMENT 1

Several studies have shown that response to amphetamine is dose dependent. Rodents, for example, show locomotion at lower and stereotyped head, limb, and mouth movements at higher doses (19,39). In the first experiment, we investigated the dose-response relationship for amphetamine in pigs.

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METHOD

Animals and Housing

Subjects were 16 Landrace × Large White (Cotswold Pig Dev. Co. Ltd., UK) male pigs, weighing between 45–69 kg. They were kept in climate-controlled rooms (20°C), with four animals to a room. They were individually housed in 1.80 × 2.30-m pens without straw. Water and food (standard growing pigs concentrate food in meal form) were continuously available.

Procedure

Pigs were randomly and independently assigned to one of three doses of amphetamine (*d*-amphetamine sulphate; Sigma Chemical Co., Dorset, UK): 0.5, 1.0, or 1.5 mg/kg, with four pigs per dose. Doses were chosen on the basis of pilot experiments. Amphetamine was dissolved in 4 ml sterile saline. Four pigs received control injections of 4 ml saline. One pig was tested each day, and pigs with different doses were tested in a random order.

Pigs were moved to the test pen on the evening prior to testing. The test room was identical and adjacent to the room with the home pens but contained one single U-shaped pen (Fig. 1). Although chain manipulation is a common stereotypic activity in sows kept in restrictive feeding and housing conditions (55), pilot experiments indicated that amphetamine did not induce this activity. A chain was therefore not present in the pen. Food was available in a trough and water from a drinking bowl. The walls of the pen consisted of vertical bars spaced 10 cm apart. The observer distinguished five different areas in the pen by cues on the wall; no boundaries were drawn on the floor (Fig. 1). Pigs were observed from behind a solid gate at one side of the room (Fig. 1). Amphetamine was injected subcutaneously at 1030 h in pen area 3, which could be closed off by a gate at either side. Observations took place between 0900 and 1500 h and consisted of 4 min of continuous observation commencing every 5 min. They included standing and sitting or lying posture and the following activities.

Amphetamine stereotypies (AMPH SS). During pilot observations, two types of amphetamine-induced movements were identified: a) head movements (up and down or sideways); b) nonlocomotory movements of the hind legs (stepping), whether lying or standing. Because of the similarity with head and limb movements induced by amphetamine in laboratory rodents, usually referred to as "amphetamine-stereotypies", the same terminology will be adopted here.

Locomotion. Forward locomotion, similar to that in non-treated pigs. Pilot observations indicated that similar to rats (12,46) nonforward locomotion (backward walking or rotation around the hind legs) could occur, but as it only occurred infrequently this behaviour was eliminated from analysis. In addition, each boundary crossing was recorded as a measure of transit.

Open eyes. Standing, sitting, or lying down with eyes open but not performing any overt activity.

Nosing objects. Standing, sitting, or lying down touching objects (trough, drinking bowl, floor, wall, or bars) with nose.

Other. Any activity other than those mentioned above.

Postures and activities were recorded as proportion of time, with the exception of AMPH SS, which were recorded as frequency of bouts. A bout of AMPH SS was defined as an uninterrupted occurrence (intervals <1 s) of head movements or stepping. In addition, the frequency of locomotory activity was recorded.

Data Analysis

Analysis was based upon logarithmic transformations of frequency and on angular transformations of proportions of time of the activities. Analysis was based on observations made between 0900 and 1400 h. Dose effects were analysed by analysis of variance (ANOVA) for repeated measures (with nested structures for pig, dose, and observation time) with two factors (dose × observation time). Increased levels of standing would be expected to be accompanied by proportional increases in activities usually performed while standing. To analyse whether changes in behaviour were specifically induced by amphetamine, or whether they were relative to increased levels of standing, the ANOVA was repeated fitting levels of standing as a covariate. Where the ANOVA indicated significant effects, the least significant difference (LSD) test was used to locate important effects.

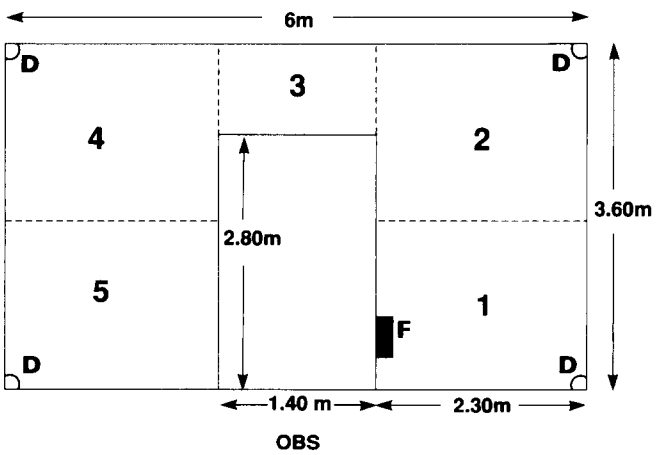

FIG. 1. Spatial arrangement of the pen used in the amphetamine test. D, drinking bowl; F, feed bowl; OBS, location from where observations took place.

RESULTS

Subjectively, amphetamine induced a behavioural syndrome that was clearly distinct from behaviour of saline-injected pigs. While at the lowest dose pigs appeared generally more active, with increasing dose the time spent alert without performing overt activities increased. At higher doses (1.0–1.5 mg/kg), pigs seemed aroused but less able to perform normal activities. Levels of nosing and rooting were reduced, and pigs were often standing rigid with open eyes, repeating head, and nonlocomotory leg movements.

Level of standing was significantly increased by amphetamine, $F(3, 12) = 3.48$, $p = 0.05$ (Fig. 2A). There was a dose × observation time interaction, $F(123, 492) = 1.36$, $p < 0.05$, with t-values of the difference indicating significantly longer levels for the highest amphetamine dose between 1.5–3 h postinjection.

Overall level of open eyes was specifically increased by the different doses of amphetamine, $F(3, 11) = 3.49$, $p = 0.05$ (Fig. 2B), with higher levels at doses 1.0 and 1.5 than at doses 0.0 and 0.5 mg/kg (LSD: $p < 0.05$). Overall level of open eyes was not correlated to level of standing, $F(1, 11) = 0.19$, NS (Fig. 3).

Although nonlocomotory movements of the hind legs in

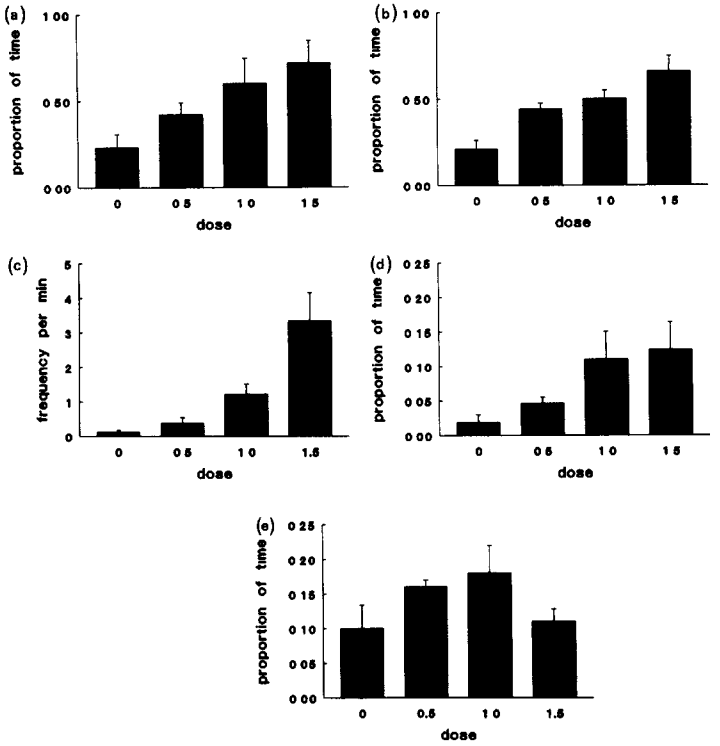

FIG. 2. Average levels (with SEM) of: (a) standing; (b) open eyes; (c) AMPH SS; (d) locomotion; (e) nosing and rooting all over a 3.5-h period after injection of different doses of amphetamine (n = 4 per treatment).

some cases occurred in a lying posture, increased level of standing was related to higher frequencies of AMPH SS, $F(1, 11) = 4.58, p = 0.056$. AMPH SS were specifically increased by amphetamine, as the effect remained significant when level of standing was fitted as a covariate, $F(3, 11) = 10.80$, $p = 0.001$ (Fig. 2C). There was a dose × observation time interaction, $F(123, 491) = 2.59$, $p < 0.001$ (Fig. 3), with animals receiving 1.5 mg/kg showing significantly higher levels of AMPH SS between 30 and 90 min postinjection than controls and other treatment groups. Again, time effects of level of standing were related to time effects of AMPH SS as shown by a significant covariate effect, $F(1, 491) = 15.46$, $p < 0.001$ (Fig. 3).

Proportion of time and frequency of forward locomotion, as well as transit, were increased by amphetamine [e.g., $F(3, 12) = 3.71$, $p < 0.05$, for both proportion of time and frequency] (Fig. 2D). These increased levels were, however, relative to increased level of standing as fitting standing as a covariate showed level of standing to be positively correlated to forward locomotion [e.g., proportion of time, $F(1, 11) = 74.85$, $p < 0.001$], and the dose effect was removed [e.g., proportion of time, $F(3, 11) = 0.70$, NS]. Similarly, time effects of level of standing also accounted for time effects of level of forward locomotion [e.g., proportion of time, $F(1, 491) = 283.48, p < 0.001$] (Fig. 3).

The overall proportion of time spent in nosing and rooting of objects was affected by amphetamine, $F(3, 12) = 3.39$, $p < 0.05$ (Fig. 2E), with significantly higher levels at doses 0.5 or 1.0 mg/kg than in controls (LSD: $p < 0.05$). Fitting level of standing as a covariate removed the dose effect, $F(3, 11) = 2.20$, NS. Time effects in level of standing could explain time effects in level of nosing and rooting, $F(1, 491) = 355.47, p < 0.001$.

Despite the overall dose effects, there were large differences between individuals receiving similar doses. For example, higher doses (1.0–1.5 mg/kg) did not increase levels of standing in all pigs; one pig, receiving 1.0 mg/kg amphetamine, responded by lying down while persistently nosing and rooting its fore legs.

DISCUSSION

Amphetamine treatment increased behavioural activation, as indicated by levels of standing, and induced a rigid standing or sitting posture with increased levels of open eyes. Other important changes included the occurrence of movements of the head and hind legs at higher doses (1.0–1.5 mg/kg).

Although amphetamine-induced rigidity has not been reported by other authors, the "awkward disjunctive posture" reported for amphetamine-treated rats (43) may be equivalent to the rigidity observed in our study.

There are similarities between amphetamine-induced behaviour in pigs and other species. Amphetamine has been reported to induce head and limb movements in species, such as rats, cats, mice, squirrel monkeys, and humans, where they are referred to as amphetamine stereotypies (19,28,39). In addition, in many species amphetamine induces oral activity, also referred to as amphetamine stereotypies, such as sniffing, licking, and gnawing in rats and mice, gnawing in guinea pigs, licking and sniffing in cats, and teeth grinding and chewing in humans (2,11,30,36,39,53). In the present study, mainly head and limb movements were observed. In another study on pigs,

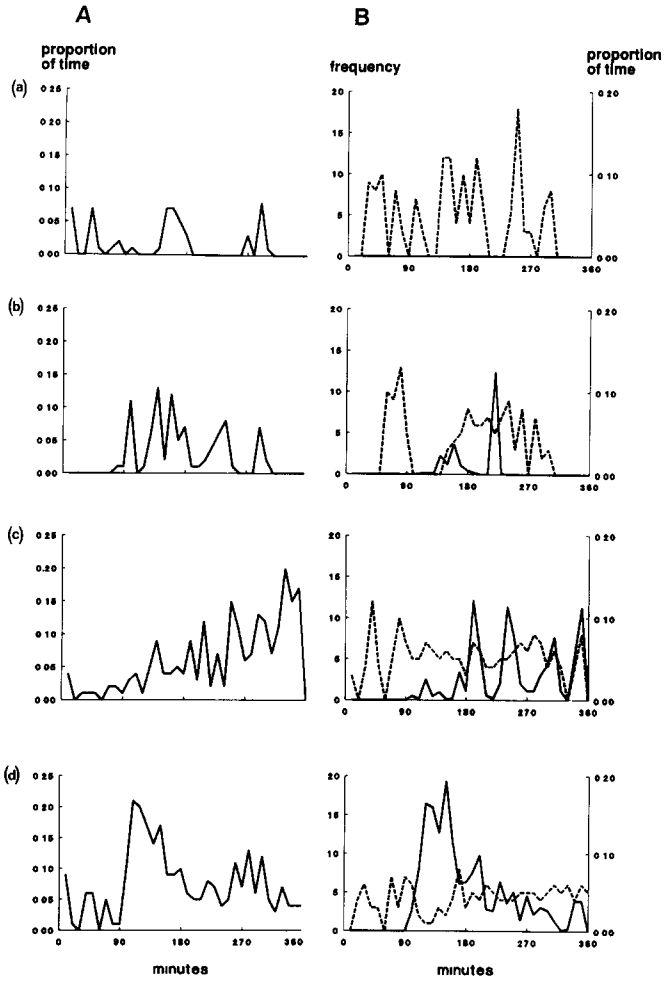

FIG. 3. Average levels of (A) open eyes and (B) locomotion and AMPH SS after injection of: (a) saline; (b) 0.5 mg/kg; (c) 1.0 mg/kg; (d) and 1.5 mg/kg amphetamine (n = 4 per treatment). Injection took place at 90 min. (B): (----), locomotion; (—), AMPH SS.

however, amphetamine induced chewing movements in addition to head and limb movements (56).

The present study also indicates some qualitative differences between pigs and other species. First, amphetamine generally reduced levels of overt activity. This effect is found in laboratory rodents only at very high doses of amphetamine, where most activities are inhibited [>10 mg/kg; (28)]. At lower doses in these species, however, amphetamine stereotypies are reportedly performed continuously (12), in contrast to pigs in our experiment, where they were performed in a discontinuous manner. Second, although amphetamine increased absolute levels of locomotion and nosing of objects, both increases were relative to increased levels of standing. Low doses of amphetamine increase locomotion in, for example, rodents and cats, but with one exception (40) we have found no reports that indicate whether the increased levels of locomotion are related to a general increase of activity or to a specific action of amphetamine (16,26,33). Neurological studies based upon specific lesions and local administration of dopamine or dopamine agonists indicate a specific role for the mesolimbic dopamine system in amphetamine-induced locomotion (15,21,37). The lack of an amphetamine-specific increase in locomotion in pigs does not necessarily indicate an absence of effect of amphetamine on the underlying neural structures, as amphetamine stereotypies may inhibit locomotion due to competition between the two behaviours (28,56).

EXPERIMENT 2

In rats, high doses of both apomorphine and amphetamine have been reported to elicit locomotion and continuous gnawing, sniffing, and licking (10,28,43,44). There are also reports of differences between the effects of the two drugs; in one study, it was found that, in contrast to apomorphine, amphetamine did not induce licking and gnawing (13). Furthermore, "snout contact fixation", where the rat keeps its snout close to surfaces, is induced by apomorphine but not by amphetamine (54). In addition, depending upon strain and history of the individual low doses of apomorphine can induce climbing in rats and mice, which has not been reported for amphetamine (5,54). The second experiment was carried out to establish which activities are induced by increasing doses of apomorphine in pigs.

METHOD

Animals and Housing

Subjects were 28 homebred British Landrace × Large White male pigs weighing 20–30 kg. They were kept in groups of four in 2 × 2 pens, on straw. A trough was fitted in one corner with food (standard growing pigs concentrate in powdered form) continuously available. A nipple drinker was fitted above the trough.

Procedure

Pigs were randomly assigned to one of five doses of apomorphine (apomorphine hydrochloride; Sigma); 0.05, 0.10, 0.30, 0.60, or 1.00 mg/kg, with four pigs per dose. In addition, four pigs received sterilised saline and four pigs received no injection. Doses were chosen based upon pilot experiments. Apomorphine was dissolved in 4 ml sterile water. Four pigs were tested each day; different doses were tested in random order.

Pigs were moved to a test pen on the evening prior to testing. The test pen was identical to the home pen and was in the same room. Straw was removed from the test pen shortly before the start of the test, while the pigs were fed. No food was available during the test. Four pigs were tested per day. Pigs were observed from a 1.5-m high platform in the middle of the room that gave a clear view over the four test pens. Observations took place between 1000 and 1400 h and consisted of 1 min of continuous observation starting every 10 min. They included: a) posture; b) activity; and c) objects on which the behaviour was performed (Table 1). The time spent in different behavioural categories was recorded. Apomorphine was injected subcutaneously at 0950 h.

Data Analysis

An ANOVA for repeated measures with a similar structure as in Experiment 1 was performed on angular transformed data. This analysis was repeated with inclusion of levels of standing fitted as a covariate to test for specific effects of apomorphine. Where significant effects were found, the LSD test was used.

TABLE 1
DESCRIPTIONS OF BEHAVIOURAL CATEGORIES

Behavioural Categories	Activities	Objects
Closed eyes	Lying down with eyes closed	
Snout contact fixation	Walking while keeping its rooting disk close to or at the floor	
Locomotion	Walking keeping its head up	
Drink	Apparently ingesting water	
Vacuum chew	Making chewing movements without having anything in its mouth	
Nose	Casually touching substrates with its rooting disk	Trough or drinker, floor or wall
Root	Making rooting movements while exerting force	Trough or drinker, floor or wall
Bite	Biting or attempting to bite in substrates	Trough or drinker, floor or wall
Lick	Licking substrates	Trough or drinker, floor or wall
Other	Urinating, defecating, yawning, stretching, scratching	

RESULTS

Subjective interpretation of the observations indicated that at the lowest dose apomorphine reduced initial levels of lying down with closed eyes. At intermediate and higher doses, apomorphine induced a behavioural syndrome characterised by persistent performance of the same behaviour pattern. This pattern started within approximately 30 min of injection and its duration appeared to be dose dependent. The pattern could be relatively simple (e.g., persistent biting of objects in one place) or more complex [walking the same route, sometimes maintaining snout contact with the floor (snout contact fixation), stopping at some point to perform the same behaviour each time]. Often, pigs appeared to be uncoordinated and on several occasions walked or fell into the food trough. This incidence could then lead to the pig walking in and out of the trough until the behaviour became more variable. Other unusual activities included standing on hind legs, with the fore legs resting on one of the walls, or jumping repeatedly into the air.

Posture

Overall levels of standing were not affected by the different doses of apomorphine, $F(6, 21) = 0.90$, NS. There was a significant treatment × observation time interaction, with a tendency for postinjection levels of standing to increase with dose, although the LSD test did not reveal significant differences between different doses at any specific time, $F(138, 482) = 1.87, p < 0.001$ (Fig. 4).

Locomotion

Apomorphine did not specifically affect overall levels of locomotion, $F(6, 20) = 1.84$, NS. However, there was a significant treatment × observation time interaction due to a specific effect of apomorphine, $F(138, 481) = 1.05$, $p < 0.001$ (Fig. 4), with increased levels of locomotion occurring within 10 min after injection with doses of greater than or equal to 0.1 mg/kg. Time effects of locomotion were also related to levels of standing, $F(1, 481) = 184.60, p < 0.001$.

Levels of snout contact fixation were specifically increased by apomorphine, $F(6, 20) = 3.71$, $p < 0.05$. This was due to high levels of snout contact fixation during the first hour postinjection induced by the dose of 1.0 mg/kg, as shown by a significant treatment × observation time interaction, $F(138, 481) = 1.28, p < 0.001$ (LSD: $p < 0.05$; Fig. 4).

Drinking

Apomorphine did not specifically affect overall drinking levels, $F(6, 20) = 0.12$. There was, however, a significant treatment × observation time interaction, $F(138, 481) = 1.66, p < 0.001$, mainly due to high levels of drinking in the first postinjection hour by pigs receiving 1.0 mg/kg (LSD: $p < 0.05$; Fig. 4).

Oral Manipulation

Absolute levels of nosing or rooting the floor or wall were not significantly affected by apomorphine [$F(6, 21) = 1.09$, NS, and $F(6, 21) = 1.58$, NS, for nosing and rooting, respectively], but levels of both behaviours were reduced relative to increased levels of standing [$F(6, 20) = 2.86, p < 0.05$, and $F(6, 20) = 3.60, p < 0.05$].

Licking the trough or drinker did not occur. No significant treatment effects were found on vacuum chewing, biting, nosing and rooting of the trough or drinker or biting or licking of the floor or wall [e.g., biting trough or drinker, $F(6, 20) = 1.36$, NS].

The lack of an overall increase of oral activities in apomorphine-treated pigs may be related to individual differences: Independently of the dose, eight pigs showed increased levels of nosing, rooting, and licking of the floor or wall and of biting and nosing the trough, with levels up to 0.65 as a proportion of time.

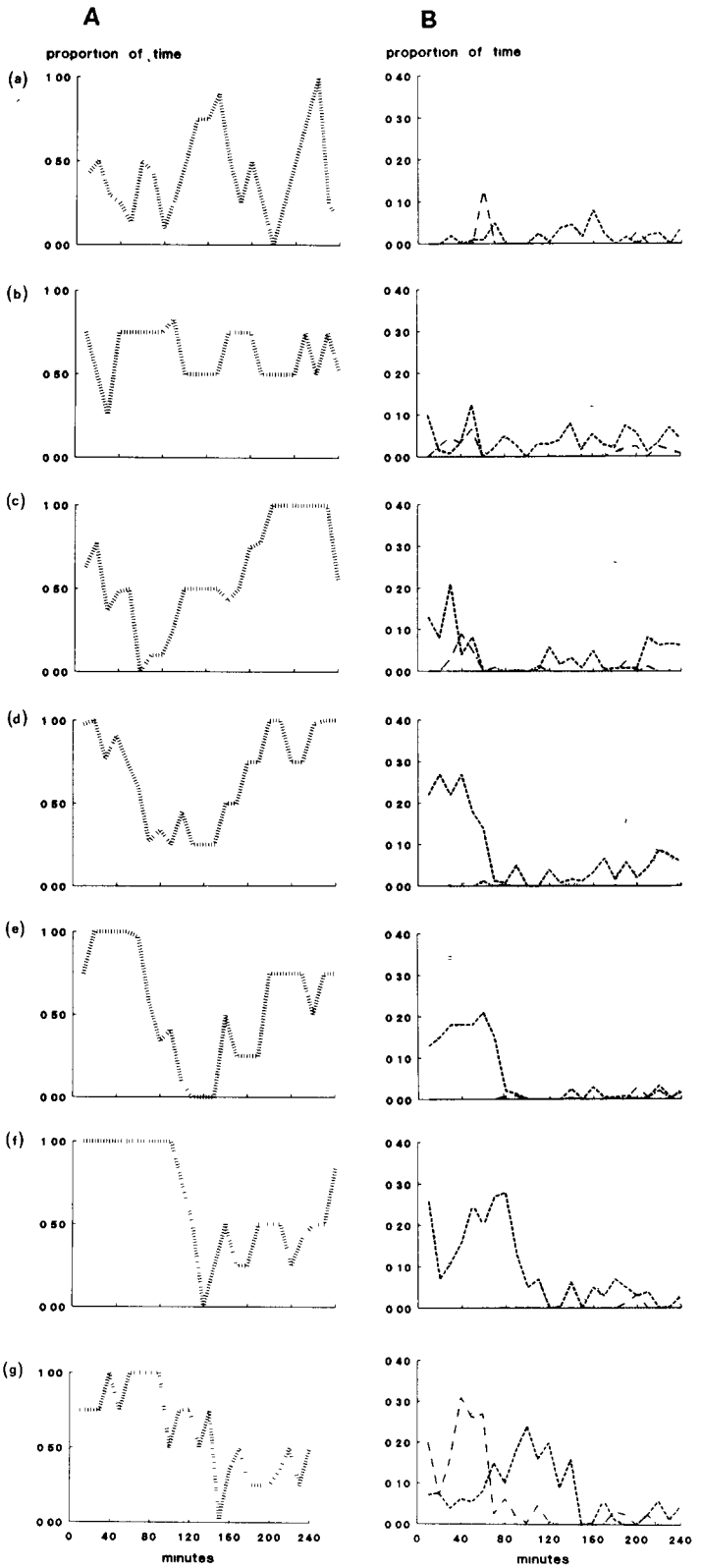

FIG. 4. Average levels of (A) standing and (B) locomotion, snout contact fixation, and drinking in: (a) controls; and after injection of (b) saline; (c) 0.05 mg/kg; (d) 0.1 mg/kg; (e) 0.3 mg/kg; (f) 0.6 mg/kg; and (g) 1.0 mg/kg apomorphine. (B) (---), locomotion; (·····), snout contact fixation;(····), drinking.

DISCUSSION

Administration of higher doses of apomorphine (0.3–1.0 mg/kg) tended to prolong the initial levels of standing. In contrast to amphetamine, it specifically induced locomotion at intermediate and higher doses (0.1–1.0 mg/kg). Furthermore, apomorphine induced snout contact fixation and increased apparent drinking. Finally, within 30 min after apomorphine administration (>0.1 mg/kg) pigs established a behaviour pattern that was repeated in a relatively invariable manner until the drug effects declined.

There are similarities between apomorphine-induced behaviour in pigs and other species. Apomorphine administered to cattle and laboratory rodents also increased locomotion (6,27,50), although in some rodent strains similar doses of apomorphine induced depression of locomotion (4,45,47). Similarly, snout contact fixation has been described as a universal aspect of apomorphine-induced behaviour in rats (54). The present results suggest that apomorphine induces snout contact fixation in pigs, although the behaviour was not observed in all individuals. Finally, it has been suggested that in rats apomorphine has a strong conditioning effect, reinforcing behaviour displayed during onset of the drug action (4). The repeated performance of a similar behaviour pattern in our experiment suggests that such an effect also takes place in pigs.

As no direct measurements of the actual amount of water ingested were made, it cannot be established whether apomorphine increased actual fluid intake in pigs; however, in a previous study on pigs we found actual amount of water ingested to be positively correlated with observed drinking (57). Although we were unable to find reports on apomorphine-induced drinking in other species than the pig, drinking induced by repeated amphetamine administration has been reported for rats, and this drinking was independent from normal water regulatory mechanisms (41).

Apomorphine induces oral activities in other species, usually referred to as apomorphine-induced oral stereotypies, such as licking, gnawing, and sniffing in rats and mice, licking in cattle, and licking and chewing in sheep (7,28,29,44,50,54). Again, these stereotypies do not occur in all individuals, and different individuals can show different forms of oral stereotypy (7,29,54). Similarly, in our experiment apomorphine increased oral activity in some, but not all, pigs and individuals differed in their expression of oral activity.

EXPERIMENT 3

In Experiment 2 it was found that within 30 min after apomorphine administration a behavioural pattern was established that was performed in a relatively invariant way until apparent waning of the drug action. Furthermore, pigs showed large individual differences in response to apomorphine. Experiment 3 was carried out to investigate the behavioural response of a larger group of pigs to a standard dose of 1.0 mg/kg apomorphine, including changes in behaviour over time. In addition, some endocrine measurements were made.

METHOD

Animals and Housing

Subjects were 16 homebred German Large White nulliparous female pigs, weighing between 100–125 kg. Each pig had been fitted with a permanent catheter in the jugular vein 2 weeks prior to the test. Prior to the operation, they were housed in two groups of eight in 8 × 2.5-m strawed pens, but were subsequently kept on straw in individual stalls. The stalls (70 cm wide) were placed in a row, with a concrete trough at the front. The troughs were filled with water outside the feeding time. The room was climate controlled (18°C). At 0830 and 1600 h, each pig received 1 kg standard concentrated food in powdered form.

Procedure

Pigs were moved to the test pen on the evening prior to testing. The test pen measured 3.5 × 3.5 m and was in the same room as where animals were housed. It had three solid walls, while one side had horizontal bars (o.d. 4 cm) spaced 30 cm apart. A 3.5-m long concrete trough was beneath the bars. A 20-cm chain was attached to the bars at 60 cm above the floor. The observer distinguished four equal sized areas in the pen by cues on the wall. No straw was available. A nipple drinker was fitted on one wall 60 cm above the floor.

Each pig received a 10-ml subcutaneous injection of sterile saline or 1.0 mg/kg apomorphine (apomorphine hydrochloride; Sigma), dissolved in 10 ml saline. Due to their size, animals had to be restrained with a rope tightened around the upper jaw. Injections were given at 0900 h and were followed by 4 h of behavioural observations. Each pig was tested once with saline and once with apomorphine in a balanced design.

Behavioural records were made as described in Experiment 2 (Table 1). In addition, snout contact in trough (walking alongside trough, with the nose on bottom of the trough) and boundary crossings (as a measure of transit) were recorded. Manipulation of the chain did not occur in this experiment.

Blood Sampling and Assays

Blood was collected in chilled EDTA-coated tubes from 11 pigs prior to injection and at 1, 2, and 4 h postinjection. Samples were centrifuged immediately at 4°C and kept at −20°C until analysis.

Plasma cortisol levels were determined by radioimmune assay as described by Ladewig and Smidt (25). Plasma adrenaline and noradrenaline levels were determined by high-pressure liquid chromatography. Each sample was extracted according to the method described by Smedes et al. (51) and modified according to Tsuchiya and Hayashi (58). Briefly, the method consists of a liquid/liquid extraction of the catecholamines with a heptan-tetraoctylammonium bromide–octanol solution after addition of diphenyl borate at pH 8.5, and reextraction with 0.08 M acetic acid. Twenty microliters of the acetic acid extract is injected for chromatography. Evaluation of the sample hormone concentration is based upon the concentration of internal standards as calculated from the area of each peak. The extraction efficiency was 93.4 ± 9.4%; the coefficient of variation were 10.1 and 3.1% for noradrenaline and adrenaline, respectively. For technical reasons, adrenaline could only be determined for 70% of the samples.

Data Analysis

An ANOVA for repeated measures on angular transformed data was performed on half-hourly averages as described in Experiment 2. Similarly, specific treatment effects were calculated as described in Experiment 2.

Preinjection hormone levels were analysed by an ANOVA for repeated measures (nested structure for pig) with one factor (day) to test whether levels differed between the first and second tests. The effect of drug treatment on hormone levels was tested by an ANOVA for repeated measures (nested struc-

tures for pig, day, and time since injection) with two factors (day and time since injection).

RESULTS

Behaviour

Apomorphine induced the performance of an invariant behaviour pattern lasting for 2–3 h (Fig. 5). Often, high levels of oral manipulation occurred, with individuals differing in type and level. No manipulation of the chain occurred.

Posture

The overall levels of standing were increased by apomorphine, $F(1, 15) = 55.55, p < 0.001$. There was a dose × observation time interaction, $F(7, 105) = 22.29, p < 0.001$, with apomorphine-injected pigs having higher levels until 2.5 h postinjection (LSD: $p < 0.01$). However, in two cases apomorphine-injected pigs were lying down for 50% of the first 1-h postinjection period, while intensively rooting the floor or trough.

Locomotion

The overall levels of locomotion were increased in apomorphine-treated pigs, $F(1, 15) = 9.60, p < 0.01$, but in contrast to Experiment 2 this effect was relative to increased standing as fitting standing as a covariate removed the effect, $F(1, 14) = 0.00$, NS, with levels of standing being significantly correlated to those of locomotion [$F(1, 14) = 23.74, p < 0.001$, across treatments]. Again, individuals differed strongly in levels of this behaviour (Fig. 6).

Locomotion while maintaining snout contact with the floor was specifically induced by apomorphine; there was a dose × observation time interaction, $F(7, 104) = 5.23, p < 0.001$, with higher levels during the first 1.5 postinjection h (LSD: $p < 0.01$, due to five pigs showing high levels of this behaviour during this period (Fig. 6).

Locomotion while maintaining snout contact with the trough was also induced specifically by apomorphine, with a significant increase in overall levels, $F(1, 14) = 25.11, P < 0.001$. This was due to three pigs showing significant amounts of this behaviour during the first hour (16, 43, and 50% of their time, respectively; Fig. 6). In apomorphine-injected pigs, the average level of total snout contact fixation was higher than that of any other behavioural category (33.3 ± 9.0% in the first postinjection hour; Fig. 6).

Overall frequency of crossovers of boundaries, a measure of total distance travelled, showed an apomorphine-specific increase, with significantly higher levels during the first 1.5 postinjection h [dose × observation time interaction, $F(7, 104) = 3.08, p < 0.001$] (LSD: $p < 0.05$).

Drinking

Overall drinking levels in apomorphine-injected pigs did not differ from saline-injected pigs, $F(1, 15) = 2.14$, NS.

Oral Manipulation

Oral manipulation of the nipple drinker was exclusively related to drinking. There were no significant effects on vacuum chewing and nosing or rooting the trough [e.g., nosing trough, $F(1, 14) = 1.29$, NS]. Overall levels of licking the trough were specifically increased by apomorphine, $F(1, 14) = 14.70, p < 0.005$ (Fig. 6). Levels of licking the floor or wall were increased in apomorphine-injected pigs, $F(1, 15) = 10.55, p < 0.005$, but this effect was relative to increased levels of standing, $F(1, 14) = 0$, NS. In apomorphine-injected pigs, average levels of licking substrates were second highest after levels of snout contact fixation (31.3 ± 7.1% in the first postinjection hour; Fig. 6). Apomorphine specifically reduced overall levels of nosing but not rooting the floor of wall, $F(1, 14) = 9.18, p < 0.01$. However, for both nosing and rooting a significant treatment × observation time interaction was found, with lower levels during the first 2-h and the first $\frac{1}{2}$-h postinjection period, respectively [$F(7, 104) = 3.79, p = 0.001$, LSD: $p < 0.05$, and $F(7, 104) = 2.54$, LSD: $p < 0.05$, for nosing and rooting, respectively]. For nosing, these effects were relative to increased levels of standing as no differences were found in absolute values [e.g., dose effect, $F(1, 14) = 1.88$, NS].

Although sometimes no significant overall effects were found for oral manipulation, individual levels could be high. Three apomorphine-injected pigs showed substantial vacuum chewing (10, 14, and 47% of time in the first postinjection hour). Rooting the trough occurred in substantial amounts of one apomorphine-treated pig (39% of the time in the first and second postinjection hour) and in lower amounts in two others (9% of the time in the first postinjection hour). Biting the trough occurred in one apomorphine-injected pig (65% of the time of the first postinjection hour).

Hormones

There was no day effect on preinjection levels on any of the hormones [e.g., $F(1, 8) = 1.66$; NS, and $F(1, 9) = 0.03$, NS, for noradrenaline and cortisol, respectively] (Fig. 7).

There was no drug or sample effect on adrenaline levels [$F(1, 6) = 1.7$, NS, and $F(3, 24) = 2.31$, NS, for drug and sample effect, respectively] (Fig. 7).

Noradrenaline levels did not vary between the samples, $F(3, 30) = 0.94$, NS, but there was a drug × sample interaction, $F(3, 26) = 2.99, p < 0.05$, with apomorphine-injected pigs having higher levels at 1 and 2 h postinjection (Fig. 7).

Cortisol levels were not affected by the drug treatment, $F(1, 9) = 0.04$. NS. However, there was a strong effect of time since injection, $F(3, 30) = 20.43, p < 0.001$, with higher levels at 1 and 2 h postinjection (Fig. 7).

DISCUSSION

As in Experiment 2, 1.0 mg/kg apomorphine elicited locomotion while maintaining snout contact with the floor. In addition, snout contact fixation occurred in the trough. While in Experiment 2 this dose also increased levels of drinking, in Experiment 3 it increased levels of licking the trough. Furthermore, some pigs showed high levels of other oral activities, such as vacuum chewing and biting or rooting the trough. Differences between Experiments 2 and 3 may be related to differences in breed, age, or test environment. The effect of breed on the behavioural response to apomorphine is not known in pigs, but strain effects on apomorphine response have been reported for mice (6,45,47). Age may affect apomorphine response in pigs, as apomorphine induced predominantly a snout-rubbing response in piglets (14). Differences in test environment between Experiments 2 and 3 may also have caused the behavioural differences. For example, while in Experiment 3 pigs had to drink from a nipple drinker in Experiment 2 they drank from the trough, which may have facilitated the behaviour.

As in Experiment 2, within 30 min after apomorphine ad-

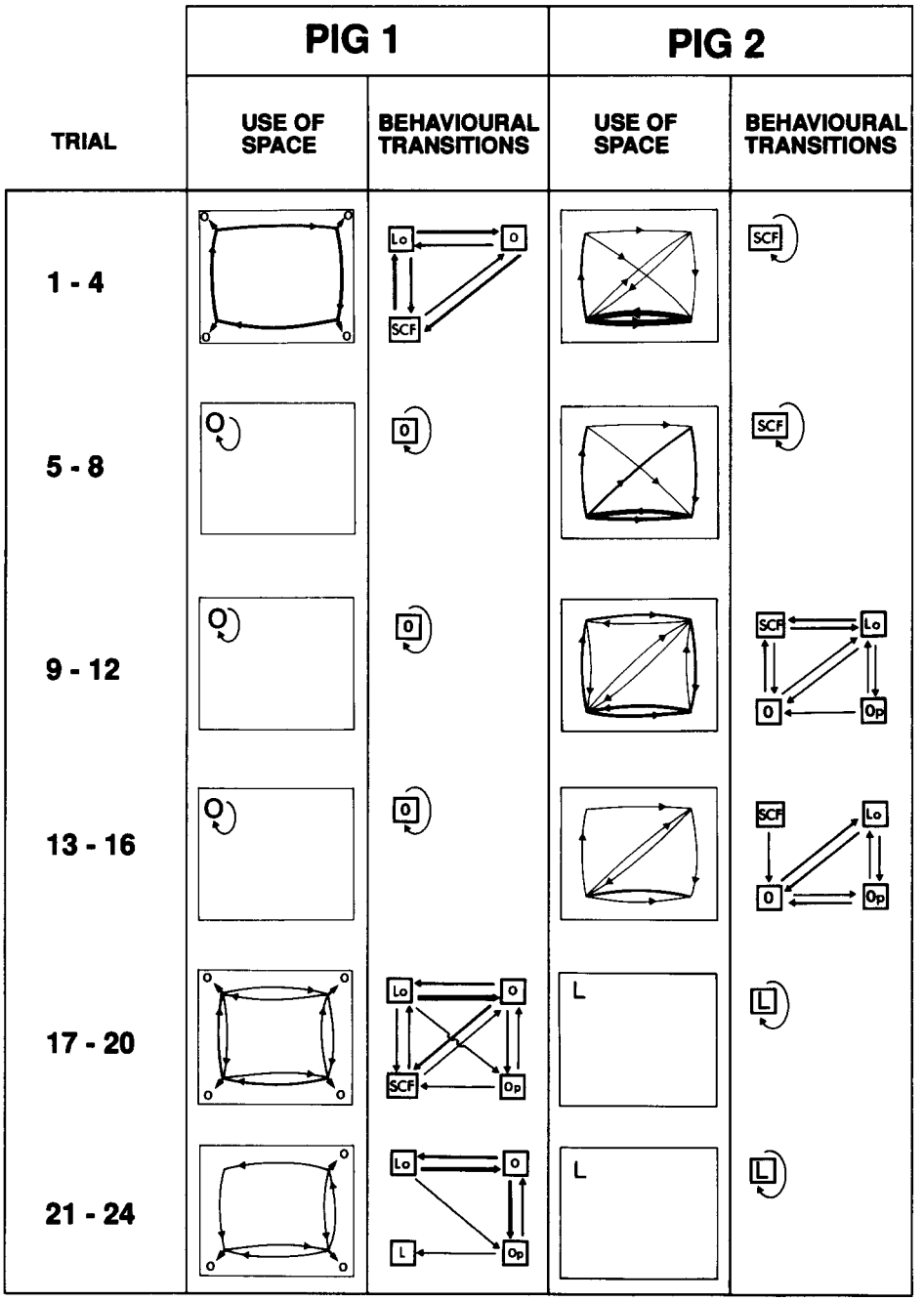

FIG. 5. Use of space and behavioural transitions in two randomly selected pigs after injection of 1.0 mg/kg apomorphine. Thickness of lines indicates frequency: range for use of space from 1–20; range for behavioural transitions from 1–8. O, oral activity; SCF, locomotion while maintaining snout contact fixation; Lo, locomotion, no SCF; Op, standing, without performing any overt activity; L, lie down. Pig 1 initially showed locomotion (with and without maintaining SCF) alongside the walls of the pen, while rooting in each corner. This was followed by 2 h of intensive rooting at one location. Eventually, other behaviours, including locomotion, reappeared. Pig 2 initially showed locomotion, while maintaining SCF, mainly between only two corners of the pen. After 1.5 h, its route became more variable and other activities appeared.

ministration an invariable behaviour pattern was established, lasting for 2–3 h, confirming previous suggestions that apomorphine reinforces behaviour displayed at the onset of the drug action (4). Thus, in addition to the direct effects of apomorphine on behaviour the reinforcing effect on behaviour forms a second route of influencing behavioural output.

The postinjection cortisol rise probably reflects the stressful aspects of the restraint that accompanied injection. The

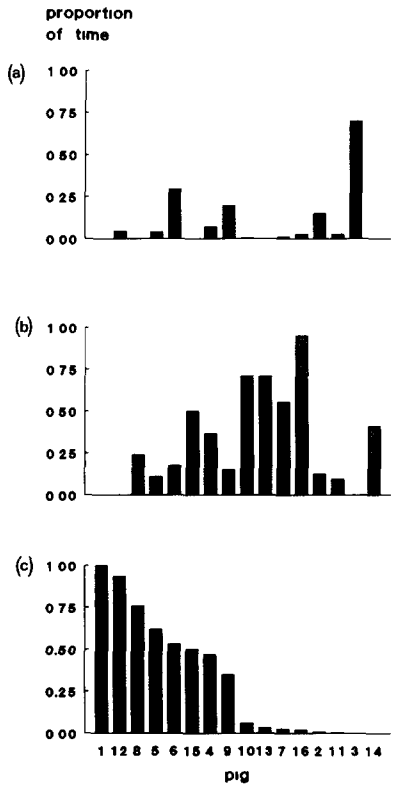

FIG. 6. Distribution of levels of: (a) locomotion; (b) licking; (c) snout contact fixation, all over the first h after injection with 1.0 mg/kg apomorphine. (b) and (c): (■), floor directed; (▩); trough directed.

increase in noradrenaline was apomorphine related and may have been related to the higher levels of physical activity in treated pigs (35).

GENERAL DISCUSSION

The present study shows that in contrast to rats, in which apomorphine and amphetamine induce stereotypy syndromes with certain similarities (10,13,28,43,44), amphetamine and apomorphine induce different responses in pigs. High doses of amphetamine induced a rigid standing, with jerking head movements and nonlocomotory movements of the hind legs, while reducing other overt activities. The occurrence of head and limb movements at a dose of 1.0 mg/kg suggests that pigs are more responsive to amphetamine than rats, where similar movements are generally reported to arise at 5–10 mg/kg (19). Apomorphine increased oral activities, locomotion, snout contact fixation, and probably drinking at similar doses (1.0 mg/kg) as those that induce oral activities in rats. In addition, apomorphine reinforced the behaviour displayed at the onset of the drug action. This reinforcing effect may have partially caused the rather large qualitative individual differences in apomorphine response, in contrast to amphetamine, where predominantly quantitative rather than qualitative individual differences were found [see also (56)]. It has been reported that in some species, including cats and dogs, amphetamine may also have conditioning effects (11).

The lack of similarity between the behavioural response to amphetamine and apomorphine may have been related to the doses used. It is possible that in pigs higher doses of apomorphine than those used in the present experiment might have elicited amphetamine-like behavioural responses. However, despite similarities in the behavioural response to amphetamine and apomorphine in rats it is well known that the two drugs' actions differ at a neurological level. In contrast to apomorphine, amphetamine stimulates not only dopamine but also noradrenaline systems in the CNS (17,24). In addition, different dopamine structures and receptors underlie different components of dopamine agonist-induced oral and nonoral activities (10,20,38,60), and may be differently affected by the two drugs. The present experiment shows that in pigs the different neurological properties of amphetamine and apomorphine are clearly reflected on a behavioural level.

It is clear that both amphetamine and apomorphine induce behavioural syndromes in pigs that are quite distinct from environmentally induced stereotypies. While amphetamine induces a rigid standing and a lack of oral manipulation, environmentally induced stereotypies consist of an uninterrupted sequence of manipulative activities and drinking (1,8,42, 56,57). Also, the head movements induced by amphetamine are discontinuous and not similar to the more fluently performed head movements, often referred to as weaving, found in pigs under restrictive feeding and housing conditions (8). As apomorphine elicits oral activities, including drinking, and stimulates continuous performance of a single behaviour pattern, its action may be more similar to the neurological alterations that occur during development of environmentally induced stereotypies, for example, licking can also occur under restrictive housing conditions. However, it is clear that there are important differences between these two classes of behaviour, such as the lack of manipulative activities (e.g., chain

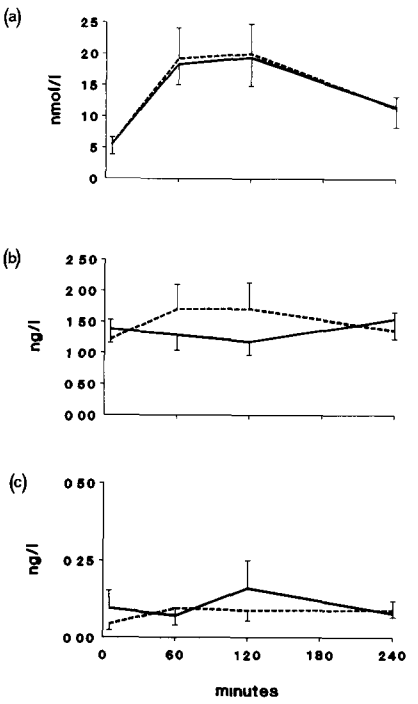

FIG. 7. Average effects of apomorphine and time on: (a) cortisol; (b) noradrenaline; (c) adrenaline. The first sample was taken prior to injection. (——), saline; (---), apomorphine.

manipulation) in apomorphine-injected pigs (Experiment 3). The differences between the three behavioural syndromes often referred to as stereotypies induced by amphetamine, apomorphine, and environmental stress show that the definition of stereotypy is rather nonspecific and that the term should be used with caution.

Summarising, amphetamine and apomorphine elicit different behavioural responses in the pig. Elements of both amphetamine- and apomorphine-induced behaviour were similar to those observed in rats and other species. Environmentally induced stereotypies appear different from both the amphetamine- and apomorphine-induced behavioural syndrome.

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