

Research report

Regional differences in feeding and other behaviors elicited by *N*-methyl-D-aspartic acid in the rodent hypothalamus: a reverse microdialysis mapping study

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Abstract

Regional differences in the feeding stimulatory actions of hypothalamically delivered *N*-methyl-D-aspartate (NMDA) were investigated. NMDA (660 μ M intraprobe) delivered by reverse microdialysis into the tuberal lateral hypothalamus (tLH) reliably elicited feeding in satiated rats. The average food intake was 8.6 g in 50 min, and during the infusion rats spent 26% of the time eating, compared to less than 1% before NMDA treatment. In contrast, NMDA did not affect feeding when reverse dialyzed into the anterior LH (aLH), posterior LH (pLH) or the medial hypothalamus (MH). NMDA had no apparent behavioral effect in the aLH; in contrast, it significantly decreased the time spent resting/sleeping when infused into each of the other three areas tested. Additionally, in the medial hypothalamus, NMDA infusions increased time spent grooming; while in the pLH only alertness was significantly increased. These data underscore the functional and anatomical heterogeneity of the hypothalamus, and implicate glutamate and NMDA receptors in different portions of the hypothalamus in the control of eating, grooming and arousal. © 2002 Elsevier Science B.V. All rights reserved.

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Topic: Ingestive behaviours

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1. Introduction

Accumulating evidence suggests that glutamate and *N*-methyl-D-aspartate (NMDA) receptors in the lateral hypothalamus (LH) may be involved in regulating food intake and body weight. Glutamate or NMDA microinjected into this region elicited dramatic increases in food intake in satiated rats, while NMDA receptor antagonists such as D-(–)-2-amino-5-phosphonopentanoic acid (D-AP5), 7-chlorokynurenic acid and ifenprodil injected there suppressed feeding elicited by NMDA [10,24–26]. Additionally, acute LH microinjections of D-AP5 suppressed nocturnal and deprivation-induced eating, and repeated injections

of this antagonist suppressed daily food intake and caused body weight loss [25]. NMDA also elicited feeding when administered into the LH by reverse microdialysis. However, in contrast to the feeding and associated behavioral hyperactivity elicited by central microinjection of this agonist, reverse microdialysis of low concentrations of NMDA produced a strong and natural-appearing eating response, without concomitant hyperactivity [6].

The orexigenic actions of glutamate and NMDA appear to be specific to the LH, since neither of these compounds had any significant effects on feeding when microinjected into surrounding brain regions [23]. However, the LH, which is bounded rostrally by the preoptic area of the hypothalamus, caudally by the ventral tegmental area, medially by the fornix and laterally by the optic tract, is a comparatively large structure. The entire rostral-caudal extent of the LH in rats is over 3 mm, and its exact size is

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difficult to determine because of the lack of clear anatomical boundaries between the LH and adjacent brain regions [16,22]. Additionally, the extent of NMDA diffusion after central microinjections is unknown. As a result, the precise region(s) of the LH involved in the control of eating behavior have not been established. In an attempt to identify subregions of the LH sensitive to the feeding-stimulatory actions of NMDA, reverse microdialysis was used to administer low concentrations of this agonist into discrete sites in LH and the medial hypothalamus (MH). We report here that the tuberal LH (tLH) is particularly sensitive to the feeding-stimulatory actions of NMDA administered by reverse microdialysis, and that NMDA has very little or no effect on feeding when dialyzed into nearby anterior or posterior portions of the LH or into the adjacent MH. Grooming and alertness were also increased by NMDA infusions, however, the locus for these effects was the MH and posterior LH (pLH), respectively. Portions of these data have been presented previously in preliminary form [5].

2. Methods

2.1. Subjects and surgery

Adult, male Sprague–Dawley rats ($n=22$) weighing from 350 to 550 g at the time of surgery were used. These rats were bred in the University of California, Riverside, Psychology Department vivarium and were descended from rats obtained from Charles River. They were individually housed in a temperature controlled room on a 12:12-h light–dark cycle and allowed free access to standard Purina rat chow pellets and water.

Under Metofane or Nembutol (50 mg/kg, i.p.) anesthesia, a unilateral, stainless-steel guide cannula (21 gauge) was stereotaxically implanted into either the anterior LH (aLH), tLH, pLH, or MH of each subject. The intended coordinates for each brain region, according to the rat brain atlas of Paxinos and Watson [17], are shown in Table 1.

Table 1
Stereotaxic coordinates for each brain region tested

Brain structure	<i>n</i>	Anterior (mm)	Lateral (mm)	Ventral (mm)
Anterior lateral hypothalamus	5	7.2	1.8	6.0 (9.5)
Tuberal lateral hypothalamus	8	6.1	1.8	6.2 (9.7)
Medial hypothalamus	4	6.8–5.8	1.0	6.5 (10.0)
Posterior lateral hypothalamus	5	5.5	1.8	6.0 (9.5)

Anterior, to interaural line; Lateral, to midline; Ventral, to skull surface. The incisor bar was 3.3 mm below the center of the earbars. The numbers in parentheses indicate the intended location of the probe tip which always extended 3.5 mm beyond the tip of the guide cannula.

The cannula and a small metal plate designed to protect the dialysis probe from damage during testing were secured to the head by skull screws and dental cement, along with a small screw for attaching a tether. Dialysis probes were designed to extend 3.5 mm beyond the cannula, putting the probe tip into the desired hypothalamic region. Rats were allowed to recover in their home cages for at least 5 days following surgery.

Three days prior to testing, the standard pellet food was replaced with a sweetened milk-mash diet consisting of Purina rat chow powder (500 g), sucrose (400 g) and Carnation Evaporated Milk (one 354 ml can). This palatable test diet was chosen because it is the standard diet that we have used in the past to study NMDA-induced feeding, and we wanted the current study to be comparable to those published previously [10,23–26]. At least 18 h prior to testing, subjects were moved to the dialysis chambers, where they were connected by a tether to a counterbalanced liquid swivel that allowed for free movement about the cage. Rats obtained food through an opening in the side of the cage that led to a food bowl connected to a scale for continuous monitoring of food intake. Rats remained in these chambers for the duration of the experiment.

2.2. Reverse microdialysis of NMDA

A more detailed account of the reverse microdialysis procedure can be found elsewhere [6]. Briefly, on the morning of the experiment, concentric dialysis probes were inserted into the guide cannula and continuously perfused at 5.0 $\mu\text{l}/\text{min}$ with standard artificial cerebrospinal fluid (aCSF). Approximately 2 h after the probe was inserted, testing began and the perfusion fluid was changed from pure aCSF to aCSF containing 660 μM NMDA (Sigma). Rats received NMDA at 5.0 $\mu\text{l}/\text{min}$ for exactly 10 min, after which the perfusion fluid was changed back to pure aCSF. Rats were given either one administration of NMDA or two administrations separated by 2–4 h.

2.3. Behavioral analysis

To determine which behaviors were elicited or suppressed by reverse microdialysis of NMDA and to assess potential behavioral hyperactivity, an observer sampled the rats' behavior at 1-min intervals. The behavioral analysis started 10 min prior to each infusion, continued during the 10 min NMDA treatment and ended 30 min later. Behavioral categories included eating, drinking, gnawing, locomotion, grooming, resting/sleeping and alertness. Alertness was defined as the animal supporting his weight on all four legs, perhaps sniffing, but without moving about the cage. Time spent engaging in each behavior was averaged over 5-min blocks and converted into a percentage of total time. Food intake in grams was measured at the end of the 50-min test period using standard Ohaus digital scales.

2.4. Histology and statistics

Animals were allowed to survive with the probe in place for 3–4 days after which time they were deeply anesthetized with Nembutol and perfused transcardially with heparinized saline followed by 4% formaldehyde in phosphate buffer. The brains were removed from the skull and placed in the same fixative for at least 24 h. They were then cryoprotected in 30% sucrose until sectioning. Frozen 50- μm thick horizontal or coronal sections were cut, mounted onto gelatin-coated slides, stained with thionin, and coverslipped for later examination under a light microscope. In all cases, relevant brain sections were traced onto sheets of paper in color using a projection microscope. Probe placements were determined by comparing these tracings along with the original sections to the rat brain atlas of Paxinos and Watson [17].

Food intake and behavioral data were analyzed using one-way ANOVA followed by Student–Newman–Keuls or Dunnett’s tests for multiple comparisons. A standard significance level of $P < 0.05$ was used for all tests.

3. Results

As described previously [6], NMDA reverse microdialyzed into the tLH of rats reliably elicited an intense eating response which typically began 5–7 min after the onset of a 10-min infusion and ended several minutes after the infusion stopped. However, systematic variations in probe placements within and adjacent to the LH dramatically altered NMDA’s effects. Specifically, as shown in Fig. 1, rats with probes located in the tLH ate an average of 8.6 g of food in the 50-min test period, significantly more than those with probes located in any of the surrounding regions tested, $F(3,18)=9.0$, $P < 0.001$. Fig. 2 shows representative photomicrographs of horizontal sections through the hypothalamus of four rats illustrating the location of the dialysis probe tip in each of the regions examined (see Table 1 for coordinates). As shown by the photomicrographs, the probe typically produced a well-defined, cylindrical tract with little apparent damage to the surrounding tissue. Fig. 3 shows that the probe tips of individual rats that ate in response to reverse dialysis of NMDA were clustered in the tLH between the fornix and the optic tract. This ‘effective’ region extended rostrally to the level of the posterior border of the paraventricular nucleus of the hypothalamus (PVN) and caudally to an area slightly anterior to the subthalamic nucleus. In contrast, probe placements 500–700 μm anterior or posterior to tLH, as well as further medial, produced very little or no NMDA-elicited feeding. Rats with probes placed in the aLH never ate in response to NMDA, and the small amounts of food eaten by rats with probes located in the pLH and MH were not significant (see Fig. 1).

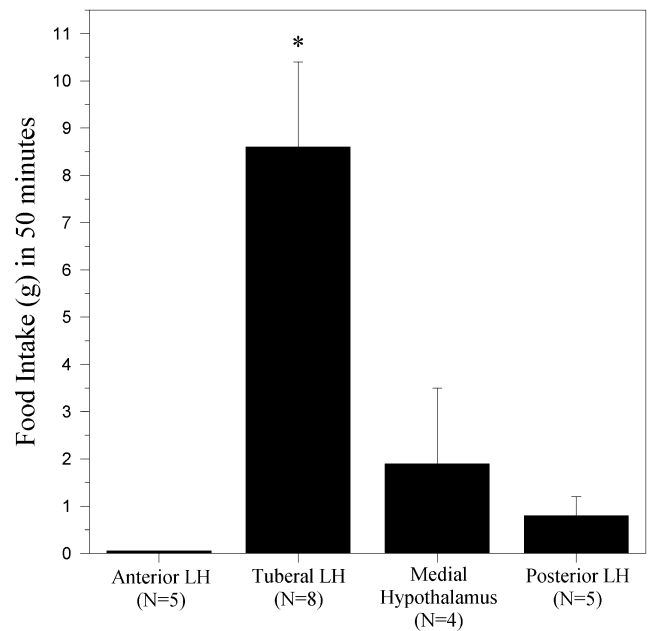


Fig. 1. Feeding elicited by reverse microdialysis of NMDA into four different hypothalamic regions. Black bars represent mean \pm S.E.M. food intake in 50 min produced by 660 μM NMDA. Asterisk (*) indicates significant differences from all other conditions, $P < 0.05$, Student–Newman–Keuls.

Behavioral analysis revealed that NMDA’s other effects also varied as a function of site of administration. Fig. 4 represents the average percentage of time spent engaged in each of seven behaviors in the 10 min during and after NMDA infusion. As mentioned above, NMDA reverse dialyzed into the tLH produced dramatic increases in food intake, along with concomitant increases in time spent eating (26 vs. <1% pre-NMDA), and decreases in time spent resting/sleeping (29 vs. 84% pre-NMDA), both of which were statistically significant (see Fig. 4). Indeed, in all regions tested, excluding the aLH, NMDA dramatically decreased time spent resting/sleeping, as compared to the pre-NMDA period, $F(4,19)=5.7$, $P < 0.005$, although there were no significant between-group differences for the tLH, pLH and MH, $F(2,9)=0.4$, $P > 0.10$. In addition, in the MH, NMDA increased time spent grooming (40%) compared to the pre-NMDA period (3%). The amount of time rats spent grooming in response NMDA treatment was significantly greater in the MH than in any other region tested, $F(4, 19)=8.6$, $P < 0.001$. NMDA in the pLH significantly increased alertness (37%) versus pre-NMDA (7%), $F(4,19)=11.8$, $P < 0.0001$. Small but significant increases in locomotion were observed after tLH NMDA infusions; however, this increase in motor activity may have been due to the animals moving towards the food bowl to eat. In contrast, NMDA reverse dialysed into the aLH had no apparent effect on any behavior, aside from non-significant decreases in resting/sleeping and increases in alertness.

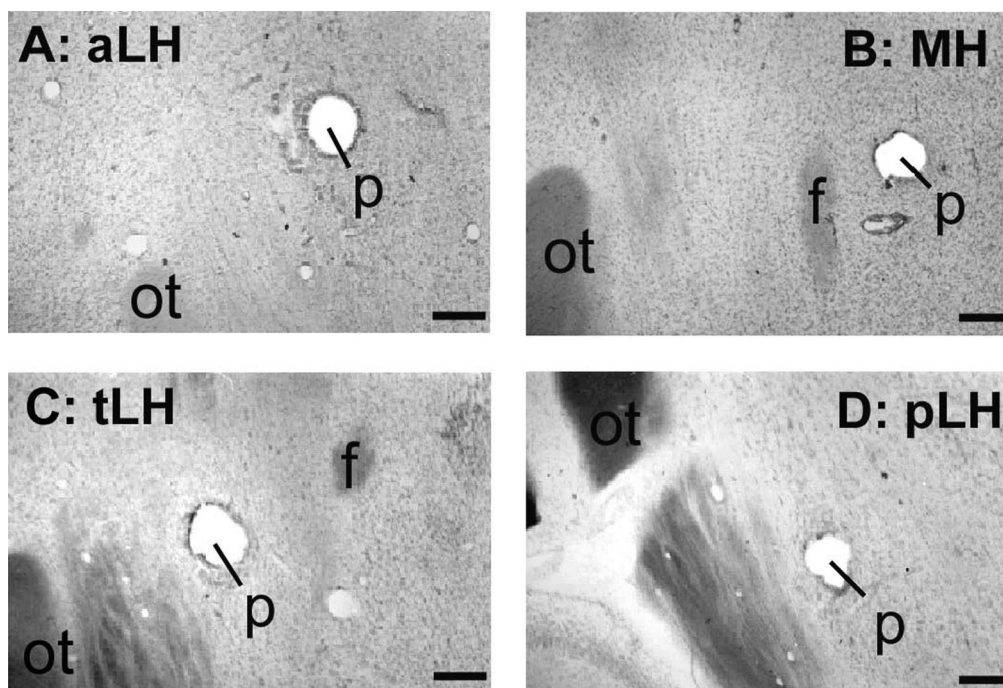


Fig. 2. Representative photomicrographs of microdialysis probe tip locations in the four hypothalamic regions tested. (A–D) Horizontal sections ($\times 50$) through the (A) aLH, (B) MH, (C) tLH, and (D) pLH. Scale bar in lower right equals 200 μm . Abbreviations: f, fornix; ot, optic tract; p, probe.

4. Discussion

Previous work by Stanley et al. [23] has shown that the LH is particularly sensitive to the orexigenic effects of injected glutamate and NMDA. More recently, we have shown that reverse microdialysis of NMDA into the same region also elicits feeding which resembles that occurring spontaneously in rats, without the marked behavioral hyperactivity associated with central microinjections [6]. The present study has confirmed these earlier findings and has more precisely delineated the region of the LH controlling the feeding response. This ‘effective site’ is located in tLH lateral to the fornix, extending from the posterior border of the PVN rostrally, to the anterior border of the subthalamic nucleus caudally (see Fig. 3); NMDA reverse dialyzed into this region was most effective in eliciting feeding in satiated rats. In contrast, NMDA had little or no effect on feeding when administered into surrounding regions as close as 500 μm , suggesting that the neurons mediating NMDA-elicited feeding are tightly clustered in this discrete region of the tLH. This region of the LH also contains the cell bodies or terminals of neurons which contain other appetite-regulating neurochemicals such as neuropeptide Y, agouti-related peptide, hypocretin/orexin (H/O) and melanin-concentrating hormone [2,7,21]. This anatomical relationship suggests the interesting possibility that LH glutamate and NMDA receptors may interact with one or more of these peptides in controlling eating.

The anatomical resolution afforded by reverse mi-

crodialysis appears to be better than that of central microinjection. Although an earlier study identified the LH as a primary locus of excitatory amino acid-elicited feeding, notable increases in food intake were observed after microinjection of NMDA in the PVN and pLH (mean of 7 g in 60 min for the pLH), presumably due to drug diffusion from these areas to the nearby LH [23]. In contrast, in the present study the maximum average feeding elicited by reverse microdialysis of NMDA into any other brain site was less than 2 g, suggesting a more limited spread of NMDA.

Although the actual extent of drug diffusion was not determined in the current experiment, diffusion of substances with reverse microdialysis is probably more restricted than with pressure injections. Jacobson and Hamberger [9] calculated that the extracellular concentration of kainic acid within a 0.5-mm radius of the dialysis probe tip could range from between 40 and 90% of that inside the probe, but noted that the actual value is probably much less because the accumulation of kainic acid in the vicinity of the probe and the presence of brain tissue would impede diffusion. Furthermore, the concentrations of KA (1–10 mM) used by Jacobson and Hamberger [9] and NMDA (3.3 mM) by Stanley et al. [23,24] were many times greater than the concentration used here with reverse microdialysis. Thus the extent of diffusion of NMDA out of the probe in the current study would be expected to be corresponding less than in either of those studies. Also unlike pressure injections, no fluid is actually delivered into the brain with reverse microdialysis. Additionally,

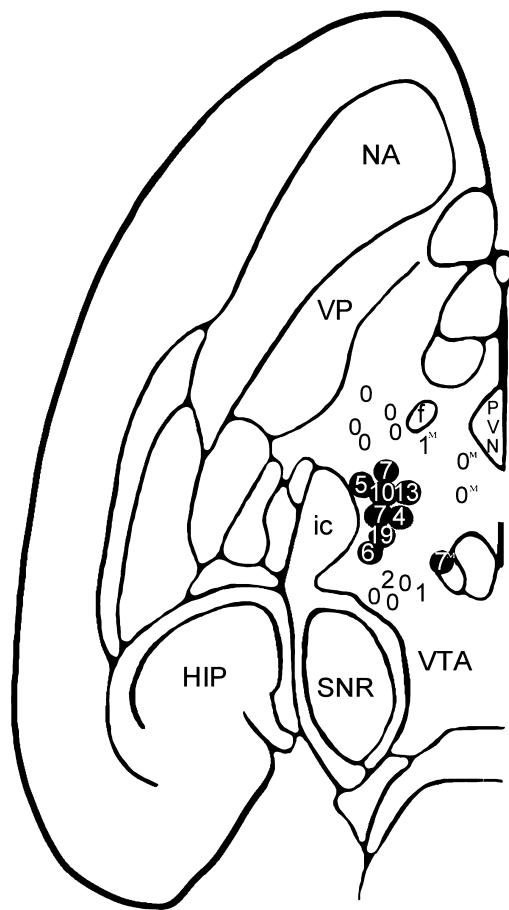


Fig. 3. Horizontal atlas section depicting food intake for each individual subject tested. Numbers indicate probe locations and elicited feeding, grams of food eaten in 50 min rounded to nearest whole number, from either one administration or the average of two administrations of 660 μ M NMDA. Scores over 3 grams are represented by filled circles. Medial group subjects denoted with an "M" next to the feeding score. Atlas section adapted from Paxinos and Watson [17]. Abbreviations: f, fornix; ic, internal capsule; HIP, hippocampus; NA, nucleus accumbens; PVN, paraventricular nucleus of the hypothalamus; SNR, substantia nigra; VP, ventral pallidum; VTA, ventral tegmental area.

other studies from our lab indicate that, when delivered by reverse microdialysis, the spread of the fluorescent tracer Fluorogold (FG) appears as an annulus about 1 mm in diameter around the probe tip [30]. This further suggests a very limited spread of NMDA, perhaps less than a 500 μ m radius, especially given that the FG concentration in the perfusate was 20 times greater than the NMDA concentration needed to elicit feeding in the same animal [30].

Was the NMDA-elicited feeding due to activation of LH NMDA receptors? Ligand binding and immunohistochemical studies have already demonstrated that NMDA receptors are present in the LH [15,18,19]. One study suggests that some LH NMDA receptors containing the NR2A and/or NR2B subunits may be of particular importance to feeding control. Specifically, Khan et al. [10] immunohistochemically localized the NR2B subunit to the LH and demonstrated that ifenprodil, an antagonist selec-

tive for NMDA receptors with NR2A/B subunits, suppressed NMDA-elicited and deprivation-induced eating. Furthermore, microinjections of the NMDA receptor antagonist D-AP5 blocked NMDA-elicited feeding without affecting feeding produced by LH microinjections of kainic acid or D,L- α -amino-3-hydroxy-5-methyl-isoxazole propionic acid [25]. These studies suggest that feeding elicited by reverse microdialysis of NMDA into the tLH is most likely due to this agonist acting specifically at NMDA receptors. Further, we have shown that *N*-methyl-L-aspartate, a less potent NMDA receptor agonist, had no effect on feeding [6], arguing that the feeding stimulation in the present study was, indeed, NMDA receptor mediated.

The dramatic differences we observed in NMDA-elicited behaviors in different regions of the hypothalamus underscore the functional heterogeneity of this brain region. That NMDA was without effect in the aLH suggests either that neurons in this region do not possess functional NMDA receptors or that the consequences of NMDA receptor activation in this region are not apparent in our tests. The latter is more likely, given the ubiquitous distribution of glutamate and NMDA receptors [18,19]. Increased grooming seen after NMDA infusion into the MH supports previous work showing that central microinjections of NMDA into the vicinity of the PVN resulted in the same behavior [20], again supporting the notion of functional heterogeneity. The increased alertness observed in response to NMDA in the pLH is intriguing, although the importance of this finding is unclear because some level of alertness or arousal is necessary for the other behaviors to be expressed. Furthermore, alertness can be characterized as a state of arousal, and in three of the four regions tested (tLH, pLH and MH), NMDA decreased resting/sleeping, or in other words, increased arousal.

Interestingly, the LH and more specifically the pLH have already been implicated in the control of sleep and sleep-related disorders. The pLH has been shown to receive direct projections from the suprachiasmatic nucleus of the hypothalamus [1], and the activity of some neurons in the rodent pLH is correlated with the different stages of sleep [27]. Furthermore, the novel neuropeptide H/O, which is produced almost exclusively by LH neurons [3,21] and was first heralded as a feeding regulatory transmitter [4,21,29], may also be involved in arousal and sleep processes. Dogs and mice with genetic mutations in the H/O system displayed narcoleptic-like symptoms [12,13], while intracerebroventricular injections of H/O increased arousal and decreased sleep in rodents [8]. Results from the current study suggest that LH glutamate and NMDA receptors may operate in conjunction with hypothalamic H/O systems in the control of sleep, given that both NMDA and H/O act to increase arousal. Evidence for this hypothesis comes from the work of van den Pol et al. [31] who demonstrated that H/O acted pre-synaptically in the hypothalamus to increase glutamate-mediated excitation of some LH neurons. Thus, hypo-

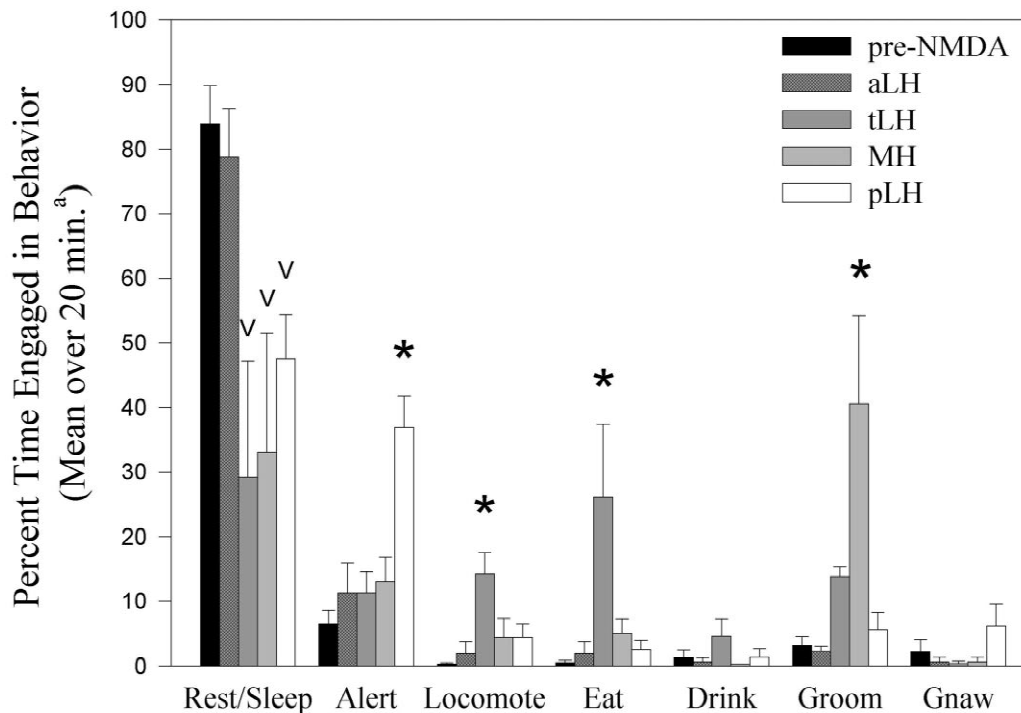


Fig. 4. Other behaviors affected by reverse microdialysis of NMDA into the hypothalamus. Vertical bars represent mean \pm S.E.M., percent time spent engaging in one of seven behaviors averaged over 20 min, 10 min of NMDA infusion and 10 min after the infusion, across four different regions tested. 'V' denotes significant difference from pre-NMDA period by Dunnett's test, $P < 0.05$. Asterisk (*) indicates significant differences from all others in same group, $P < 0.05$, Student–Newman–Keuls. *Note, pre-NMDA is 10 min average for behaviors occurring during the period before NMDA treatment for all groups.

thalamic H/O may increase glutamate transmission in the pLH leading to increases in alertness or arousal as seen in the present study.

With the behavioral consequences of NMDA being so different across the hypothalamic regions tested, particularly in the LH itself, it is likely that the neuroanatomical substrates mediating these behaviors are also different. Some evidence already exists suggesting that aLH, tLH and pLH may have different efferent projection patterns [22,32]. Preliminary work from our lab suggests that regions of the LH where NMDA elicits feeding may receive a different pattern of afferent inputs than regions of the LH where NMDA is without effect. FG reverse microdialyzed into the tLH 'effective site' resulted in reliable retrograde labeling in several areas of the brain including in the nucleus accumbens (NA) and median preoptic hypothalamus, two areas poorly labeled in rats with aLH, pLH or MH probe placements [5]. We are also examining efferent connections using fos protein expression as a neuronal marker, and preliminary results indicate that NMDA-elicited eating in the LH is also associated with increases in fos-like immunoreactivity in the NA [11]. A functional feeding circuit including the NA and the LH has already been described [14,28], and according to the authors, glutamate and GABA are important neurochemical constituents of this circuit. Thus the tLH, along with the NA and other structures, may be part of a larger

feeding circuit utilizing glutamate as one possible neurotransmitter. LH glutamate and NMDA receptors may also be involved in arousal and sleep regulation, a possibility deserving of future study.

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