

## Limited anxiolytic-like effects of non-benzodiazepine hypnotics in rodents

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The present experiments compared the anxiolytic-like effects of the benzodiazepine (BZD) hypnotic triazolam with those of four non-BZD hypnotics including one non-selective (zopiclone) and three  $\omega_1$ -BZD selective (zolpidem, zaleplon and SX-3228) receptor ligands, in classical animal models including conflict tests (punished lever pressing and punished drinking tests in rats) and exploratory models (elevated plus-maze test in rats and light/dark choice test in mice), and a recently developed mouse defence test battery (MDTB) which has been validated for the screening of anxiolytic drugs. Results from both conflict procedures showed that zopiclone (0.3–10 mg/kg) produced anxiolytic-like effects comparable to those of triazolam (0.1–3 mg/kg), whereas the selective  $\omega_1$ -BZD receptor hypnotics zolpidem (0.3–3 mg/kg), zaleplon (0.1–3 mg/kg) and SX-3228 (0.1–1 mg/kg) displayed weaker and/or non-specific anxiolytic-like effects. Similarly, in the light/dark test in mice, zolpidem (0.1–1 mg/kg), zaleplon (0.3–10 mg/kg) and SX-3228 (0.03–0.3 mg/kg) showed a reduced potential to produce anxiolytic-like effects as compared to the non-selective  $\omega$ -BZD receptor hypnotics triazolam (0.03–1 mg/kg) and zopiclone (1–30 mg/kg). In the elevated plus-maze test, zopiclone (1–10 mg/kg), zolpidem (0.1–1 mg/kg), zaleplon (0.3–3 mg/kg) and SX-3228 (0.1–1 mg/kg) displayed anxiolytic-like activity at doses close to those producing behavioural impairment, whereas triazolam (0.03–1 mg/kg) exhibited anxiolytic-like effects over a wide dose range in the absence of decreases in general activity. In the MDTB, zaleplon (0.3–10 mg/kg) decreased all defensive responses, a profile which was similar to that of triazolam (0.03–1 mg/kg), while zopiclone (1–30 mg/kg), zolpidem (0.3–10 mg/kg) and SX-3228 (0.03–1 mg/kg) had fewer effects on defensive behaviours with several effects occurring only at motor-impairing doses. Taken together, these results demonstrate that, although selective  $\omega_1$ -BZD receptor hypnotics display anxiolytic-like activity, the effects are generally weaker than those observed with non-selective  $\omega$ -BZD receptor selective hypnotics such as triazolam or zopiclone. In particular, the anxiety-reducing potential of the  $\omega_1$ -BZD receptor selective compounds is limited to certain anxiety measures and may be confounded and/or masked by behavioural suppression.

**Key words:** anxiety; conflict procedures; hypnotics; SX-3228; triazolam; zaleplon; zolpidem, zopiclone

### Introduction

Sleep disorders such as insomnia, sleep interruption and poor sleep efficacy are often associated with anxiety disorders (Lader, 1986; Freeman, 1996). Until recently, benzodiazepines (BZDs), which have both anxiolytic and hypnotic effects, were by far the most widely used hypnotics (Dingemans, 1995). However, these compounds have come under critical review because of the problems of drug dependence, tolerance, muscle relaxation, suppression of rapid eye movement (REM) sleep, rebound insomnia and amnesia. Over the last few years, a search has been undertaken for compounds chemically unrelated to BZDs, which may produce fewer unwanted effects, but retain hypnotic properties. Typical of such

compounds are the imidazopyridine zolpidem and the cyclopyrrolone zopiclone (for reviews, see Langtry and Benfield, 1990; Stutzmann *et al.*, 1992).

Zopiclone and zolpidem, although structurally unrelated to BZDs, act at BZD sites [also designated as  $\omega$  (Langer and Arbilla, 1988)] associated with GABA<sub>A</sub> receptors. However, zolpidem has marked selectivity for the  $\omega_1$ -BZD receptor subtype (Pritchett and Seeburg, 1990; Faure-Halley *et al.*, 1993), while zopiclone interacts with high affinity and high efficacy at both  $\omega_1$ -BZD and  $\omega_2$ -BZD receptor subtypes (Blanchard *et al.*, 1979). Zolpidem and zopiclone have been successfully used in the treatment of insomnia (e.g. Chaudoir *et al.*, 1990; Priest *et al.*, 1997) and there is evidence that both drugs have fewer side-effects than BZD hypnotics (e.g. less

residual effects upon waking, no depression of REM sleep) (Monti, 1989; Kerr *et al.*, 1995). However, less is known about the anxiolytic potential of these novel hypnotics. Although one clinical study showed that zolpidem and the BZD hypnotic triazolam displayed comparable efficacy in improving anxiety states of insomniac patients (Pagot *et al.*, 1993), studies in animals are less clear. Several reports have shown that zolpidem either produced weaker anxiolytic-like effects than BZDs (Depoortere *et al.*, 1986; Griebel *et al.*, 1996c,d) or was devoid of such activity (Sanger and Zivkovic, 1988; Perrault *et al.*, 1990; Sanger, 1995; Griebel *et al.*, 1996d). For example, in the elevated plus-maze test in rats and in the light/dark test in mice, the magnitude of the anxiolytic-like effects of zolpidem was smaller than those of BZDs such as diazepam and chlordiazepoxide (Griebel *et al.*, 1996c,d). In addition, in these studies, the weak anxiolytic-like action of zolpidem was evident only at doses which reduced locomotor activity, indicating that these effects may have been non-specific. A few studies have investigated the anxiolytic-like effects of zopiclone in animals. Although the drug was inactive in one study using the Vogel conflict test in rats (Kataoka *et al.*, 1991), most reports indicated that zopiclone displayed anxiolytic-like effects in conflict procedures in rats (Goldberg *et al.*, 1983; Sanger *et al.*, 1985; Ueki, 1987) and monkeys (Goldberg *et al.*, 1983; Barrett *et al.*, 1986), and in tests based on food consumption in an unfamiliar environment (Yamamoto and Ueki, 1987; Perrault *et al.*, 1990). Clinical studies revealed that zopiclone was effective in improving anxiety symptoms of patients affected by insomnia and generalized anxiety disorders (GAD) (Inanaga *et al.*, 1982; Mizuki *et al.*, 1983; Agnoli *et al.*, 1989).

The aim of the present study was to compare the effects of the BZD hypnotic triazolam with those of several non-BZD hypnotics under identical test conditions in classical animal models of anxiety including conflict procedures (punished lever pressing and punished drinking tests in rats) and exploratory models (elevated plus-maze test in rats and light/dark choice test in mice), and in a recently developed mouse defence test battery (MDTB) which was found to be useful for the screening of anxiolytic drugs (Griebel *et al.*, 1995). In addition, a more ethological-orientated scoring method was used with the elevated plus-maze and the light/dark choice tests as there is increasing evidence that sensitivity to drug effects may be increased when such techniques are employed (Rodgers and Cole, 1994; Griebel *et al.*, 1997a). The non-BZD drugs used were the clinically effective hypnotics zopiclone and zolpidem, and two novel potential hypnotic agents, zaleplon (Day *et al.*, 1992; Beer *et al.*, 1994) and SX-3228 (Ohta, 1996). Recent findings have shown that zaleplon and zolpidem share similar pharmacological properties (e.g. anticonvulsant activity, central depressant effects, lack of BZD discriminative stimulus action), and  $\omega_1$ -BZD receptor selectivity (Day *et al.*, 1992; Vanover and Barrett, 1994; Sanger *et al.*, 1996). Zaleplon, like zolpidem, was also reported to show little or no anxiolytic-like activity in a test of punished responding (Sanger, 1995) and in the elevated plus-maze (Griebel *et al.*, 1996d). Little has been published on the preclinical pharmacology of SX-3228, but it has been reported to be selective for  $\omega_1$ -BZD receptors (Ohta, 1996), and as potent and effective as diazepam in a conditioned suppression of drinking procedure (Bayley *et al.*, 1996).

## Methods

### Animals

Male Wistar rats (Charles River, Saint-Aubin-les-Elbeuf, France) were used in the punished lever pressing procedure. They weighed 180–200 g at the beginning of training and 400–500 g at the time of drug testing. Male Sprague-Dawley rats (Iffa Credo, L'Arbresle, France and Charles River) weighing 180–230 g at time of testing were used in the punished drinking and the elevated plus-maze tests. Male Long Evans rats (400–500 g) (Iffa Credo) were used as threat stimulus in the MDTB. BALB/c mice (7 weeks old) and male Swiss mice (10 weeks old) (both supplied by Iffa Credo) were used in the light/dark test and in the MDTB, respectively. Rats used in the elevated plus-maze and in the Vogel drinking tests were housed in groups of eight, whereas those used in the punished lever pressing procedure were housed singly. BALB/c mice were housed in groups of six and Swiss mice were isolated 1 week prior to testing. All animals were maintained under standard laboratory conditions (22–23 °C) and kept on a 12 h : 12 h light : dark cycle with light onset at 07.00 hours. Rats used in the punished lever pressing procedure were restricted to the food obtained during sessions and a daily ration of 15–20 g of standard laboratory chow given at the end of each weekday and over the weekend. With the exception of the rats used in the punished lever pressing test, animals were only tested once.

### Drugs

All drugs were prepared as solutions or suspensions in physiological saline containing one or two drops of Tween 80. They were injected in a volume of 2 ml/kg (rats) or 20 ml/kg (mice). The drugs used were triazolam, zolpidem, zopiclone, SX-3228 (synthesized by the Chemistry Department, Synthélabo Recherche) and zaleplon (CL 284,846) (courtesy of Dr B. Beer, American Cyanamid, USA). Drugs were given i.p. 30 min before experiments. Testing was performed between 09.00 and 15.00 hours. Doses are expressed as the bases.

### Punished lever pressing

The procedure was a modification of that described previously (Sanger *et al.*, 1985). Animals were tested in standard rat operant test chambers (MED Associates, Inc., Georgia, USA) placed in sound-attenuated boxes that were well ventilated. Each chamber was fitted with a stainless steel grid floor. Electric shocks could be delivered to each grid by a shock generator and scrambler (MED Associates, Inc.). A total of 11 rats were trained initially to press a lever for food reward (45-mg precision food pellets, PJ Noyes, Inc., Lancaster, USA). As training progressed, schedule parameters were gradually changed to a variable interval (VI 30 sec) schedule of food reinforcement during daily 15-min sessions. After several sessions of VI 30-sec responding, five 60-sec periods of a visual stimulus were presented during a 25-min session. Each visual stimulus consisted of three stimulus lights situated above the food pellet dispenser and to the right of the response lever, which flashed at a rate of 1 sec on, 1 sec off. In this component, a footshock punishment schedule consisting of two independent VI schedules (VI 30 sec for food, VI 10 sec for shock) was in operation. Footshock was initially set at 0.1 mA. The first

stimulus presentation started 5 min after the beginning of the session, and each following stimulus commenced 150 sec after the end of the preceding stimulus. The magnitude of footshock was individually titrated for each rat (shock levels ranged from 0.3–0.65 mA) to obtain stable baselines of responding (i.e. an average lever pressing rate of  $8 \pm 2$  presses in each 1-min punished responding period). To obtain stable levels of responding, an average of approximately 30 sessions after initiation of the punishment contingency was necessary. Once stable baselines of responding were obtained, drug studies were initiated.

Drug injections were given once or twice each week with at least two non-drug days intervening between two drug administrations. Vehicle was injected on all non-drug days. Drugs and doses were given in a mixed order. The effects of drugs were assessed on punished and unpunished response rates. The former corresponds to those recorded during the presentation of the visual stimulus, whereas the latter were taken from the 60-sec periods immediately preceding and immediately following each stimulus presentation. The mean values of punished and unpunished rates recorded during the non-drug session preceding the drug injection sessions were used as the control values. Thus, drug effects were analysed statistically by comparing performances after drug administration with the mean values taken from appropriate control sessions using a Friedman's ANOVA.

### Punished drinking

The procedure was a modification of the technique described by Vogel *et al.* (1971). At the beginning of the experiment, rats, deprived of water for 48 h prior to testing, were placed in cages ( $27 \times 22 \times 21$  cm) with a stainless steel grid floor. Each cage contained a drinking tube connected to an external 50-ml buret filled with tap water. Trials were started only after the animal's tongue entered into contact with the drinking tube for the first time. An electric shock (0.3 mA) was delivered to the tongue after every 20 licks. The number of shocks was recorded automatically during a 3-min period. Because of inter-individual variability, results were analysed by the non-parametric Kruskal–Wallis test.

### Elevated plus-maze

The test apparatus is based on that described by Pellow *et al.* (1985). All parts of the apparatus were made of dark polyvinylplastic with a black rubber floor. The maze was elevated to a height of 50 cm with two open ( $50 \times 10$  cm) and two enclosed arms ( $50 \times 10 \times 50$  cm), arranged so that the arms of the same type were opposite each other, connected by an open central area ( $10 \times 10$  cm). To prevent rats falling off, a rim of Plexiglas (1-cm high) surrounded the perimeter of the open arms. The illumination in the experimental room consisted of a red neon tube fixed on the ceiling, so that experiments were performed under dim light conditions. The light intensity on the central platform was 10 lx. At the beginning of the experiment, rats were placed in the centre of the maze, facing one of the enclosed arms, and observed for 4 min. The apparatus was equipped with infrared beams and sensors capable of measuring time spent in open arms, number of open-arm entries and number of closed-arm entries (defined

as entry of all four limbs into an arm of the maze). In addition, rats were observed via video link by an observer located in an adjacent room. This permitted the recording of the more ethologically-orientated measures. (a) Attempt: attempt at entry into open arms followed by avoidance responses. This includes stretched attend posture (the rat stretches forward and retracts to original position). (b) Head-dipping: protruding the head over the edge of an open arm and down towards the floor (this response can occur while the animal's body is in a closed arm, central square or on an open arm). The results were expressed as mean ratio of time spent in open arms to total time spent in both open and closed arms, mean ratio of entries into open arms to total entries into both open and closed arms, mean total number of closed arm entries, mean total number of attempts and mean total number of head-dips. The experimenter was unaware of the drug treatment. Data were analysed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

### Light/dark choice test

The test apparatus is based on that described by Misslin *et al.* (1989). It consisted of two polyvinylchloride boxes ( $20 \times 20 \times 14$  cm) covered with plexiglass. One of these boxes was darkened. A neon tube fixed on the ceiling provided the room illumination so that the light intensity in the centre of the illuminated box was 150 lx. An opaque plastic tunnel ( $5 \times 7 \times 10$  cm) separated the dark box from the illuminated one. At the beginning of the experiment, a mouse was placed in the illuminated box, facing the tunnel. Recording started when the animal entered the tunnel for the first time. The apparatus was equipped with infrared beams and sensors capable of recording the following three parameters during a 4-min period: (a) time spent by mice in the lit box; (b) attempt at entry into the lit box followed by avoidance responses. This includes stretched attend posture; (c) total number of tunnel crossings. Data were analysed with the Kruskal–Wallis test.

### MDTB

The procedure has been extensively described in a previous paper (Griebel *et al.*, 1997b). The test was conducted in an oval runway,  $0.40 \times 0.30 \times 4.4$  m, consisting of two 2-m straight segments joined by two 0.4-m curved segments and separated by a median wall ( $2.0 \times 0.30 \times 0.06$  m). The apparatus was elevated to a height of 0.80 m from the floor. All parts of the apparatus were made of black plexiglass. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with video cameras mounted above the apparatus. In addition, the apparatus was equipped with infrared beams and sensors capable of measuring the velocity of the animal during the chase/flight test. The room illumination was provided by a red neon tube fixed on the ceiling and two desk lamps with red bulbs placed respectively on two tables (elevated to a height of 1 m) located 1 m away from the runway. The light intensity in the runway was 7 lx. The experimenter was unaware of the drug treatment.

The following procedures were undertaken. (a) Pre-test: a 3-min familiarization period, a subject was placed into the runway for a 3-min familiarization period, in which line

crossings, wall rears, wall climbs and jump escapes were recorded (min 1 to 3). (b) The rat avoidance test (min 4 to 6), immediately after the 3-min familiarization period, the experimenter introduced a hand-held dead rat (killed by CO<sub>2</sub> inhalation just before the beginning of the experiment) five times at one end of the runway and brought up to the subject at a speed of approximately 0.5 m/sec. Approach was terminated when contact with the subject was made or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) was recorded. (c) The chase test (min 7 to 8), the hand-held rat was brought up to the subject at a speed of approximately 2.0 m/sec. Flight speed (measured while the subject was running straight) and the number of stops (pause in movement) during the chase were recorded. (d) The straight alley test (min 9 to 11), after the chase was completed, the runway was converted to a straight alley by closing two doors (60 cm from each other). The dead rat was placed in one end of the straight alley and the number of approach/withdrawal responses (subject must move more than 0.2 m forward from the closed door, then return to it) was measured during a 30-sec period. Stops and approach/withdrawal responses are described as RA activities (Griebel *et al.*, 1995). (e) The forced contact test (min 12 to 13), finally, the experimenter brought the rat up to contact the subject in the straight alley. For each such contact, defensive threat and attack responses (i.e. bites and upright postures) were noted, this was repeated three times. (f) Post-test contextual defence, immediately after the forced contact test, the rat was removed and the doors were opened. Escape attempts (wall rears, wall climbs and jump escapes) were recorded during a 3-min session (min 14 to 16).

Data from the pre-test (line crossings), the rat avoidance test (avoidance distance) and flight speed during the chase were analysed with a one-way ANOVA, whereas risk assessment activities (stops and approaches/withdrawals) and escape attempts (post-test) were analysed with the non-parametric Kruskal–Wallis test. In those cases where parametric statistics were employed, subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test. Pre- vs post-test differences were evaluated with the Wilcoxon matched paired test.

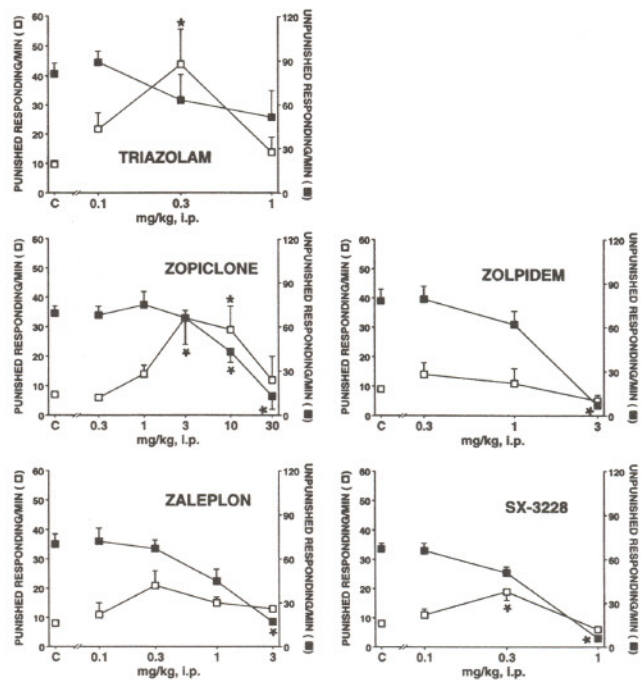
**Results**

**Punished lever pressing**

Figure 1 shows that the rates of responding decreased by the punishment contingency were significantly increased by triazolam ( $\chi^2=7.95$ ;  $p<0.05$ ), zopiclone ( $\chi^2=23.03$ ;  $p<0.001$ ) and SX-3228 ( $\chi^2=13.69$ ;  $p<0.01$ ), but not by zolpidem or zaleplon. These effects occurred at doses which did not modify unpunished responding. However, Fig. 1 shows that these latter responses were significantly decreased by zopiclone ( $\chi^2=26.29$ ;  $p<0.001$ ), zolpidem ( $\chi^2=16.35$ ;  $p<0.001$ ), zaleplon ( $\chi^2=23.6$ ;  $p<0.001$ ) and SX-3228 ( $\chi^2=17.25$ ;  $p<0.001$ ) at higher doses.

**Punished drinking**

Figure 2 shows that all compounds significantly modified the number of shocks received, triazolam (KW = 15.81;  $p<0.01$ ), zopiclone (KW = 13.51;  $p<0.01$ ), zolpidem (KW = 14.21;

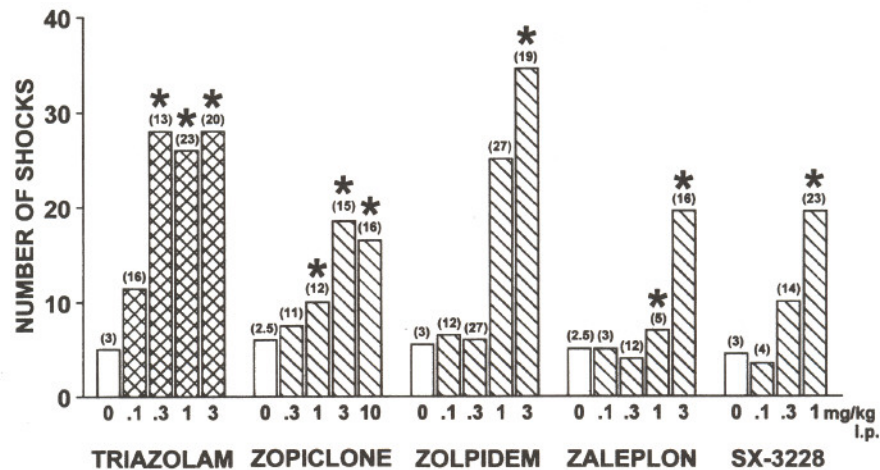


**Figure 1** Effects of one benzodiazepine (BZD; triazolam) and four non-BZD (zopiclone, zolpidem, zaleplon and SX-3228) hypnotics on rates of punished and unpunished lever pressing in rats. Drugs were administered i.p. 30 min before testing. Data represent mean  $\pm$  SEM.  $n=8$ ; \* $p<0.05$  (Friedman test vs vehicle control)

$p<0.01$ ), zaleplon (KW = 17.79;  $p<0.01$ ) and SX-3228 (KW = 5.88;  $p<0.05$ ). *Post-hoc* analysis indicated that while triazolam (0.3–3 mg/kg) and zopiclone (1–10 mg/kg) significantly increased punished responding over a wide dose-range, zolpidem (3 mg/kg), zaleplon (1 and 3 mg/kg) and SX-3228 (1 mg/kg) produced similar effects at the highest doses only.

**Elevated plus-maze**

Table 1 shows that all drugs significantly modified both the percentage of time spent, triazolam [ $F(4,30)=4.41$ ;  $p<0.01$ ], zopiclone [ $F(3,28)=7.4$ ;  $p<0.001$ ], zolpidem [ $F(3,28)=5.65$ ;  $p<0.01$ ], zaleplon [ $F(3,31)=6.6$ ;  $p<0.01$ ] and SX-3228 [ $F(3,44)=3.07$ ;  $p<0.05$ ] and the percentage of entries made, triazolam [ $F(4,30)=3.69$ ;  $p<0.05$ ], zopiclone [ $F(3,28)=10.08$ ;  $p<0.001$ ], zolpidem [ $F(3,28)=9.44$ ;  $p<0.01$ ], zaleplon [ $F(3,31)=6.54$ ;  $p<0.01$ ] and SX-3228 [ $F(3,44)=8.27$ ;  $p<0.001$ ] into open arms. *Post-hoc* analysis indicated that triazolam (0.1–1 mg/kg), zopiclone (1–10 mg/kg) and zaleplon (0.3–3 mg/kg) significantly increased activity in open arms over a wide dose range. SX-3228 produced a similar effect at 0.3 and 1 mg/kg, whereas zolpidem significantly increased open-arm time and entries at the highest dose only (1 mg/kg). With respect to the ethologically derived measures, all compounds modified the number of attempts at entry into open arms followed by avoidance responses, triazolam [ $F(4,30)=20.78$ ;  $p<0.001$ ], zopiclone [ $F(3,28)=15.63$ ;  $p<0.001$ ], zolpidem [ $F(3,28)=8$ ;  $p<0.001$ ], zaleplon, [ $F(3,31)=44.59$ ;  $p<0.001$ ]



**Figure 2** Effects of one benzodiazepine (BZD; triazolam) and four non-BZD (zopiclone, zolpidem, zaleplon and SX-3228) hypnotics in the punished drinking conflict test in rats. Drugs were administered i.p. 30 min before testing. Data represent median (inter-quartile range).  $n = 7-10$ ; \* $p < 0.05$  (Kruskal-Wallis test, vs vehicle control)

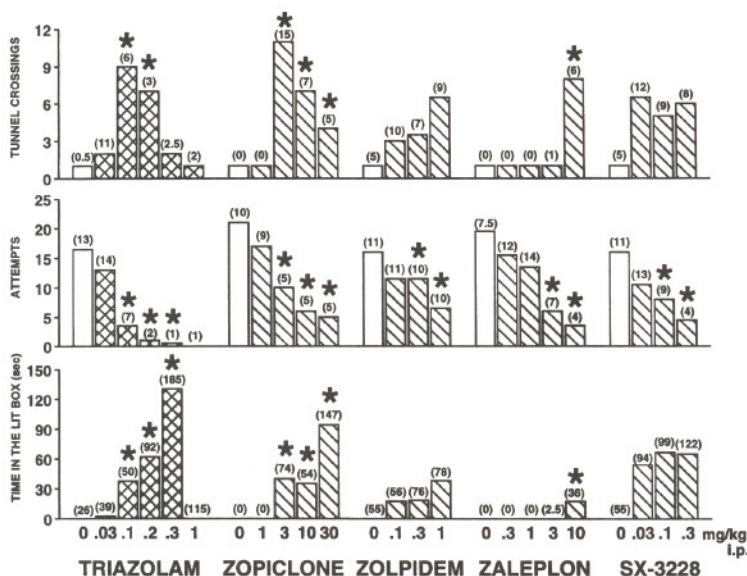
**Table 1** Effects of one benzodiazepine (BZD; triazolam) and four non-BZD (zopiclone, zolpidem, zaleplon and SX-3228) hypnotics on the behaviour of rats on the elevated plus-maze

Drug	Dose (mg/kg)	% time in open arms	% entries in open arms	Attempts	Head-dippings	Closed-arm entries
Triazolam	0	13.2 ± 5.0	12.6 ± 4.4	10.1 ± 0.6	4.3 ± 0.9	9.7 ± 0.9
Triazolam	0.03	31.5 ± 5.3	30.6 ± 5.2	8.1 ± 1.1	8.4 ± 2.1	9.1 ± 0.7
Triazolam	0.1	32.5 ± 5.8	32.8 ± 5.0*	6.6 ± 1.5*	10.7 ± 2.1*	10.9 ± 1.8
Triazolam	0.3	49.5 ± 9.3*	43.9 ± 9.3*	1.0 ± 0.4*	13.6 ± 1.9*	7.9 ± 1.6
Triazolam	1	47.1 ± 8.2*	33.4 ± 4.0*	0.9 ± 0.5*	5.4 ± 1.3	6.0 ± 1.3
Zopiclone	0	16.4 ± 6.6	17.0 ± 6.7	9.5 ± 1.1	3.9 ± 0.9	8.9 ± 0.5
Zopiclone	1	33.8 ± 6.9	36.0 ± 6.4*	5.0 ± 0.8*	10.5 ± 2.2*	7.8 ± 0.5
Zopiclone	3	57.4 ± 5.9*	59.0 ± 4.9*	2.6 ± 0.7*	14.8 ± 1.9*	5.6 ± 1.0*
Zopiclone	10	46.7 ± 6.5*	52.0 ± 5.3*	2.3 ± 0.7*	9.5 ± 1.9*	4.6 ± 0.9*
Zolpidem	0	18.0 ± 4.2	20.1 ± 3.8	8.4 ± 0.7	3.0 ± 0.4	8.9 ± 0.8
Zolpidem	0.1	18.1 ± 4.8	18.6 ± 3.8	7.3 ± 0.7	3.1 ± 0.5	8.5 ± 0.5
Zolpidem	0.3	15.2 ± 4.8	13.0 ± 4.8	8.8 ± 0.7	2.1 ± 0.4	8.5 ± 0.5
Zolpidem	1	46.8 ± 3.9*	45.4 ± 1.2*	3.9 ± 0.5*	6.0 ± 0.8*	7.1 ± 0.8
Zaleplon	0	7.5 ± 2.8	12.4 ± 3.6	9.1 ± 0.5	4.8 ± 0.8	7.8 ± 0.5
Zaleplon	0.3	34.4 ± 6.2*	36.2 ± 5.3*	4.6 ± 0.7*	8.9 ± 1.4	7.6 ± 0.7
Zaleplon	1	37.1 ± 10.6*	43.6 ± 7.3*	0.8 ± 0.3*	8.1 ± 2.0	4.1 ± 0.7*
Zaleplon	3	53.1 ± 7.4*	43.0 ± 6.3*	1.3 ± 0.5*	7.5 ± 1.4	4.5 ± 1.0*
SX-3228	0	17.0 ± 5.7	15.6 ± 4.8	8.5 ± 1.0	3.2 ± 0.6	9.5 ± 0.8
SX-3228	0.1	36.0 ± 4.0	33.1 ± 2.9	6.7 ± 0.7	8.1 ± 1.2*	8.3 ± 0.5
SX-3228	0.3	41.3 ± 5.1*	46.2 ± 5.1*	3.8 ± 0.5*	8.2 ± 1.1*	5.7 ± 0.6*
SX-3228	1	41.2 ± 7.5*	48.6 ± 6.2*	0.8 ± 0.3*	5.3 ± 1.1	4.3 ± 1.0*

Drugs were administered i.p. 30 min before testing. Data represent mean ± SEM.  $n = 7-12$ ; \* $p < 0.05$  (Dunnett's *t*-test, vs vehicle control).

and SX-3228 [ $F(3,44) = 24.44$ ;  $p < 0.001$ ]. *Post-hoc* analysis showed that triazolam (0.1–1 mg/kg), zopiclone (1–10 mg/kg), zaleplon (0.3–3 mg/kg) and SX-3228 (0.3–1 mg/kg) significantly reduced attempts at several dose levels, whereas zolpidem decreased this behaviour at the highest dose only (1 mg/kg). In addition, triazolam [ $F(4,30) = 4.86$ ;  $p < 0.01$ ], zopiclone [ $F(3,28) = 6.39$ ;  $p < 0.001$ ], zolpidem [ $F(3,28) = 5.9$ ;  $p < 0.001$ ] and SX-3228 [ $F(3,44) = 5.77$ ;  $p < 0.01$ ] modified directed exploration (head-dippings). *Post-hoc* analysis revealed that this response was significantly increased by

triazolam (0.1–0.3 mg/kg), zopiclone (1–10 mg/kg) and SX-3228 (0.1–0.3 mg/kg) at several doses, whereas it was increased by zolpidem at the highest dose only (1 mg/kg). Although the effect of zaleplon did not reach statistical significance for this measure, a tendency to an increase was observed. Finally, zopiclone [ $F(3,28) = 6.79$ ;  $p < 0.01$ ], zaleplon [ $F(3,31) = 7.4$ ;  $p < 0.001$ ] and SX-3228 [ $F(3,44) = 10.7$ ;  $p < 0.001$ ] significantly decreased the number of closed-arm entries. These effects reached statistical significance for zopiclone (3 and 10 mg/kg), zaleplon (1 and 3 mg/kg), and SX-3228 (0.3 and 1 mg/kg).



**Figure 3** Effects of one benzodiazepine (BZD; triazolam) and four non-BZD (zopiclone, zolpidem, zaleplon and SX-3228) hypnotics in the light/dark test with BALB/c mice. Drugs were administered i.p. 30 min before the beginning of the experiment. Data represent median (inter-quartile range).  $n=12-15$ ;  $*p<0.05$  (Kruskal-Wallis test vs vehicle control)

**Light/dark choice test**

Figure 3 shows that triazolam, zopiclone and zaleplon, but not zolpidem or SX-3228 significantly modified time spent by mice in the lit box, triazolam [KW=14.98;  $p<0.05$ ], zopiclone [KW=19.94;  $p<0.001$ ], zaleplon [KW=19.69;  $p<0.001$ ] and total number of tunnel crossings, triazolam [KW=15.97;  $p<0.01$ ], zopiclone [KW=13.25;  $p<0.05$ ] and zaleplon [KW=19.31;  $p<0.001$ ]. *Post-hoc* analysis indicated that triazolam (0.1–0.3 mg/kg) and zopiclone (3–30 mg/kg) increased both parameters at several doses, whereas zaleplon produced a similar effect at the highest dose only (10 mg/kg). The number of attempts at entry in the lit box was significantly affected by all compounds, triazolam [KW=46.06;  $p<0.001$ ], zopiclone [KW=41.13;  $p<0.001$ ], zolpidem [KW=9.73;  $p<0.05$ ], zaleplon [KW=25.54;  $p<0.001$ ] and SX-3228 [KW=18.67;  $p<0.001$ ]. While triazolam (0.1–1 mg/kg) and zopiclone (3–30 mg/kg) significantly reduced attempts over a wide dose range, zolpidem (0.3 and 1 mg/kg), zaleplon (3 and 10 mg/kg) and SX-3228 (0.1–3 mg/kg) decreased this measure at the two highest doses only.

**MDTB**

*Pre-test: motor activity before exposure to the rat (Table 2)*  
 Statistical analyses revealed that triazolam [ $F(4,25)=5.4$ ;  $p<0.01$ ], zopiclone [ $F(4,35)=10.77$ ;  $p<0.001$ ] and zolpidem [ $F(4,35)=11.18$ ;  $p<0.001$ ], but not zaleplon or SX-3228 significantly modified the number of line crossings. *Post-hoc* analysis showed that triazolam (0.3 and 1 mg/kg) and zopiclone (10 and 30 mg/kg) reduced this measure at the two highest doses, whereas zolpidem reduced pre-test line crossings at 10 mg/kg.

*Rat avoidance test (Table 2)*

With the exception of zopiclone, the drugs significantly affected the stimulus-subject distance at which avoidance occurred, triazolam (KW=11.18;  $p<0.05$ ), zolpidem

(KW=11.8;  $p<0.05$ ), zaleplon (KW=11;  $p<0.05$ ) and SX-3228 (KW=14.2;  $p<0.01$ ). Triazolam (0.03–0.3 mg/kg) and SX-3228 (0.03–1 mg/kg) significantly reduced avoidance over a wide dose range. Zolpidem reduced this behaviour in a non-dose-dependent manner at 1 and 10 mg/kg, and zaleplon decreased avoidance at 10 mg/kg.

*Chase/flight test (Table 2)*

All drugs significantly modified flight speed, triazolam [ $F(4,25)=13.51$ ;  $p<0.001$ ], zopiclone [ $F(4,35)=3.9$ ;  $p<0.05$ ], zolpidem [ $F(4,35)=4.39$ ;  $P<0.01$ ], zaleplon [ $F(4,35)=6.93$ ;  $p<0.01$ ] and SX-3228 [ $F(4,35)=3.51$ ;  $p<0.05$ ] and the number of stops, triazolam [KW=14.5;  $p<0.01$ ], zopiclone [KW=17.4;  $p<0.01$ ], zolpidem [KW=11.6;  $p<0.05$ ], zaleplon [KW=11.9;  $p<0.05$ ] and SX-3228 [KW=12.8;  $p<0.05$ ]. *Post-hoc* analysis revealed that while triazolam significantly reduced both parameters at all dose levels (0.03–1 mg/kg), all other drugs decreased them at the highest doses only.

*Straight alley test*

None of the drugs significantly modified the number of approaches to the rat followed by withdrawal responses (data not presented).

*Forced contact test (Table 2)*

Triazolam, zopiclone, zaleplon, but not zolpidem and SX-3228 significantly modified upright postures, triazolam (KW=18.1;  $p<0.01$ ), zopiclone (KW=17.2;  $p<0.01$ ) and zaleplon (KW=16;  $p<0.01$ ) and defensive biting upon forced contact with the rat, triazolam (KW=13.7;  $p<0.01$ ), zopiclone (KW=24.4;  $p<0.001$ ) and zaleplon (KW=20.3;  $p<0.001$ ). *Post-hoc* analysis indicated that triazolam significantly reduced

**Table 2** Effects of one benzodiazepine (BZD; triazolam) and four non-BZD (zopiclone, zolpidem, zaleplon and SX-3228) hypnotics on several behavioural responses displayed by Swiss mice before (locomotor activity) and during (flight, risk assessment and defensive threat/attack) exposure to a Long Evans rat in the mouse defence test battery

Drug	Dose (mg/kg)	Locomotor activity		Flight		Risk assessment	Defensive threat and attack	
		Line crossings	Avoidance distance (cm)	Speed (m/sec)	Stops	Upright postures	Bitings	
Triazolam	0	132.0 ± 7.7	109.8 (44.5)	0.95 ± 0.13	13.5 (4)	3 (0)	2.5 (1)	
	0.03	132.3 ± 10.3	60.4 (31.2) <sup>†</sup>	0.60 ± 0.03*	2.5 (5) <sup>†</sup>	2.5 (2)	1 (2) <sup>†</sup>	
	0.1	80.0 ± 12.4	27 (0) <sup>†</sup>	0.39 ± 0.04*	2.5 (2) <sup>†</sup>	1 (3) <sup>†</sup>	0 (0) <sup>†</sup>	
	0.3	69.3 ± 16.2*	25.5 (9) <sup>†</sup>	0.36 ± 0.03*	2 (2) <sup>†</sup>	0 (0) <sup>†</sup>	0 (0) <sup>†</sup>	
	1	68.5 ± 20.6*		0.40 ± 0.06*	2.5 (1) <sup>†</sup>	0 (0) <sup>†</sup>	0 (0) <sup>†</sup>	
Zopiclone	0	146.3 ± 12.7	95.2 (38.4)	0.81 ± 0.11	11.5 (4.5)	3 (0)	2 (1.5)	
	1	138.5 ± 12.2	106.8 (43.5)	0.79 ± 0.12	10 (4.5)	3 (0)	1.5 (2)	
	3	135.4 ± 8.2	81.9 (24.6)	0.75 ± 0.05	9.5 (8)	3 (0)	1.5 (1)	
	10	101.9 ± 12.4*	74.5 (33.5)	0.54 ± 0.06*	5.5 (2) <sup>†</sup>	0 (1.5) <sup>†</sup>	0 (0) <sup>†</sup>	
	30	61.5 ± 6.7*	55.8 (62.8)	0.45 ± 0.04*	4 (6.5) <sup>†</sup>	0.5 (1.5) <sup>†</sup>	0 (0) <sup>†</sup>	
Zolpidem	0	126.3 ± 8.7	90.1 (26.2)	0.79 ± 0.07	11 (1.5)	3 (0)	1.5 (2)	
	0.3	120.4 ± 17.3	74.8 (34.4)	0.86 ± 0.08	6.5 (3)	3 (0)	1 (2)	
	1	103.0 ± 11.5	54.5 (35.5) <sup>†</sup>	0.65 ± 0.06	7.5 (5.5)	3 (0)	1 (2)	
	3	89.4 ± 9.5	71 (7.9)	0.60 ± 0.05	9 (4.5)	3 (0)	0 (2)	
	10	29.3 ± 8.8*	41 (74.4) <sup>†</sup>	0.50 ± 0.08*	5 (1.5) <sup>†</sup>	2 (3)	0 (0.5)	
Zaleplon	0	132.5 ± 12.6	111.1 (27.7)	1.04 ± 0.18	11 (5.5)	3 (0)	2 (1)	
	0.3	107.3 ± 13.2	97.3 (44.4)	0.72 ± 0.09	9 (6)	3 (1)	1.5 (1.5)	
	1	119.8 ± 13.9	132 (82.3)	0.95 ± 0.10	11.5 (7.5)	3 (0)	1.5 (1.5)	
	3	102.0 ± 13.0	70 (10) <sup>†</sup>	0.43 ± 0.04*	6.5 (6.5)	1 (3) <sup>†</sup>	0 (1) <sup>†</sup>	
	10	84.0 ± 13.7	60 (39)	0.46 ± 0.03*	4 (5.5) <sup>†</sup>	0 (1) <sup>†</sup>	0 (0) <sup>†</sup>	
SX-3228	0	121.8 ± 16.4	101 (25.2)	0.98 ± 0.13	10 (5.5)	3 (0)	2.5 (1.5)	
	0.03	126.9 ± 8.8	72 (41.4) <sup>†</sup>	0.81 ± 0.12	9.5 (7)	3 (1)	2 (2)	
	0.1	126.5 ± 9.3	83.3 (33.5) <sup>†</sup>	0.71 ± 0.06	1 (4.5)	3 (0)	2.5 (1.5)	
	0.3	121.0 ± 9.7	50.7 (8.5) <sup>†</sup>	0.73 ± 0.07	6.5 (4) <sup>†</sup>	3 (0)	3 (2.5)	
	1	102.6 ± 13.3	39 (39) <sup>†</sup>	0.52 ± 0.04*	6 (6) <sup>†</sup>	1.5 (2.5)	0.5 (1)	

Drugs were administered i.p. 30 min before the beginning of the experiment. Data represent mean ± SEM or in the case of non-parametric statistics median (inter-quartile range).  $n = 6-8$ ; \* $p < 0.05$  (Dunnett's *t*-test vs vehicle control); <sup>†</sup> $p < 0.05$  (Kruskal-Wallis test vs vehicle control).

defensive threat and attack responses over a wide dose-range (0.1–1 mg/kg), whereas zopiclone (10 and 30 mg/kg) and zaleplon (3 and 10 mg/kg) decreased these responses at the two highest doses only.

#### Post-test escape attempts

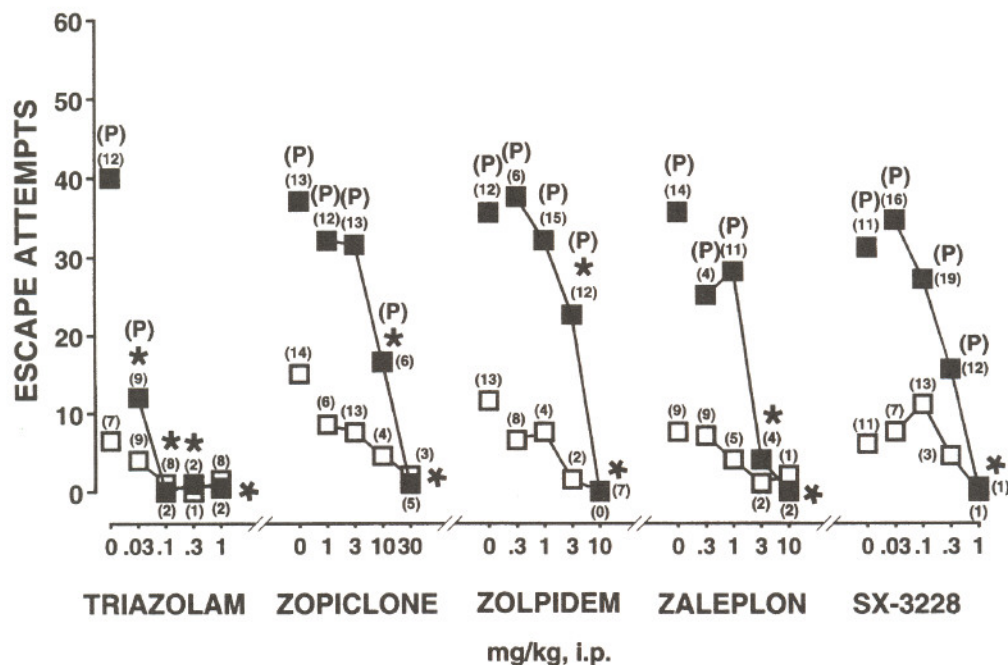
Figure 4 shows that escape attempts were significantly increased in all control groups following the removal of the threat stimulus from the runway apparatus. Triazolam prevented the increase in this behaviour from 0.1–1 mg/kg, whereas the other compounds modified the response at the highest doses only. Comparisons between control mice and drugged animals revealed that all compounds significantly reduced post-rat escape attempts, (triazolam (KW = 21.9;  $p < 0.001$ ), zopiclone (KW = 27.9;  $p < 0.001$ ), zolpidem, (KW = 24;  $p < 0.001$ ), zaleplon (KW = 30.6;  $p < 0.001$ ) and SX-3228 (KW = 23.4;  $p < 0.001$ ). Triazolam decreased escape attempts at all doses (0.03–1 mg/kg), while the other drugs reduced this behaviour at the highest doses only.

## Discussion

The results of this study showed that non-BZD hypnotics produced anxiolytic-like effects in rodents. However, differ-

ences in terms of the efficacy and the specificity of the effects observed were noted between these agents and the BZD hypnotic triazolam.

In the punished lever pressing and the punished drinking conflict tests in rats, triazolam produced an increase in rates of responding suppressed by punishment. The lack of significant modification of unpunished responding in the lever pressing procedure indicated that these effects were specific, although at 1 mg/kg a tendency to a decrease was observed. This may explain the loss of antipunishment action in the lever pressing test. In the punished drinking test, where triazolam displayed anxiolytic-like effects from 0.3–3 mg/kg, one can assume that motor deficits interfered less with responding so that anticonflict effects were still detectable. Among the non-BZD hypnotics, zopiclone, SX-3228, but not zolpidem and zaleplon produced an increase in punished responding in the lever pressing test, while all compounds displayed anticonflict activity in the punished drinking procedure. In the lever pressing test, these effects occurred in the absence of significant decreases in unpunished responding, suggesting that the drugs produced specific antipunishment effects. Importantly, the magnitude of the increase in punished responding with SX-3228 was much smaller than that produced by triazolam and zopiclone, indicating a weaker anxiolytic-like activity. In the punished drinking test, zopiclone produced anxiolytic-like



**Figure 4** Effects of one benzodiazepine (BZD; triazolam) and four non-BZD (zopiclone, zolpidem, zaleplon and SX-3228) hypnotics on escape attempts by mice from the runway cage before pre-test (open symbols) and after (solid symbols) exposure to a dead Long Evans rat. Data represent median (inter-quartile range). (P)  $p < 0.05$  (vs pre-test); \* $p < 0.05$  (vs vehicle control)

effects at doses (1–3 mg/kg) which did not impair unpunished response rates in the lever pressing test, suggesting that this action was specific. In contrast, the selective  $\omega_1$ -BZD receptor compounds zolpidem, zaleplon and SX-3228 produced anxiolytic-like effects at doses which decreased unpunished responding, consistent with the suggestion that the anticonflict action of such compounds may have been confounded by behavioural suppression (Zivkovic *et al.*, 1988; Sanger, 1995). Taken together, the findings from conflict procedures indicate that zopiclone produced anxiolytic-like effects comparable to those of triazolam, whereas the selective  $\omega_1$ -BZD hypnotics displayed weaker and/or non-specific anxiolytic-like effects. The results obtained with zopiclone, zolpidem and zaleplon are consistent with earlier findings showing that the former displayed potent anticonflict activity in rats (Goldberg *et al.*, 1983; Sanger *et al.*, 1985; Ueki, 1987) and monkeys (Barrett *et al.*, 1986), and that the two  $\omega_1$ -BZD receptor selective compounds failed to modify rates of lever pressing suppressed by punishment in rats (Sanger, 1995).

In the elevated plus-maze test in rats, all drugs showed anxiolytic-like activity on all behavioural measures. Thus, on traditional behavioural indices, they increased percentage of time spent in open arms and number of open-arm entries. Regarding the ethologically derived measures, all compounds markedly decreased attempts and increased head-dippings, but this latter effect was not statistically significant for zaleplon. The reason for this is unclear, as we have previously found that zaleplon can increase head-dippings (Griebel *et al.*, 1996d). Together, these 'risk assessment' data are consistent with traditional indices of anxiety in indicating anxiolytic-like activity. However, it is important to note that anxiolytic-like activity appeared with non-BZD hypnotics at doses which

were close to those producing impairment of motor activity as revealed by the data on closed-arm entries. These results corroborate recent findings from the elevated plus-maze with zolpidem, zaleplon and BZD anxiolytics (diazepam, chlordiazepoxide and clorazepate) showing that decreased anxiety produced by selective  $\omega_1$ -BZD compounds appeared at doses which were the same as those producing behavioural suppression (Griebel *et al.*, 1996d).

Previous studies with the light/dark test demonstrated that the administration of BZDs (e.g. alprazolam, diazepam, chlordiazepoxide) increased time spent by mice in the illuminated part of the apparatus and the number of tunnel crossings, while the number of aborted attempts at entry in the lit box were decreased (Griebel *et al.*, 1996c). These effects are consistent with an anxiolytic-like action in this test. The results obtained in this study with triazolam agree with these data as the drug displayed a similar behavioural profile in this test. Among the non-BZD hypnotics, only zopiclone showed clear evidence for reduced anxiety-related responses. The drug produced effects comparable to those observed with the BZDs over a wide dose range (3–30 mg/kg). The selective  $\omega_1$ -BZD hypnotics zolpidem and SX-3228 reduced attempts, but failed to modify the two other behavioural measures in a significant manner, thereby indicating a weaker anxiolytic-like activity in this test. These results are in agreement with a recent study which reported weak effects of zolpidem and two other selective  $\omega_1$ -BZD receptor ligands (i.e. CL 218,872 and abecarnil) in the light/dark test (Griebel *et al.*, 1996c). Surprisingly, zaleplon at 10 mg/kg displayed a behavioural profile which is more akin to that of zopiclone and the BZDs, since it modified all behavioral parameters. However, it can be speculated that the significant increase in time spent in the lit



box and in tunnel crossings may merely be due to low baseline performance. Moreover, the magnitude of the effects of zaleplon on the time measure was small in comparison to the non-selective  $\omega_1$ -BZD receptor compounds. Together, these findings indicate that hypnotics which selectively activate  $\omega_1$ -BZD receptors have a reduced potential to produce anxiolytic-like effects in the light/dark test in mice compared to non-selective  $\omega_1$ -BZD receptor hypnotics.

In the MDTB, triazolam markedly reduced all defensive behaviours over a wide dose range (0.03–1 mg/kg). The drug affected both flight measures as it reduced the avoidance distance when the rat was placed into the runway and the flight speed during the chase test. In this latter situation, the drug also decreased risk assessment activities (i.e. stops). Furthermore, upon forced contact with the rat, triazolam reduced defensive upright postures and bitings. Finally, after the rat was removed from the test area, the BZD counteracted the potentiation of escape attempts from the runway apparatus. All these effects are consistent with an anxiolytic-like action in this test (Griebel *et al.*, 1995). However, it is important to note that triazolam also decreased locomotor activity before the exposure to the threat stimulus at 0.3 and 1 mg/kg, suggesting that effects on defensiveness may have been non-specific at these doses.

Compared to triazolam, results obtained with zopiclone, zolpidem and SX-3228 showed that they affected a narrower range of defensive responses. By contrast, zaleplon, like triazolam, attenuated all behavioural responses in the absence of significant effects on locomotor activity. Although zopiclone reduced flight speed, risk assessment, defensive threat and attack reactions, and post-test escape attempts at 10 and 30 mg/kg, the effects may have been contaminated by behavioural suppression since the drug decreased locomotor activity during the pre-test at these doses. Zolpidem also reduced flight speed and risk assessment only at a motor-impairing dose (10 mg/kg), but decreases in avoidance distance (1 mg/kg) and escape attempts (3 mg/kg) appear to be specific as no deficit in locomotor activity was evident at these doses. However, the effects on avoidance distance were not dose-dependent and zolpidem failed to reduce significantly this measure at 3 mg/kg. Finally, SX-3228 specifically reduced avoidance distance at all doses (0.03–1 mg/kg), risk assessment at 0.3 and 1 mg/kg, flight speed and escape attempts at 1 mg/kg, but failed to modify defensive threat and attack reactions.

It is noteworthy that, unlike the other models used in this study, the MDTB revealed differences between selective  $\omega_1$ -BZD hypnotics. SX-3228 primarily affected avoidance distance, whereas zaleplon displayed a behavioural profile comparable to that of triazolam. Finally, zolpidem generally failed to affect defensive behaviours in a specific manner. The extensive behavioural and pharmacological evaluation of the MDTB has demonstrated that this test may model different emotional states (Griebel *et al.*, 1996a,b). Thus, panic-modulating compounds specifically affect animals' flight responses, whereas classical anxiolytic agents such as BZDs (e.g. chlordiazepoxide) have inconsistent effects on flight, but they affect risk assessment, defensive threat/attack reactions and escape attempts, thereby suggesting that these latter defense responses more likely relate to certain aspects of GAD (Griebel *et al.*, 1995, 1996e). Based on these findings, the

present results with triazolam and zopiclone are in line with clinical data showing that the drugs improved patients with GAD (Agnoli *et al.*, 1989). Little is known about the clinical efficacy of zolpidem against anxiety disorders, but the drug was found to improve anxiety scores of anxious insomniac patients (Pagot *et al.*, 1993). No data are available for the anti-panic potential of triazolam, zopiclone and zolpidem. Finally, on the basis of the effects of zaleplon and SX-3228 in the MDTB, we can anticipate the potential efficacy of zaleplon in GAD and of SX-3228 in panic disorders.

In conclusion, this study showed that selective  $\omega_1$ -BZD hypnotics displayed anxiolytic-like activity, but these effects were generally weaker than those observed with zopiclone and the BZD hypnotic triazolam. In particular, the anxiety-reducing potential of the selective  $\omega_1$ -BZD receptor compounds may be confounded and/or masked by behavioural suppression and is limited to a narrower range of indices of anxiety.

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