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Marmoset Conspecific Confrontation: An Ethologically-Based Model of Anxiety

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CILIA, J. AND D. C. PIPER. *Marmoset conspecific confrontation: An ethologically-based model of anxiety.* PHARMACOL BIOCHEM BEHAV **58**(1) 85–91, 1997.—A method of measuring confrontation-induced behavioural changes in common marmosets (*Callithrix jacchus*) together with automated monitoring of locomotor activity has been developed as a possible model of anxiety. Recording both affiliative and agonistic behaviours between male/female pairs of marmosets and using diazepam as a reference drug, it has been possible to define a profile of behavioural changes which could be regarded as representing an anxiolytic response. Unfamiliar male/female pairs of marmosets were brought into close (non-contact) proximity in a controlled environment, in which their locomotor activity was recorded automatically. Simultaneously, their interactive behaviour was assessed by an independent observer via closed-circuit television. The following behaviours were analysed: aggressive postures, allogrooming, scratching, anxiety-related behaviours, social contact and self-grooming. Administration of diazepam at 1 and 3.5 mg/kg PO induced a significant (compared to control) reduction in scratching, aggressive behaviours, anxiety-related behaviours and an increase in allogrooming without affecting locomotor activity during confrontation. Differing responses dependant on gender were not found, nor did gender influence the effect of treatment on behaviour. Habituation to repeated confrontation did not occur. The results from this study demonstrate that this method can be used to measure anxiolytic activity in an objective manner. © 1997 Elsevier Science Inc.

Common marmoset Conspecific Confrontation Diazepam Aggression Anxiety Gender

THE behavioural repertoire of common marmosets (*Callithrix jacchus*) has been studied in detail (8,9,13,22,23) and it has been shown that when in a state of anxiety marmosets display characteristic behaviours. Such behaviours include slit-stare, anogenital presentation, piloerection, bouncing gate and a form of vocalisation known as geckering (23). Scratching, self grooming, wet-dog shake and scent marking may also occur. Slit-stare and anogenital presentation (see Method section for definitions) are aggressive displays that are normally aimed at the source of the threat and warn of an impending attack. Piloerection and scent marking are non directed displays that reflect both the aggression and anxiety associated with the situation.

Self grooming, scratching and wet-dog shake have been classified by many authors as displacement activities, i.e., a behaviour that is irrelevant to the situation the animal is in (1,5,7,14,19,26). Such behaviours may also form part of a coping strategy. Similarly, social contact (huddling) is known to increase as a consequence of anxiogenic stimuli (24). These behaviours are thought to be an indication of the emotional state of the animal, becoming apparent or increased during stressful situations. These behaviours are therefore of great importance in a confrontational model, as is the affiliated behaviour, allogrooming. Grooming of a partner is one of the parameters measured in social interaction; the well established rodent model of anxiety (10). It has been demonstrated that a reduction in social interaction occurs when rats are placed in aversive conditions or when treated with anxiogenic compounds. Thus, the occurrence of allogrooming after the administration of a drug may reflect an anxiolytic property of the compound being studied.

Some anxiety disorders are more prevalent in females than males (6). Gender differences in the response of squirrel monkeys following social separation and human challenge tests (both are models of anxiety) has been demonstrated (17). It is therefore important, in a study such as this, to take account of this factor in both the design and the analysis of the data generated. Thus, the effect of gender on aggression and anxiety-related behaviours was analysed. These categories were considered the most important measures of anxiolytic activity in this model. Another factor which was taken into consideration when designing this model was the effect of time, i.e.,

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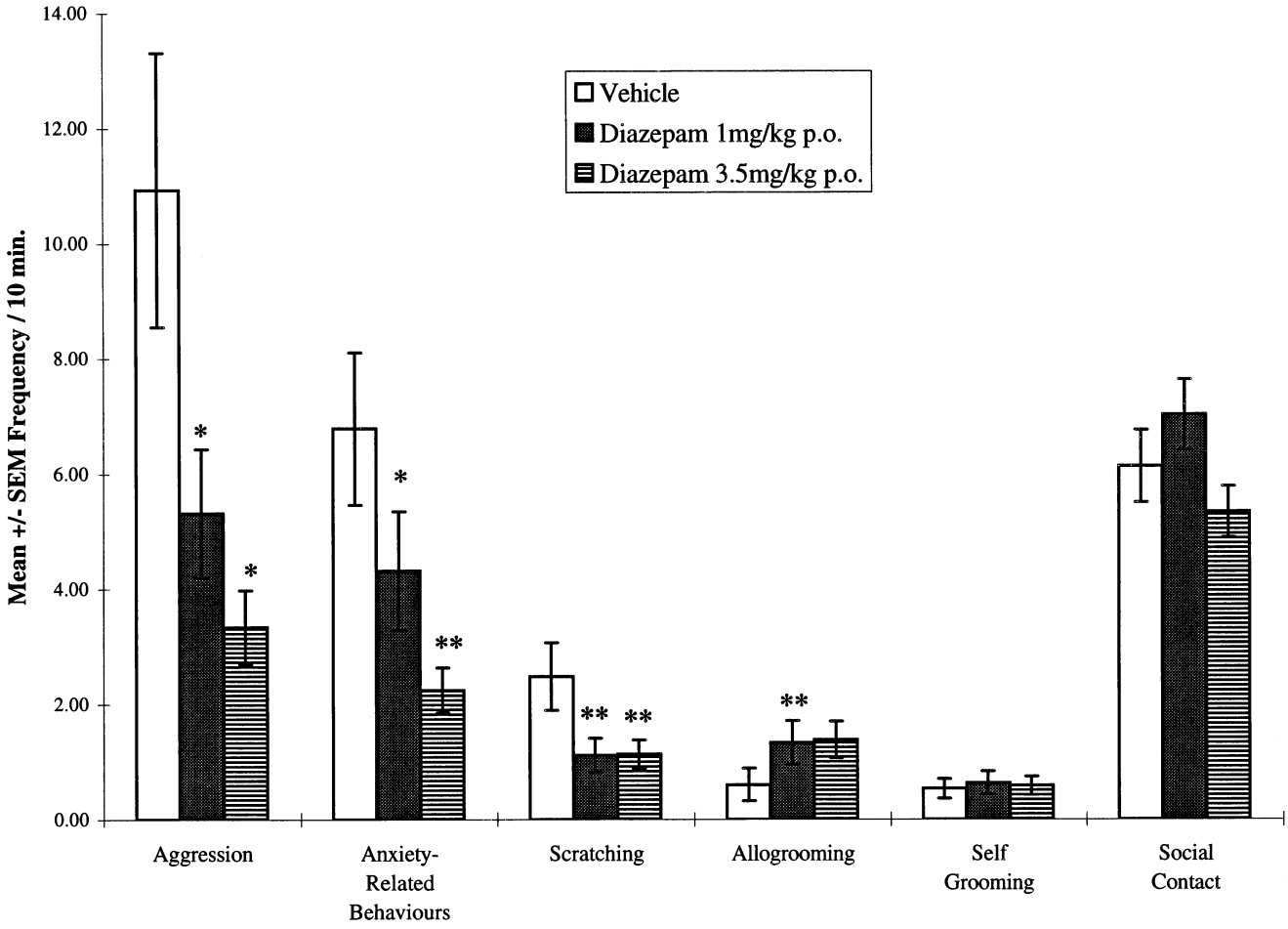

FIG. 1. Mean frequency ± SEM of marmoset behaviour during confrontation by conspecifics following vehicle and diazepam treatment ($n = 20$). Significantly different from vehicle; $^*p < 0.05$, $^{**}p < 0.01$.

the effect of repeated exposure of the marmosets to the same conspecifics during a test session. Too many confrontational periods may induce a conditioning effect which could confound the results. For this reason the confrontational periods were kept to a maximum of three, 10 min periods half an hour apart; 10 min was considered long enough to establish a response and 20 min long enough to recover from the experience. From prior experience it was known that from an overall test session of 2 h, with behaviours being sampled at 30, 60 and 90 min post-dose, information on the onset and duration of action of the compound being studied can be obtained. To ensure that repeated exposure did not affect the results this factor was also accounted for in the analyses. Experiments were also 7 days apart to prevent any familiarisation occurring during the course of the study.

Although predator and conspecific intruder studies (11,15) have been carried out using non-human primates, there have been no reported studies on the effect of anxiolytic agents upon the response to confrontation by unfamiliar conspecifics. The aim of this study was to examine the behaviour of marmosets during confrontation by unfamiliar conspecifics and to measure the change in behaviour following the acute administration of the benzodiazepine, diazepam.

METHODS

Animals

Twenty common marmosets (*Callithrix jacchus*) normally housed in male/female pairs (all males had been vasectomised) were used in this study. Eighteen of these marmosets were experienced experimental animals. The age of these marmosets ranged from 18 months to 5 years. All were bred in house and weighed between 400–500g. Care and use were in accordance with the UK Animals Scientific Procedures Act 1986.

Equipment

The apparatus used in this study comprised of two locomotor activity/observation cages constructed from 2cm^2 wire mesh, measuring 60 cm × 45 cm × 60 cm, surrounded by 24 infra-red photobeams and containing 3 horizontal perches, 1 central ladder and 1 shelf running around 3 sides of the cage. The infra-red beams were directed vertically and horizontally to provide optimal detection of movement, and were linked to PC's running purpose-designed software to record beam-breaks. These cages were housed in an observation

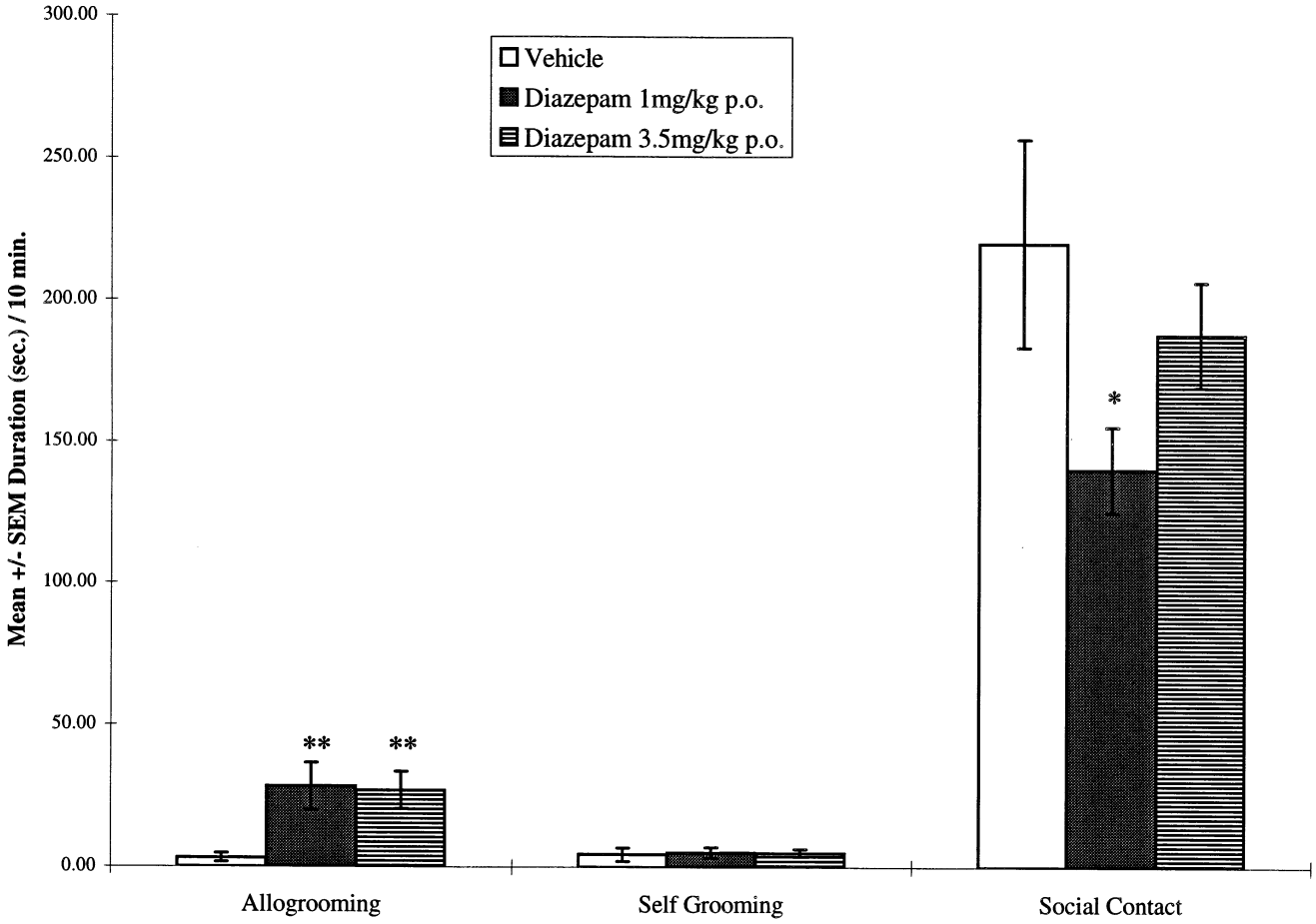

FIG. 2. Mean duration (s) ± SEM of marmoset behaviour during conspecific confrontation following vehicle and diazepam treatment ($n = 20$). Significantly different from vehicle; $*p < 0.05$, $**p < 0.01$.

room which was equipped with video cameras for closed-circuit television monitoring of behaviour.

Two keypads were used to score the behaviours, each keypad had 12 buttons (one pad for each pair, 6 buttons for each marmoset) and each button could measure both the frequency and duration of all behaviours.

PROCEDURE

Two pairs of male/female marmosets, taken from different holding rooms so that they had no previous auditory or visual contact with one another, were placed in adjacent locomotor activity/observation cages following the acute administration of vehicle or the compound of interest. These cages were separated by an opaque barrier which created a distance of approximately 15 cm between the cages. This distance also prevented any physical contact between the confronting pairs of marmosets. Locomotor activity recording was started immediately after dosing and continued for a period of 2 h. Thirty min after dosing the animals, the barrier was removed and the behaviours of both pairs of marmosets were measured for a 10 min period, after which the barrier was replaced. This procedure was repeated at the 60 and 90 min postdose time points. While the confronting pairs of marmosets did not have visual contact with each other during the non-confrontational periods, they were able to establish and maintain auditory contact.

The following behaviours were observed remotely via close circuit television and recorded using the keypads:

1. The frequency of aggressive behaviours, i.e., anogenital presentation, slit-stare and piloerection.
2. The frequency of anxiety-related behaviours, i.e., scent marking, head weaving and wet-dog shake.
3. The frequency of scratching.
4. The frequency and duration of allogrooming.
5. The frequency and duration of self grooming.
6. The frequency and duration of social contact.

Food and water were available through out the experiment, at the end of which the marmosets were returned to their home cages.

The combination of confronting pairs remained the same through out the study and the marmosets were given a period of at least 7 days between testing. Testing occurred between 0900–1100 and 1400–1600. For each marmoset pair, the test day and the time of testing remained the same through out the study. Confronting pairs were given the same treatment, the order of which was randomised so that each set of confronting marmosets received each treatment in a different order.

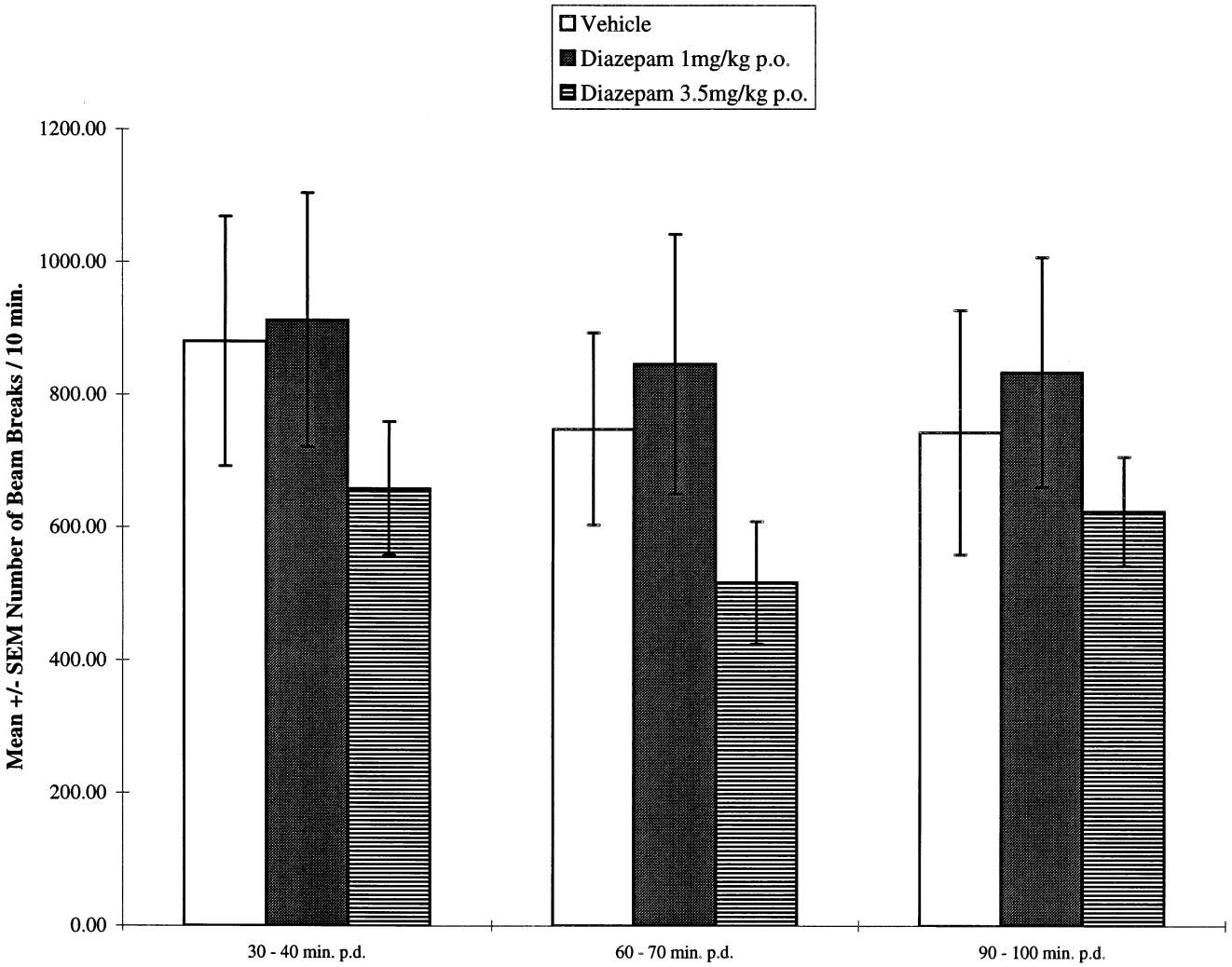

FIG. 3. Locomotor activity of marmosets during confrontation by conspecifics following vehicle and diazepam treatment (n = 10 pairs).

This procedure prevented biasing of the results due to the possible occurrence of familiarisation.

The observer was blind to all treatments.

Definition of Behaviours

1. Allogrooming–The grooming of one marmoset by another.
2. Anogenital Presentation–Raising of the tail to display the anogenital region.
3. Head Weaving–Darting movement of the head from side to side. Displayed mainly by juveniles and subordinates in response to a threat.
4. Piloerection–Erection of body pelage. This may be localised, i.e., involving just the tail or the anterior portion of the body.
5. Scent Marking–Rubbing of the anogenital or sternal region along an object.
6. Scratching–Repeated movement of the hand or foot during which the claws are rapidly drawn across the individual's fur.
7. Self Grooming–Also known as autogrooming. Differs from scratching in that it involves deliberate parting of the pelage to pick at the skin beneath.
8. Slit-Stare–Narrowing of the eyes to a slit, accompanied by flattening of the ear tufts.
9. Social Contact–Passive contact made between two marmosets, excluding allogrooming.
10. Wet-dog Shake–Shaking movement of the whole body which appears to start with the head.

Drugs

Diazepam (Courtin and Warner, UK) or its vehicle (1% methyl cellulose water) were administered by oral gavage in a dose volume of 1 ml.

Data Analysis

Time point analysis of all behaviours and locomotor activity was carried out on non-transformed data using repeated measures ANOVA followed by Dunnett's t-test if normally distributed or Wilcoxon signed rank test for non-normally distributed data.

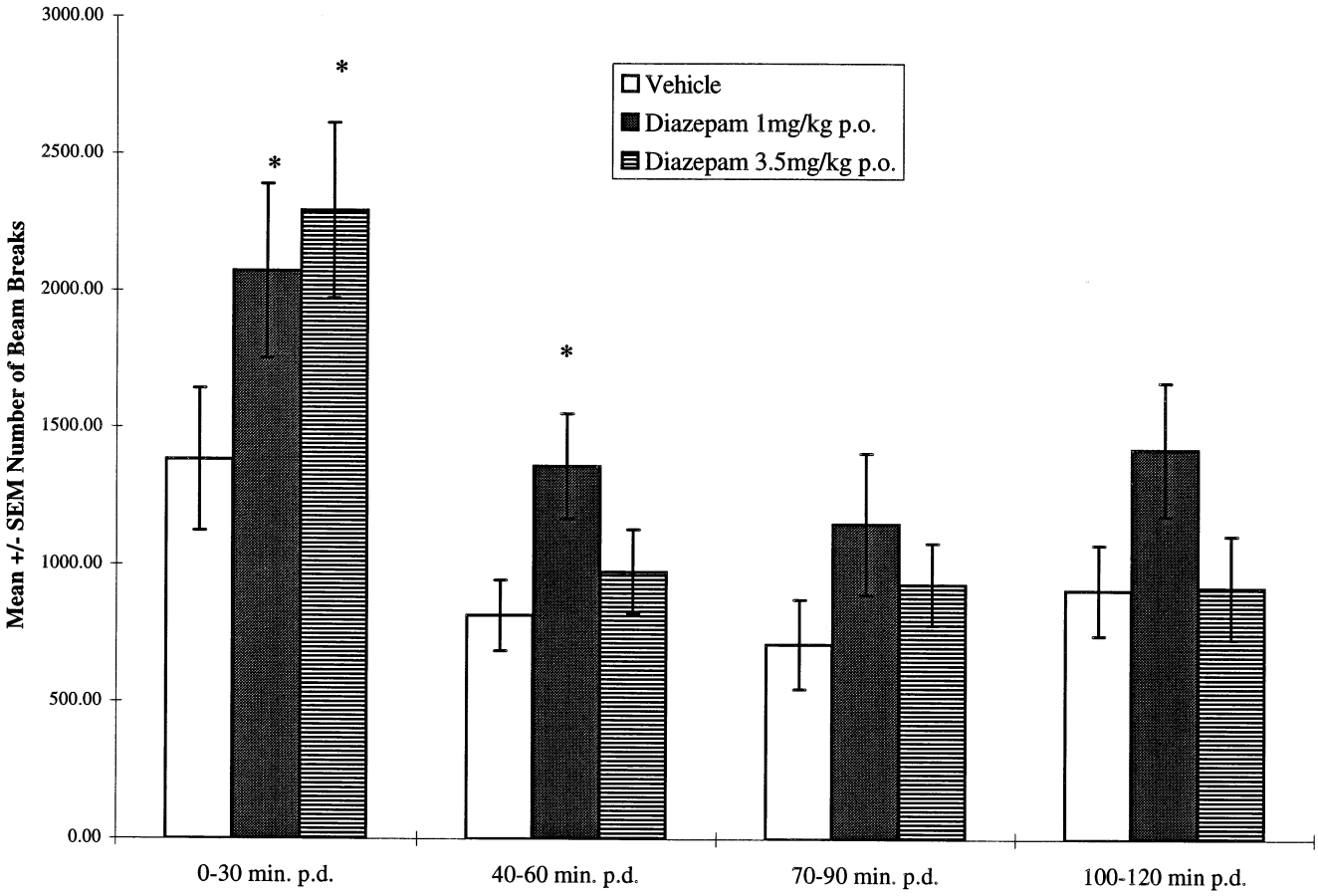

FIG. 4. Locomotor activity of marmosets during non confrontational periods following vehicle and diazepam treatment (n = 10 pairs). Significantly different from vehicle; *$p < 0.05$.

Data was meaned across the 3 confrontational time points for each marmoset. Normally distributed data was analysed by 1-way ANOVA followed by a Dunnett's t-test. Non-normally distributed data was analysed using Friedman's test followed by a Wilcoxon signed rank test. NB: aggression, duration of self grooming and locomotor activity during confrontation was $\log_{10}$ transformed to obtain normality.

Multifactorial ANOVA analysis with gender and time as factors was carried out on aggressive and anxiety-related behaviours.

All analyses were carried out using the SAS © –RA statistical package (SAS Institution Inc., USA).

RESULTS

Diazepam at 1 and 3.5 mg/kg PO induced a significant reduction, compared to vehicle, in the frequency of aggression ($F = 4.10, p < 0.0001$), anxiety-related behaviours ($\chi^2 = 10.18$, $p = 0.017$) and scratching ($\chi^2 = 8.66$, $p < 0.03$; Fig.1). Both doses of diazepam also increased the frequency and duration of allogrooming. At 1 mg/kg both the frequency and duration of allogrooming was significantly increased, relative to vehicle, as was the duration of this behaviour following the administration of 3.5 mg/kg of diazepam (frequency: $\chi^2 = 10.39$, $p = 0.012$; duration: $\chi^2 = 25.25$, $p = 0.0001$, Fig. 2). The increase in the frequency of allogrooming at 3.5 mg/kg PO just failed to reach statistical significance ($p = 0.06$, Wilcoxon signed rank test Fig. 1). Diazepam at 1 mg/kg PO also induced a significant reduction compared to vehicle, in the duration of social contact ($\chi^2 = 7.98$, $p = 0.046$), this may be a result of the increase in allogrooming. Repeated measures analysis showed that the reduction in aggression induced by 1 mg/kg of diazepam was significant at 30–40 min post-dose ($p < 0.05$, Dunnett's t-test) and the increase in allogrooming was significant at 60–70 min postdose (frequency only, $p < 0.01$, Wilcoxon signed rank test) and 90–100 min post-dose (frequency, $p < 0.01$ Wilcoxon signed rank test; duration, $p < 0.05$, Dunnett's t-test).

Both 1 mg/kg and 3.5 mg/kg of diazepam had no effect on locomotor activity during confrontation (Fig. 3). Repeated measures analysis revealed that both doses of diazepam significantly increased locomotor activity at 0–30 min postdose ($p < 0.05$, Dunnett's t-test, Fig. 4). This effect also occurred during the 40–60 min non-confrontational period following 1 mg/kg of diazepam. At 0–30 min postdose the marmosets have yet to experience confrontation by conspecifics, thus this data suggests that diazepam has an initial stimulatory effect on locomotor activity.

Multifactorial analysis revealed that there were no differences between the aggressive and anxiety-related behaviour of males and females (aggression: $F = 2.01$, $p = 0.19$; anxiety-related: $F = 0.004$, $p = 0.94$, Figs. 5 and 6). Nor did gender

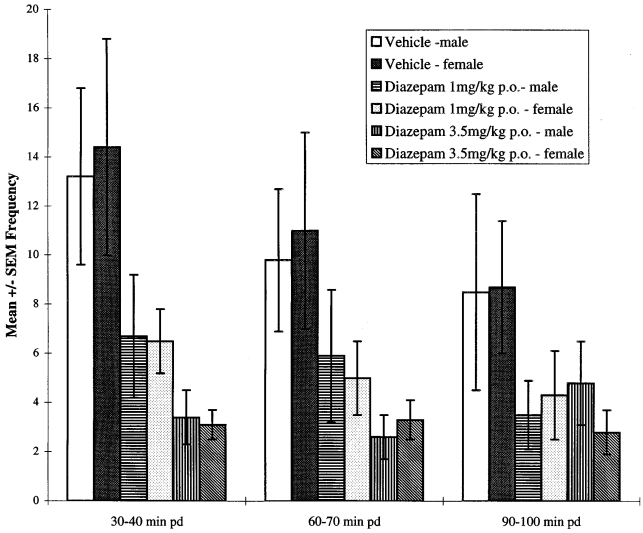

FIG. 5. Mean frequency ± SEM of male and female marmoset aggressive behaviour during conspecific confrontation following vehicle and diazepam treatment (n = 10).

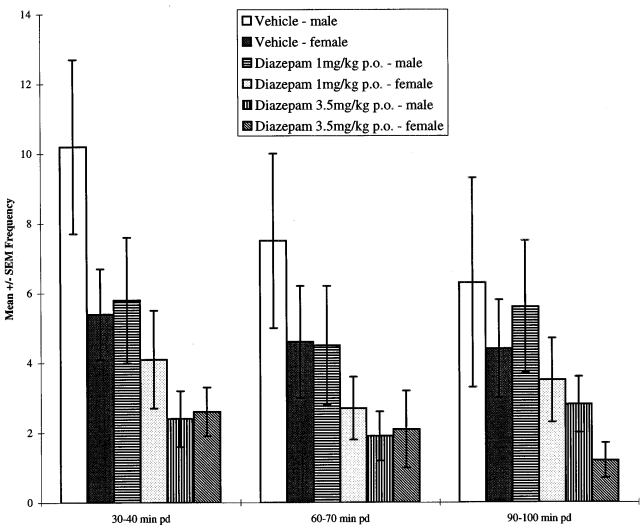

FIG. 6. Mean frequency ± SEM of male and female marmoset anxiety-related behaviours during conspecific confrontation following vehicle and diazepam treatment (n = 10).

influence the effect of treatment on these 2 behavioural categories (aggression: $F = 2.00$, $p = 0.13$; anxiety-related: $F = 0.23$, $p = 0.79$). This analysis also enabled us to look at the effect of time on these behaviours and treatments. Time had no influencing effect on treatment for either aggression or anxiety-related behaviour (aggression: $F = 0.55$, $p = 0.69$; anxiety-related: $F = 0.06$, $p = 0.94$), indicating that it was valid to sample these behaviours at the time points stated and that this data could then be pooled for analysis. This was further enhanced by the lack of statistically significant differences between the 3 time points (aggression: $F = 1.83$, $p = 0.16$; anxiety-related: $F = 2.77$, $p = 0.06$). Because the p value for anxiety-related behaviour was close to significance, a Dunnett's t-test was carried out on the pooled data. This analysis showed a significant difference between the 30–40 min and 90–100 min confrontational periods for this behavioural category, indicating a downward trend over the 3 time points.

Emesis and muscle relaxation (measured as the inability to co-ordinate movement) was demonstrated by several of the marmosets following both doses of diazepam (2 marmosets displayed emesis at 1 mg/kg and 12 at 3.5 mg/kg PO; muscle relaxation: 14 marmosets at 1 mg/kg and all at 3.5 mg/kg PO (two of which also demonstrated eyelid closure)). Following both doses of diazepam the number of emetic episodes ranged from 1–6 per animal, with the onset ranging from 9–76 min post-dose.

DISCUSSION

The human/marmoset threat model developed by Costall and co-workers has shown that anxiolytic agents such as zacopride, diazepam, ondansetron, 8-OH-DPAT and buspirone induce a decrease in the number of postures monitored in response to a human threat, i.e., anogenital presentation, slit-stare, scent marking and piloerection. In this model of anxiety, Costall et al. (2,3,4) demonstrated diazepam to be active at a dose as low as 10 μg/kg SC, while Walsh et al. (27) have shown chlordiazepoxide to be active, in a similar model, over a dose range of 0.3–3 mg/kg SC. The aggressive behaviour of rhesus monkeys, induced by the introduction of a pole through the bars of the home cage, is inhibited by diazepam (5–20 mg/kg) (25). Similar work with cynomolgus monkeys also demonstrated a reduction in aggression following the acute administration of diazepam at 2.5 mg/kg (12,18). All these models used the response to the presentation of an imposing threat as a selection criterion, whereas in the study reported here pre-selection was not necessary. The raw data revealed that all the marmosets used in this study demonstrated varying levels of aggressive and anxiety-related behaviours (following vehicle administration) therefore, pre-selection was not required. Because of concern over the possibility of habituation of response following repeated exposure to the same pair of marmosets during a test session and over the course of the study, experiments were designed to minimise this effect. Thus, the number of confrontational periods per session, were kept to a minimum, treatments were randomised and there was always one week between test sessions. The appropriateness of this design was confirmed by the fact that time had no influencing effect, although there appeared to be a downward trend in anxiety-related behaviours across the 3 time points, this was independent of treatment.

In this study diazepam (1 and 3.5 mg/kg PO) induced a number of marked changes in the behaviour of marmosets during confrontation with conspecifics. For example, reduced aggression (anogenital presentation, slit-stare and piloerection), anxiety-related behaviours (scent marking, head weaving and wet-dog shake), scratching and increased allogrooming. As mentioned above, a reduction in agonistic behaviours following the administration of anxiolytic agents has been demonstrated by many workers. The data analyses employed in this study have enabled us to demonstrate that the changes seen in aggression and anxiety-related behaviours is solely due to the effect of the treatment and is not influenced by gender or time. Scratching, widely reported as a displacement behaviour that is increased during anxiety provoking events (5,7,14,19), is reduced following benzodiazepine administration (16,20,21). Diazepam, at both doses, significantly reduced scratching. The increase in allogrooming, an affiliative behav-

iour, seen following diazepam at 1 and 3.5 mg/kg is a strong indication of an anxiolytic effect, as is the reduction in the duration of social contact seen following the 1 mg/kg dose. The latter two effects, together with no change in locomotor activity also indicate that the muscle relaxation and emesis demonstrated in this study did not confound the results.

The behavioural profile obtained with diazepam in this study is characteristic of that which would be predicted for an anxiolytic agent in such a paradigm. These findings strongly support our suggestion that marmoset conspecific confrontation may be an experimental model of anxiety. Investigation of the effects of other psychotropic drugs, e.g., antidepressant, antipsychotic and neuroleptic agents, is required to fully characterise and validate the model.

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