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Behavioural Effects of Anxiogenic Agents in the Common Marmoset

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CAREY, G. J., B. COSTALL, A. M. DOMENEY, D. N. C. JONES AND R. J. NAYLOR. *Behavioural effects of anxiogenic agents in the common marmoset.* PHARMACOL BIOCHEM BEHAV **42**(1) 143–153, 1992.—The effects of the anxiogenic agents FG7142, caffeine, pentylenetetrazole, and amphetamine were assessed in two anxiety situations in the marmoset, first in an "anxiogenic" test based on the animal's response to a human observer standing in front of the home cage and second in a low-anxiety situation where animals behaviour was videotaped in the absence of the observer. In response to the human observer, the anxiolytic agent diazepam (0.1–2.5 mg/kg, SC) was shown to reduce the intensity of behaviours such as postures, while increasing time spent on the cage front. In this test, with the exception of amphetamine, which only modified responding at stereotypic doses, the anxiogenic agents failed to modify marmoset behaviour. In contrast, in the low-anxiety filming protocol the anxiogenic agents consistently reduced measures of locomotor activity while increasing the amount of time animals spent in the nest box. It is suggested that the low-anxiety protocol may be useful to evaluate drug-induced anxiogenesis and in studies of withdrawal from chronic anxiolytic treatment or drugs of abuse.

Anxiety Anxiolytics Caffeine Pentylenetetrazole Amphetamine Marmoset

THE detection of novel anxioselective agents remains dependent to a great extent on the use of animal models of anxiety. Some models may also allow an examination of the mechanisms involved in anxiety and an ideal model would be one reproducing all features of human anxiety. However, it remains clear that in most animal experiments the attempt has been to model rather than reproduce human anxiety, the data generated being interpreted in terms of analogy rather than homology. Yet, similar features of descent and characteristics between human and nonhuman primates has focussed interests on primates in the development of anxiety models that may be more relevant.

The presence of a human to induce anxiety in primates has been the basis of several tests, including the "taming" models in rhesus and cynomolgus monkeys (24) and the behavioural responses of dominant male baboons toward group subordinates (3). The marmoset human threat test, developed in the primate laboratories at Bradford (8), is based upon the measurement of the behavioural response of marmosets to a human observer in which the amount of time marmosets spend at the front of the cage in confrontation with the observer and the exhibition of characteristic postures directed at the observer were measured. The response of this group of marmosets to the human threat was found to be consistent over at least 5 months (9). Some physiological evidence that this was an anxiety-type response by the marmosets was provided by the detection of a significant increase in plasma cortisol immediately following the period of threat. Pharmacological evidence was provided by the ability of the anxiolytic agents diazepam and buspirone to reduce the time spent forward and postures. Interestingly, a range of other pharmacological agents including the 5-hydroxytryptamine-3 (5-HT_3) receptor antagonists, 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OHDPAT), pentobarbitone, and tiapride also displayed an anxiolytic profile of action.

The major aims of the present studies were to further characterise the response of the marmoset to a human threat and develop a methodology to detect the behavioural changes induced by agents reported to be anxiogenic. This was attempted in two ways. The first approach was to assess the influence of agents reported to induce anxiety in both humans and animals on the response of marmosets to a human threat. This was designed to provide information about the possibility of a bidirectional property of the experimental protocol, allowing detection of the distinct actions of both anxiolytic and anxiogenic agents.

The second approach was the development of a methodology that would allow a quantification of marmoset behaviour in the absence of human threat and in a manner lacking the aversive properties of the human threat test (i.e., a "low-anxiety" protocol). This was achieved using a remote-controlled videocamera system, the difference in the behaviour of threatened and nonthreatened animals providing further evidence that behaviours measured during the human threat test are a

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consequence of the threat itself. A range of anxiogenic agents and diazepam were used in the low-anxiety protocol to identify those behaviours indicative of anxiogenesis and investigate whether anxiogenic agents could produce similar behavioural changes to those caused by the aversive presence of threat.

The agents utilised in the present study have all been reported to induce anxiety-like effects in both man and animals and include: β-carboline-3-carboxylic acid methyl amide (FG7142) (13), caffeine (2), yohimbine (6), pentylenetetrazole (PTZ) (18), and amphetamine (20). The behaviour of marmosets, following administration of these agents, in the presence or absence of a human threat is described below.

METHOD

Animals

Twelve adult marmosets (*Callithrix jacchus*, 310–405 g) were housed in single-sex pairs in cages of dimensions 76 (high) × 50 (wide) × 61 cm (deep). The sides, bottom, back, and roof of each cage were made of solid metal, while the removable cage front consisted of a hinged grid. A nestbox (25 × 18 × 18 cm), suspended from the cage ceiling, provided a place for marmosets to sleep and which to retreat. The cage also contained two wooden perches. The front perch was positioned near to the cage front (22 cm from the cage floor and 14 cm from the cage front) and the rear was positioned at the back of the cage (47 cm from the cage floor and 18 cm from the cage back). The holding rooms were maintained at 25 ± 1°C at a humidity of 55%. Rooms were illuminated on a 12L:12D cycle, lights being on between 7:00 a.m. and 7:00 p.m. Simulated dawn and twilight periods were programmed to occur 0.5 h before and after main lights came on or went off. During the 12-h dark period, a single 60-W red bulb was illuminated to avoid complete darkness.

Animals were given free access to food and water (Mazuri primate diet, SDS Ltd., Essex) that they received in the morning. The remainder of the diet (fruit, malt loaf, and brown bread) was given between 4:00–5:00 p.m.

Influence of anxiogenic agents upon the response of marmosets to a human threat. The protocol utilised in this study was identical to that first reported by Costall and coworkers (8). The experimenter, who was later to assess the behaviour, entered the holding room to catch and remove marmosets to an anteroom where dosing took place. This group of animals was always handled with a pair of heavy leather gauntlets (International Market Supply, Cheshire). Marmosets appeared to find this particularly aversive and it is believed that the association of this procedure with the experimenter was a major contribution to the development of the animals' response to the "human threat." The experimenter reentered the room 45 min subsequent to dosing to commence behavioural assessment.

Behavioural assessment. To assess behaviour, the experimenter stood approximately 40 cm from the cage front and made eye contact with one of the marmoset pair throughout the 2-min test period. The behaviour of the marmoset was recorded utilising a small, hand-held electronic key pad connected to a BBC microcomputer, printer (Epson RX50), 5-in. disc drive (Pace), and monitor (Philips computer monitor 80) that were situated in an adjacent room. The following parameters were recorded.

1. The number of "postures" exhibited. The most commonly observed postures were:
   - *Tail posture*—when the marmoset turns its back on the observer and raises its tail to expose the genital region.
   - *Scent marking*—Marmosets most commonly marked with the circumanal/genital scent glands, which was observed as the pressing of the anal and genital region against the surface to be marked.
   - *Slit stare*—The marmoset momentarily stares at the observer with its eyes reduced to slits while simultaneously flicking down its ear tufts, which lie flat against the head.
   - *Arched pilo*—The marmoset adopts an "arched" back stance and with full body piloerection walks to and fro along the perch or cage floor.

   The parameter "postures" refers to the sum of the frequencies of all these postures.
2. The time (in seconds) spent by the marmoset at the cage front.
3. The number of jumps from the back to the front of the cage.
4. The time (in seconds) spent inside the nest box.

In addition to these parameters, any deviation from the "normal" behavioural repertoire was noted. Upon completion of the 2-min test period, the experimenter moved to another pair of animals and assessed the behaviour of one marmoset. Once a single animal from each pair had been assessed, the observer returned to the original cage and assessed the second marmoset of the pair and so on until all remaining animals had been assessed. All behavioural assessments took place between 2:00 and 3:00 p.m.

Influence of anxiogenic agents upon the behaviour of marmosets in the absence of a human threat (low-anxiety situation). To habituate animals to the presence of the camera equipment, the camera was placed in the holding room for a period of approximately 30–60 min each day for at least 2–3 weeks prior to commencement of the study. Behaviour was remotely filmed by means of a 20-m extension cable connected to a videorecorder (Panasonic, N1/100 VHS) and a TV monitor in an adjacent room. This procedure was designed to acclimatise the marmosets to the introduction of this novel object. To facilitate video recordings of marmoset pairs in their home cages, the wire cage front was replaced by a Perspex sheet (58 × 62 × 0.5 cm) through which animals could be clearly filmed. To habituate the marmosets to this Perspex sheet, it was left in position for approximately 45–75 min each day. The final stage in the habituation procedure was to position the camera directly in front of the cage (1 m away) with the Perspex cage front in place. Although no objective behavioural parameters were measured at this stage, it was apparent that this step did not produce any gross behavioural changes indicative of stress or anxiety, such as piloerection or scent marking. The equipment was left in position for approximately 60 min. This procedure was carried out at least five times over a 2-week period.

To reduce stress induced by handling and dosing to a minimum, several steps were included in the habituation protocol. The group of marmosets used in this particular part of the study were always handled with thin surgical gloves. Throughout the entire procedure of habituation, marmosets were caught and removed to an anteroom at least once a day. Once a week, these animals were injected subcutaneously with saline (1 ml/kg).

Behavioural assessment. The behaviour of pairs of marmosets in the home cage were recorded over two 15-min periods. The initial pretreatment recording followed a 30-min period of habituation to the camera equipment and Perspex sheet and was, for the purposes of the present study, termed "normal"

behaviour. Subsequent to this first recording, animals were caught and removed to an anteroom and returned to the home cage (control) or dosed with drug or vehicle before returning. Pretreatment time for all agents was 45 min; therefore, 15 min after dosing the cage door was replaced again with the Perspex sheet. Thirty minutes subsequently, the second behavioural recording was made.

From the videotapes, the following behavioural parameters were measured for each marmoset:

1. The number of postures—these were as described for the human threat test.
2. The time spent at the cage front—this measure composed of the time spent in contact with the Perspex sheet, that is, leaning forward from the front perch or hanging from the top or the side of the cage.
3. The number of jumps.
4. The number of line crossings—this parameter measured the number of crossings from one side of the cage to the other, thus crossing a line drawn onto the TV monitor that vertically divided the cage into two equal halves.
5. The time spent inside the nestbox.

Any unusual changes in behaviour were also noted and are described in the Results section. All behavioural assessments took place between 11:30 a.m. and 3:30 p.m. on weekdays.

Experimental Design

Throughout these studies, an experimenter-blind, cross-over design for drug administration was utilised. Each animal in a pair received the same treatment. Diazepam was included in both sets of studies as a "positive control."

In studies utilising the human threat protocol, marmosets were tested no more than twice per week to avoid the risk of habituation and allow an adequate drug washout period. Between each study (a study comprising the testing of several doses of a single agent), marmosets were given at least 2 weeks break from testing. Each study commenced following a test when animals had been treated with saline alone. The response of drug-treated marmosets was compared with that following vehicle treatment using a matched-pairs *t*-test (Statworks® package, MacIntosh SE).

In the second set of studies utilising the low-anxiety filming protocol, marmosets received a treatment only once per week and were allowed at least 2 weeks break between studies. Each study commenced with at least one sham recording, that is, equipment was set up and removed after 45 min. This was designed to minimise any association being made between the testing equipment and injection procedure.

Because of the highly variable nature of the spontaneous behaviour recorded using the filming protocol, it was necessary to analyse treatment-induced changes in behaviour as a function of baseline behaviour (normal) before comparing with effects of vehicle treatments. Initial studies failed to reveal any statistically significant differences between control (handling alone) or vehicle treatment. This was achieved by calculating the difference between pretreatment (normal) and postreatment behavioural recordings for each parameter. These values were compared with those obtained for control and vehicle treatments using a Wilcoxan signed-rank test (Statworks package, MacIntosh SE). However, for all descriptions and illustrations of the data the postreatment behaviour is expressed as a percentage of the pretreatment behaviour.

Drugs

Yohimbine HCl (Sigma, UK), caffeine (Sigma), pentylenetetrazole (Sigma), and amphetamine sulphate (Sigma) were dissolved in sterile saline (0.9% w/v). FG7142 (R.B.I.) was dissolved in the minimum quantity of polyethylene glycol and diluted with sterile saline. Injectable diazepam (Diazmul®, Wyeth) was diluted with sterile saline and a vehicle control was provided by the use of Intralipid,® which was similarly diluted.

RESULTS

Response of Saline-Treated Marmosets to a Human Threat

General observations. When approached by the experimenter, saline-treated marmosets ceased all normal activity and fixed their attention upon the observer. During the test period, marmosets spent 13.2–22.7% of the test period directly on the cage front. In general, marmosets spent little time in the nestbox and this appeared to show individual variation; for this reason, this data is not presented for drug treatments. The marmosets made 7.2–10.5 postures, the majority of which were scenting behaviour, and spent a large proportion of the time engaged in locomotor activity (7.2–16 jumps). It is important to note that at no time during the test period did the experimenter observe any active social interaction between the animal pair, although occasional bouts of passive interaction were observed.

Influence of diazepam. Administration of diazepam (0.1 and 0.25 mg/kg, SC) altered the response of marmosets to a human threat in a manner consistent with the findings of earlier studies (9) (Fig. 1A). Diazepam increased the time marmosets spent directly on the cage front from 16.1 ± 5.5 s to 32 ± 6.7 s (0.1 mg/kg, SC, $p < 0.05$). In addition, diazepam (0.25 mg/kg, SC) caused a significant reduction in the number of postures from 10.4 ± 2 to 3.2 ± 1.3 ($p < 0.01$), while failing to modify locomotor activity.

Influence of anxiogenic agents. Administration of FG7142 (5 and 10 mg/kg, SC), yohimbine (0.5 and 1 mg/kg, SC), pentylenetetrazole (10 and 20 mg/kg, SC), or caffeine (5, 10, and 20 mg/kg, SC), administered 45 min previously, had no significant influence upon the response of marmosets to a human threat and induced no apparent behavioural changes; only data for FG7142 is shown (Fig. 1B).

Following administration of amphetamine (0.5, 1, and 2 mg/kg, SC), there was a dose-dependent decrease in the number of postures that achieved statistical significance at the 1 mg/kg (SC) dose ($p < 0.05$) (Fig. 1C). However, it should be noted that a reduced level of posturing was observed only in four of the six animals who also exhibited stereotyped behaviours. In three marmosets, this was seen as a repetitive "pseudoscratching" movement, in which marmosets exhibited behaviour consistent with scratching but failed to make full skin contact. The fourth marmoset showed some bouts of pseudoscratching, but mainly exhibited bouts of "checking" behaviour (small, rapid head movements in the horizontal plane) of greatly increased frequency.

At the highest dose tested (2 mg/kg, SC), amphetamine induced stereotyped behaviour in all six marmosets that ranged from constant checking and pseudoscratching movements to stereotyped head movements. One marmoset spent the entire test period moving its head, in a circular fashion, from a position looking over its left shoulder back toward the right side of its chest and back again. Posturing behaviour was virtually abolished in all six marmosets ($p < 0.01$) and the number of jumps was reduced significantly from 13.7 ± 4 to 5.3 ± 2.9 ($p < 0.05$). In one animal pair, this dose of

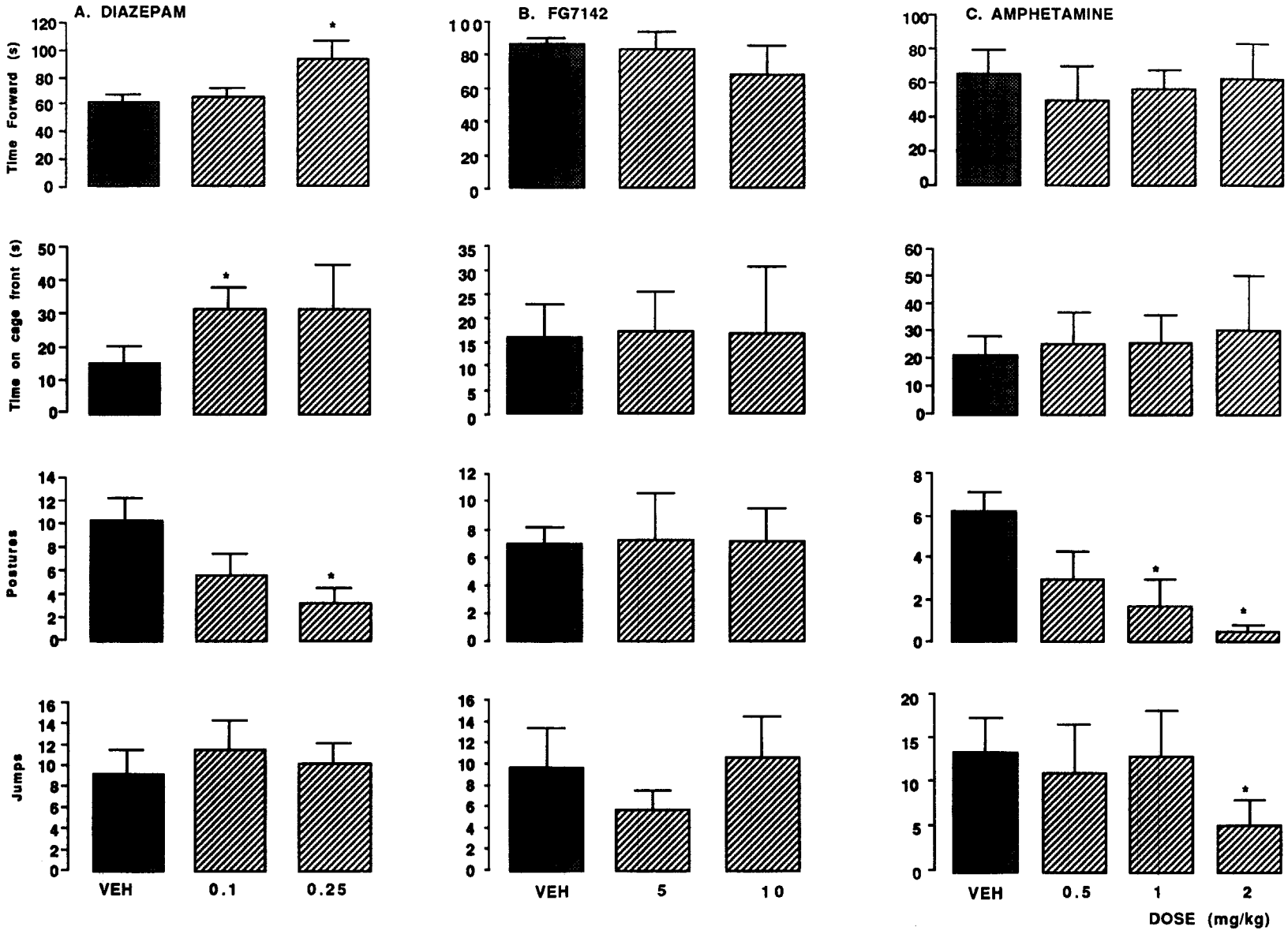

FIG. 1. Behavioural response of marmosets to a 2-min period of human threat following pretreatment with (A) diazepam (0.1–0.25 mg/kg, SC), (B) FG7142 (5–10 mg/kg, SC), and (C) amphetamine (0.5–2 mg/kg, SC). Vehcile or drug treatments were administered 45 min prior to testing. $n = 6$. Significant differences between treatment groups and vehicle are shown as $^*p < 0.05$ (matched-pairs t-test).

amphetamine appeared to cause a reduction in social interaction; indeed, marmosets positioned themselves as far away from each other as possible ("spacing" behaviour).

Behaviour of Common Marmosets Filmed in a Low-Anxiety Situation

General observations. The behaviour of marmosets filmed in the absence of a human threat differed markedly from that observed during the human threat test. In general, there was a trend for the values for all parameters measured to increase during the second behavioural assessment (except for the number of postures). However, the changes that did occur were consistent and so provided a relatively stable baseline against which the behaviour of drug-treated marmosets could be compared. The data for the behavioural parameters used during this study is given in Table 1. This data compares the behaviour of nontreated with saline-treated marmosets.

While a much wider range of behaviours were exhibited, these were too variable or individualistic to be used as valid measures of drug-induced changes.

Some general observations can, however, be made. For example, marmoset pairs interacted with each other in a manner not observed when a human was present. Marmosets spent a mean of 45.5 s (20.9–73.4 s) of the 15-min period engaged in active social interaction that was most often exhibited as allogrooming, playful wrestling, or chasing. Passive interaction, when marmosets were positioned in very close contact with each other but not behaviourally interacting, was observed for 191.7 s (116.4–273.4 s) of each 15-min period. Feeding and autogrooming were also regularly observed, although these behaviours were particularly variable.

Marmosets spent a substantial part of each 15-min period engaged in locomotor activity that was qualitatively different from that observed during a human threat test. In the human threat test, "normal locomotion" was the predominant form of locomotor activity (travelling from one place to another). In the absence of a human threat, a large proportion of locomotor activity consisted of "somersault/bouncing gait" activity. It was observed, in one pair of marmosets at least, that long bouts of "somersault/bouncing gait" activity, where marmosets move with an exaggerated springy movement and bounce off cage surfaces, triggered the cage mate to join in. Marmosets displayed postures very infrequently during each 15-min period (see Table 1) and the majority of these were scent-marking behaviours. The time spent in the nestbox was again, as with the human threat, variable and individualistic in nature. However, some marked increases in this parameter were observed with certain doses of drug treatment and these are reported in the Results section but not illustrated.

Influence of diazepam. Administration of diazepam (0.1 and 0.25 mg/kg, SC) 45 min before the second behavioural recording appeared to cause a trend toward increased locomotor activity. This trend reached significance following diazepam (0.25 mg/kg, SC) when the number of line crossings increased to 161.2% of normal behaviour compared with vehicle treatment (96%, $p < 0.05$, Fig. 2). This dose of diazepam also appeared to increase the time spent directly on the cage front, although this failed to achieve statistical significance ($p = 0.058$).

There was a trend for treatment with diazepam (0.25 mg/kg, SC) to induce a greater level and range of activity and an increase in bouncing gait locomotion and playful behaviour. This was reflected by an approximate twofold increase in active interaction in at least two marmoset pairs compared with vehicle-treated marmosets.

Influence of putative anxiogenic agents. The behavioural changes induced by the administration of FG7142, caffeine, yohimbine, PTZ, and the lowest dose of amphetamine (0.5 mg/kg, SC) appeared to be qualitatively similar and were the reverse of those changes produced by diazepam, that is, reduced time at cage front and reduced locomotor activity. However, in all cases posturing behaviour remained unaltered by any of the treatments.

FG7142 (1–20 mg/kg, SC). FG7142 (5, 10, and 20 mg/kg, SC) caused significant reductions in the time spent directly on the cage front from 117% (vehicle) to 64, 50, and 56%, respectively ($p < 0.05$, Fig. 2).

All doses of FG7142 tested caused increases in time spent by the marmosets inside the nestbox. However, this achieved statistical significance only after administration of FG7142 (5 mg/kg, SC), when this parameter increased from 186.3% (vehicle) to 430% ($p < 0.05$).

Administration of FG7142 (1, 5, and 10 mg/kg, SC) pro-

TABLE 1

A COMPARISON OF BEHAVIOURS UNDER LOW-ANXIETY CONDITIONS FOLLOWING CONTROL (TWO STUDIES) OR SALINE TREATMENT (FOUR STUDIES)

	Control			Saline		
Parameter	Pre	Post	% Change	Pre	Post	% Change
Time forward (seconds)	451 (403–499)	478.1 (462–494)	117.3 (92.6–142)	478.1 (409.5–578)	513.9 (440–543)	109.2 (93.8–132)
Time on cage front (seconds)	133.7 (63–190)	146.7 (66.3–166.7)	110 (76–142)	117.0 (62–134)	113.3 (82–158)	96.8 (65.8–118)
Time in nestbox (seconds)	14.3 (11.4–17.2)	16.9 (15.6–18.2)	121.5 (106–137)	31.3 (17.5–61.6)	11.2 (4.4–23.3)	36.9 (17.7–70)
No. of postures	4.8 (4.2–5.4)	3.5 (3.4–3.6)	74.4 (63–85.7)	3.2 (1.3–6.7)	2.9 (1.5–4.1)	106.9 (61–154.9)
No. of jumps	33.5 (33.1–33.8)	39.1 (34.3–43.9)	116.8 (101–132.6)	20.9 (15–31.5)	28.5 (20.3–39.4)	139.5 (110–195)
No. of line crossings	66.9 (65.3–68.5)	72.5 (50.5–94.5)	107.5 (77–138)	43.3 (24–59.1)	64.6 (43.8–88.4)	153.5 (142.6–182.5)

Data is given as the mean plus the range of data (in parentheses). $n = 6$–10.

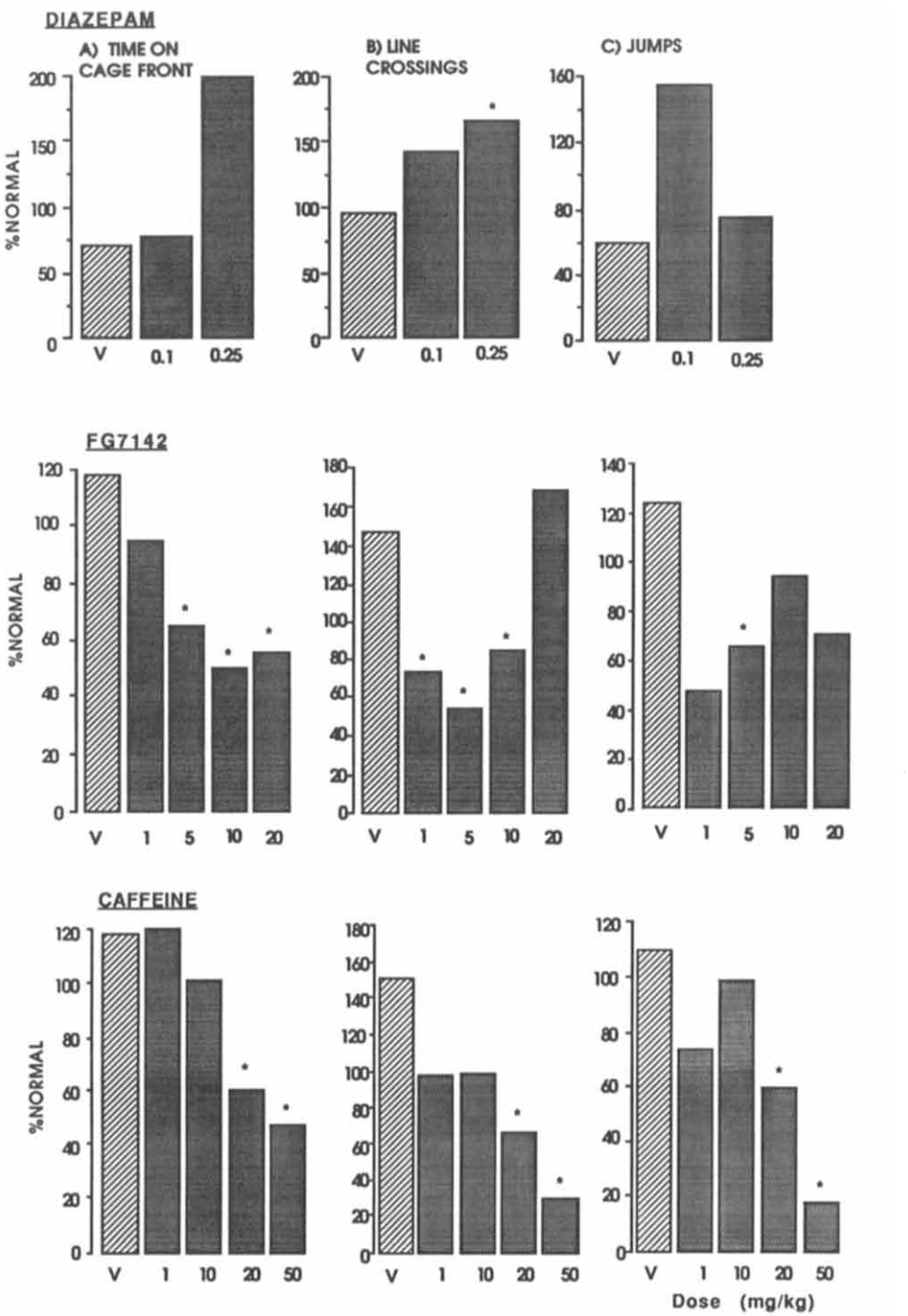

FIG. 2. Effect of diazepam (0.1–2.5 mg/kg, SC), FG7142 (1–20 mg/kg, SC), and caffeine (1–50 mg/kg, SC) on the behaviour of marmosets in the absence of a human threat. Data is expressed as a percentage of behaviour exhibited in a predrug period (normal behaviour). V represents the response of vehicle-treated animals. $n = 6$. SEMs 12–184% calculated on the difference between pre- and posttreatment recordings. Significant differences to vehicle treatment are shown as $*p < 0.05$ (Wilcoxon signed-rank test, calculated on original data).

duced significant reductions in the number of line crossings from 147.4% (vehicle) to 72.9, 54.8, and 84.9%, respectively ($p < 0.05$). Following administration of 20 mg/kg (SC), however, the number of line crossings was no different from that of vehicle-treated marmosets.

The number of jumps was also reduced by FG7142, although this only achieved significance following administration of 5 mg/kg (SC). This parameter was reduced from 123.7% (vehicle) to 65% (5 mg/kg, SC, $p < 0.05$).

Caffeine (1–50 mg/kg, SC). Following administration of caffeine (20 and 50 mg/kg, SC), there was a reduction in the time spent directly on the cage front from 119% (vehicle) to 60 and 47%, respectively ($p < 0.05$, Fig. 2).

There appeared to be a dose-dependent increase in the time spent by marmosets inside the nestbox. This effect reached significant levels following administration of 50 mg/kg SC ($p < 0.05$). All doses of caffeine appeared to reduce locomotor activity, although significant changes were seen only at the highest doses tested (20 and 50 mg/kg, SC, $p < 0.05$). These changes included line crossing (150 for vehicle to 66 and 29% respectively) and the number of jumps (110 for vehicle to 58.7 and 17.7%, respectively).

It is important to note that 6 of the 10 marmosets who received the highest dose of caffeine (50 mg/kg, SC) exhibited behaviour consistent with nausea, which consisted of nose-rubbing and eye closure. In four animals, this was accompanied by at least one episode of vomiting during the test period.

Yohimbine (0.5–5 mg/kg, SC). Following administration of 5 mg/kg (SC), marmosets spent significantly less time directly on the cage front. This parameter was reduced from 65.2% (vehicle) to 7.4% (5 mg/kg, $p < 0.05$, Fig. 3).

Yohimbine (1 mg/kg, SC) increased time spent in the nestbox from 70% (vehicle) to 567% ($p < 0.05$). Following administration of the higher dose of yohimbine (5 mg/kg, SC), this fell to control values.

All doses of yohimbine tested appeared to cause a reduction in locomotor activity. However, this reduction only became significant following administration of 5 mg/kg (SC). In at least one marmoset, this dose of yohimbine induced a type of locomotor activity very similar to bouncing gait locomotor activity, but much jerkier. In the same animal, bouts of jerky, backward movements were observed that were initiated from a sitting position.

It should be noted that at the 5-mg/kg (SC) dose yohimbine appeared to cause peripheral cardiovascular effects that manifested as marked and sustained "blushing" in at least four of the marmosets tested.

PTZ (10 and 20 mg/kg, SC). Administration of PTZ (10 mg/kg, SC) caused a significant reduction in time spent directly on the cage front from 95.7% (vehicle) to 23.3% ($p < 0.05$, Fig. 3).

Treatment with PTZ (20 mg/kg, SC) caused a marked increase in the time spent in the nestbox from 22% (vehicle) to 438%. Because of the large degree of variability in this data, however, this change was not statistically significant.

PTZ administration produced a dose-dependent decrease in locomotor activity. These changes reached significance following treatment with 20 mg/kg (SC), such that the number of line crossings was reduced from 142.6% (vehicle) to 52.2% ($p < 0.05$) and the number of jumps was reduced from 117.8% (vehicle) to 42.4% ($p < 0.05$).

Amphetamine (0.5–2 mg/kg, SC). Administration of amphetamine (0.5 mg/kg, SC) caused a significant reduction in the time spent directly on the cage front from 138% (vehicle) to 51.5% ($p < 0.05$, Fig. 3).

Treatment with amphetamine (0.5 mg/kg, SC) also caused reductions in locomotor activity, although these were not statistically significant.

At this dose of amphetamine, some bouts of stereotyped behaviours were evident in two of the six animals tested, although these were mild in nature, that is, they did not appear to influence the behavioural profile of these marmosets. One animal exhibited infrequent bouts of enhanced checking behaviour, while the other exhibited stereotyped scratching (15 of 17 bouts of autogrooming appeared to be stereotyped). There was a dose-dependent increase in the intensity of stereotyped behaviours following administration of the higher doses of amphetamine (1 and 2 mg/kg, SC). Treatment with amphetamine at 1 mg/kg appeared to induce stereotyped behaviour in at least three of the six marmosets tested. These were manifest as intense bouts of stereotyped pseudoscratching, checking behaviour, or head movements. At the highest dose tested (2 mg/kg, SC) all six animals exhibited intense stereotyped behaviours that ranged from continual stereotyped scratching or checking to stereotyped upper-body movements. One marmoset spent virtually the entire 15-min period moving back and forth along the bottom of the cage front. In one pair of animals, this dose of amphetamine appeared to induce the "spacing" behaviour observed during the human threat test (see earlier), although this was not accompanied by the aggressive vocalisation observed previously.

The induction of stereotyped behaviours at the higher doses of amphetamine precluded other behaviours and so the behavioural profile obtained differed from that of the other putative anxiogenics.

DISCUSSION

The claim for validity of the human threat test as a model for the assessment of anxiolytic agents is supported by ethological, physiological, and pharmacological evidence (9).

In the present study, there were some quantitative differences in the response of marmosets to a human threat compared with previous studies. In the original studies of Costall et al., marmosets were selected for inclusion in the study if they exhibited a minimum of eight postures and spent less than 25% of the time at the cage front. However, in the present studies only minimal animal selection was utilised. As a result, the mean number of postures exhibited by saline-treated marmosets during the test period ranged from 7.2–10.5 and marmosets spent 46–87 s near to the cage front, of which 15.8–27.2 s was spent directly on the cage front. Nonresponding animals, that is, those marmosets who appeared indifferent to the presence of a human or those timid animals who consistently hid in the nestbox, were excluded.

It is important to demonstrate that the response of the marmosets included in this study to a human threat could be influenced by a clinically active anxiolytic, such as diazepam. This agent could be shown to attenuate the response of this group of marmosets to the human threat such that they spent a significantly greater period of time on or near the cage front and exhibited fewer postures, with no apparent influence on locomotor activity. These findings would suggest that, qualitatively at least, this group of marmosets reacted to the human threat in essentially the same way as the high-responding animals previously utilised.

Administration of agents reported to induce anxiety, such as FG7142, yohimbine, caffeine, and pentylenetetrazole, had no influence upon the response of marmosets to a human

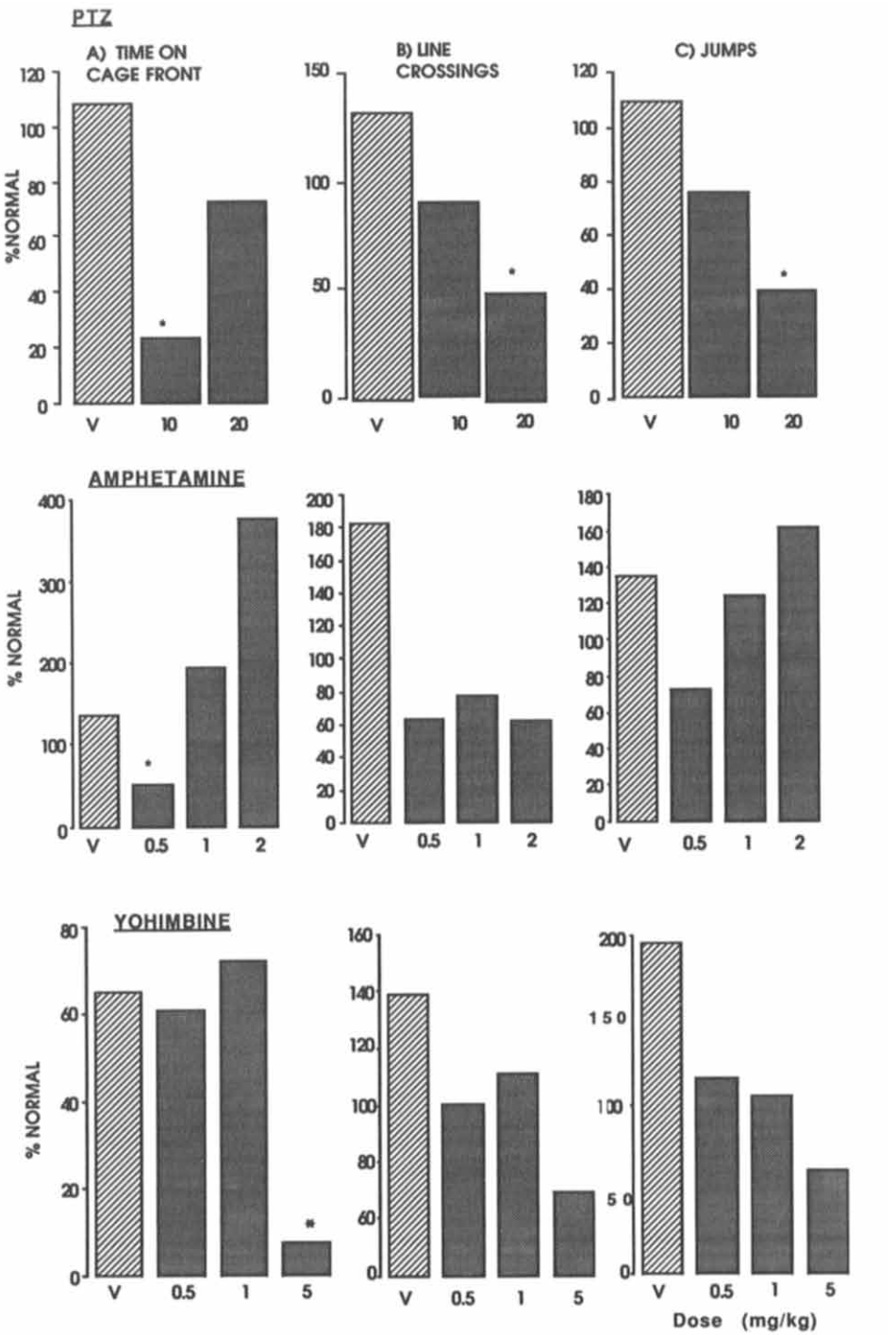

FIG. 3. Effect of pentylenetrazole (10–20 mg/kg, SC), yohimbine (0.5–5 mg/kg, SC), and amphetamine (0.5–2 mg/kg, SC) on the behaviour of marmosets in the absence of a human threat. Data is expressed as a % behaviour exhibited in a predrug period (normal behaviour). V represents the response of vehicle-treated animals. $n = 6$. SEMs 12–170% calculated on the difference between pre- and posttreatment recordings. Significant differences to vehicle treatment are shown as $*p < 0.05$ (Wilcoxon signed-rank test, calculated on original data).

threat. Thus, it would appear that the human threat test is not able to detect agents reported to induce anxiety in other animals models. These findings have implications for the usefulness of this protocol in studies where the measurement of enhanced anxiety levels is required, for example, in the study of behavioural consequences of the intake and subsequent withdrawal of drugs of abuse or for determination of the ability of novel agents to suppress such anxiogenesis. A bidirectional measurement property, that is, the ability to measure reductions and increases in anxiety levels, has been claimed for several rodent models of anxiety. These include the social interaction test (16), the elevated plus-maze (23), the mouse light:dark test (10), and conflict models (26). However, the ability of the anxiogenic agents to clearly produce the opposite behavioural responses to those produced by anxiolytics in these models has been disputed (28).

The ability of amphetamine to reduce posturing behaviour, assumed to be indicative of an anxiolytic effect in the human threat test, may be explained by the dose-dependent increases in stereotyped behaviours, causing behavioural displacement, such as increased checking behaviour, pseudoscratching or repetitive head movements (1,25). Stereotyped behaviours were apparent in all six marmosets at the highest dose of amphetamine tested and these behaviours were accompanied by a reduction in locomotor activity. Following administration of the high dose of amphetamine, there was a reduction in interaction between marmosets that was very clear for one pair studied. This effect was described previously by Annett et al. (1). In one marmoset, however, the behaviour resembled more closely the vocal threat described by Lipp (21), that is, chatter vocalisation, full body piloerection, with slow body swaying. Consequently, the failure of the human threat test to detect false positives provides further evidence for the anxioselective nature of this model.

In the second approach adopted, marmosets were filmed in conditions designed to be as nonaversive as possible, that is, in the animals' home cage, in the absence of a human threat, and subsequent to prolonged periods of habituation to the novel equipment. The behaviours observed were perhaps indicative of the success of this protocol. For example, marmoset pairs were observed to spend approximately one third of the test period engaged in social interaction, which was either passive (marmosets in close contact with each other, but generally inactive, "huddling") or active (grooming, hugging, taking food from each other, or playful wrestling) in nature. Other behaviours observed included feeding and autogrooming. Such behaviours were not observed during the human threat test. Subjectively, the quality of the locomotor activity was also different. In the low-anxiety protocol, a large proportion of the locomotor activity was of the bouncing gait type, previously described by Stevenson and Poole (27). This type of locomotor activity is suggested to represent more than just a means of getting from one place to another but to have a signalling function and to be a form of playful behaviour. However, the most striking difference between the behaviour exhibited by marmosets in the low-anxiety and human threat protocols was in the number and type of postures. In the low-anxiety protocol, the frequency of postures was very low and consisted almost entirely of scent-marking behaviour. Other postures, such as tail postures or slit stares were only occasionally observed and appeared to be directed toward marmosets in other cages. The general observations made above are in agreement with those of other workers (4,14,27) and provide further evidence that the behaviours observed during the human threat test are a result of the human threat. Conversely, the behaviours observed under low-anxiety filming conditions resemble the "normal" activity described by other workers, although these studies generally utilised family groups.

From the list of general observations made, it was necessary to select parameters that would provide an accurate index of the anxiety state of the marmosets. The difficulties associated with the systematic measurement of spontaneous behaviour in species such as primates has been outlined by Berry et al. (3). The problem of the great variability in the behaviour between individual animals encountered by these workers was also encountered in this study. Behaviours such as feeding and different types of active interaction were particularly variable. Box (4) stressed the great temperamental differences between groups of marmosets and this was found to be equally true for the marmoset pairs included in the present study. The parameters chosen had to reflect activities in which all marmosets were involved, that were easily identified using the video assessment, and that did not require the use of a behavioural rating scale. The parameters selected measured the marmoset's cage position (directly on the cage front, in the nestbox) and locomotor activity (jumps, line crossings).

Administration of the anxiogenic agents FG7142, caffeine, pentylenetetrazole, yohimbine, and a low dose of amphetamine all caused qualitatively similar changes in the behaviour of marmosets in a low-anxiety situation. Namely, reductions in the time spent forward of the front perch (particularly directly upon the cage front), increased time spent inside the nestbox, and reduced locomotor activity. In contrast, administration of diazepam at doses that were effective in the human threat test produced a trend of behavioural changes in the opposite direction, that is, increased time spent near the cage front and increased locomotor activity.

The reduction in the locomotor activity induced by treatment with anxiogenic agents appeared to be the most consistent behavioural change, and it raises the question that this action may be the result of sedation. However, there is evidence to argue against this. First, diazepam, which itself can produce sedation, caused an increase in locomotor activity. Second, none of the so-called anxiogenic agents produced any signs of sedation in the human threat test, which has been shown to be sensitive to changes in locomotor activity (9). Finally, the inclusion in the present study of caffeine, previously reported to stimulate locomotor activity (22), would appear to provide compelling evidence that the effects observed were not the result of sedation. It may be argued that the reduction in locomotor activity is the result of a behavioural displacement, for example, time spent in the nestbox is time that cannot be spent in locomotor activity. However, by the same argument the diazepam-induced increase in time spent directly on the cage front should have resulted in a reduction in locomotor activity. In addition, yohimbine and caffeine both reduced locomotor activity at doses that had no effect upon the time spent in the nestbox. Therefore, it is proposed that the changes in locomotor activity observed in the present study represent a measure of the ability of anxiolytic and anxiogenic agents to alter anxiety levels in the marmoset. However, the ability of such agents to influence locomotor activity per se must be borne in mind when utilising the protocol described.

Treatment with the anxiogenic agents also increased the time marmosets spent in the nestbox and peering out of its entrance. This behaviour has been described previously by

Lipp (21) and is termed "lurking." However, these workers reported that lurking behaviour was usually elicited by stimuli that startled the group, such as the sudden entrance of a human into the holding room. However, in the present studies entrance into the nestbox was not hurried and did not appear to be the result of any particular event that affected the holding room in the same way. It is suggested that this behaviour was an attempt by the marmoset to "take cover" (14) and is not a consequence of, for example, sedation for the reasons outlined earlier. In addition, earlier studies in our laboratories showed that marmosets, when sleeping during the day, were usually positioned on the back perch in close contact (huddling) with their cagemates. Speculatively, the increased time spent in the nestbox and reduced time spent near the cage front could indicate a reduced willingness or ability of the marmoset to interact with the rest of the colony following administration of agents reported to be anxiogenic. By the same reasoning, interaction with the colony (or the test conditions) may have some stress-inducing effects itself as diazepam was able to increase the time spent on or near the cage front. However, such suggestions require more extensive investigation.

The validity of using putative anxiogenic agents to define behaviours associated with anxiety has received support from studies in humans, rodents, and primates. In humans, for example, pentylenetetrazole has been reported to induce feelings of "catastrophe" [see (20)]. Similarly, following the administration of FG7142 subjects reported feelings of "impending doom and annihilation" that were accompanied by increases in plasma cortisol, prolactin, and growth hormone, increases in blood pressure, and profuse sweating (13). Similar findings have been reported for yohimbine (7) and caffeine (15). Other reports of the anxiety-inducing potential in humans of the agents utilised in the present study are reviewed by Lader and Bruce (19). In primates, agents such as β-carboline-3-carboxylic acid ethyl ester (β-CCE), a β-carboline structurally related to FG7142, have been used to induce anxiety-like behavioural and physiological changes (11,12,17): β-CCE was shown to increase plasma cortisol, corticotropin and catecholamines, heart rate, and blood pressure and induce behavioural changes the authors claimed to be analogous to behaviours exhibited by rhesus monkeys in naturalistic "anxiety-provoking" situations. These effects were shown to be sensitive to the actions of diazepam and clonidine (12).

In conclusion, it is suggested that the low-anxiety filming protocol utilised in the present study may provide a model in which the actions of anxiogenic drugs can be detected. This protocol may also be of value for the detection of the behavioural effects following the intake and withdrawal of drugs of abuse and those with dependence potential and may provide a model in which to assess the potential of novel anxiolytic agents to combat a drug-induced anxiogenic situation.

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