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Research report

# The hedonic impact and intake of food are increased by midazolam microinjection in the parabrachial nucleus

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**Abstract**

Benzodiazepines have been reported to induce eating when administered into the brainstem of rats (either the fourth ventricle or the parabrachial nucleus). Benzodiazepines in the brainstem also have been reported to enhance the hedonic impact of taste, as measured by hedonic/aversive taste reactivity patterns, when administered to the fourth ventricle. The present study examined whether the parabrachial nucleus in particular is a brainstem site of the benzodiazepine-produced enhancement of eating and palatability. Food intake (cereal mash) was measured after brainstem microinjections of midazolam or vehicle (0.0, 7.5, and 15.0 μg) into the parabrachial nucleus, the nucleus of the solitary tract, the pedunculopontine tegmental nucleus, or the fourth ventricle (60 μg). We used the taste reactivity paradigm to measure hedonic/aversive affective reactions elicited from rats by oral infusions of a bittersweet solution (7% sucrose–0.01% quinine). Positive hedonic reactions and negative aversive reactions to sucrose–quinine were also measured after microinjections of midazolam (0.0, 7.5, and 15 μg) into the parabrachial nucleus. Midazolam increased food intake and selectively enhanced positive hedonic taste reactivity patterns to the bittersweet solution when microinjections were delivered to the parabrachial nucleus. When administered to the other brainstem sites at the same doses, however, midazolam had no effect. We therefore conclude that the parabrachial nucleus can mediate the benzodiazepine-induced enhancement of the hedonic impact of taste as well as mediating the enhancement of eating behavior.


*Theme:* Neural bases of behaviour

*Topic:* Ingestive behaviours

*Keywords:* Parabrachial nucleus; Nucleus of the solitary tract; Pedunculopontine tegmental nucleus; Fourth ventricle; Food intake; Microinjection; Midazolam; Benzodiazepine; GABA; Palatability; Hedonic; Aversive; Taste; Pleasure; Reward

## 1. Introduction

Benzodiazepines are well known to elicit increased eating behavior in animals, and the enhancement of food intake appears to be separate from either the anxiolytic or sedative effects of benzodiazepine agents [8,14,16–18,55,69]. Administration of benzodiazepines has also been reported to facilitate food intake in humans, which is consistent with a role for benzodiazepine receptors in appetite [23,33].

The appetite-enhancing action of benzodiazepines appears to be due in part to a specific enhancement of the perceived taste of food [6,8,13,14,16,17,71]. The hypothesis of palatability enhancement was originally proposed by Cooper and Estall [16], and has been supported by the results of several subsequent studies of affective taste reactivity patterns, which is a behavioral measure of the hedonic impact of taste that can be applied to human infants and to animals [7,30,32]. These taste reactivity studies have found that administration of chlordiazepoxide, diazepam, or midazolam enhances the positive or hedonic affective reaction patterns that are elicited from rats by oral infusions of sucrose or other sweet tastes, but does not enhance aversive reactions elicited by bitter quinine [9,26,49,51,65,67,68]. The benzodiazepine-induced enhancement of hedonic taste reactivity patterns to sucrose can also be blocked by benzodiazepine antagonist such as Ro 15-1788 or the inverse agonist CGS 8216 [68,20].

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Given these findings, the palatability effect seems to be benzodiazepine receptor specific.

Little is known about the precise neuroanatomical location of the receptors underlying taste palatability and feeding effects of benzodiazepines. Forebrain microinjections of benzodiazepines are relatively ineffective compared to other agents in eliciting eating behavior or hedonic palatability enhancement. For example, Söderpalm and Berridge [66] found that nucleus accumbens microinjections of diazepam failed to increase food intake, although muscimol microinjections did facilitate food intake at that forebrain site, as do other GABA-A and GABA-B agonists [39,41,62,63]. Thus even though diazepam and muscimol both act by potentiating GABA-A receptors [15,40,43,60], only muscimol may act in the nucleus accumbens to facilitate food intake, whereas diazepam seems to act elsewhere. Evidence indicates that opioid receptors in the nucleus accumbens and other forebrain sites also may mediate feeding behavior and the enhancement of taste palatability [19,21,42,44,52–54].

By contrast to the forebrain site of hyperphagic action for opioid agonists and direct GABA agonists, the available evidence points to a site in the brainstem for benzodiazepine-induced enhancement of food intake and hedonic impact. A role for brainstem receptors and circuitry in the effects of benzodiazepines on taste reactivity and food intake was first indicated by the report that systemic chlordiazepoxide still increased positive taste reactivity patterns even after midbrain decerebration [4]. Subsequent microinjection studies in normal rats supported the hypothesis of a brainstem substrate. Peciña and Berridge [52] showed that microinjections of diazepam into the fourth ventricle of intact rats are more effective than microinjections into the lateral ventricles at enhancing positive hedonic taste reactivity patterns and at enhancing food intake. Most relevant to the current study, Higgs and Cooper [35,36] further identified a candidate site of action within the brainstem, the parabrachial nucleus of the pons, by showing that midazolam delivered into the fourth ventricle or directly into the parabrachial nucleus was sufficient to increase food intake. The parabrachial nucleus is the second gustatory relay nucleus, receiving ascending gustatory inputs from the nucleus of the solitary tract [34,56], and is thus well positioned to modulate taste processing and eating behavior.

Other brainstem sites besides the parabrachial nucleus are also candidates to mediate the hyperphagic and palatability effects of benzodiazepines. The nucleus of the solitary tract is the primary gustatory relay nucleus in vertebrate brains [45–48], and plays an important role in food intake and responses to taste [24,38]. The nucleus of the solitary tract contains GABA-A receptors [11,37] that are sensitive to benzodiazepine agents [22], and these GABA receptors have been shown in electrophysiological studies to play a role in gustatory processing [10]. The pedunculopontine tegmental nucleus is another brainstem site that has been identified as a critical substrate for food reward in rats, as well as for opiate reward and brain stimulation reward [2,3,61,70]. Regarding food reward in particular, van der Kooy and colleagues have shown that lesions of the pedunculopontine tegmental nucleus block saccharin-conditioned place preferences and food-conditioned place preferences, as well as attenuating the unconditioned intake of saccharin [3,61].

The present series of experiments aimed to confirm Higgs's and Cooper's [36] finding that the parabrachial nucleus is an effective site for increasing food intake by benzodiazepine administration, and to further examine whether the parabrachial nucleus mediates enhancement of the hedonic impact of a sweet taste caused by benzodiazepine administration. We compared the effects of midazolam microinjections when the benzodiazepine was delivered to either the parabrachial nucleus (PBN), the nucleus of the solitary tract (NTS), the pedunculopontine tegmental nucleus (PPT), or the fourth ventricle. The first experiment compared the effect on food intake of microinjections at these various sites. The second experiment examined the effect on hedonic and aversive taste reactivity patterns elicited by oral infusions of a bittersweet sucrose–quinine solution, and to examine whether palatability was actually enhanced by the PBN microinjections of midazolam that were found to increase feeding in experiment 1.

## 2. Experiment 1

### 2.1. Materials and methods

#### 2.1.1. Subjects

Male Sprague–Dawley rats (born at the University of Michigan) were housed in pairs, on a 12 h light–dark cycle (lights off 8 a.m. on 8 p.m.), in a temperature-controlled room. Behavioral testing was conducted during the dark phase of the light cycle between 09.00–13.00 h. Rats had ad libitum access to food (Purina chow pellets) and water in their home cages throughout the experiments. Their weight was 250–350 g at the beginning of each experiment.

#### 2.1.2. Surgical procedure

Rats were pretreated with atropine methyl nitrate (1 mg/kg, i.p.) and anesthetized with sodium pentobarbital (8 mg/kg, i.p.). They were then implanted for experiment 1 with bilateral intracranial guide cannulae aimed either at the PBN ($N=12$), NTS ($N=9$), or the PPT ($N=10$). An additional group of rats received a single cannula aimed at the fourth ventricle ($N=6$) to provide a non-specific brainstem administration comparison group. The animals were placed in a stereotaxic device, with bregma and lambda in a horizontal plane, and bilateral intracranial stainless steel guide cannulae (22 gauge, 14 mm long)

were surgically placed using coordinates from the Paxinos and Watson [50] atlas. The stereotaxic coordinates for PBN were (relative to bregma): anterior/posterior (AP)=−9.4 mm; lateral (L)=±2.0 mm; and ventral (V)=−5.8 mm. Coordinates for NTS were: AP=−12.3 mm; L=±1.5 mm; and V=−7.7 mm. Coordinates for PPT were: AP=−8.0 mm; L=±1.8 mm; and V=−7.0 mm. Coordinates for the fourth ventricle were: AP=−11.5 mm; L=±0.0 mm; and V=−6.0 mm. The cannulae were fixed to the skull using dental cement and skull screws. After surgery, dummy cannulae were placed into the microinjection guides to prevent occlusion.

Rats were also implanted in the same surgery with a chronic oral cannula for taste reactivity tests. While a rat was placed on its back on a heating pad with its mouth open a beveled end of 14-G needle was placed just anterolateral to the first upper molar and advanced such that it protruded on the top of the head between the ears. The catheter (a 5 cm length of PE60 tubing) was then inserted through the needle, which was subsequently pulled out. The intraoral end of the catheter had a heat-flared end, behind which a small silicone disc (1-mm thick, 3-mm in diameter; Silastic Medical Adhesive, Dow Corning, USA) had been threaded. The end of the catheter was secured to the skull with skull screws and dental cement. The catheter was flushed with a small amount of water each day. These oral cannulae do not interfere with the eating behavior of the animal, and they allow the direct infusion of solutions into the mouth for taste reactivity testing. After surgery, the animals were housed in pairs during the recovery period before behavioral testing.

*2.1.3. The microinjections*

Microinjections were made with a 0.5-μl Hamilton syringe connected to a syringe pump. The volume infused into PBN, NTS, and PPT was 0.25 μl on each side. For the fourth ventricle 0.5 μl was injected. During the microinjections the animals were handheld gently while the injection cannulae tip was lowered through the guide cannulae (the microinjector tip extended 1 mm below the tip of the guide cannulae for the PBN site, 0.5 mm for the NTS site, 0.5 mm for PPT site, and 2 mm for the fourth ventricle site). Drug or vehicle was then delivered over a period of 30 s, in each hemisphere, by a microsyringe pump connected by 20PE tubing to the injection cannulae. The injectors were left in place for 1 min to allow diffusion. Afterwards the rats were immediately put in their test cages. Two days prior to testing each animal had the microinjector tip lowered through the guide cannula to familiarize it to the procedure.

*2.1.4. Drugs and doses*

The water soluble benzodiazepine agonist midazolam maleate (generously supplied by Hoffmann-La Roche) was dissolved fresh every day in sterile 0.9% saline. Experiment 1 doses: midazolam total dose: 7.5 μg in 0.5 μl and vehicle 0.5 μl (0.9% saline; PBN, PPT, NTS, fourth ventricle), plus 60 μg of midazolam in 0.5 μl and vehicle 0.5 μl for the animals in the separate fourth ventricle group (similar to the supra-threshold fourth ventricle dose of diazepam found by Peciña and Berridge to be required to elicit feeding and hedonic enhancement). Experiment 2 doses: midazolam 7.5 μg in 0.5 μl, 15 μg in 0.5 μl, and vehicle 0.5 μl. Since bilateral microinjections were always given in both the left and the right sides of PBN, NTS, and PPT, each unilateral microinjection contained one-half the stated total dose and volume (i.e., the 7.5-μg bilateral dose consisted of two unilateral microinjections that each contained 3.75 μg).

*2.1.5. Behavioral food intake test*

After the animals recovered from surgery (5 days) in experiment 1, they were habituated to the food intake test procedure over the next 5 days. Each day the rats were transported in their home cages to the test room and placed into test chambers similar to their home cages. The rats were habituated to eat a palatable cereal mash made of baby cereal (banana taste flavor, Gerber Products Company Fremont, MI, USA) mixed with an equal amount of water. The meals were freshly made every day and the cereal mash and water were available ad lib in the test cages for 2 h. The mash was pre-weighed before the test and the amount of food eaten was measured by weight after 15 min, 1 h and 2 h. The rats received the cereal mash twice a day, once during the time in the test cages and the second meal again around 5 p.m. every day in their home cages (to further habituate the rats to the mash, and to obtain stable baseline intake levels), in which they at the same time also had access to ordinary chow and water.

*2.1.6. Procedure: food intake after microinjections of midazolam or vehicle into the PBN, NTS, PPT, and the fourth ventricle*

Rats received microinjections of either vehicle or midazolam (0, 7.5, or 15 μg) into their brainstem site, and the order of doses and vehicle was balanced across rats. Each rat only received one microinjection and intake test per day with 48 h between treatments. The doses were chosen based on earlier reports of elicited feeding at that dose range [36]. Immediately after the microinjection rats, were tested for voluntary intake of a palatable cereal mash in a 2 h test as described above. Rats that had a guide cannula in the fourth ventricle also received, in a separate test afterwards, a high dose of midazolam (60 μg) or vehicle in counterbalanced order (Fig. 1). The 60-μg dose was chosen based on earlier reports from Peciña and Berridge [52] who reported an increase in both feeding and in hedonic reactions to sucrose after injections of diazepam in the fourth ventricle at that dose. It was used here in order to compare the *magnitude* of food intake evoked by fourth ventricle administration at a dose that was clearly above the threshold for eating behavior to the magnitude of

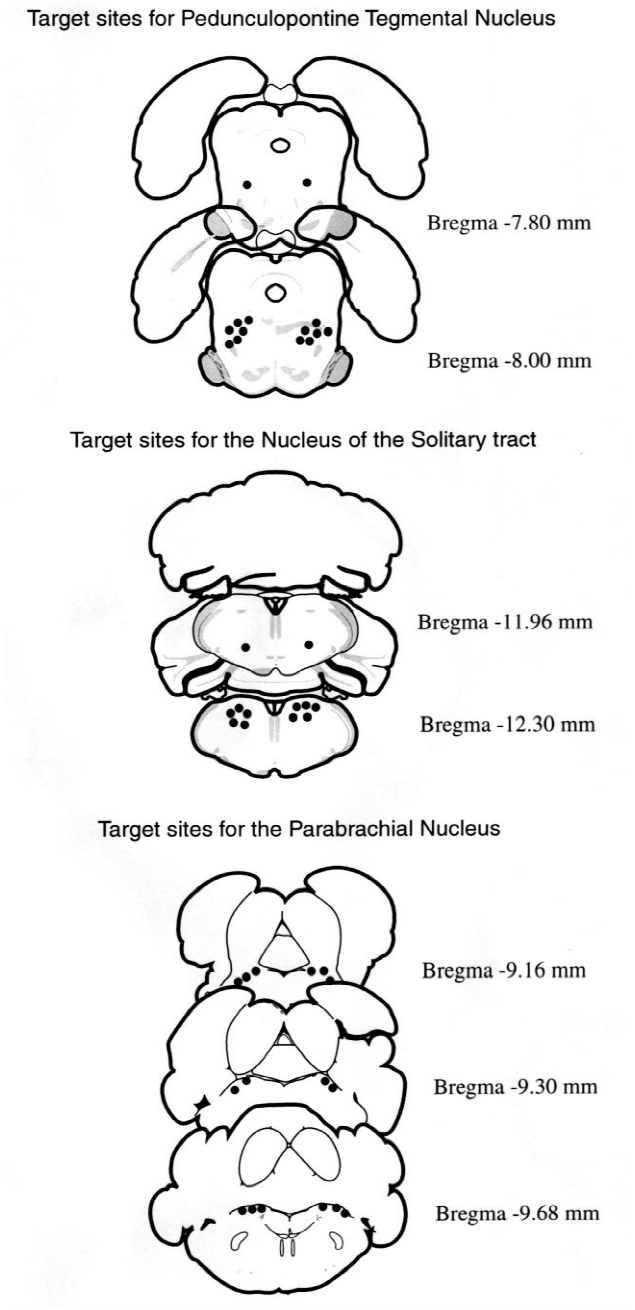


Fig. 1. The distribution of injection sites in the parabrachial nucleus (PBN), nucleus of the solitary tract (NTS), and pedunculopontine nucleus (PPT) for rats used in experiment 1. Sites are shown bilaterally. Sections are redrawn from Paxinos and Watson [49] and from Swanson's [64] atlas. Section number refers to millimeters from bregma.

intake obtained from specific brainstem sites at the lower doses.

*2.1.7. Verification of the cannulae placement*

After each experiment the rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.). Each animal was then perfused via the heart with saline followed by a 10% formalin solution. The brains were removed and put in formalin for a period of at least 1 week. The brains were then frozen, sliced in a cryostat, stained with cresyl violet, and the cannulae placements were verified histologically (Fig. 1). Animals with off-target placements were eliminated from the study (leaving groups of: PBN $N=8$; NTS $N=6$, PPT $N=7$; fourth ventricle $N=6$). All the histology was conducted blind with respect to the behavioral data. The distribution of injection sites are presented in Fig. 1.

*2.1.8. Statistical analysis*

Results were analyzed with an ANOVA mixed between/within factors design, two-way and one-way repeated measure ANOVAs. The post hoc comparisons were made using LSD and Newman-Keuls tests.

*2.2. Results*

An overall ANOVA mixed between/within subjects design (PBN, NTS, PPT, fourth ventricle) revealed that there was both an overall effect of dose ($F(2,46)=3.78$, $P<0.03$), and a microinjection site×dose two-way interaction ($F(6,46)=5.64$, $P<0.0001$). Two-way ANOVAs conducted for each site showed that midazolam microinjection (7.5 and 15 μg doses) increased feeding over vehicle levels in the PBN group ($F(2,28)=9.0$, $P<0.003$), but not in the NTS group ($F(2,20)=2.68$, $P>0.12$), the PPT group ($F(2,24)=1.65$, $P>0.23$), or the fourth ventricle group ($F(2,24)=2.1$, $P>0.2$).

Midazolam significantly increased food intake only for the PBN site group, and the increase occurred after both the 7.5 μg PBN dose of midazolam ($F(3,23)=8.3$, $P<0.001$) and the 15 μg PBN dose ($F(3,23)=7.1$, $P<0.001$; Figs. 2 and 3). For the PBN group, the midazolam elicited significantly more food intake than vehicle at every time tested after these doses: 15 min ($F(3,23)=5.0$, $P<0.008$),

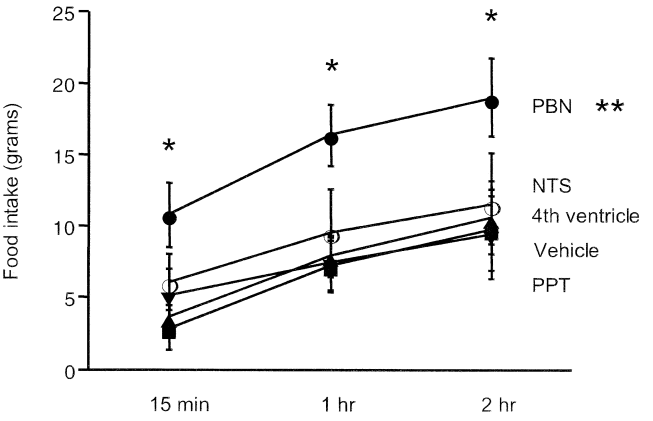


Fig. 2. Microinjections of midazolam (7.5 μg) into the parabrachial nucleus (PBN), nucleus of the solitary tract (NTS), pedunculopontine nucleus (PPT), and the fourth ventricle (experiment 1). Amount of food consumed after the time course 15 min, 1 h and 2 h. Midazolam (7.5 μg) into the PBN increased food intake over NTS, PPT, and the fourth ventricle ($P<0.001$). The results are presented as mean±S.E.M. An asterisk denotes significant elevations in food intake relative to vehicle.

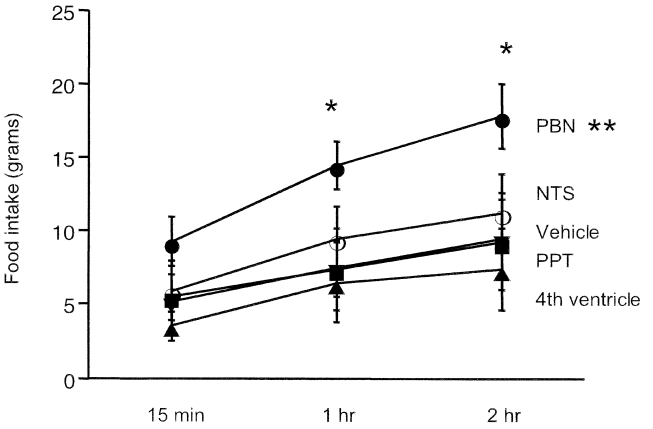


Fig. 3. Microinjections of midazolam (15 μg) into the parabrachial nucleus (PBN), nucleus of the solitary tract (NTS), pedunculopontine nucleus (PPT), and the fourth ventricle (experiment 1). Amount of food eaten after the time course 15 min, 1 h and 2 h. Midazolam (15 μg) into the PBN increased food intake over NTS, PPT, and the fourth ventricle ($P<0.001$). The results are presented as mean±S.E.M. An asterisk denotes significant elevations in food intake relative to vehicle condition.

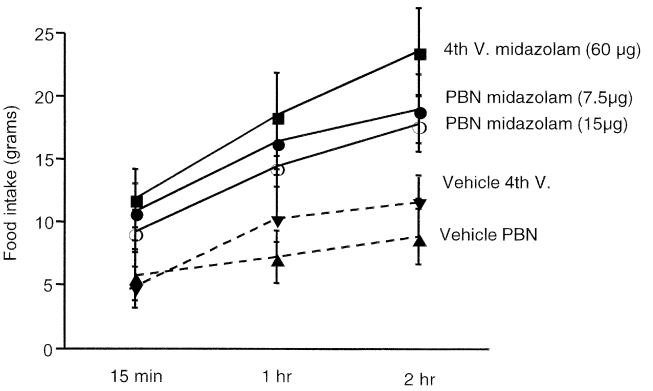


Fig. 4. Microinjections of midazolam into the parabrachial nucleus (PBN; 7.5 and 15 μg) and a high dose of midazolam (60 μg) into the fourth ventricle (experiment 1). Amount of food consumed after the time course 15 min, 1 h, and 2 h. There were no differences in food intake between the two groups. The PBN animal's intake was similar to the animals that received the higher fourth ventricle dose. The results are presented as mean±SEM.

1 h ($F(3,23)=5.3$, $P<0.006$), and 2 h ($F(3,23)=3.1$, $P<0.05$).

By contrast, feeding was not increased by midazolam at either of these doses in the NTS group ($F(2,20)=2.68$, $P>0.12$), the PPT group ($F(2,24)=1.65$, $P>0.23$), or the fourth ventricle group ($F(2,24)=2.1$, $P>0.2$). A two-way ANOVA (site×dose) revealed that intake differed by site at 15 min ($F(6,46)=2.9$, $P<0.02$), 1 h ($F(6,46)=6.9$, $P<0.0002$), and 2 h ($F(6,46)=4.6$, $P<0.001$).

For explicit comparison of the PBN site to other microinjection sites, an LSD post-hoc test revealed that the PBN group ate significantly more than all the other groups after midazolam at 15 min ($P<0.05$ each) and 1 h ($P<0.05$ each). At 2 h after the midazolam microinjection the PBN group had eaten more than either the PPT group ($P<0.03$) or the fourth ventricle group ($P<0.01$), and marginally more than the NTS group ($P=0.08$).

#### *2.2.1. High dose (60 μg) fourth ventricle versus low dose PBN (7.5 and 15 μg) comparison*

Animals in the fourth ventricle group were also tested for a high midazolam dose (60 μg) to compare the magnitude of feeding enhancements produced by suprathreshold doses given respectively to the fourth ventricle and the PBN, and provide initial information on the relative role of the PBN in feeding produced by fourth ventricle benzodiazepine administration. For example, if the magnitude of feeding produced by 60 μg in the fourth ventricle was much greater than that produced by 7.5 μg in the PBN, it would suggest that other brainstem sites besides the PBN also contribute to fourth ventricle effects. However, when compared in a separate one-way ANOVA there were no significant differences in the magnitude of food intake enhancement produced by the 60 μg dose of midazolam given in the fourth ventricle versus the 7.5 μg dose of midazolam given in the PBN animals after 15 min (Fig. 4; $F(1,12)=0.1$, n.s.), 1 h ($F(1,12)=0.4$, n.s.) and 2 h ($F(1,12)=1.2$, n.s.). Similar results were found after administration with the 15 μg dose (15 min ($F(1,12)=0.9$, n.s.), 1 h ($F(1,12)=1.5$, n.s.), or 2 h ($F(1,12)=2.1$, n.s.). Thus a low 7.5 μg or 15 μg dose of midazolam administered to the PBN produced a similar magnitude of increase in eating behavior as a much greater 60 μg dose administered to the fourth ventricle.

### *2.3. Discussion*

Food intake was increased by microinjections of midazolam (either 7.5 or 15 μg) into the parabrachial nucleus of the pons, which is consistent with the earlier report of Higgs and Cooper [36]. The increase in feeding behavior at these low doses was fully equivalent to the increase produced by a much higher 60 μg dose administered to the fourth ventricle. The ability of a 7.5 μg dose administered to the PBN to increase food intake as much as a 60-μg dose administered to the fourth ventricle suggests that the fourth ventricle microinjection may not recruit multiple other brain sites of action in addition to the PBN, at least not in a way that boosts eating behavior over and above what can be achieved by PBN receptor activation. Instead it suggests that activation of benzodiazepine receptors in the PBN alone may be able to increase food intake as much as a microinjection that reaches a broader anatomical distribution of brainstem sites. It would be important to compare actual dose–response curves obtained from these two sites in order to have strong confidence in this conclusion, but the present data are at least suggestive. The conclusion that the PBN may be especially important relative to other brainstem sites in mediating increases in food intake produced by benzodiazepine administration is also supported by the finding

that food intake was not increased by the 7.5- or 15-μg midazolam doses when administered to either NTS or PPT. These results taken as a group support the hypothesis that the PBN is a chief brainstem site for mediating benzodiazepine-induced increases in food intake.

A question that remains is whether low-dose brainstem microinjections into PBN that are able to increase food intake do so by increasing the hedonic impact of the taste of food. In other words, do rats 'want' and eat food more because PBN administration of benzodiazepine makes them 'like' food more? If so, then any microinjection of midazolam into PBN that increases food intake should also increase hedonic affective reactions to a sweet solution in the taste reactivity test. This was tested in experiment 2 using the taste reactivity technique for measuring the hedonic impact of food reward (for review of taste reactivity as a measure of hedonic impact see Ref. [7]). Experiment 2 focused on the PBN site, since the PBN was the only brainstem site that showed an enhancement of feeding behavior after low doses of midazolam in experiment 1.

## 3. Experiment 2

### *3.1. Method*

#### *3.1.1. Procedure: affective reactions to a bittersweet mixture of sucrose (7%) and quinine (0.01%) after microinjections of midazolam into the PBN*

Rats that showed an increase in food intake in experiment 1, those who received microinjections in the PBN ($N=8$), were selected to be tested for hedonic and aversive taste reactivity patterns to oral infusions of a bittersweet stimulus after the same microinjection treatment used in the feeding test. The taste stimulus used for oral infusions was a bittersweet solution made of 7% sucrose and 0.01% quinine hydrochloride (1 ml), infused into the mouth at a rate of 1 ml/min. A mixture of sucrose–quinine was used in order to elicit both hedonic and aversive reactions in a single taste reactivity test [5], so that the effect of midazolam could be observed simultaneously on both positive and negative affective valence with a minimum number of intracranial microinjections.

After habituation to the taste reactivity-test apparatus each rat received microinjections of either vehicle or midazolam (0, 7.5, or 15 μg) over a course of 6 days (48 h between each drug treatment). Each rat received only one drug and one behavioral test per day (consisting of three oral infusions of 1 ml each) and the order of drug administration and dose was randomized across subjects.

#### *3.1.2. The taste reactivity test and scoring*

The taste reactivity testing took place in Plexiglas cylinder (10 inches in diameter and 15 inches in height) resting on a transparent plastic base. The animal's face was videotaped through a mirror positioned beneath the base of the transparent chamber. The PBN group in experiment 1 had been habituated to the test cage on 3 days (2 h periods/day) before the first test. On habituation sessions the subject received three intraoral infusions of water, 1 ml/min, to get used to the procedure. Immediately after a microinjection, the rat's oral cannulae were connected to a length of PE60 tubing, and it was placed in the test apparatus. After 15 min, a 1 ml infusion of the sucrose–quinine solution (20°C) was delivered to the rat's mouth by a syringe pump at a constant rate during a 1-min period. Taste reactivity patterns were videotaped for subsequent slow-motion analysis. This procedure was repeated 45 min later (1 h after the microinjection) and again at 2 h after the microinjection.

The behavior of each rat was scored in slow motion (1/30 to 1/10 normal speed) for the occurrence of hedonic and aversive taste reactivity components [4,8,30]. Hedonic reaction patterns were paw licking, lateral tongue protrusions (non-rhythmic lateral tongue extensions), and tongue protrusions (rhythmic forward tongue extensions). Aversive reactions were gapes (triangular wide opening of the mouth), chin rubs (bringing the mouth in contact with the floor while projecting the body forward), face washing (either a single wipe over the face with the paws or a bout of several wipes), forelimb flails (rapid side to side flailing of the forepaws), paw treading (planting of the limbs on the floor and alternating forceful strikes forward and back), and head shakes (rapid shaking of the head from side to side). For the purpose of quantifying the number of responses emitted, discrete actions such as lateral tongue protrusions, gapes, chin rubs, forelimb flails, head shakes, bouts of face washing, paw treading, and locomotion were counted each time they occurred. Continuous actions that typically persist for relatively long periods were counted as follows [30]: paw licks, mouth movements, face washing, and locomotion were counted in 5-s bins (any occurrence of these behaviors up to 5-s in duration was counted as a single occurrence). Rhythmic tongue protrusions were scored in the same way in 2-s bins. Use of this procedure allows different reactions to be compared, and to make proportionate contributions to a combined hedonic total or to a combined aversive total.

### *3.2. Results*

Fig. 5 shows that microinjections of midazolam into PBN significantly enhanced the number of hedonic reactions (tongue protrusions, lateral tongue protrusions, and paw licking) elicited by the oral infusion of the sucrose–quinine solution ($F(2,28)=5.9$, $P<0.01$; drug×dose×time after microinjection). Post hoc comparisons revealed that the hedonic reactions were higher than vehicle both after the 7.5-μg dose ($P<0.02$) and after the 15-μg dose ($P<0.02$). There was a significant enhancement of hedonic reaction patterns by midazolam at 15 min after the

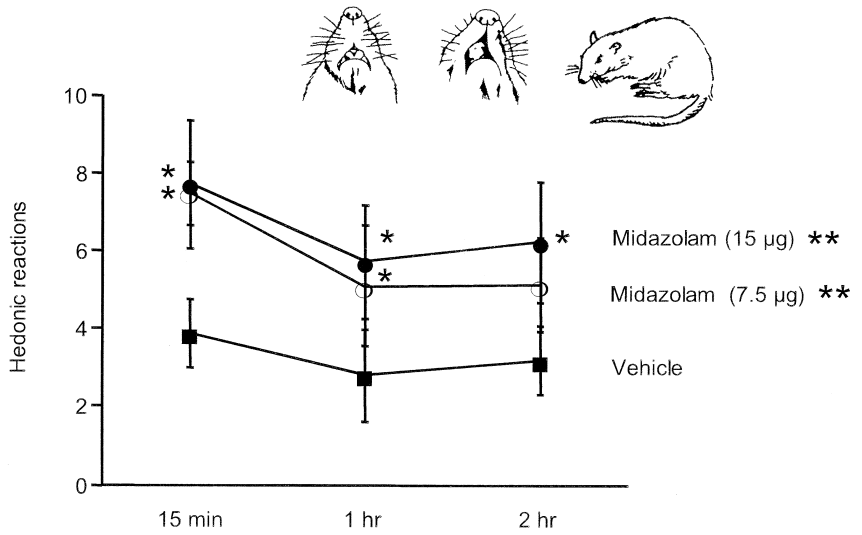


Fig. 5. Hedonic enhancement stimulated by microinjections of midazolam (7.5 and 15 μg) into the parabrachial nucleus (PBN) (experiment 2). Hedonic reactions of rats (rhythmic and lateral tongue protrusions, paw licking, etc.) emitted to oral infusions of a bittersweet solution (7% sucrose–0.01% quinine) after microinjections of midazolam or vehicle into PBN. Midazolam enhanced the hedonic reactions to the test solution over the time course 15 min to 2 h ($P<0.01$). The results are presented as mean±S.E.M. An asterisk denotes significant elevations over vehicle levels.

microinjection ($F(2,14)=6.6$, $P<0.01$; one-way ANOVA) after both the 7.5-μg dose ($P<0.009$) and the 15-μg dose ($P<0.02$). Similarly, at 1 h there was a significant enhancement of hedonic reaction patterns by midazolam ($F(2,14)=3.73$, $P>0.05$) after the 7.5-μg dose ($P<0.03$) and after the 15-μg dose ($P<0.03$). By 2 h after the microinjection, however, there was no longer any difference in the number of hedonic reactions elicited by sucrose–quinine after midazolam compared to vehicle ($F(2,14)=2.5$, $P>0.1$). Aversive reactions did not increase or change after midazolam administration at any dose (Fig. 6).

## 4. General discussion

Our findings support the report by Higgs and Cooper [36] that the pontine parabrachial nucleus is a primary site within the brainstem where benzodiazepines elicit increases in food intake [35,36]. We found that 7.5 μg or 15 μg of midazolam into the parabrachial nucleus increased the intake of a palatable mash (baby cereal), whereas the same doses had no effect when delivered to the hindbrain nucleus of the solitary tract or to the tegmental pedunculopontine nucleus.

The increase in food intake produced by microinjections

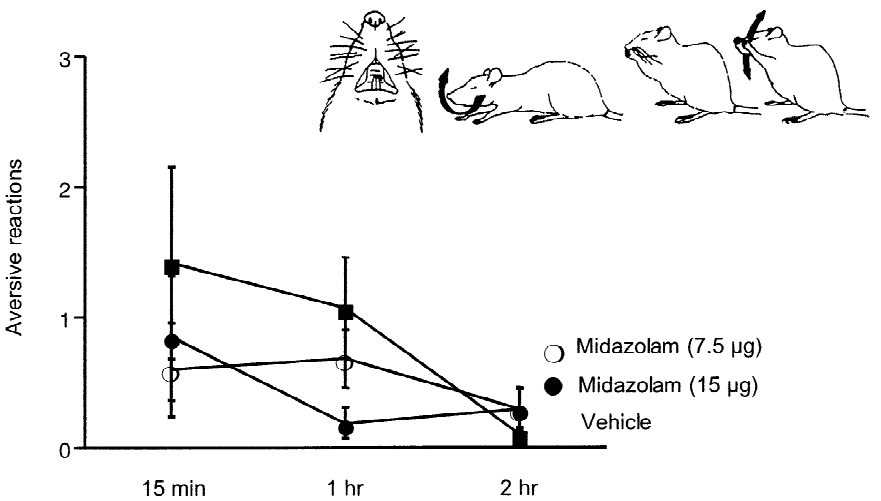


Fig. 6. Aversive reactions after microinjections of midazolam (7.5 and 15 μg) into the parabrachial nucleus (PBN) (Expt.2). Aversive reactions of rats (gapes, chin rubs, face washing, forelimb flails, paw treading, head shakes) emitted to oral infusions of a bittersweet solution (7% sucrose–0.01% quinine) after microinjections of midazolam or vehicle into PBN. Midazolam did not change the aversive reactions to the test solution over the time course 15 min to 2 h. The results are presented as mean±S.E.M.

of low 7.5- and 15-μg doses of midazolam into the PBN was fully equivalent in magnitude to the increase produced by a much larger 60-μg dose administered to the fourth ventricle, which would have influenced a much wider distribution of brainstem sites. This suggests that increases in food intake produced by benzodiazepines acting on the brainstem may be largely mediated by receptors within or near the PBN.

Further, our current results indicate that the hedonic impact of taste is also enhanced by midazolam microinjections in the parabrachial nucleus. The same 7.5- and 15-μg doses that increased voluntary eating also produced an increase in hedonic reaction patterns elicited by the sucrose component of a bittersweet taste after microinjection into the PBN. Aversive reaction patterns to the quinine component of the taste were not enhanced by midazolam in the PBN, indicating that the benzodiazepine had caused a selective increase in the positive hedonic palatability of the taste. Under some conditions, PBN midazolam might possibly reduce the number of aversive reactions (for example, if the vehicle baseline of aversion were higher), and it would be interesting in future experiments to examine whether midazolam into the PBN would suppress the number of aversive reaction elicited by a more concentrated quinine taste.

The time course of the hedonic palatability enhancement paralleled the time course of the eating enhancement. These results suggest that activation of benzodiazepine receptors in the parabrachial nucleus may cause increases in eating behavior partly because it causes food to be perceived as more palatable. This conclusion is compatible with the 'enhancement of palatability' hypothesis to explain the effects of benzodiazepines on food intake that was originally advanced by Cooper and Estall [16].

Benzodiazepine receptors alter neuronal function by promoting the function of GABA-A receptor channels [40]. The PBN serves as a gustatory relay nucleus in the rat, and has been reported to contain both GABA-A and GABA-B receptors [1,12,37]. Lesion studies of the parabrachial nucleus have shown to disrupt aspects of feeding behavior and to similarly disrupt hedonic and aversive taste reactivity patterns. For example, Flynn et al. [24,25] and Spector [58] showed that bilateral lesions of the PBN disrupted intake preference tests, disrupted the ability to form conditioned taste aversions, and abolished the normal effect of sodium deficiency on taste reactivity patterns to NaCl. The results of those and subsequent experiments have been interpreted by Norgren, Spector, and their colleagues to indicate that the PBN is an important site for processing of taste related to eating behavior and palatability [27–29,31,57,59].

Our results do not rule out the possibility that brainstem sites other than the PBN also contain receptors that contribute to the hyperphagia and palatability effects of benzodiazepines. It is even possible that the other sites we investigated here might participate in the hyperphagic and hedonic effects of benzodiazepines, even though our present data are negative regarding their role in food intake. Further studies with other benzodiazepines and with other doses will be needed to provide a conclusive answer as to whether brainstem sites outside the PBN make any contribution to benzodiazepine-induced enhancement of ingestive behavior. However, our present data suggest that midazolam, at least, is more effective at increasing food intake in the PBN than in those other sites.

In conclusion, it seems that the PBN is an especially important brainstem site underlying both the increase in food intake and the increase in hedonic impact of sweet tastes caused by benzodiazepine administration. The fact that enhancement of 'liking' for food can be mediated by the parabrachial nucleus at the same doses that increase 'wanting' for food, suggests that palatability enhancement is one psychological mechanism through which benzodiazepine stimulation of the PBN causes an increase in eating behavior.

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