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Selective stimulation of central GABA_Aα2,3,5 receptors increases intake and motivation to consume sucrose solution in rats.

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Abstract

Benzodiazepines are one of the most commonly prescribed anxiolytic drugs in America, and between 2006 and 2015 prescription rates increased by an estimated 27.1%. Weight gain is a common side effect of these drugs and it may result from increased feeding caused by drug-enhanced food palatability. We investigated the role of specific GABA_A receptor subtypes involved with benzodiazepine-induced food consumption through third ventricle injections of L-838,417, a partial agonist of GABA_A α2, α3, and α5 subunits, and a full antagonist of the α1 receptor subunit. A microanalysis of the licking behavior of adult male rats to a sucrose solution was used to isolate drug effects on specific consummatory behaviors that include: hedonic taste evaluation, food approach behavior, and oromotor function. L-838,417 dose-dependently increased intake through increases in the motivation to approach the solution (shorter pause intervals between bouts of licking) and through enhancement of measures associated with hedonic taste evaluation. Oromotor depressant effects previously associated with broad-spectrum benzodiazepine receptor agonists were not observed. These results indicate that nuclei in proximity to the ventricles respond to GABA_A α2, α3, or α5 activation to induce motivation to feed, absent of α1 receptor subunit activation. Furthermore, activation of the α1 subunit is not necessary for benzodiazepine hyperphagia and may instead contribute to the oromotor depressant and sedative properties of classic benzodiazepine agonists. Hypothalamic nuclei such as the paraventricular nucleus may be involved in the benzodiazepine-increased motivation to feed, while

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CONTRIBUTIONS

Tyler Nelson, John-Paul Baird and David Pittman conceived and designed the study. Tyler Nelson, Jennifer Swick, Heather Allen and Sarah Holstein participated in collecting data. Tyler Nelson and David Pittman performed the statistical analyses. Tyler Nelson drafted the manuscript and Tyler Nelson, David Pittman and John-Paul Baird revised the manuscript. All authors read and approved the final manuscript.

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DECLARATIONS OF INTEREST

None.

the parabrachial nucleus of the hindbrain could contribute to benzodiazepine-induced enhancement of taste palatability.

Keywords

GABA-A; hyperphagia; benzodiazepine; L-838417

Introduction

Reports of benzodiazepine-induced hyperphagia can be traced back to the early work of Randall and his colleagues (1960) with the first synthesized benzodiazepine, chlordiazepoxide. They reported that this novel benzodiazepine compound resulted in an “appetite-stimulant” effect in rats and dogs leading to increased food consumption, or hyperphagia (Randall, 1960; Randall et al., 1960). Benzodiazepines increase the inhibitory neurotransmission of gamma-aminobutyric acid (GABA) (Costa and Guidotti, 1979; Haefely, 1978) through specific benzodiazepine receptors located on the modulatory subunits of most GABA_A receptor proteins (Schoch et al., 1985). GABA_A receptors are heteropentameric structures with 19 variations of homologous subunits that combine to form a channel through which Cl⁻ ions are permeable (Olsen and Sieghart, 2009). Canonical GABA_A receptors contain two α subunits, two β subunits, and a γ subunit. Benzodiazepines bind at the $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$ (not $\alpha 4$ or $\alpha 6$), and the $\gamma 2$ (not $\gamma 1$ or $\gamma 3$) subunits (Rudolph and Knoflach, 2011). All of these subunits are differentially expressed across brain regions, leading to varied influences on behavior, physiology, and other functions (Olsen et al., 1990; Olsen and Sieghart, 2009). With the prescription rate of benzodiazepine drugs increasing by an estimated 27.1% over the last decade (Garcia et al., 2018), understanding the specific GABA_A receptor subunits involved in mediating benzodiazepine-induced hyperphagia has become increasingly important.

GABA_A receptor agonists, antagonists, and inverse agonists have been useful in helping to unravel the role of the specific GABA_A receptor subunits in feeding. For example, the compound CGS 17867A binds to $\alpha 1$, $\alpha 2$, and $\alpha 3$ subunits with significantly higher efficacy at the $\alpha 2$, and $\alpha 3$ subunits than the $\alpha 1$ subunit, and intraperitoneal injection of CGS 17867A increased consumption of both highly palatable food and a normally aversive hypertonic NaCl solution in rodents (Cooper and Desa, 1988; Yerbury and Cooper, 1989). This suggests that benzodiazepines with a high affinity for $\alpha 2$ and $\alpha 3$ receptor subunits and low affinity for $\alpha 1$ subunits are most likely to induce hyperphagia (Cooper, 2005). In this regard it is worth noting that zolpidem (Ambien[®]) exhibits selective activation for the GABA_A $\alpha 1$ receptor subunit, and it did not elicit hyperphagia in a palatable food consumption test (Yerbury and Cooper, 1989). However, this result may be species-specific as zolpidem treatment has been reported to increase food intake in non-human primates (Griffiths et al., 1992) and squirrel monkeys (Duke et al., 2006). Likewise, the $\alpha 5$ receptor subunit is not believed to be involved in hyperphagia because treatment with the $\alpha 5$ agonist, QH-ii-066, did not increase sucrose pellet consumption in squirrel monkeys (Duke et al., 2006). It is also worth noting that abecarnil, an agonist for GABA_A receptors containing either $\alpha 1$ and

$\alpha 3$ subunits, was shown to increase sucrose intake after intraperitoneal injection (Cooper and Ridley, 2005).

In the current study, we explored the role of the novel GABA_A agonist, L-838,417, on consumption of a palatable sucrose solution in rats after microinjection into the third ventricle. L-838,417 is a partial GABA_A agonist that activates GABA_A receptors containing $\alpha 2$, $\alpha 3$, and/or $\alpha 5$ subunits, while it is a full antagonist for those expressing the $\alpha 1$ receptor subunit (McKernan et al., 2000). Little research has used L-838,417 to assess the role of specific GABA_A receptor subtypes in hyperphagia. L-838,417 injected via the intraperitoneal route into $\alpha 2$ subunit knockout mice found that L-838,417 enhanced food intake and feeding behavior, leading the authors to conclude that the $\alpha 2$ is not necessary for hyperphagia and to suggest that the $\alpha 3$ receptor subunit may be key (Morris et al., 2009). We sought to further this work by directly injecting L-838,417 into the third ventricle to access several periventricular structures implicated in GABAergic feeding responses, such as paraventricular nucleus and the parabrachial nucleus. It is well known that non-selective GABA_A agonists increase feeding behavior after intracerebroventricular (ICV) microinjections (Baldwin et al., 1990; Berridge and Pecina, 1995; Bungo et al., 2003). For example, injection of the GABA_A agonist muscimol into the third ventricle increased feeding in a dose related fashion (Pu et al., 1999). To our knowledge, this is the first study to explore direct CNS stimulation effects of the more selective L838,417 on feeding behavior. We therefore chose an ICV route to provide access to the broadest anatomical range of brain structures. To provide further understanding of the role of central GABA_A $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits in feeding behavior, we measured the effects of L-838,417 injection on the pattern of licking for a palatable sucrose solution during a 90 min test session. A pattern analysis of licking can help to discern the effects of activating GABA_A $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits on oromotor function, hedonic taste evaluation, and other ingestive behaviors that influence food intake.

EXPERIMENTAL PROCEDURES

Animals

Adult, male Sprague-Dawley rats ($n = 25$, body weight 300–400 g, Charles River Laboratories) were individually housed in transparent plastic cages in a temperature-controlled colony room on a 12:12 h light:dark cycle. Animals had free access to Harlan Teklad 8604 rodent chow and water throughout the duration of the study. All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Wofford College.

Surgery

Subjects were anesthetized with 0.35 ml ketamine (100 mg/ml) and xylazine (10 mg/ml) and secured into a stereotaxic apparatus (David Kopf). A 22 gauge 11 mm guide cannula was inserted into the third ventricle of each rat using coordinates relative to bregma (anterior-posterior -2.30 mm, medial-lateral 0.0 mm, dorsal-ventral -8.5 mm). Two anchor screws were then placed in the skull and the cannula was fixed with dental cement. Animals had a

minimum of 48 hours post-surgical recovery period before verification of cannula placement. Subjects were excluded from the study on the basis of failure of third ventricle cannula placement verification task, signs of infection that was nonresponsive to carprofen treatment, or failure of histological confirmation of third ventricle placement. No rats received carprofen on a testing day.

Verification of Third Ventricle Cannula Placement

Prior to experimental testing, viability of the third ventricle cannula placement was assessed by administering 50 ng / 5 μ L angiotensin II (ANGII) into the cannula and measuring water consumption for 30 minutes following the injection. Third ventricle infusions of ANGII are well known to induce drinking in rats (Epstein et al., 1970; Franci et al., 1989). The cannula was assumed to be within the third ventricle if the rat consumed > 5 g of water within the 30-minute period following ANGII administration. Rats consuming less than 5 g of water were excluded ($n = 6$).

Drug and Taste Stimuli

Tastants and drug infusions were mixed from reagent grade chemicals (Sigma Aldrich and VWR). The GABA_A agonist L-838,417 was dissolved in dimethyl sulfoxide (DMSO) at a 100 nM concentration and then diluted into 1 nM and 10 nM concentrations before all concentrations were stored frozen in 2 μ L aliquots. We selected these doses based on several factors. First, the K_i for L838,417 for GABA_A receptor α_2 , α_3 , and α_5 subunits is 0.67 nM, 0.67 nM, and 2.25 nM, respectively (McKernan et al., 2000). It has been reported that binding saturation for L838,417 in brain tissue corresponds to approximately 0.7nM binding sites (Stevens and Pollack, 2005). Third, extracellular concentrations ranging from 1nM to 10nM produced maximal electrophysiological activation of GABA_A receptors in vitro (McKernan et al., 2000). Finally, one prior study reported that 1nM injection of L838,417 directly into the amygdala was sufficient to alter behavioral anxiety responses (Mathiasen et al., 2007).

We used a counterbalanced, within-subjects design in which either 2 μ L aliquots of L-838,417 dissolved in DMSO (experimental) or 2 μ L of DMSO alone were delivered into the third ventricle via cannula at a rate of 120 μ L / hr using a 10 μ L Hamilton Syringe, PE20 tubing and KD Scientific microinjector. A 1-minute post-injection wait period before removing the microinjector tip allowed for drug diffusion into the third ventricle. Animals were placed in the testing chamber immediately after the microinjection and the 90-minute test sessions were begun within 10 minutes following the microinjection.

Behavioral Testing

Prior to the start of testing, rats received four consecutive training days in the AC-108 single spout lickometer (DiLog Instruments, Tallahassee, FL) with 90-minute access to 0.5 M sucrose for two training days followed by two training days of 0.3 M sucrose. All rats achieved the performance threshold of > 1500 licks to 0.3 M sucrose in the final training session. Testing sessions were carried out on consecutive days following the training sessions. In a counterbalanced design, rats received one of the four drug conditions (DMSO, 1 nM, 10 nM, and 100 nM L-838,417) via third ventricle infusion. Immediately after third

ventricle infusion, rats were placed into the AC-108 lickometer to record licking (1 ms resolution) for 0.3 M sucrose for a 90-minute test session. Rats were given two days in-between each test session to allow drug wash out and prevent carryover effects. On the two days between testing, rats were given daily 90-minute access to 0.3 M sucrose in the AC-108 single spout lickometer to maintain consistency and stimulus control.

Histology

After completing the test sessions, we microinjected 2 μ L of Chicago blue ink using the same infusion parameters as the testing phase. Ink administration through the cannulas is a common practice to indicate drug infusion patterns but we cannot rule out that drug may have spread in a dissimilar manner. Animals were perfused immediately following the ink infusion and brains were stored in a 10% formalin solution. The brains were dissected by midsagittal cuts and ink diffusion was photographed and assessed. Three experimenters blind to the results of the study reviewed the histology and determined whether the ink had fully circulated in the third ventricle. Some subjects showed ink diffusion which extended from the third ventricle partially into the fourth ventricle ($n = 17$), while other subjects only had ink within the third ventricle ($n = 8$). Subjects with incomplete ink diffusion in the 3V were excluded ($n = 8$). There were no statistical differences observed when results from the third and fourth ventricle diffusion group were compared to the third ventricle only group. Therefore, the data from both groups were combined (Fig 1A & 1B).

Data Analysis

A microstructural analysis of the pattern of licking behavior during the 90-minute test session grouped the licks for each rat into meals, initiated by a burst of 5 licks within 1 s and terminated with a pause of 10 minutes or longer in which no licking bursts were expressed (Spector et al., 1998). Every subject completed at least one meal with approximately half of the subjects initiating a second meal during the 90-minute test session. An analysis of the patterns of licking during the first meal of the test session produced a variety of whole-meal measures (meal lick count, latency to initiate the meal, meal duration) and intrameal licking patterns (number of bursts, average licks per burst, mean burst duration, mean pause duration, and average lick rate). These metrics allow analysis of both taste mediated behaviors and postingestive feedback sensitive licking measures (Baird et al., 2005; Davis and Levine, 1977; Pittman et al., 2012; Spector et al., 1998). Drug effects on intrinsic oromotor functions were assessed via measuring interlick intervals (ILIs) and average lick rate during bursts.

We defined a licking burst as the total number of licks until a 1 s pause in licking. The mean burst size is the total number of licks in the meal divided by the number of bursts in the meal. The mean burst duration is the average length of time for each licking burst within the meal and the mean pause duration is the average length of time from the termination of the previous burst until the initiation of the next licking burst. To determine the number of licks per second (average lick rate), we divided the meal licks by the meal duration. Interlick interval distributions were analyzed via calculating the average duration of the ILIs in the range 50–250 ms and from 250–2000 ms. This results in a ILI ratio (%) that reflects the

number of ILIs in the 250–2000 ms range, which increases with natural or conditioned aversive taste responses (Pittman et al., 2012).

For each measure a repeated measures analysis of variance was used to determine a significant main effect of L-838,417 across doses. Post hoc Dunnett's tests were performed to control for multiple comparisons between L-838,417 doses. Significance was defined as p -value < .05. All statistical analyses and graphs were created in *Prism 7.0*.

RESULTS

Whole Meal Variables

As shown in Fig. 2A, there was a main effect of the drug condition [$F(3,72) = 3.671$, $p = .016$]; the total number of licks in the first meal increased in a dose-dependent manner, with the 100 nM concentration of L-838,417 producing significantly more total licks compared to the control condition, DMSO ($p = .037$). Although the adjusted p value did not reach significance, mean data values suggest that the 10 nM concentration induced an intermediate increase in licking compared to the DMSO, whereas DMSO and 1 nM drug conditions were not different. There was no effect of the L-838,417 on the latency to initiate the first meal (Fig. 2B) or for meal duration (data not shown).

Intrameal Variables

The increase in total meal licks was due to two primary factors. There was an increase in the number of bursts in the meal [Fig. 3A; $F(3,72) = 3.146$, $p = .030$] combined with an increase in the duration of bursts [Fig. 3B; $F(3,72) = 4.061$, $p = .010$]. Given that meal duration did not change, the increased durations of bursts corresponded with a decrease in the average pause time during the meal [Fig. 3C; $F(3,72) = 4.788$, $p = .004$]. The L-838,417 treatment significantly increased the average rate of ingestion [Fig. 3D; $F(3,72) = 3.370$, $p = .023$] meaning that the more numerous and longer bursts combined with a reduction of pause time between bursts to produce faster/denser licking during the meal on average. Collectively, these changes suggest an increased motivation to consume sucrose.

Analysis of licking at the start of the meal can be reflective of orosensory-driven behavior independent of postingestive feedback (Davis and Levine, 1977; Spector et al., 1998). As shown in Fig. 4, L-838,417 significantly increased both the number of licks in the first minute of the meal [$F(3,72) = 2.846$, $p = .044$] and the number of licks in the first burst of the meal [$F(3,72) = 3.405$, $p = .022$].

Oromotor Function Variables

Previous studies have shown that though benzodiazepines increase the number of licks expressed, they also prolong the intrinsic lick cycle time through increases in interlick interval periods (Pittman et al., 2012), suggesting an oromotor depressant effect that is independent of food acquisition motivation. In contrast, we found that L-838,417 treatment did not affect variables associated with oromotor function. Fig. 5A shows the highly consistent licking rate during bursts. Intraburst licking is a highly stereotyped behavior producing consistent interlick intervals of approximately 150 ms between each lick. As

shown in Fig. 5B, the average interlick intervals within bursts were highly consistent between the control and all L-838,417 doses. Previous taste reactivity tests have also shown that when rats consume palatable taste stimuli such as sucrose the rats will occasionally interrupt their stereotypical licking to perform tongue protrusions and lateral tongue movements (Grill and Norgren, 1978). These mimetic responses create interlick intervals longer than 250 ms but shorter than the 1 s pause criterion to terminate a burst. As shown in Fig. 5C, less than 6% of the interlick intervals fell into this category which is a value normally expected for palatable taste stimuli, and L-838,417 had no effect on the proportion of these longer interlick intervals.

DISCUSSION

We found that third ventricle microinjection of the GABA_A receptor agonist L-838,417, a partial agonist at $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits, and a full antagonist at the $\alpha 1$ receptor subunit, significantly increased the number of licks for sucrose in non-deprived rats. A comprehensive licking analysis revealed that this compound increased the motivation to consume the sucrose solution, as well as positive hedonic taste evaluation.

These results indicate that brain structures along the third and fourth ventricles express cognate GABA_A $\alpha 2$, $\alpha 3$, and/or $\alpha 5$ subunits that influence food intake. It is also possible that antagonism of constitutive activity in cognate $\alpha 1$ -containing GABA_A receptors also contributed to the increased intake, however this interpretation is disfavored when one considers that the $\alpha 1$ -containing GABA_A receptors more predominantly contribute to the amnesic and sedative side-effects of benzodiazepines (Cooper, 2005).

We believe that the results of this study have three main implications. First, our results provide further support for the growing body of literature that $\alpha 2, \alpha 3, \alpha 5$ -containing GABA_A receptors play a role in benzodiazepine-induced hyperphagia (Knoflach et al., 1993; Pribilla et al., 1993; Yerbury and Cooper, 1989). Prior benzodiazepine agonist studies also stimulated, in a nonselective manner, GABA_A $\alpha 1$ receptors, making it impossible to distinguish which receptor subtypes contributed to the hyperphagic effects. Given the antagonistic actions of L-838,417 at the GABA_A $\alpha 1$ receptor, our results support the more recent work of Morris et al. who used L-838,417 in GABA_A $\alpha 2$ knockout mice and concluded that it is the $\alpha 3$ - and/or $\alpha 5$ -containing GABA_A receptors, independent of those containing $\alpha 2$ subunits that induce hyperphagia (Morris et al., 2009). Given that the GABA_A $\alpha 5$ agonist, QH-ii-066, did not increase sucrose pellet consumption in squirrel monkeys (Duke et al., 2006), our results suggest that further research focused on receptors expressing the $\alpha 3$ subunit would be propitious.

Our second finding is that the microanalysis of licking behavior suggested that L-838,417 increases hedonic taste evaluation and the motivation to eat. Many studies attribute benzodiazepine hyperphagia to the enhancement of taste palatability (Berridge, 1988; Berridge and Pecina, 1995; Soderpalm and Hansen, 1998), and our results are consistent with this interpretation because L-838,417 increased the initial rate of licking, the size of the first burst of licking, and the mean burst duration; all measures that correlate with hedonic taste reactivity (Spector et al., 1998). Our data also point to an increase in food approach

motivation (appetitive behavior), suggesting that multiple processes produce the hyperphagia and these processes might vary as a function of different brain regions with GABA_A $\alpha 2/\alpha 3/\alpha 5$ receptor access to the ventricle system. Specifically, rats in this study exhibited a significant increase in first meal size after third ventricle injection of 100 nM L-838,417, which was due to an increase in the number of bursts and the average burst duration coupled with a decrease in pause duration. Combined, these effects result in an increased ingestion rate during the meal, all of which are indicative of increased motivation to consume. However, meal duration was not affected, suggesting there were no effects of L-838,417 on satiation or satiety-related processes (Baird et al., 2006a; Baird et al., 2006b), or other behaviors such as brief consumption inactivity or exploration. In fact, pauses between the bursts of licking were shorter, indicating that rats returned more quickly to the spout, resulting in a greater density of ingestive behavior within the meal period.

On the other hand, behavioral effects previously observed after broad-spectrum benzodiazepine agonist treatment in other studies were noticeably absent after L-838,417 treatment. Prior studies have reported that broader agonists such as chlordiazepoxide (CDP) increase meal latency, likely due to sedative effects, and that the intrinsic lick cycle is prolonged, suggesting a depressant oromotor effect, perhaps related to effects on hindbrain oromotor nuclei (Cooper, 2005; Pittman et al., 2012). Our third major finding is that we did not observe any increases in meal latency, meal duration, the pauses between lick bursts, or the intervals between individual licks, all outcomes that would have indicated soporific or depressant effects. Given that L-838,417 does not activate $\alpha 1$ -expressing GABA_A receptors, we conclude that activation of the $\alpha 1$ subunit is not necessary for benzodiazepine-induced hyperphagia.

As this is (to our knowledge) the first study to explore the effects of direct CNS application of L-838,417 on feeding behavior, we selected an ICV route of injection in order to maximize the number brain structures stimulated, which is a common approach in studies exploring the CNS effects of novel ligands. We observed that 3V injections often penetrated the 4V (Fig 1D), which suggests that proximal brain structures were likely exposed to the strongest concentrations of L838,417. Given the positive findings reported here, prior studies that used central application of other broader-spectrum benzodiazepine ligands suggest some brain structures in proximity to the ventricle system that could be targeted for future investigation.

One nucleus favored for further research is the paraventricular hypothalamic nucleus (PVN), which lies adjacent to the third ventricle and has long been implicated in autonomic functions, energy homeostasis, and feeding (Broberger et al., 1998a; Broberger et al., 1998b; Qin et al., 2018). A diffuse staining of $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$ receptor subtypes was found within the neuropil of the PVN (Backberg et al., 2003), and several neurons that express feeding-related neuropeptides such as Neuropeptide Y (NPY) / Agouti Related Peptide (AgRP) / GABA containing neurons from the arcuate nucleus, target the PVN nucleus and have been shown to induce feeding (Pu et al., 1999). Nonspecific GABA_A agonist muscimol infusions into the PVN have been shown to increase food intake in non-deprived rats (Stratford and Wirtshafter, 2013). Recently, it has been demonstrated that disruption of GABA release from Pdx1-cre-positive hypothalamic neurons into the PVN (Kim et al.,

2015), lateral hypothalamic neurons into PVN (Wu et al., 2015), or NPY/AgRP/GABA neurons into PVN (Meng et al., 2016) all potentially reduced feeding behaviors in mice. Furthermore, optogenetic activation of GABAergic lateral hypothalamic fibers in the PVN produced monosynaptic IPSCs in PVN neurons and dramatically increased feeding behaviors (Wu et al., 2015). Therefore, increased consumption of a palatable sucrose solution in rats in this study may be mediated in part via activation of $\alpha 2$, $\alpha 3$, or $\alpha 5$ -expressing GABA_A receptors within the PVN which could be tested in a future study with direct intra-PVN injections of L-838,417.

We have found that both L-838,417 and systemic CDP increase intake through two mechanisms: increased hedonic taste evaluation and increased food-sampling (appetitive) motivation, with one key difference being a lack of lick-motor depressant effects after L838,417, as was observed after CDP treatment (Pittman et al., 2012). The first central relay for taste processing in the hindbrain is the nucleus of the solitary tract (NST), which lies subjacent to the 4V. However, previous work suggests that another structure proximal to the 4V, the parabrachial nucleus, may be more likely to contribute to benzodiazepine hyperphagia: injection of broad-spectrum benzodiazepines to the PBN increased food intake and orofacial taste responses (taste reactivity), but similar doses delivered to the NST, pedunclopontine nucleus, and 4V did not (Higgs and Cooper, 1996a, b; Soderpalm and Berridge, 2000). In addition, GABA_A receptors in the NST have been reported to exhibit little responsiveness to benzodiazepines (Kasparov et al., 2001).

In the parabrachial nucleus, benzodiazepine-responsive GABA_A receptors are found in the gustatory “waist” region and the viscerosensitive external lateral subnucleus (Guthmann et al., 1998). Preliminary work within our laboratory showed that benzodiazepines injected into the parabrachial nucleus, the second relay of the gustatory taste pathway, increased the initial lick rate and licks per burst. This suggests that GABA_A receptors within the parabrachial nucleus likely contribute to enhanced hedonic taste evaluation (Pittman et al., 2014), but not appetitive food-sampling motivation. It is possible that L-838,417 stimulation of the portion of the parabrachial nucleus that accesses the ventricles (lateral subnuclei) could have contributed to the increases in hedonic taste evaluation observed in this study. The results also suggest that L-838,417 acted within extra-parabrachial nuclei (such as the PVN) to induce the appetitive effects, perhaps the same nuclei as those affected by systemic benzodiazepines.

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Highlights

- The novel compound L-838,417 is a partial agonist of GABA_A α 2, α 3, and α 5 and a full antagonist of the α 1 receptor subunit.
- Dose-dependent ICV injections of L-838,417 increase hedonic taste evaluation and the motivation to consume sucrose.
- L-838,417 did not affect satiation or satiety-related processes.
- α 2/ α 3/ α 5-containing GABA_A receptors play a role in benzodiazepine-induced hyperphagia.

Fig 1: Stereotaxic atlas coronal (A) representation of cannula placement and drug diffusion based off stereotaxic coordinates. Site of infusion is indicated by red dot. All animals underwent histological verification of ink diffusion and some animals had ink contained within the third ventricle (B, n = 8), while others had ink diffusion in both the third and fourth ventricles (C, n = 17).

Fig. 2.

Average total licks in the first meal (A) increased with 100 nM ICV application of L838 with a concurrent no effect on the latency to initiate the first meal (B).

Fig. 3.

The effect of ICV injection of L838 on the licking patterns to sucrose within the first meal. Average number of bursts (A) and burst duration (B) increased with the 100 nM L838 dose. Pause duration between bursts (C) decreased across L838 doses (** indicates $p < .01$) with a corresponding increase in the average ingestion rate for the first meal (D) across L838 doses (* indicates $p < .05$).

Fig. 4. ICV injection of L838 increased measures of hedonic value of the sucrose stimulus. Number of licks in the first minute of the meal (A) and number of licks in the first burst (B) significantly increased with the 10 nM L838 dose ($p < .05$).

Fig. 5.

There was no effect of ICV injection of L838 on measures of oromotor function such as average lick rate during bursts (A); average interlick intervals less than 250 ms (B); and the percent of interlick intervals 250–2000 ms (C).