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Evidence for zolpidem-induced hyperphagia, but not anxiolysis, in a successive negative contrast paradigm

Colin P. Mitchell*,[1], Mary Leszczuk Ost, Charles F. Flaherty

Department of Psychology, 152 Frelinghuysen Rd., Piscataway, NJ 08854, United States

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Abstract

Zolpidem is an imidazopyridine which binds to certain benzodiazepine receptor types with varying degrees of affinity. The effect of zolpidem on successive negative contrast was investigated in three experiments. In each experiment, a contrast group was given brief access to 32% sucrose for 10 days, then shifted to 4% sucrose for 2 days; a procedure that elicits anxiety primarily on the second postshift day. One control group was given only 4% sucrose. Experiments 2 and 3 included a 2% sucrose group as an intake rate-dependent control. In Experiment 1, zolpidem (4.0 and 0.5 mg/kg) dose-dependently reduced contrast on the two postshift days. Contrast occurred during the first postshift consummatory burst. Zolpidem prolonged the first postshift burst equally in both shifted and unshifted groups, suggesting a general facilitation of intake masked by a ceiling effect in controls. In Experiment 2, zolpidem's (4.0 mg/kg) anti-contrast action was equivalent to its hyperphagic effect in the 2% control group. Zolpidem prolonged the first postshift burst equally in all three groups, again consistent with general intake facilitation. In Experiment 3, 8.0 mg/kg zolpidem produced an anti-contrast effect not present in 2% controls on both postshift days. This does not appear attributable to anxiolysis, however, as the effect was equivalent during stressful and non-stressful phases of the postshift period, and zolpidem extended the duration of the first postshift burst equally in all three sucrose groups. Thus, unlike benzodiazepines, zolpidem is not anxiolytic in this paradigm.

Keywords: Incentive relativity; Contrast; Imidazopyridine; Benzodiazepine; Reward; Hyperphagia

An animal's response to reward is influenced by both the absolute and relative values of that reward (Flaherty, 1982, 1996; Williams, 1983, 2002). For example, when rats are initially allowed access to a 32% sucrose solution and then shifted to 4% sucrose, intake of the 4% sucrose falls significantly below the intake level of rats who have only experienced the 4% solution. This propensity for responding to decrease below the level of unshifted control subjects is referred to as successive negative contrast (SNC), and it is a usual consequence of reward reduction, within certain parametric constraints (Flaherty, 1996; Williams, 1983). SNC is often attributed to a frustration-like emotion that interferes with goal-directed behavior (Crespi, 1942; Flaherty, 1996; Gray, 1987). Evidence supporting this interpretation includes findings that a variety of anti-anxiety drugs, including benzodiazepines (BDZs), ethanol, and sodium amobarbital, promote a reduction in contrast which can be dissociated from the hyperphagic and memory-modulating effects of these agents (Flaherty, 1996; Flaherty and Driscoll, 1980; Becker and Flaherty, 1982; Flaherty et al., 1986; Flaherty et al., 1990; Gray, 1987; Flaherty et al., 1996).

However, a simple interpretation of contrast in terms of a causal effect of emotion is questionable because the anti-contrast actions of BDZs and ethanol are not observed until the second postshift day. These drugs are not effective on the first postshift day, when SNC is typically maximal (e.g., Becker and Flaherty, 1982; Flaherty, 1996; Flaherty et al., 1986, 1990, 1980). Additionally, the initial postshift session does not elicit

* Corresponding author. Tel.: +1 317 402 5314; fax: +1 317 876 1898.
E-mail address: colinmitchell2@hotmail.com (C.P. Mitchell).
[1] Current address: Eli Lilly and Co., Lilly Corporate Center DC 6156, Indianapolis, IN 46285, United States.

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an increase in plasma corticosterone, but corticosterone is reliably elevated in shifted animals both immediately prior to and for an extended period after the second postshift session (Flaherty et al., 1985; Mitchell and Flaherty, 1998). Further, a factor analytic study demonstrated that SNC loaded with other indices of anxiety, such as context-conditioned fear and emergence into an open field, on the second, but not the first postshift day (Flaherty et al., 1998).

The differences in the effects of BDZs and ethanol on the initial and later response to reward reduction, the delayed activation of the HPA axis, and the selective loading of SNC with behavior in other animal models of anxiety have led to the hypothesis that different psychological processes are involved in initial and subsequent responses to the reduced reward (e.g., Flaherty, 1996). For example, the initial response to reward reduction may involve detection and evaluation of the reward difference, in comparison with the memory of the "old" reward, and search for the "missing" reward (Flaherty, 1996; Pecararo et al., 1999). The subsequent response to the reduced reward appears to be frustration-related stress, which is modulated by BDZs and ethanol and stimulates glucocorticoid secretion. Alternatively, the emotional response may be proactive in nature. The elevation in corticosterone that occurs prior to the encounter with the reduced reward on the second postshift day suggests some form of anticipatory emotional response (Mitchell and Flaherty, 1998). Or there may be a sequence of different emotional reactions to the reduced reward. Views of sequential emotional reactions to non-reward have been used with profit in the understanding of reward loss in runway and operant tasks (e.g., Amsel, 1992; Gray, 1987; Papini and Dudley, 1997).

The current series of studies was conducted to broaden the pharmacological profile of the successive negative contrast paradigm, to further evaluate the hypothesis of a multi-stage psychological reaction to reward reduction, and to further understanding of the behavioral effects of the BDZ-receptor ligand zolpidem. Whereas classical BDZs bind to both "type I" ($GABA_A$ receptors containing the α_1 subunit) and "type II" ($GABA_A$ receptors containing α_2, α_3, or α_5 subunits) BDZ receptors, zolpidem, an imidazopyridine, displays greater affinity for the "type I" BDZ receptor (e.g., Pritchett and Seeburg, 1990; Benavides et al., 1993; Criswell et al., 1997). Studies using mice with specific point mutations in α_1 (Rudoloph et al., 1999; McKernan et al., 2000), α_2, or α_3 (Low et al., 2000) subunits, which confer insensitivity to BDZ receptor ligands, have demonstrated that sedative properties of BDZ receptor ligands are mediated by α_1-containing receptors, while anxiolytic properties of BDZ receptor ligands are mediated by α_2-containing receptors. Congruently, preclinical studies with zolpidem have shown that it fails to alter several indices of anxiety which are sensitive to BDZs, including activity in an open field/novel environment, punished operant responding, time spent in a brightly lit compartment, and defensive behaviors (e.g., Sanger and Zivkovic, 1988; Kleven and Koek, 1999; Griebel et al., 1996b,c).

However, some studies have indicated that zolpidem produces anxiolytic effects in other paradigms. One such paradigm is the elevated plus maze (e.g., Auta et al., 1993; Davies et al., 1994; Griebel et al., 1996a), a test widely used in studies of anxiolytic agents, although zolpidem-induced anxiolysis is generally weak compared to BDZs and tends to occur at doses which produce sedation (Auta et al., 1993; Davies et al., 1994; Griebel et al., 1996a; Moy et al., 1997; Depoortere et al., 1986; Massotti et al., 1991; Sanger and Zivkovic, 1988; Vanover et al., 1999). Our pilot studies indicated that zolpidem decreased SNC, and therefore could potentially exhibit anxiolytic properties in this paradigm as well. Factor analytic studies have demonstrated that SNC, and presumably the underlying affective processes, differs qualitatively from behavior in the elevated plus maze (Flaherty et al., 1998), and thus could yield insight into possible anxiolytic properties of zolpidem.

The effect of zolpidem on negative contrast was determined on both the first and second postshift days to distinguish pharmacological activity during hypothesized nonstressful and stressful stages of contrast. Microstructural analysis of individual consummatory bursts and pauses was conducted to precisely determine the initial timepoint of efficacy for zolpidem. The ability of zolpidem to produce hyperphagia was also investigated with the use of controls that received only 2% sucrose, which elicits lick totals similar to rats shifted from 32% to 4% sucrose (Flaherty et al., 1985; Rowan and Flaherty, 1987). The appetite-stimulating properties of BDZs are well known (e.g., Wise and Dawson, 1974; Cooper and Estall, 1985; Berridge and Pecina, 1995), but studies utilizing zolpidem have produced mixed results in regard to appetite-stimulation (Stanhope et al., 1993; Yerbury and Cooper, 1989; Cooper and Desa, 1998; Cooper and Yerbury, 1988; Davies et al., 1994; Sanger and Zivkovic, 1988).

1. Materials and methods

1.1. Subjects

Male Sprague–Dawley rats were obtained from Harlan (Indianapolis, IN) and were experimentally naïve. Subjects were food deprived for 1 week before experimentation and maintained at 82% of ad lib feeding weight via once per day feeding of Lab Diet chow (#5012). Water was freely available in the home cage. Subjects' weights ranged between 310 and 400 g prior to deprivation. The rats were housed individually in a temperature-controlled colony on a 14/10-h light/dark cycle. All procedures strictly adhered to

the policies on animal welfare of the National Institutes of Health and Rutgers University.

1.2. Apparatus

Six identical Plexiglas boxes measuring 26.5×31.5×28 cm equipped with motors that advanced and retracted sucrose solutions were utilized. Solutions were presented in Nalgene cylinders with a metal spout embedded in rubber cork. The spout was advanced to just outside an access hole located 7 cm above the grid floor and the spout location was adjusted to prevent subjects from making continuous contact with it. Licks were recorded via a contact relay circuit and microprocessors.

1.3. Procedure

In each experiment, subjects received one 5-min habituation session to the apparatus, without sucrose available, prior to the initiation of the sucrose access days. Throughout all experiments, subjects were weighed daily, carted to a dimly lit running room where white noise was present, and given access to sucrose for 5 min. The total number of licks, number of licks per burst, and number of bursts generated were measured using a conventional definition of burst as a series of licks without a pause greater than 0.5 s (e.g., Grigson et al., 1993). Subjects were returned to the colony immediately following the 5-min session and fed Lab Chow approximately 30 min later. In all experiments, subjects within each sucrose condition were matched according to lick totals on Days 9 and 10 and randomly subdivided into drug treatment groups. All drug administration occurred on Days 11 and 12.

1.3.1. Experiment 1

In the first experiment, 60 rats were randomly divided across two sucrose conditions. Group 32-4 received 5-min access/day to 32% sucrose for 10 consecutive days and then 4% sucrose on Days 11 and 12; Group 4-4 received access to 4% sucrose on all 12 days. Rats in each sucrose condition were divided into five sub-groups of six rats each. Groups 0,0 received saline injections on both Days 11 and 12. Groups 0.5,0 and 4,0 received a 0.5- or 4-mg/kg dose of zolpidem on Day 11, respectively, and saline on Day 12. Groups 0,0.5 and 0,4 received saline on Day 11 and 0.5 or 4 mg/kg zolpidem on Day 12, respectively.

1.3.2. Experiment 2

In a second experiment, 30 rats were randomly separated into three sucrose conditions. As in the first experiment, Group 32-4 received daily 5-min access periods to 32% sucrose for the first 10 days and was then shifted to 4% sucrose for Days 11 and 12, while Group 4-4 received 4% sucrose throughout the study. Additionally, a group receiving 5 min daily access to 2% sucrose (Group 2-2) was included to control for hyperphagic effects of zolpidem expressed in a rate-dependent manner. Rats in each sucrose condition were subdivided into two drug groups receiving either saline (Groups SAL) or 4 mg/kg zolpidem (Groups ZOLP) on both Days 11 and 12. The 4-mg/kg dose of zolpidem was used because of its efficacy in the first experiment.

1.3.3. Experiment 3

In a third experiment, the effect of a higher dose of zolpidem (8 mg/kg) was investigated. Several previous reports have indicated that anxiolytic effects produced by zolpidem are most robust at doses that also produce mild sedation, thus a higher dose was used in a further attempt to dissociate anxiolytic and hyperphagic effects. Sixty rats were again given 5-min daily access periods to either 32% sucrose for 10 days followed by 4% on Days 11 and 12 (Group 32-4), 4% sucrose for all 12 days (Group 4-4), or 2% sucrose for all 12 days (Group 2-2). Subjects in each sucrose condition were divided into three subgroups. Groups 0,0 (n=20) were administered saline on Days 11 and 12, Groups 8,0 (n=19) received 8 mg/kg zolpidem on Day 11 and saline on Day 12, and Groups 0,8 (n=21) received saline on Day 11 and 8 mg/kg zolpidem on Day 12.

1.4. Drugs

Zolpidem (RBI, Natick, MA) was dissolved in sterile saline within 15 min prior to its administration. In each experiment, zolpidem was administered via ip injections in a volume of 1 ml/kg. A pretreatment time of 30 min was used.

1.5. Data analysis

In each experiment, measures of lick frequency, number of licking bursts, and number of licks per burst were analyzed via analysis of variance (ANOVA). Sucrose condition and Drug condition were factors in all analyses. In all studies, the effect of these factors across the terminal preshift (Day 10) and two postshift Days (Days 11 and 12) (Sucrose×Drug×Day), across Minutes within a given day (Sucrose×Drug×Minute), and across the first burst on Day 11 (Sucrose×Drug) was investigated. Significant differences among group means were followed up with Fisher's LSD tests using a significance level of $p<0.05$. In Experiment 1, the Sucrose×Day interaction narrowly missed significance, even though the effect of Sucrose Condition was clearly restricted to Days 11 and 12. Thus, in this experiment Day 10 was analyzed separately from Days 11 and 12. Data are described in text as Mean±S.E.M. Bursts consisting of fewer than five licks were excluded from the analysis, as these frequently reflect non-lick contacts with the spout; the occurrence of these did not differ among groups in any experiment.

2. Results

2.1. Experiment 1

On Day 10 (the last preshift day), no group differed from any other in lick rates across either sucrose or drug conditions (Sucrose: $F(1,50)<1.00$). However, there was a reliable negative contrast effect on each of the two postshift days: shifted rats (Group 32-4) in each drug condition licked less than the unshifted rats (Group 4-4) on Days 11 and 12 [Sucrose: $F(1,100)=97.16$, $p<0.0001$]. These data are illustrated in Fig. 1 (unshifted groups in all drug conditions were virtually identical, thus they have been collapsed in the figure).

2.1.1. First postshift day

Both doses of zolpidem (0.5 and 4 mg/kg) reduced the magnitude of SNC on the first postshift day, but the 4 mg/kg dose produced a more robust effect [Sucrose×Drug×Day: $F(8,100)=3.5$, $p<0.001$] (Fig. 1). The increase in consummatory behavior by zolpidem was selective for the shifted rats (Group 32-4), as the unshifted rats (Group 4-4) were statistically equivalent in lick totals across drug conditions on the first postshift day. Minute×minute analysis of Day 11 yielded a reliable Sucrose×Drug interaction [$F(1,50)=4.37$, $P<0.01$] which revealed the same pattern of drug effects as the total lick analysis. The lack of a Drug×Minute [$F<1.0$] or Sucrose×Drug×Minute [$F<1.0$] interaction suggests that zolpidem reduced contrast equivalently throughout the entire 5-min period.

Microstructural analysis of Day 11 revealed that rats in Group 32-4 initiated more bursts than rats in Group 4-4 in the first minute, but fewer bursts than Group 4-4 in minutes 2–5 [Sucrose×Minute: $F(5,200)=17.43$, $p<0.0001$]. Group 32-4 also produced fewer licks per burst throughout the session than Group 4-4 [Sucrose: $F(1,50)=11.51$, $p<0.01$]. Neither of these measures was affected by zolpidem.

In order to determine if zolpidem influenced the time required for the rats to detect that the postshift solution was different from the memory of the preshift solution, the duration of the first licking burst on the first postshift day was analyzed. This analysis revealed that the contrast effect was reliable in the first consummatory burst following the shift (Group 32-4: 9.0±2.17 s; Group 4-4: 30.2±5.7 s) [Sucrose: $F(1,50)=14.01$, $p<0.001$]. Zolpidem increased burst duration in a dose-dependent fashion [Drug: $F(4,50)=3.58$, $p<0.05$], such that 4.0 mg/kg (39.18±9.25 s)>0.5 mg/kg (22.3±9.48 s)>vehicle (12.17±1.83 s) (Fig. 2). This appeared to reflect a general hyperphagic action, however, as the burst-lengthening effect of zolpidem was not greater in shifted rats than unshifted controls [Sucrose×Drug: $F(4,50)<1.0$].

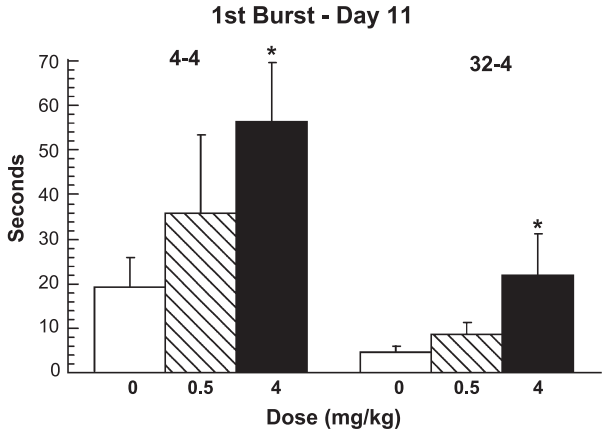

Fig. 2. Mean duration of the first consummatory burst on the first postshift day (Day 11) for unshifted (4-4) and shifted (32-4) subjects receiving vehicle (0), 0.5 mg/kg, or 4 mg/kg zolpidem. *p=0.01 for main effect of Drug.

2.1.2. Second postshift day

Both doses of zolpidem also reduced contrast on the second postshift day; there was no difference between the two doses in the size of the effect. That is, shifted (32-4) rats receiving zolpidem (0.5 or 4.0 mg/kg) licked more than shifted vehicle controls, but less than the unshifted (4-4) drug and vehicle groups [Sucrose×Drug×Day: $F(8,100)=3.5$, $p<0.01$] (Fig. 1). Once again, this contrast reduction was due to the effect of the drug on the shifted rats, as there was no difference between the unshifted drug groups. Minute×minute analysis revealed that this effect

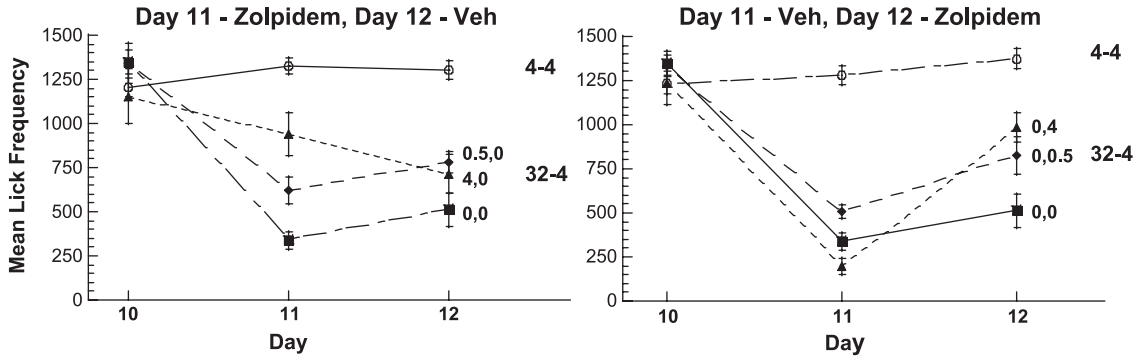

Fig. 1. Mean lick totals for Day 10 (the last Preshift Day), Day 11 (the first Postshift Day), and Day 12 (the second Postshift Day) for shifted (32-4) and unshifted (4-4) subjects. There were no differences between drug conditions in the unshifted animals, thus all unshifted groups are pooled in the figure.

was distributed across the entire 5-min session [Drug×Minute: $F<1.0$; Sucrose×Drug×Minute: $F<1.0$]. Microstructural analysis showed that zolpidem did not specifically affect either licks per burst or the total number of bursts.

These results show that zolpidem possesses anti-contrast actions, but that the drug is effective on both the first and second postshift days, a pattern distinctly different from that observed with BDZs (Flaherty et al., 1980, 1986).

2.2. Experiment 2

Groups 32-4 and 4-4 licked more than Group 2-2 on Day 10, prior to the shift [Sucrose×Day: $F(4,48)=14.56$, $p<0.0001$]. There was no difference in the intake of 32% versus 4% sucrose on this day.

Contrast effects occurred on both Days 11 and 12. That is, the shift from 32% to 4% sucrose led to a decline in lick frequency in Group 32-4 to a level equivalent to Group 2-2 and substantially below the contrast control- Group 4-4 [Sucrose×Day: $F(4,48)=14.56$, $p<0.0001$]. These data are illustrated in Fig. 3.

Zolpidem (4 mg/kg) enhanced lick frequency on both Days 11 and 12 [Drug×Day: $F(2,48)=4.25$, $p<0.02$]. This was a general hyperphagic effect which occurred equally in all sucrose groups, as evidenced by the lack of a Sucrose×Drug interaction [$F<1.0$], although there was a strong tendency toward greater increases in licking in Groups 32-4 and 2-2 than in Group 4-4 (Fig. 3). Minute×minute analyses of Days 11 and 12 also revealed a general zolpidem-induced hyperphagic effect, which, as in Experiment 1, was equally distributed across all 5 min on each day. Microstructural analysis did not reveal any systematic effect of zolpidem on either licks per burst [Drug: $F(2,24)<1.0$] or the number of bursts initiated [Drug: $F(2,24)=2.18$, $p>0.05$]. Duration of the first burst on the first postshift day was greater in Group 4-4 than in Groups 32-4 and 2-2, which did not differ [Sucrose: $F(2,24)=8.05$, $p<0.01$]. The duration of the first postshift burst was again lengthened by zolpidem [Drug: $F(1,24)=20.32$, $p<0.01$). However, as in Experiment 1, the lengthening of the first burst by zolpidem was equivalent in the shifted and unshifted groups and thus did not relate to contrast [Sucrose×Drug: $F(2,24)=1.86$, $p>0.05$) (Fig. 4).

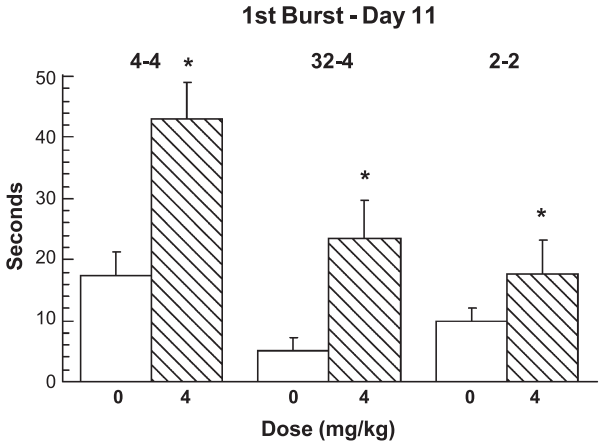

Fig. 4. Mean duration of the first consummatory burst on the first postshift day (Day 11) for unshifted (4-4), shifted (32-4), and rate-dependent controls (2-2) receiving saline or zolpidem (4 mg/kg). *$p<0.001$ for main effect of Drug.

These data show that zolpidem increases licking in shifted animals, consistent with Experiment 1. However, the data suggest that this action is attributable to a general potentiation of consummatory behavior in all sucrose groups, rather than an effect which antagonizes contrast specifically (e.g., anxiolysis). Again, this is unlike the reduction of contrast produced by BDZs (Flaherty et al., 1980, 1986).

2.3. Experiment 3

Six subjects were sedated by the 8-mg/kg dose of zolpidem and failed to lick sucrose; these subjects were dropped from the analysis (2 animals from Group 2-2, 3 from Group 4-4, and 1 from Group 32-4). In addition, four other animals were dropped due to equipment failure.

On the last preshift day (Day 10), Group 32-4 licked more than Group 4-4, which licked more than Group 2-2 [Sucrose×Day: $F(4,82)=34.02$, $P<0.0001$].

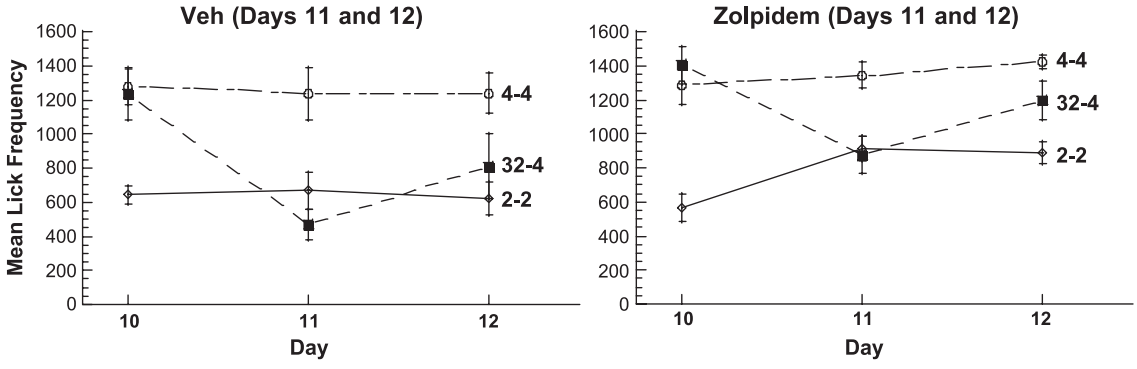

Fig. 3. Mean lick totals for Day 10, Day 11, and Day 12 (the last preshift day and two postshift days) for shifted (32-4), unshifted (4-4), and rate-dependent control (2-2) subjects. Subjects received either saline (left Panel) or 4 mg/kg zolpidem (right Panel) on both Days 11 and 12.

A reliable contrast effect occurred on both postshift days in the saline injected rats [Sucrose×Drug×Day: $F(8,82)=4.26$, $p<0.001$]. On both days, rats in the saline group that were shifted from 32% to 4% sucrose licked less than unshifted controls (Group 4-4) and at a level equivalent to Group 2-2 (Fig. 5).

2.3.1. First postshift day

On Day 11, all sucrose groups that received 8 mg/kg zolpidem (Groups 2-2, 4-4, and 32-4) licked at equivalent rates—an effect brought about by increased lick frequencies in Group 32-4 and decreased lick frequencies in Group 4-4, the contrast control group, relative to saline-treated animals. Thus, there was no contrast effect in subjects receiving zolpidem. [Sucrose×Drug×Day: $F(8,82)=4.26$, $p<0.001$] (Fig. 5). There were no differences among drug conditions within Group 2-2, the rate-dependent control. Minute by minute analysis revealed that the effect of zolpidem did not differ across minutes [Drug×Minute: $F<1.0$; Sucrose×Drug×Minute: $F<1.0$]. The duration of the first postshift burst was again greater in Group 4-4 than in Groups 32-4 and 2-2, which did not differ [Sucrose: $F(2,41)=6.15$, $p<0.01$]. Zolpidem again increased the duration of the first postshift burst [Drug: $F(2,41)=3.94$, $p< 0.05$], but this effect was not selective for the shifted group [Sucrose×Drug: $F(4,41)< 1.0$] and thus appears to reflect a general facilitation of intake (Fig. 6).

2.3.2. Second postshift day

On the second postshift day, contrast was reliable in shifted rats in both vehicle conditions (Groups 0/0 and 8/0), but there was no contrast effect among the groups given zolpidem [Sucrose×Drug×Day: $F(8,82)=4.26$, $P<0.001$]. Thus, as in Experiment 1, the effect of zolpidem given on the first postshift day was temporary. All groups given zolpidem on the second postshift day (32-4, 4-4, 2-2) licked at equivalent rates [Sucrose×Drug: $F(8,82)=4.26$, $p<0.001$] (Fig. 5).

Within Group 32-4, animals receiving zolpidem licked more than animals receiving saline. The tendency for zolpidem to decrease lick totals in Group 4-4 was not statistically significant, nor were there any differences among drug conditions in Group 2-2. A minute×minute analysis showed that the effect of zolpidem was exerted equally across the 5-min period [Drug×Minute: $F(16,164)= 1.53$, $p>0.05$; Sucrose×Drug×Minute: $F<1.0$].

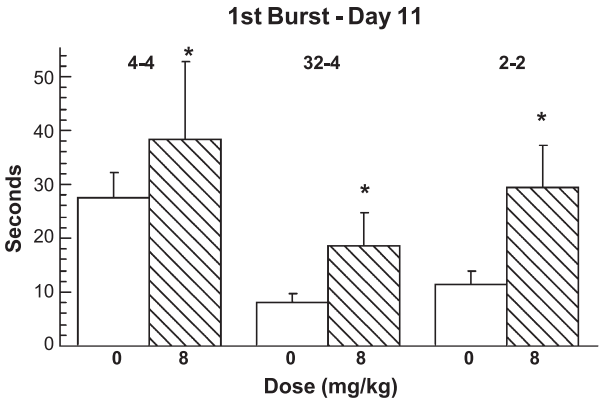

Fig. 6. Mean duration of the first consummatory burst on the first postshift day (Day 11) for unshifted (4-4), shifted (32-4), and rate-dependent controls (2-2) receiving saline or zolpidem (8 mg/kg). *$p<0.05$ for main effect of Drug.

Thus, the actions of a high (8.0 mg/kg) dose appear to be specific for the shifted group (32-4) across the entire 5-min access period, although zolpidem increased the duration of the first postshift burst equally in all sucrose groups. Moreover, unlike BDZs, zolpidem once again displayed anti-contrast properties equally on the first and second postshift days.

3. Discussion

Classical benzodiazepines, such as chlordiazepoxide and midazolam, robustly antagonize contrast on the second postshift day, but are inefficacious on the first postshift day in food deprived rats (Flaherty, 1996). Abundant data support the interpretation of this pattern as an anxiolytic BDZ effect over mnemonic, motoric, or enhanced palatability interpretations; BDZ anxiolysis is revealed during a delayed aversive emotional response, which follows initial responses such as detection and search for the preshift

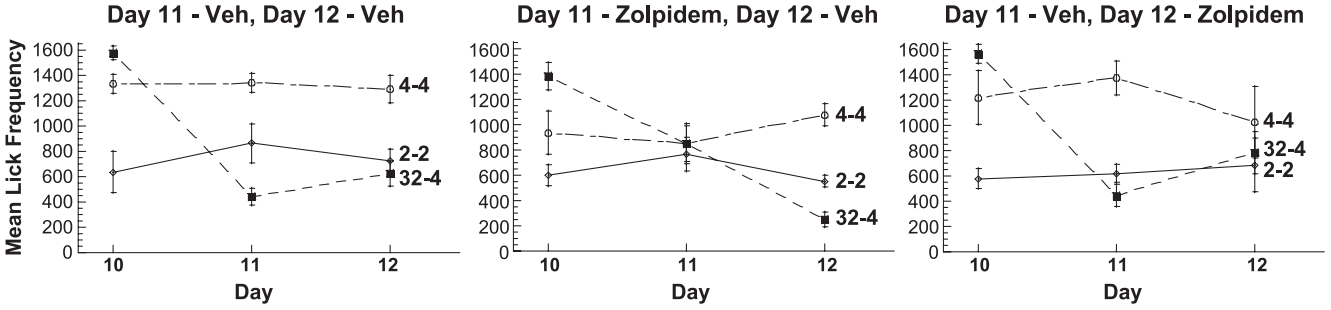

Fig. 5. Mean lick totals for Day 10, Day 11, and Day 12 (the last preshift day and two postshift days) for shifted (32-4), unshifted (4-4), and rate-dependent control (2-2) subjects. Subjects either received vehicle on both Days 11 and 12 (left Panel), 8 mg/kg zolpidem on Day 11 and vehicle on Day 12 (Middle Panel), or vehicle on Day 11 and zolpidem on Day 12 (right Panel).

reward (Flaherty, 1996; Pecararo et al., 1999; Flaherty et al., 1985; Mitchell and Flaherty, 1998; Flaherty et al., 1998).

The results of Experiment 1 show that zolpidem (0.5 and 4.0 mg/kg) increases lick totals in food-deprived rats shifted from 32% sucrose to 4% sucrose on both the first and second postshift trial. Zolpidem was effective immediately, increasing the duration of the first postshift burst, and remained effective across all 5 min of access during each session. Thus, the pattern of zolpidem efficacy differs from that of classical BDZs (Flaherty et al., 1986, 1990). The immediate efficacy of zolpidem following reward downshift demonstrates that its actions are not contingent upon the putative delayed emotional response. These data could be interpreted as reflecting: (1) an enhancement of consummatory behavior/palatability; (2) an impairment of the memory of the preshift reward; or (3) a reduction in motor (e.g., search) activity. The observation that the first consummatory burst on the first postshift day was prolonged in unshifted animals as well as shifted subjects suggested a general hyperphagic effect, which could potentially be masked in individual minutes or trials in unshifted rats by ceiling effects on lick totals.

In Experiment 2, 4.0 mg/kg zolpidem increased lick totals in unshifted rate-dependent (Group 2-2) and contrast (Group 4-4) controls, as well as in the shifted group. In fact, this general stimulation of consummatory behavior by zolpidem was evident in all three groups in the first burst following the shift. One limitation of this study is that, unlike Experiments 1 and 3, zolpidem was administered to the same animals on both postshift trials. This introduces a potential confound wherein administration of the drug on the first postshift trial could influence the effect of the drug on the second postshift trial, although this did not appear to be the case. These data further suggest that the apparent anti-contrast actions of this dose of zolpidem are more reasonably interpreted as a hyperphagic effect, unlike the effect of classical BDZs.

The use of a higher dose of zolpidem (8 mg/kg) in Experiment 3 provided some evidence of a contrast-attenuating effect that was independent of the general lick-enhancing properties of zolpidem. Within Group 32-4, subjects receiving zolpidem licked more than subjects receiving saline; there was no difference between drug conditions within Group 2-2. However, a couple of observations cast doubt upon an interpretation of these data as reflecting anxiolytic actions of zolpidem.

First, zolpidem produced a robust increase in the duration of the first postshift burst that was equivalent in all three sucrose groups. Thus, zolpidem initially produced a dramatic increase in intake that was not selective for shifted animals. The failure of this effect to persevere in Groups 2-2 and 4-4 may be attributable to the sedative effects of zolpidem; indeed, several subjects had to be dropped due to drug-induced catatonia. Other studies in our lab utilizing runway or radial arm maze paradigms have suggested that shifted subjects are less susceptible to drug-induced sedation, perhaps because of the arousal produced by the shift (Flaherty, unpublished observations).

Secondly, zolpidem was equally effective in Group 32-4 on both the first and second postshift days. As noted above, plasma corticosterone is not elevated in shifted rats until the second postshift day (Flaherty et al., 1985; Mitchell and Flaherty, 1998), and SNC loads on factors with other indices of anxiety on the second, but not first postshift day (Flaherty et al., 1998). These observations, together with the delayed efficacy of BDZs, are most easily interpreted as reflecting a delayed aversive emotional response to reward downshift (Flaherty, 1996). Thus, zolpidem shows no selectivity for the putative anxiety stage of contrast.

Finally, zolpidem failed to increase licking in shifted subjects (32-4) relative to rate-dependent controls (2-2). That is, although zolpidem increased lick totals in Group 32-4 relative to saline-injected subjects in Group 32-4, and failed to increase licking in Group 2-2 relative to Group 2-2 saline-injected subjects, zolpidem did not increase licking in Group 32-4 relative to Group 2-2. The decrease in licking in Group 4-4 produced by 8.0 mg/kg zolpidem and the number of subjects rendered catatonic by this dose again raises the possibility that hyperphagia was obscured in Group 2-2 by a sedative effect, but that subjects in Group 32-4 were less vulnerable to sedation because of arousal produced by the shift.

Perhaps the most reasonable interpretation of our results is that low doses of zolpidem produced hyperphagic effects, but failed to produce anxiolytic effects. A higher dose (8 mg/kg), which produced heavy sedation in some subjects, increased licking in shifted subjects, although the effect was not specific to the putative delayed stress/anxiety stage and thus may also reflect facilitation of consummatory behavior. However, the evidence supporting this interpretation is not entirely conclusive. For instance, it does appear that there was some room for zolpidem to enhance lick rates in Group 4-4 in Experiment 1, yet it did not. Secondly, while the magnitude of the effect of zolpidem did not differ significantly between Groups 2-2 and 32-4 in Experiment 2, zolpidem was numerically more efficacious in Group 32-4. Thus, the possibility remains that our results are not entirely attributable to zolpidem-induced hyperphagia, although this explanation seems to best fit the data.

Other studies have yielded conflicting reports on the ability of zolpidem to induce hyperphagia. A lack of zolpidem-induced ingestion has been reported using saccharin, sweetened mash, and both sweetened and unsweetened chow (Cooper and Yerbury, 1988; Yerbury and Cooper, 1989; Davies et al., 1994; Sanger and Zivkovic, 1988). A failure to observe zolpidem-induced increases in the consumption of a familiar hypertonic saline solution by rehydrating rats has also been reported (Cooper and Desa, 1998). However, in a subsequent study where subjects were less familiar with the test solution, higher doses of zolpidem did increase intake of hypertonic saline in water-deprived rats (Lobarinas and Falk, 2000). Zolpidem has also been

observed to increase intake of a glucose–saccharin mixture (Stanhope et al., 1993). These results may suggest that zolpidem possesses hyperphagic properties which are contingent upon factors such as the perceived palatability of available food and the duration of the test session.

The apparent failure of zolpidem to reduce SNC via anxiolytic actions in the current experiments is consistent with the failure of this drug to display anxiolytic properties in other paradigms, such as punished operant responding in rats (Sanger and Zivkovic, 1988) and pigeons (Kleven and Koek, 1999), the murine free exploration test (Griebel et al., 1996b), and the murine light/dark test (Griebel et al., 1996b). The data reported here are also consistent with "knock-in" mouse studies which provide evidence that sedative properties of BDZ receptor ligands are mediated by α_1-containing receptors, while anxiolytic properties of BDZ receptor ligands are mediated by α_2-containing receptors (Rudoloph et al., 1999; McKernan et al., 2000; Low et al., 2000).

Zolpidem-induced anxiolysis has been consistently reported in the elevated plus maze (Auta et al., 1993; Davies et al., 1994; Griebel et al., 1996a; Moy et al., 1997) and punished drinking paradigms (Depoortere et al., 1986; Massotti et al., 1991; Vanover et al., 1999). However, the magnitude of these anxiolytic actions are generally smaller than those produced by classical BDZs and tend to occur at doses which produce sedation. This difference in the anti-anxiety pattern of zolpidem in an elevated plus maze and in the contrast paradigm is consistent with data from factor analyses showing that the behavior of individual animals is not related in these two paradigms (Flaherty et al., 1998).

In summary, the effect of zolpidem on successive negative contrast in the dose ranges investigated here (0.5, 4.0, 8.0 mg/kg) is not congruent with the effects of BDZs on contrast. Zolpidem increases licking in a manner that is not specific to any of the putative psychological responses to reward reduction, which appears, at least in part, attributable to generalized hyperphagia rather than anxiolysis.

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