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## Alprazolam withdrawal and tolerance measured in the social conflict test in mice

**Abstract** *Rationale:* It is difficult to assess withdrawal from benzodiazepines, and preclinical assessment of behaviour during social conflict offers the opportunity to quantify tolerance and withdrawal by measuring aggressive, defensive and social behaviour. The relationship between benzodiazepine withdrawal symptoms and the development of tolerance is not well understood. Are withdrawal symptoms dependent on the development of tolerance? *Objective:* The aim of the present study was to compare the development of tolerance to alprazolam effects on the behavioural repertoire during the social conflict test in mice, and to determine whether or not behavioural changes during alprazolam withdrawal are correlated with the development of tolerance. *Methods:* An experimental model consisting of interactions of pairs of singly housed male mice with non-aggressive group-housed male mice was used. Alprazolam (1 mg/kg) was given orally once or repeatedly (twice daily) for 8 or 21 days. Behaviour was measured, based on videoanalysis, in aggressive mice before treatment, 30 min or 3 days after the last dose, respectively. *Results:* A single administration of alprazolam significantly reduced aggressive activities and increased social investigation without changing locomotion or other behaviour. Tolerance developed to the inhibitory effects of alprazolam on aggressive behaviour but not to the effects of alprazolam to increase social investigation. When withdrawn from alprazolam, mice exhibited less social investigation and

locomotion while aggression tended to be increased. *Conclusions:* Tolerance to the alprazolam effects on aggressive and social behaviour developed at different rates suggesting that they are differentially regulated. Furthermore, the evidence of withdrawal responses appearing in a behaviour to which tolerance had not developed does suggest that tolerance and withdrawal phenomena are dissociated in benzodiazepines.

**Keywords** Benzodiazepine · Withdrawal · Tolerance · Alprazolam · Aggression · Anxiety

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### Introduction

Benzodiazepines are widely used therapeutic agents with sedative, anxiolytic, anticonvulsant and muscle relaxant effects in humans and animals. The use of these agents is limited by the development of tolerance to their effects and the risk of developing dependence (File and Andrews 1993; File 1985b, 1990; Olivieri et al. 1986; Dorow and Duka 1986). Dependence to benzodiazepines can be also manifested by a withdrawal syndrome which may include symptoms such as tremors, sweating, sleep disturbance, lowered seizure threshold, increased tension and anxiety, irritability, difficulty in concentration etc. (Petursson 1994; Woods et al. 1987; Schweizer and Rickels 1998).

Behavioural studies of benzodiazepine withdrawal in animal models have been mostly focused on detection of signs of increased anxiety. For example, reduced exploration of open arms in the elevated plus maze, reduced social behaviour in the social interaction test or increased ultrasonic “distress” vocalizations were reported in rats or mice after withdrawal from benzodiazepines (File et al. 1987, 1991; Kulkarni and Sharma 1993; Miczek and Vivian 1993; Vivian et al. 1994; Andrews et al. 1997; Ward and Stephens 1998). However, it is possible that behavioural changes after benzodiazepine withdrawal are more diverse and that the expression of anxiety is not the only and predominant behavioural response

to benzodiazepine withdrawal. To test this possibility, a behavioural model engendering a broad spectrum of behavioural measures was employed in the present study. The ethologically oriented model is based on the analysis of offensive, defensive-escape, social, locomotor and other behavioural acts and postures occurring during social conflict in mice. Anxiolytic-like, anxiogenic-like, anti-aggressive, aggressogenic, sedative and other behavioural changes are readily detected in this model after acute administration of agonists or inverse agonists at benzodiazepine receptors (Kršiak 1975, 1979; Kršiak et al. 1984; Kršiak and Sulcova 1990; Sulcova et al. 1992). In a preliminary study (Kršiak et al. 1998), withdrawal from relatively short (8 days) treatment with alprazolam reduced social investigation, moderately increased aggression but did not produce marked signs of anxiety in the present model of social conflict in mice. The first aim of the present study was to explore whether withdrawal from longer (21 days) alprazolam treatment produces more marked increase of aggression and other activities, particularly increased defensive-escape behaviour as a putative measure of increased anxiety.

The development of tolerance to several benzodiazepine effects has been studied extensively in various experimental models (e.g. File 1985a, 1990; Shumsky and Lucki 1994; Fernandes et al. 1999). It has been shown that tolerance develops at different rates to diverse behavioural effects of benzodiazepines (File 1985a). For example, tolerance to the sedative effects develops rapidly (Fernandes et al. 1996) while tolerance to the anxiolytic effects is slow or absent (File 1985a; Fernandes et al. 1999). However, the development of tolerance has been mostly measured in different tests and animals, which could influence the rate of development of tolerance. Therefore, the second aim of the present paper was to compare the development of tolerance to alprazolam effects on different behaviours occurring during the same test in the same animals.

The relationship between benzodiazepine withdrawal symptoms and the development of tolerance is not well understood. Are withdrawal symptoms dependent on the development of tolerance? Are they a mirror image of acute effects of benzodiazepines? Several benzodiazepine studies reported withdrawal responses in a behaviour to which tolerance had not developed (File and Wilks 1990; van der Laan et al. 1993; Shumsky and Lucki 1994; Andrews et al. 1997) but these phenomena have been mostly measured in separate tests and animals. Thus, the third purpose of the present study was to determine in the same test and animals whether behavioural changes during alprazolam withdrawal are correlated with, or independent of, the development of tolerance.

## Materials and methods

### Subjects

Male albino random-bred mice derived from ICR strain (Velaz, Prague, Czech Republic) weighing 18–20 g at the beginning of the experiment were used. They were housed singly in self-cleaning cages or in groups of ten. The self-cleaning cages used for the individual housing were made of solid metal walls 13 cm high with wire-mesh floors (8×17 cm) which were placed 3 cm above trays with wood shavings. This wire-mesh floor ensured that the isolates were not handled throughout the period of single housing. The group-housed mice were housed in large standard plastic cages (26×42×15 cm) with floors covered with wood shavings. All mice were housed under room lighting (with lights on from 6 a.m. to 6 p.m.) and under temperature ranging from 22 to 24°C. Food and water were available ad libitum.

The mice were observed in transparent cages (20×30×20 cm) with wood shavings on the floor and tops covered with transparent covers with apertures for air. The observations were performed under room lighting from 8 a.m. to 1 p.m.

Experiments were approved by the Expert Committee for Protection of Experimental Animals of the 3rd Faculty of Medicine and were performed in accordance with the Animal Protection Act of the Czech Republic (No. 246/1992 Sb).

### Procedures

Social interactions always involved one singly-housed and one group-housed mouse, being placed as pairs in the observational cages. Each isolate was paired with the same group-housed partner throughout the experiment. The isolates were allowed 30-min adaptation in the observational cages before the group-housed partners were introduced; the interaction ended after 4 min. This procedure, which suppresses aggression in group-housed mice and reduces their social behaviour, facilitates active social behaviour in isolates. The observational cages were cleaned and their floors were covered with new wood shavings after each interaction.

Three experiments were performed and all of them consisted of three phases. In the first phase, mice were housed singly or in groups for 3 weeks. In the second phase, singly housed mice (isolates, the test mice) were given drugs daily for a specific time (1, 8 or 21 days). Group-housed mice (the stimulus animals) remained untreated. The third phase consisted of withdrawal from alprazolam treatment for 72 h.

In the first experiment, alprazolam (1 mg/kg) or the vehicle were administered orally only once ( $n=16$  each). In the second experiment, alprazolam (1 mg/kg) or the vehicle were administered twice daily for 8 days and on day 9 an additional dose of alprazolam (1 mg/kg) ( $n=19$ ) or the vehicle ( $n=18$ ) were given. In the third experiment, alprazolam (1 mg/kg) or a vehicle were administered twice daily for 21 days and on day 22 an additional dose of alprazolam (1 mg/kg) or the vehicle was given ( $n=16$  or 17, respectively).

Altogether, three social interactions were performed in each experiment. The first interaction was performed 1 day before the alprazolam treatment started (pretreatment interaction) and served for classification of animals. The second interaction was conducted 30 min after the last dose of alprazolam (treatment interaction) and the third interaction was performed 72 h after the last dose of alprazolam (the withdrawal interaction). According to our pilot experiments and published findings (Lopez et al. 1990), an interval of 48–96 h after discontinuation of alprazolam administration appears to be most appropriate for ascertainment of behavioural and receptor changes produced by the withdrawal.

The behaviour of animals during the interaction was recorded on videotape. Next, the tapes were analyzed by an observer with no knowledge of the drug treatment. This was done with a keyboard that was connected to a standard PC and software for behavioural analysis (Donat 1991).

## Measures

The frequency, total duration and latency of a number of aggressive, defensive-escape (timid), social and locomotor activities derived from the ethogram of mice (Grant and Mackintosh 1963) and described in detail previously (Kršiak 1975; Kršiak et al. 1984) were recorded. In short, the acts and postures evaluated in the present paper were defined as follows:

### *Sociable activities (social investigation)*

*Social sniff* (Sn) – also referred to as naso-nasal and ano-genital contacts, sniffing the partner's head, body, genitals or tail; *climb* (Cl) – the mouse places its forepaws on the partner's back, mostly in the shoulder region, and usually sniffs this area at the same time (Grant and Mackintosh called this Attempted Mount); *follow* (Fo) – following the partner by quiet walking.

### *Aggressive activities*

*Attack* (At) – a fierce lunging at the partner often associated with biting; *threat* (Th) – a sideways or an upright stance with head and forebody movements toward the partner, and trying to bite the partner (offensive sideways or upright posture); *tail rattle* (Tr) – rapid vibrations of the tail.

### *Timid activities*

*Defense* (De) – the mouse responds to the partner's social behaviour by raising forepaws, hunching the back (defensive upright posture) or by some rotation of the body bringing the legs closest to the other animal off the ground (defensive sideways posture); *escape* (Es) – a rapid running or jumping away from the partner; *alert posture* (Al) – a sudden interruption of all movements with eyes and ears being directed toward the partner.

### *Locomotor activities*

*Walk* (Wa) – any walking across the cage which is not apparently related to the partner; and *rear* (Re) – the mouse stands only on his hind legs and usually sniffs air or walls at the same time.

Duration was not measured for escapes and attacks because of momentary character of these acts (i.e. measurement of duration was not considered accurate enough and meaningful in these acts).

The inter-observer reliability of the recorded items was satisfactory as determined by several observers independently scoring a videotaped record of behaviour of 70 mice in interactions lasting 4 min each. The correlation ranged from  $r=0.83$  to  $0.97$ .

## Drug

Alprazolam (Léčiva, Prague) was dissolved in distilled water with two drops of Tween 80 and administered orally in a volume 0.1 ml/10 g body weight. After administration, isolated mice were always returned to their home cages (except for the last administration when they were placed into the observational cage).

## Data analysis

Mice exhibiting attacks (aggressive mice) in the pretreatment interaction were selected for the analysis (the number of non-aggressive mice was too small for evaluation).

Behavioural elements, their frequency, duration and latency were summed in four behavioural categories (sociable, aggressive, locomotor and timid) for the statistical analysis. Statistical evaluation and interpretation of timid activities is limited due to their

rare frequency in aggressive mice and we present this data for integrity. The behavioural categories were evaluated by a three-way repeated measures ANOVA (Wilks Lambda) with the factors treatment (control and alprazolam), duration of treatment (1, 8 and 21 days) and observation period (pretreatment, last day of treatment and withdrawal). Subsequent analysis was performed using Bonferroni *t*-test to reveal significant differences between the control and the alprazolam treated group of mice. All statistical tests used two-tailed criteria, with an alpha level of  $P<0.05$ .

## Results

### Aggressive activities

A three-way repeated measures ANOVA showed a significant effect of treatment [ $F(2,97)=7.652$ ,  $P<0.001$ ], duration of treatment [ $F(4,194)=5.109$ ,  $P=0.001$ ] and observation period [ $F(2,97)=6.522$ ,  $P=0.002$ ] in the number of aggressive acts (attacks, threats, tail rattles). Next, a three-way repeated measures ANOVA showed a significant effect of observation period [ $F(2,196)=4.920$ ,  $P=0.008$ ] in the time spent in aggressive acts and a significant effect of treatment [ $F(2,196)=10.225$ ,  $P<0.001$ ], duration of treatment [ $F(4,196)=24.064$ ,  $P<0.001$ ] and observation period [ $F(2,196)=12.829$ ,  $P<0.001$ ] for latencies to aggressive acts.

### Sociable activities

A three-way repeated measures ANOVA showed a significant effect of treatment [ $F(2,97)=26.321$ ,  $P<0.001$ ], duration of treatment [ $F(4,194)=8.916$ ,  $P<0.001$ ] and observation period [ $F(2,97)=20.466$ ,  $P<0.001$ ] in the number of sociable acts (social sniffs, climbs, follows). Next, a three-way repeated measures ANOVA showed a significant effect of treatment [ $F(2,196)=19.679$ ,  $P<0.001$ ], duration of treatment [ $F(4,196)=13.725$ ,  $P<0.001$ ] and observation period [ $F(2,196)=36.964$ ,  $P<0.001$ ] in the time spent in sociable acts and a significant effect of treatment [ $F(2,196)=8.039$ ,  $P<0.001$ ], duration of treatment [ $F(4,196)=7.609$ ,  $P<0.001$ ] and observation period [ $F(2,196)=8.205$ ,  $P<0.001$ ] for latencies to sociable acts.

### Locomotor activities

A three-way repeated measures ANOVA showed a significant effect of treatment [ $F(2,97)=7.084$ ,  $P=0.001$ ] and observation period [ $F(2,97)=28.168$ ,  $P<0.001$ ] in the number of locomotor acts (walks, rears), effect of treatment [ $F(2,196)=13.580$ ,  $P<0.001$ ] and observation period [ $F(2,196)=14.668$ ,  $P<0.001$ ] in the time spent in locomotor acts and a significant effect of observation period [ $F(2,196)=4.786$ ,  $P=0.009$ ] for latencies to locomotor acts.

**Table 1** Mean number of frequency, duration and latency ( $\pm$ SEM) of behavioural categories after acute, 8 days and 21 days of alprazolam treatment (1 mg/kg b.i.d.)

Duration of treatment	1 day	8 days	21 days
Control mice	<i>n</i> =16	<i>n</i> =18	<i>n</i> =17
Alprazolam-treated mice	<i>n</i> =16	<i>n</i> =19	<i>n</i> =16
Sociable acts (social sniff, climb, follow)			
Number of acts ( $\pm$ SEM)			
Control	8.13 $\pm$ 1.73	8.78 $\pm$ 1.53	5.47 $\pm$ 0.96
Alprazolam	14.50 $\pm$ 1.66*	18.58 $\pm$ 2.82**	12.06 $\pm$ 1.67**
Time (s) spent in acts ( $\pm$ SEM)			
Control	17.23 $\pm$ 4.39	22.08 $\pm$ 4.95	16.64 $\pm$ 4.90
Alprazolam	71.37 $\pm$ 9.96***	44.81 $\pm$ 7.44	60.71 $\pm$ 11.76**
Latencies (s) to acts ( $\pm$ SEM)			
Control	393.39 $\pm$ 40.57	372.60 $\pm$ 49.27	458.75 $\pm$ 39.34
Alprazolam	199.83 $\pm$ 46.33**	257.47 $\pm$ 46.55*	315.94 $\pm$ 46.84
Aggressive acts (attack, threat, tail rattle)			
Number of acts ( $\pm$ SEM)			
Control	126.69 $\pm$ 13.75	65.06 $\pm$ 13.44	73.41 $\pm$ 12.77
Alprazolam	37.00 $\pm$ 13.78***	48.79 $\pm$ 10.69	64.00 $\pm$ 15.85
Time (s) spent in acts ( $\pm$ SEM)			
Control	40.80 $\pm$ 6.00	34.32 $\pm$ 5.81	33.91 $\pm$ 7.73
Alprazolam	18.41 $\pm$ 5.41**	37.75 $\pm$ 5.88	37.78 $\pm$ 9.43
Latencies (s) to acts ( $\pm$ SEM)			
Control	54.45 $\pm$ 24.55	278.83 $\pm$ 62.65	220.86 $\pm$ 66.44
Alprazolam	328.59 $\pm$ 86.28***	278.68 $\pm$ 50.42	283.68 $\pm$ 92.97
Locomotor acts (rear, walk)			
Number of acts ( $\pm$ SEM)			
Control	43.38 $\pm$ 4.11	66.78 $\pm$ 6.39	44.65 $\pm$ 3.95
Alprazolam	39.69 $\pm$ 8.21	57.74 $\pm$ 5.98	29.00 $\pm$ 5.85*
Time (s) spent in acts ( $\pm$ SEM)			
Control	114.16 $\pm$ 8.08	125.75 $\pm$ 7.95	126.28 $\pm$ 6.56
Alprazolam	85.19 $\pm$ 12.23	109.23 $\pm$ 8.25	76.51 $\pm$ 10.33***
Latencies (s) to acts ( $\pm$ SEM)			
Control	60.58 $\pm$ 14.03	43.54 $\pm$ 13.11	45.59 $\pm$ 10.82
Alprazolam	83.13 $\pm$ 22.66	46.71 $\pm$ 10.10	144.92 $\pm$ 37.58
Timid acts (defence, escape, alert)			
Number of acts ( $\pm$ SEM)			
Mean	0.38 $\pm$ 0.30	0.94 $\pm$ 0.41	2.12 $\pm$ 1.16
SEM	0.06 $\pm$ 0.06	0.58 $\pm$ 0.23	0.94 $\pm$ 0.30
Time (s) spent in acts ( $\pm$ SEM)			
Control	1.95 $\pm$ 1.89	2.51 $\pm$ 1.35	16.90 $\pm$ 8.62
Alprazolam	0.14 $\pm$ 0.14	3.17 $\pm$ 1.87	7.64 $\pm$ 2.61
Latencies (s) to acts ( $\pm$ SEM)			
Control	683.55 $\pm$ 35.29	639.66 $\pm$ 37.13	626.65 $\pm$ 54.14
Alprazolam	712.48 $\pm$ 7.29	657.14 $\pm$ 27.41	586.53 $\pm$ 47.57

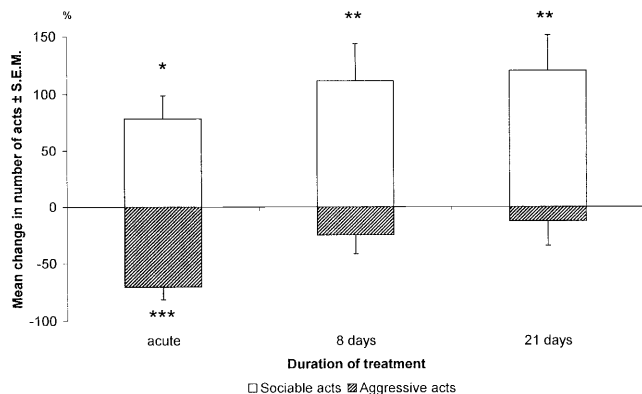
A three-way repeated measures ANOVA with subsequent comparison with Bonferroni *t*-test, \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 when compared alprazolam treated to the control group

## Subsequent analysis

### *Behaviour after alprazolam administration*

*Aggressive activities.* Subsequent Bonferroni *t*-test showed a significant reduction of the number of aggres-

sive activities after a single administration of alprazolam (1 mg/kg PO) (*t*=4.105, *df*=92, *P*<0.001) compared to the control group of mice (Table 1, Fig. 1). The acute treatment with alprazolam also shortened the total duration of aggressive activities (*t*=3.134, *df*=96, *P*=0.002) and prolonged the latency to aggressive activities

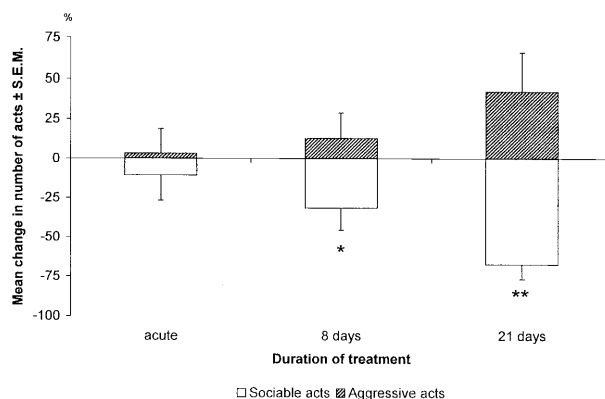


**Fig. 1** Changes in aggression and social investigation after alprazolam administration. Alprazolam or the vehicle was given once (acute treatment 1 mg/kg), for 8 or 21 days (1 mg/kg b.i.d.). Behaviour was measured 30 min. after the last dose of alprazolam. Changes are expressed as the percent difference between the number of aggressive and sociable acts in the alprazolam treated and control groups. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  for the difference between the control and the alprazolam-treated group of mice, a three-way repeated measures ANOVA with the subsequent Bonferroni  $t$ -test

( $t = 5.548$ ,  $df = 96$ ,  $P < 0.001$ ). After the 8-day and the 21-day treatment, the number and the duration of aggressive activities was reduced and the latency was lengthened to a lesser degree and these changes were not significantly different from the control values (Table 1, Fig. 1). There was a large decline in the number of aggressive acts (but not in the duration of these acts) and a marked prolongation of the latency to their first occurrence in the control mice given vehicle for 8 or 21 days, which was probably due to adaptation to handling of the animals during the repeated administrations of the vehicle.

**Sociable activities.** When alprazolam was administered once, the number and duration of sociable activities was increased significantly ( $t = 2.416$ ,  $df = 92$ ,  $P = 0.018$  and  $t = 3.766$ ,  $df = 92$ ,  $P < 0.001$ , respectively) and the latency to their first occurrence was shortened ( $t = 2.879$ ,  $df = 92$ ,  $P = 0.005$ , Table 1, Fig. 1). Similarly, the number of sociable activities was increased after 8 days treatment with alprazolam ( $t = 3.083$ ,  $df = 92$ ,  $P < 0.003$ ) and the latency to their first occurrence was shortened ( $t = 2.145$ ,  $df = 92$ ,  $P = 0.036$ ). Also, after 21 days of alprazolam treatment, the number and duration of sociable activities were increased significantly ( $t = 2.782$ ,  $df = 92$ ,  $P = 0.007$  and  $t = 3.116$ ,  $df = 92$ ,  $P = 0.002$ , Table 1, Fig. 1).

**Locomotion and timid activities.** No significant changes were found in any of the measures of timid and locomotion activities except of the mean number and total duration of locomotion activities which was decreased after the 21 days of alprazolam treatment ( $t = 2.257$ ,  $df = 92$ ,  $P = 0.026$ ,  $t = 4.191$ ,  $df = 92$ ,  $P < 0.001$ , respectively).



**Fig. 2** Changes of aggression and social investigation after alprazolam withdrawal. Alprazolam or the vehicle was given once (acute treatment 1 mg/kg), for 8 or 21 days (1 mg/kg b.i.d.). Behaviour was measured 72 h after the last dose of alprazolam. Changes are expressed as the percent difference between the number of aggressive and sociable acts in the alprazolam treated and control group. \* $P < 0.05$ , \*\* $P < 0.01$  for the difference between the control and the alprazolam-treated group of mice, a three-way repeated measures ANOVA with the subsequent Bonferroni  $t$ -test

#### Behaviour after alprazolam withdrawal

Social investigation was reduced while aggression tended to be increased after withdrawal from alprazolam (Table 2, Fig. 2).

**Sociable activities.** The number and the duration of sociable activities was decreased significantly ( $t = 2.390$ ,  $df = 92$ ,  $P < 0.019$ ,  $t = 2.383$ ,  $df = 92$ ,  $P < 0.019$ , respectively) already after withdrawal from 8 days treatment with alprazolam. This decrease was still more pronounced after 21 days of treatment ( $t = 2.731$ ,  $df = 92$ ,  $P = 0.008$  for the number and  $t = 2.895$ ,  $df = 92$ ,  $P = 0.005$  for the duration, Table 2, Fig. 2).

**Aggressive activities.** Although aggressive activities appeared to be increased directly in proportion to the length of previous alprazolam treatment, only the total duration after withdrawal from 21 days of alprazolam treatment was significantly increased ( $t = 2.128$ ,  $df = 92$ ,  $P = 0.036$ ).

**Timid and locomotor activities.** No significant changes were found in any measure of timidity after alprazolam withdrawal. The number but not the duration of locomotor activities tended to be reduced directly to the length of the alprazolam treatment. When compared to the control group of mice, the number and the duration of locomotion activities was reduced significantly in mice withdrawn from alprazolam given for 21 days ( $t = 3.133$ ,  $df = 92$ ,  $P = 0.002$ ,  $t = 2.808$ ,  $df = 92$ ,  $P = 0.006$ , respectively, Table 2).

**Table 2** Mean number of frequency, duration and latency ( $\pm$ SEM) of behavioural categories after 72 h withdrawal from acute, 8 days and 21 days of alprazolam treatment (1 mg/kg b.i.d.)

Duration of treatment	1 day	8 days	21 days
Control mice	<i>n</i> =16	<i>n</i> =18	<i>n</i> =17
Alprazolam-withdrawal mice	<i>n</i> =16	<i>n</i> =19	<i>n</i> =16
Sociable acts (social sniff, climb, follow)			
Number of acts ( $\pm$ SEM)			
Control	6.44 $\pm$ 1.67	11.83 $\pm$ 1.81	7.29 $\pm$ 1.33
Alprazolam withdrawal	5.75 $\pm$ 1.03	8.11 $\pm$ 1.66*	2.38 $\pm$ 0.69**
Time (s) spent in acts ( $\pm$ SEM)			
Control	13.63 $\pm$ 4.70	21.83 $\pm$ 4.40	20.13 $\pm$ 4.55
Alprazolam withdrawal	13.79 $\pm$ 3.10	14.34 $\pm$ 3.51*	7.71 $\pm$ 2.75**
Latencies (s) to acts ( $\pm$ SEM)			
Control	426.08 $\pm$ 50.42	349.29 $\pm$ 37.91	461.85 $\pm$ 34.43
Alprazolam withdrawal	400.56 $\pm$ 44.59	373.38 $\pm$ 56.17	586.14 $\pm$ 28.94
Aggressive acts (attack, threat, tail rattle)			
Number of acts ( $\pm$ SEM)			
Control	85.63 $\pm$ 9.50	60.78 $\pm$ 12.98	65.00 $\pm$ 10.92
Alprazolam withdrawal	88.44 $\pm$ 13.28	68.63 $\pm$ 9.72	92.38 $\pm$ 15.59
Time (s) spent in acts ( $\pm$ SEM)			
Control	32.41 $\pm$ 3.24	33.76 $\pm$ 5.78	26.34 $\pm$ 4.30
Alprazolam withdrawal	43.96 $\pm$ 6.80	33.88 $\pm$ 4.80	*39.43 $\pm$ 5.75
Latencies (s) to acts ( $\pm$ SEM)			
Control	87.56 $\pm$ 43.59	240.27 $\pm$ 47.95	230.35 $\pm$ 72.27
Alprazolam withdrawal	129.54 $\pm$ 62.20	162.19 $\pm$ 35.81	206.05 $\pm$ 58.29
Locomotor acts (rear, walk)			
Number of acts ( $\pm$ SEM)			
Control	47.94 $\pm$ 4.05	79.28 $\pm$ 7.37	64.12 $\pm$ 3.83
Alprazolam withdrawal	48.31 $\pm$ 3.42	72.95 $\pm$ 5.44	** 40.25 $\pm$ 4.04
Time (s) spent in acts ( $\pm$ SEM)			
Control	130.59 $\pm$ 6.20	129.06 $\pm$ 9.05	148.96 $\pm$ 5.28
Alprazolam withdrawal	121.63 $\pm$ 6.16	122.97 $\pm$ 6.14	114.49 $\pm$ 8.81**
Latencies (s) to acts ( $\pm$ SEM)			
Control	37.61 $\pm$ 4.65	42.78 $\pm$ 14.94	27.35 $\pm$ 4.43
Alprazolam withdrawal	27.01 $\pm$ 4.34	20.22 $\pm$ 3.38	67.14 $\pm$ 15.61
Timid acts (defence, escape, alert)			
Number of acts ( $\pm$ SEM)			
Control	1.25 $\pm$ 0.81	0.39 $\pm$ 0.18	1.76 $\pm$ 0.87
Alprazolam withdrawal	0.81 $\pm$ 0.50	1.21 $\pm$ 0.56	1.94 $\pm$ 0.92
Time (s) spent in acts ( $\pm$ SEM)			
Control	7.85 $\pm$ 6.49	1.04 $\pm$ 0.52	8.51 $\pm$ 4.25
Alprazolam withdrawal	3.78 $\pm$ 2.32	4.18 $\pm$ 1.97	22.21 $\pm$ 10.86
Latencies (s) to acts ( $\pm$ SEM)			
Control	674.25 $\pm$ 24.09	663.20 $\pm$ 33.17	633.99 $\pm$ 48.45
Alprazolam withdrawal	672.26 $\pm$ 25.05	632.78 $\pm$ 32.59	613.63 $\pm$ 44.87

A three-way repeated measures ANOVA with subsequent comparison with Bonferroni *t*-test, \**P*<0.05, \*\**P*<0.01, when compared alprazolam withdrawal to the control group

## Discussion

In the present study, social investigation was reduced while aggression tended to be increased after withdrawal from alprazolam. These behavioural changes correlated with the length of alprazolam treatment. The reduction

of social investigation appears to be in agreement with inhibition of social interaction in the social interaction test as an index of increased anxiety reported after withdrawal from benzodiazepines (File et al. 1991; Andrews et al. 1997). However, the reduction of "sociable" behaviour in terms of components of social investigation such

as social sniffing, climbing and following partners found in the present study does not seem to reflect anxiety. In the present model, anxiogenic activity is manifested by increased defensive-escape behaviour, which can be accompanied (in aggressive animals), with reduced aggression. Such behavioural changes were produced by anxiogenic drugs such as inverse agonists at benzodiazepine receptors (beta-CCE and FG 7142; Sulcova et al. 1992). In the present study, however, no significant increase of defensive and escape behaviour on alprazolam withdrawal was found even after 21 days of treatment with the drug. Thus, no evidence of increased anxiety during withdrawal from alprazolam was found in aggressive mice.

Although aggression during alprazolam withdrawal appeared to be increased directly in proportion to the length of alprazolam treatment, only the increase in total duration of aggressive behaviour was significant on alprazolam withdrawal after 21 days of treatment with the drug. Only a few reports deal with changes in aggressive behaviour during benzodiazepine withdrawal. Increased frequency of threats was observed in a few monkeys following withdrawal from diazepam precipitated with flumazenil (Grant et al. 1985). On the other hand, no changes in aggressive behaviour were found during social conflict in mice after withdrawal from 21 days of lorazepam (File and Wilks 1990).

The increase in social investigation and reduction of aggressive behaviour found after alprazolam treatment in the present study is in good agreement with a number of previous studies using this behavioural model (Kršiak 1975, 1979; Kršiak and Sulcova 1990). In general, benzodiazepines have a biphasic effect on aggressive behaviour; low doses enhance this behaviour and higher doses decrease it (Miczek 1974, 1987; Miczek and Kršiak 1979; Olivier et al. 1991; Ferrari et al. 1997). However, evidence for this effect with alprazolam is scarce (Mos and Olivier 1989).

Tolerance developed to the inhibitory effects of alprazolam on aggressive behaviour but it had not developed to the stimulatory effects of alprazolam on social investigation. Tolerance did not develop to the slight inhibitory effect of alprazolam on locomotion either. In fact, locomotion was reduced only after 21 days of treatment with alprazolam. Tolerance developed at different rates to diverse actions of benzodiazepines in separate tests. For example, tolerance developed rapidly to the anticonvulsant effects (File and Wilks 1990; Byrnes et al. 1993), to motor-impairing effects in the rotarod test (Shumsky and Lucki 1994) or to sedative effects measured in the hole-board test (File 1981; Lister et al. 1983; Fernandes et al. 1999) but little or no tolerance developed to hypothermic or amnesic effects of benzodiazepines (Shumsky and Lucki 1994). Tolerance developed to suppressive effects on unpunished responding, but not to increases in punished responding following chronic administration of oxazepam or chlordiazepoxide (Margules and Stein 1968; McMillan and Leander 1978). Thus, tolerance appears to develop more rapidly to the inhibitory effects of benzo-

diazepines than to their disinhibitory or stimulating effects. Different rates of development of tolerance to behavioural effects of benzodiazepines suggest that they are differentially regulated. The decrement of antiaggressive activity of alprazolam after 8 and 21 days of treatment does not seem to be due to the decrease of control (basal) level of aggressivity because it occurred also in a parameter (the time spent in aggressive acts) where control values remained comparable. The rapid development of tolerance to the inhibitory effects of alprazolam on aggressive behaviour is in agreement with a study using lorazepam, where tolerance to anti-aggressive effects had developed after 7 days of administration of the drug (File and Wilks 1990).

In contrast to the marked and rapid tolerance to the inhibitory effects of alprazolam on aggressive behaviour, only moderate increases of aggression were detected after withdrawal of alprazolam treatment. Social investigation was reduced upon alprazolam withdrawal, although tolerance had not developed to this behavioural effect. Reduction of social investigation appears to be a consistent consequence of withdrawal from benzodiazepines during social conflict in mice. It was found also after withdrawal from lorazepam (File and Wilks 1990), diazepam (Podhorná and Kršiak 1995; Andrews et al. 1997) and chlordiazepoxide (Baldwin and File 1989). Reduction of locomotion represents another behavioural change occurring upon withdrawal from alprazolam to which tolerance had not developed. Locomotion was reduced after withdrawal after 21 days alprazolam treatment, while acute and 8 days of treatment did not change locomotion. Alternatively, no changes in locomotion were observed after withdrawal of low doses of lorazepam although tolerance developed to the inhibitory effect of the drug on locomotion (van der Laan et al. 1993). The evidence of withdrawal responses appearing for a behaviour to which tolerance had not developed does suggest that benzodiazepine tolerance and withdrawal phenomena can be dissociated.

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