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Anxiolytic effects of the GABA_A receptor partial agonist, L-838,417: Impact of age, test context familiarity, and stress★

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Abstract

The partial $\alpha 2,3,5$ GABA_A receptor agonist, L-838,417 has been reported to have anxiolytic effects in adult rodents. Although maturational differences exist for the GABA_A receptor subunits, the anxiolytic effects of L-838,417 have not been tested in younger animals. The goal of the present experiments was to determine whether L-838,417 reverses anxiety-like behavior induced by either an unfamiliar environment (Experiment 1) or repeated restraint stress (Experiment 2) differentially in adolescent and adult, male and female Sprague–Dawley rats using a modified social interaction test. In Experiment 1, rats were injected with 0, 0.5, 1.0, 2.0, or 4.0 mg/kg L-838,417, i.p. and tested 30 min later in an unfamiliar test context for 10 min. In Experiment 2, rats were exposed to restraint stress (90 min daily for 5 days). Immediately after the last restraint session, animals were injected with L-838,417 and placed alone for 30 min in the test apparatus to familiarize them to this context prior to the 10 min social interaction test. In Experiment 1, L-838,417 produced anxiolytic effects in adults at 1.0 mg/kg, as indexed by a transformation of social avoidance into preference and an increase in social investigation. In adolescents, a dose of 2.0 mg/kg eliminated social avoidance, but had no anxiolytic effects on social investigation. Testing under familiar circumstances (Experiment 2) after repeated restraint stress eliminated age differences in sensitivity to L-838,417, with 0.5 mg/kg reversing the anxiogenic effects of prior stress regardless of age, but with doses ≥ 1 mg/kg decreasing social investigation, an effect possibly due in part to locomotor-impairing effects of this compound. Although locomotor activity was suppressed in both experiments, higher doses of L-838,417 were necessary to suppress locomotor activity in Experiment 1. Thus, anxiolytic effects of L-838,417 were found to be context-, age-, and stress-dependent.

Keywords

L838417; Anxiety; Adolescents; Restraint stress; Novel environment; Social interaction

1. Introduction

Adolescence is a developmental period associated with high levels of peer-directed social interactions that are observed in humans (Berndt, 1982; Csikszentmihalyi et al., 1977) as well as in rodent models (Varlinskaya and Spear, 2002, 2004, 2009). The increases in social interactions observed during this time are believed to be beneficial for the organism's successful transition into adulthood (Spear, 2000). For example, among human adolescents, social interactions have been reported to positively influence their self-esteem as well as to

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help them practice social skills for the future (Berndt, 1982; Connolly et al., 1987), whereas peer-directed social interactions among adolescent rodents have been suggested to help develop and improve their communication signals (Trezza et al., 2010; Vanderschuren et al., 1997). Not only is adolescence a period of increased social interactions, but adolescents also appear to find the opportunity to interact with peers more rewarding than do adults (Douglas et al., 2004). Given evidence that many of the hormonal, behavioral, and neural changes of adolescence has been conserved across a variety of mammalian species (Spear, 2000, 2010), experiments using laboratory animals have proved useful to model certain basic aspects of adolescence. For example, Sprague–Dawley rats are particularly social animals, and this strain of rats has been used extensively for investigation of socially facilitating, socially inhibiting, and anxiolytic drug effects (Morales et al., 2011; Varlinskaya and Spear, 2002, 2006, 2010).

The social interaction test has been widely used as a model for testing anxiety-like behavior in animals (File, 1980; File and Hyde, 1978). For example, the social behavior of animals is suppressed under anxiogenic circumstances (e.g., under bright light or in an unfamiliar test environment), an effect that can be reversed with anxiolytic compounds (File and Seth, 2003). Although total time spent in all forms of social activity has traditionally been measured in the social interaction test (File and Hyde, 1978; Sanders and Shekhar, 1995), in prior work we have found specific forms of social behavior to be especially sensitive to anxiogenic manipulations and anxiolytic compounds (Doremus-Fitzwater et al. (2009) Varlinskaya et al., 2010; Varlinskaya and Spear, 2012). These measures include social investigation (e.g., sniffing of the social partner) and a measure of social preference/avoidance (indexed via a coefficient reflecting approaches toward versus away from the non-manipulated test partner) (Varlinskaya et al., 1999). For example, both social investigation and the preference/avoidance coefficient were reduced by testing rats in an unfamiliar environment (Varlinskaya and Spear, 2002) or following repeated restraint stress (Doremus-Fitzwater et al., 2009; Varlinskaya et al., 2010), with these anxiogenic effects reversed by acute ethanol administration under both circumstances (Varlinskaya et al., 2010; Varlinskaya and Spear, 2002). Ethanol-related reductions in anxiety-like behaviors following anxiogenic manipulations in the social interaction test have been observed in both adolescent and adult rats, although when tested in an unfamiliar environment adolescents tend to be less sensitive than adults to ethanol's anxiolytic effects (Varlinskaya and Spear, 2002).

Anxiety assessed in the modified social interaction test may be modulated in part by GABA_A receptors. The GABA_A system has been implicated in anxiolytic properties of ethanol Eckardt et al. (1998) and GABA agonists reduce anxiety in animals tested under anxiogenic circumstances (File and Hyde, 1978; Gardner and Guy, 1984; Pellow and File, 1984). Indeed, benzodiazepines enhance GABA function and have been among the more commonly prescribed drugs for treatment of anxiety disorders, often despite undesired sedative effects (Griffiths and Weerts, 1997; Licata et al., 2010) thought to be mediated by the $\alpha 1$ subunit (McKernan et al., 2000). In the pursuit for non-sedative anxiolytics, a variety of GABA-related compounds have been developed. Of particular interest is L-838,417, a compound thought to act as an antagonist at the $\alpha 1$ subunit and as a partial agonist at $\alpha 2,3,5$ subunits (Atack, 2003) and to produce anxiolytic effects without compromising motor activity (McKernan et al., 2000). Mice with a point mutation for the $\alpha 1$ subunit that rendered the subunit insensitive to benzodiazepines demonstrated a resistance to the sedative effects of benzodiazepines, whereas the anxiolytic properties were left intact (Rudolph et al., 1999). In contrast, in another study involving selective mutations of the $\alpha 2$ and $\alpha 3$ subunits, benzodiazepines were found to be ineffective as anxiolytics in mice with disrupted $\alpha 2$ but not the $\alpha 3$ subunit, suggesting a role for the $\alpha 2$ subunit in mediating the anxiolytic effects of benzodiazepines (Low et al., 2000).

Despite the fact that the median age of onset for anxiety disorders is around the age of 11 (Kessler et al., 2005) and that childhood anxiety disorders put an individual at risk for psychiatric disorders in adulthood (Ramsawh et al., 2010), most studies of pre-clinical models of anxiety have not included younger populations. Examining younger animals is important because they may respond differently than adults to drugs that target the GABA receptor, given that GABAergic innervation is a prolonged developmental process. For example, in the rat primary visual cortex, peak GABA innervation occurs during mid-adolescence plateauing thereafter (Morales et al., 2002). Notable maturational differences are also evident in terms of subunit expression within the neurons of the developing rat cortex and thalamus, and to a lesser extent, the cerebellum (Henschel et al., 2008; Laurie et al., 1992; Liu and Wong-Riley, 2005). For example, expression of $\alpha 1$ mRNA is only observed postnatally and increases with age, peaking to adult levels by postnatal day 30 (Yu et al., 2006) whereas $\alpha 3$ mRNA is abundantly expressed in the embryo and subsequently declines to reach the low levels typically seen in adults by P12 (Laurie et al., 1992). Given that expression of mRNA for the GABA subunits differs notably between developing and adult rats, the slowly maturing GABA system may result in differences in sensitivity to drugs that affect this system.

Due to the lack of research examining the anxiolytic effects of compounds in younger animals despite evidence that GABA_A receptor subunit expression changes developmentally (Yu et al., 2006), the present series of experiments examined the effectiveness of L-838,417 as an anxiolytic compound in adolescent and adult male and female Sprague–Dawley rats. Female rats were included in the current experiments because females appear to be at greater risk for most anxiety disorders (McLean and Anderson, 2009). Rats in Experiment 1 were tested in an unfamiliar environment whereas animals in Experiment 2 were tested in a familiar environment following 5 days of restraint stress. These two test circumstances may reflect different forms of anxiety, given that adolescents typically express a resistance to the anxiolytic effects of ethanol compared to adults when tested in an unfamiliar environment (Varlinskaya and Spear, 2002) but not when tested in a familiar environment after repeated (Varlinskaya et al., 2010) or acute restraint stress (Varlinskaya and Spear, 2012). Indeed, the anxiety induced by external cues associated with testing in an unfamiliar and uncertain environment has been suggested to provide an index of generalized anxiety (File and Hyde, 1978) whereas the social suppression seen following repeated stressors may reflect anxiety resulting from internal cues associated with the prior stressor and may perhaps provide a model of social anxiety (Varlinskaya and Spear, 2012). The goal of the current experiments was to explore the possibility that age-related differences in the anxiolytic properties of ethanol observed in these tests of anxiety are related to GABA_A subunit expression by investigating whether the partial $\alpha 2,3,5$ GABA_A receptor agonist might induce a similar pattern of anxiolytic effects.

2. General methods

2.1. Subjects

A total of 460 male and female adolescent and adult Sprague–Dawley rats derived from our breeding facility served as experimental subjects for the current experiments. An equal number of animals served as partners. On postnatal day (P) 1, all litters were culled to 10 pups (5 males, 5 females) and were group-housed with their same-sex littermates at weaning (P21). Rats were given ad libitum access to food (Purina Lab chow, Lowell, MA) and water, and were maintained in a temperature-controlled (22 °C) vivarium with a 12:12 h light-dark cycle (lights on at 0700 h). At all times animals were treated in accordance with guidelines for animal care established by the National Institutes of Health under protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

2.2. Drug administration

The selective GABA_A $\alpha_{2,3,5}$ partial agonist, L-838,417 (Tocris) was dissolved in a (2-Hydroxypropyl)- β -cyclodextrin solution and administered intraperitoneally (i.p.) at a volume of 2 ml/kg.

2.3. Experimental design

The purpose of Experiment 1 was to assess the anxiolytic effects of L-838,417 when animals were tested in an unfamiliar social environment. Thus, the design for Experiment 1 was a 2 age (adolescent: P35, adult: P70) $\times$ 2 sex (male, female) $\times$ 5 L-838,417 dose (0, 0.5, 1.0, 4.0 mg/kg) factorial, with 7 animals placed into each of the 20 groups. Experiment 2 examined the potential anxiolytic effects of L-838,417 following repeated restraint stress, and used a 2 age (adolescent, adult) $\times$ 2 sex (male, female) $\times$ 2 stress (non-stressed [NS], repeated restraint stress [RS]) $\times$ 5 L-838,417 dose (0, 0.5, 1.0, 2.0, 4.0 mg/kg) factorial design, with 8 animals placed into each of the 40 groups. No more than one subject/sex from a given litter was placed into an experimental group, reducing the possibility of litter effects (Holson and Pearce, 1992).

2.4. Experimental procedure

In Experiment 1, experimental animals were taken from their home cage and injected i.p. with one of the five doses of L-838,417 (0, 0.5, 1.0, 2.0, 4.0 mg/kg). Immediately after injection, each experimental animal was marked with a vertical black line across the back to differentiate it from its test partner, and placed alone in a novel holding cage for 30 min to increase levels of social behavior. At 30 min post-injection, each test animal was taken from the holding cage and simultaneously placed with an unfamiliar partner of the same age (adolescent: P35, adult: P70) and sex for the 10 min social interaction test in a novel social testing apparatus.

In Experiment 2, experimental animals were exposed to restraint stress (90 min/day) from P31–35 (adolescent) or P66–70 (adult). Immediately after the final restraint session (i.e., P35 or P70), each rat was injected with one of the doses of L-838,417 (0, 0.5, 1.0, 2.0, 4.0 mg/kg), marked with a vertical black line, and placed into the social interaction apparatus for 30 min, thus familiarizing it to the testing environment. After 30 min, an unfamiliar partner of the same age and sex was placed into the apparatus for the 10 min social interaction test.

2.5. Testing apparatus

The social interaction apparatus was composed of clear Plexiglas (Binghamton Plate Glass, Binghamton, NY) and size-scaled for each test age: 30 $\times$ 20 $\times$ 20 cm for adolescents and 45 $\times$ 30 $\times$ 20 cm for adults. The apparatus was divided on the long axis into two equal sized compartments by a wall containing an aperture measuring 7 $\times$ 5 cm for adolescents and 9 $\times$ 7 cm for adults, thereby allowing animals to move between compartments either toward (crossovers to) or away from (crossovers from) the partner. Before placing an animal into the social testing apparatus, the floor and walls were wiped clean with a 3% hydrogen peroxide solution and the floor was covered with fresh pine shavings.

2.6. Behavioral measures

During each 10-min test, behavior of the test animal and its partner was videotaped (Panasonic model AF-X8, Secaucus, NJ), with real time recorded directly onto the video (Easy Ready II Recorder; Telcom Research TCG 550, Ontario, Canada). Tapes were later scored for social behaviors by 2 experimenters blind to the experimental condition of each animal, with at least 90% inter-experimenter agreement. Given that the social investigation

and the social preference/avoidance coefficient are two measures that have been shown to be particularly sensitive to alterations in anxiety levels (Doremus-Fitzwater et al., 2009; Varlinskaya et al., 2010; Varlinskaya and Spear, 2002, 2012), the scoring focused on these behaviors. Social investigation is defined as the sniffing of any body part of the partner and was scored in terms of frequency. A social preference/avoidance coefficient was calculated as $[\text{Coefficient (\%)} = (\text{crossovers to the partner} - \text{crossovers away from the partner}) / (\text{crossovers to the partner} + \text{crossovers away from the partner}) \times 100]$ and was used as an index of social motivation toward the partner, with positive scores indicating a preference and negative scores indicating relative avoidance of the partner. Additionally, an index of locomotor activity was calculated by summing the number of crossovers between compartments made by the experimental animal during the test session to examine possible motor impairing effects of the compound under these test conditions.

2.7. Data analysis

Prior to analysis, data were checked for outliers and data from any animals that were 3 standard deviations above or below the mean were removed. Only one animal (Experiment 1: adolescent male, 1 mg/kg dose) met this criterion and was removed prior to data analysis. Data were analyzed using factorial ANOVAs. In order to avoid inflating the possibility of type II errors on tests with at least 3 factors (Carmer and Swanson, 1973), Fisher's planned pairwise comparison test was used to explore significant effects and interactions. Effect sizes were calculated using η^2 to account for variance between 2 or more factors, with 0.04, 0.25, and 0.64 representing "small," "medium," and "large" effects, respectively (Ferguson, 2009). Significance was set at $p < 0.05$ and all data are expressed as mean $\pm$ standard error (M $\pm$ SEM).

3. Results

3.1. Experiment 1: can the GABA_A $\alpha 2,3,5$ partial agonist, L-838,417 reverse the anxiogenic effects of testing in an unfamiliar environment?

The ANOVA of the preference/avoidance coefficient revealed a main effect of dose [$F(4,119) = 6.38$, $p < 0.001$, $\eta^2 = .16$], as well as an interaction of dose with age [$F(4,119) = 3.17$, $p < 0.05$, $\eta^2 = 0.08$]. The 1 mg/kg dose, L-838,417 transformed social avoidance into social preference among adult animals, whereas adolescents required a higher dose (2 mg/kg) to attenuate their social avoidance (Fig. 1). The ANOVA of social investigation yielded a main effect of dose [$F(4,119) = 3.27$, $p < 0.05$, $\eta^2 = 0.09$], and a dose $\times$ age interaction [$F(4,119) = 2.87$, $p < 0.05$, $\eta^2 = 0.08$]. An anxiolytic effect of L-838,417 emerged in adults at the 1 mg/kg dose, whereas no anxiolytic effect was evident at any dose in adolescents (Fig. 2). Analysis of locomotor activity under these social testing circumstances indexed via total number of crossovers revealed a main effect of age [$F(1,119) = 7.35$, $p < 0.01$, $\eta^2 = 0.05$] and dose [$F(4,119) = 6.17$, $p < 0.001$, $\eta^2 = 0.16$]. Overall, adolescents were more active than adults, and activity was suppressed at the highest dose (4 mg/kg) of L-838,417 regardless of age (Fig. 3).

3.2. Experiment 2: can the GABA_A $\alpha 2,3,5$ partial agonist, L-838,417 reverse the anxiogenic effects following repeated restraint stress?

The ANOVA of the preference/avoidance coefficient yielded a main effect of dose [$F(4,278) = 3.66$, $p < 0.01$, $\eta^2 = 0.04$], as well as an interaction of dose with stress condition [$F(4,278) = 3.44$, $p < 0.01$, $\eta^2 = 0.04$]. Post-hoc analyses conducted on data collapsed across age revealed that restraint stress had an overall anxiogenic effect, as indicated by lower values of the coefficient in stressed than non-stressed animals among the vehicle-injected groups. The lowest dose of L-838,417 (0.5 mg/kg) was sufficient to reverse the anxiogenic effects of repeated restraint as reflected by a significant increase in the coefficient relative to vehicle-

treated animals. The only effect of L-838,417 in non-stressed animals was a decrease in the coefficient seen following the highest dose of L-838,417 (Fig. 4).

The ANOVA for social investigation revealed main effects of stress [$F(1,278) = 40.13$, $p < 0.0001$, $\eta^2 = .10$] and dose [$F(4,278) = 14.32$, $p < 0.0001$, $\eta^2 = .14$]. An anxiogenic effect of restraint stress was evident regardless of dose and age, with stressed subjects demonstrating less social investigation than non-stressed animals. Challenge with L-838,417 also decreased the frequency of social investigation after doses of 1 mg/kg and higher—an effect that did not interact with age or stressor condition (Fig. 5).

Analysis of locomotor activity under social circumstances revealed a main effect of dose [$F(4,278) = 63.39$, $p < 0.0001$, $\eta^2 = .44$] and an interaction of age with stress [$F(1,278) = 11.56$, $p < 0.001$, $\eta^2 = .02$]. Stressed adolescents had significantly lower levels of activity (15.4 ± 1.15) than non-stressed adolescents (19.5 ± 1.45), whereas the stressor did not affect locomotor activity in adults (stressed: 19.6 ± 1.05 ; non-stressed: 17.5 ± 1.20). When data were collapsed across age and stress to explore the main effect of dose, significant reductions in locomotor activity were seen following all doses of L-838,417 (Fig. 6).

4. Discussion

The results from the present series of experiments demonstrate that the anxiolytic effects of L-838,417 are context-, age-, and stress-dependent, with adolescents being less sensitive to these effects than adults when tested under unfamiliar anxiogenic circumstances but not when anxiety was induced via prior repeated stressors. When testing occurred in an unfamiliar environment (Experiment 1), L-838,417 reversed the social avoidance in adults at a lower dose than was necessary to attenuate this social avoidance in adolescents. Furthermore, adults also demonstrated an anxiolytic response to L-838,417 when indexed via increases in social investigation whereas adolescents were insensitive to this anxiolytic effect. In the familiar test context of Experiment 2, 5 days of repeated restraint stress reduced social preference and social investigation of animals at both ages, with this decrease in social preference similarly reversed by 0.5 mg/kg L-838,417 at both ages. Social investigation was reduced following administration of L-838,417 at doses of 1 mg/kg and above, an effect possibly due to motor-impairing properties of the drug. In both experiments, locomotor activity was reduced by L-838,417 similarly at both ages.

Although when adults were tested in the elevated plus maze and using conflict test procedures, L-838,417 was found to have anxiety-reducing effects without impairing motor performance (McKernan et al., 2000; Rowlett et al., 2005), in the present series of experiments, L-838,417 not only reduced anxiety in a social interaction test but also lowered locomotion under these test conditions. These results, like several other more recent studies (Hofmann et al., 2012; Mathiasen et al., 2008), suggest that L-838,417 does not display anxi-selective properties, under at least some test circumstances. Differences in the way locomotion was assessed across studies may account for the differing findings regarding L-838,417's locomotor effects. For example, our measure of locomotor activity could be considered as "passive" given that animals can choose the extent to which they walk back and forth between compartments. In the studies of McKernan et al. (2000) and Rowlett et al. (2005), locomotor activity was indexed using the rotarod and chain pulling tests which can be viewed as "active" measures of motor activity, with animals having to balance themselves to prevent falling in the former and to pull on a chain to receive a food reward in the latter. This, however, cannot fully account for the discrepant findings. For example, in a study investigating the potential anxiolytic and motor-impairing effects of L-838,417 (3–30 mg/kg) in Sprague–Dawley rats, Hofmann et al. (2012) found a dose-dependent decline in

locomotor activity when indexed via both the open field (“passive”) and beam walking tests (“active”), but not in the rotarod test (“active”) (Hofmann et al., 2012).

Test-specific effects of L-838,417 on locomotor activity were even seen in the current study, with locomotor activity being significantly reduced after the highest dose only (4 mg/kg) in Experiment 1, whereas in Experiment 2, all doses significantly decreased activity. Thus, in an unfamiliar, anxiety-provoking context (Experiment 1), L-838,417 induced anxiolytic-specific effects at doses lower than those suppressing locomotion, whereas when testing in a familiar environment following repeated stress (Experiment 2), locomotor suppressing effects were evident at anxiolytic dose levels when indexed via the social preference/avoidance coefficient. Somewhat similar test circumstance dependency was observed in our laboratory with ethanol-treated adolescent and adult rats (Varlinskaya and Spear, 2002). Varlinskaya and Spear (2002) demonstrated that adolescent and adult rats tested in an unfamiliar environment showed no evidence for ethanol-induced decreases in locomotor activity at any dose tested whereas animals tested in a familiar environment showed suppressed locomotor activity at the highest dose, suggesting that testing in the unfamiliar environment decreased sensitivity to the motor-impairing effects of ethanol. The ability for stress to change drug sensitivity has been reported elsewhere (Ojima et al., 1995, 1997), with for example, isolate-housed mice having shorter pentobarbital-induced sleep times than group-housed mice (Ojima et al., 1995). However, the ability for stress to change the pharmacological response of a drug appears to be stressor-specific, given that the stress associated with testing in an unfamiliar environment versus following prior restraint stress produced different patterns of sensitivity to the locomotor suppressant effects of L-838,417 in the present studies.

In Experiment 1, adolescents required a higher dose of L-838,417 to induce an anxiolytic effect than adults, and this anxiolytic effect was evident only for the social preference/avoidance coefficient and not the social investigation measure that was also sensitive to L-838,417 in adults. These results support existing evidence that adolescents are less sensitive than adults to anxiety-reducing compounds that target the GABA receptor (e.g., diazepam, ethanol). For example, while P35 and P60 rats were shown to display lower levels of social interaction when tested in an unfamiliar environment, only adults showed an anxiolytic effect of diazepam whereas adolescents were unaffected (Primus and Kellogg, 1990). A similar ontogenetic insensitivity was observed for both social investigation and the social preference/avoidance coefficient following an acute challenge of ethanol in rats tested in an unfamiliar environment (Varlinskaya and Spear, 2002). The relative insensitivity observed in adolescents to anxiolytic effects of drugs that target the GABA system may be due to ongoing developmental changes in this neural system that persist throughout adolescence and into adulthood (Yu et al., 2006). Evidence shows that the GABA system and drug interactions within the GABA system may not be fully developed during adolescence (Fleming et al., 2007). For example, GABA_A tonic inhibition in the dentate gyrus is greater in adults than in adolescent animals—indicating that the inhibitory network within the dentate gyrus may still be developing (Fleming et al., 2007). Ethanol was also found to increase GABA_A receptor-mediated inhibitory postsynaptic currents (sIPSCs) in hippocampal pyramidal cells of adults but not adolescents (Li et al., 2006). Interestingly, ethanol was found to produce greater tonic GABA_A inhibition in the dentate gyrus of adolescents than adults (Fleming et al., 2007), supporting the need for more research to determine how maturational differences in the brain GABA_A receptor system influence the increased pharmacological response to GABAergic drugs in adolescents versus adults.

Developmental differences in sensitivity to L-838,417 were eliminated by the test circumstances of Experiment 2, with anxiolytic effects evident only for social motivation in both adolescent and adult animals tested in a familiar environment after 5 days of restraint

stress. A similar finding was recently reported for ethanol: acute ethanol challenge effectively reversed decreases in social preference and social investigation induced by 5 days of restraint stress in adolescent as well as adult animals (Varlinskaya et al., 2010). One difference, however, between the results of Varlinskaya et al. (2010) and the present study was that stressed adolescents and adults in the former study showed an actual ethanol-induced increase in social investigation beyond basal levels, reminiscent of the ethanol-induced social facilitation typically seen after low doses in non-stressed adolescents (but not adults) tested in a familiar environment (Varlinskaya and Spear, 2002, 2012). Such social facilitation was not evident here in either adolescents or adults following challenge with L-838,417 after repeated stress. Perhaps different mechanisms underlie drug-induced anxiolysis versus social facilitation per se, with stronger GABA_A involvement in the former than the latter whereas other neural systems (e.g., μ opioid receptor, NMDA receptor) may be involved in regulating play (Siviy et al., 1995; Vanderschuren et al., 1995; Varlinskaya and Spear, 2009).

Although anxiolytic effects of L-838,417 were observed for the social preference/avoidance coefficient in Experiment 2, doses of 1 mg/kg and higher decreased levels of social investigation. This effect of L-838,417 on social investigation may be related in part to locomotor suppressing effects of the drug that may have been sufficient to disrupt social investigative behavior. These results demonstrate that while L-838,417 does have anxiety-relieving effects (i.e., increased social motivation in Exp. 1 and 2; increased social investigation in Exp. 1), it unfortunately also has locomotor-suppressing effects. Thus, while this compound was originally thought to be a useful anxiolytic because it did not alter motor activity, similar to other GABAergic compounds used to treat anxiety, it has sedative side effects as well. However, suppression of locomotor activity alone cannot account for this effect, given that locomotor activity was decreased following all doses of L-838,417 (i.e., 0.5 mg/kg and higher) whereas social investigation was significantly reduced only at doses of 1 mg/kg and higher. Furthermore, although it is a rare occurrence, there have been reports of paradoxical side effects of benzodiazepine treatment in patients, including increases in anxiety (Ashton, 1986; Hall and Zisook, 1981; Lader, 1999). Therefore, while the results from Experiment 2 provide evidence for locomotor-impairing effects, it is still possible that the reductions in social investigation observed may be due to anxiety elicited by L-838,417.

Given the greater prevalence of anxiety disorders in females than males (Breslau et al., 1995; Palanza, 2001), it is surprising that with the exception of sex-specific research, males have been the primary focus for pre-clinical research in a variety of anxiety-related paradigms (File and Hyde, 1978; McDermott and Kelly, 2008; Violle et al., 2009). Unfortunately, the few studies that have included both males and females in anxiety tests have yielded inconsistent findings. For example, male and female Long-Evans rats demonstrated similar levels of anxiety reduction in both the elevated plus maze and defensive prod-burying tests following various doses of diazepam and ethanol (Wilson et al., 2004). The lack of sex differences in the anxiolytic effects of diazepam has been confirmed in some (Stock et al., 2000), but not all (Fernandez-Guasti and Picazo, 1997) studies. We have generally observed a lack of sex differences in the anxiolytic effects of ethanol in the social interaction test (Varlinskaya and Spear, 2002, 2012). While the data from Experiments 1 and 2 failed to reveal sex differences, future research should include females given the current lack of consensus in the animal literature regarding efficacy of anxiolytic compounds.

The results from Experiments 1 and 2 demonstrate that L-838,417 does produce some anxiety-reducing effects; however, it is not an “anxio-selective” compound given that locomotor suppressing effects were evident in both experiments. These data also point to ontogenetic differences that are only observed in non-stressed animals during testing in an

unfamiliar environment, suggesting that the anxiety-provoking effects of testing in a novel context are different from those induced by repeated restraint stress. It is possible that these two paradigms reflect different anxiety states. For example, manipulating external cues by changing light level and/or familiarity of the test apparatus has been well established as a model of generalized anxiety disorder (Cheeta et al., 2000; File and Hyde, 1978). This model is thought to reflect generalized anxiety because it is associated with ambiguity (unfamiliarity) and fear (bright light) associated with the test context. In contrast, the anxiety exhibited following 5 days of restraint stress by rats in Experiment 2 that were tested in a familiar, dimly lit (and hence presumably low anxiety-provoking) environment may model social anxiety induced by internal cues associated with pre-test stress (Varlinskaya and Spear, 2012). Prior work examining anxiety-provoking effects of pre-test stressors on social interactions has typically tested animals in unfamiliar (i.e., stressful) test conditions, and has not found restraint stress to decrease social interactions (Breese et al., 2004; Gregus et al., 2005), perhaps because the testing environment itself may have been sufficiently stressful to mask anxiogenic effects of prior stress. In contrast, when animals are tested in a relatively low-anxiety provoking environment, restraint stress has been shown to produce decreases in social interactions (Doremus-Fitzwater et al., 2009; Gehlert et al., 2005; Sajdyk et al., 2006; Varlinskaya et al., 2010). Although modification of external cues has been well established as a model for generalized anxiety disorder, more research is needed that focuses on the internal anxiety-state of the animal induced by prior stressor exposure as a potential model for social anxiety. By testing under test conditions that dissociate external versus internal sources of anxiogenesis, effectiveness of L-838,417 and other anxiolytic compounds in attenuating these separable types of anxiety can be examined.

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Fig. 1.

Preference/avoidance coefficient for adolescent and adult animals tested in a novel environment. L-838,417 effectively reversed the social avoidance observed when animals were tested in an unfamiliar environment with adults demonstrating a reversal of social avoidance after 1 mg/kg but adolescents requiring a higher dose (2 mg/kg) to attenuate this effect (*significantly different from same-age vehicle counterpart).

Fig. 2. Social investigation (frequency) for adolescent and adult animals. Adolescents were unaffected by any dose tested; however, L-838,417 (1 mg/kg) was anxiolytic in adults (*significantly different from same-age vehicle counterpart).

Fig. 3.

Locomotor activity as indexed by total number of crossovers in adolescents and adults.

Overall, adolescents were more active than adults; however, activity was equally suppressed among both ages following the highest dose (4 mg/kg) of L-838,417 († significant difference between adolescents and adults; *significantly different from vehicle).

Fig. 4.

Preference/avoidance coefficient for adolescent and adult animals tested in a familiar environment. Data are collapsed across age due to a lack of age differences in the analysis of these data. Repeated restraint stress (90 min/5 day) resulted in social avoidance in vehicle-treated animals that was reversed by 0.5 mg/kg L-838,417. Among non-stressed animals, an anxiogenic effect emerged at the highest dose (4 mg/kg) (# significant difference between stressed and non-stressed vehicle-treated animals; *significantly different from vehicle).

Fig. 5.

Social investigation was significantly reduced following restraint stress relative to non-stressed animals. L-838,417 (0.1 mg/kg) decreased social investigation in animals, regardless of age or stress condition (# significant difference between stressed and non-stressed animals; *significantly different from vehicle).

Fig. 6.

Total number of crossovers after the different doses of L-838,417. Data collapsed across age and stress condition show that all doses of L-838,417 reduced crosses relative to vehicle-treated animals. As shown in the inset, stressed adolescents exhibited significantly fewer crosses than non-stressed adolescents, with no difference observed between adults. (*significant difference from vehicle-treated animals; #significant difference between stressed and non-stressed animals of the same age group).