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Research report

Behaviorally specific versus non-specific suppression of accumbens shell-mediated feeding by ipsilateral versus bilateral inhibition of the lateral hypothalamus

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HIGHLIGHTS

- The behavioral phenotype of LH inhibition that suppresses AcbSh feeding is unclear.
- Bilateral LH D-AP5 or muscimol halts AcbSh feeding by inducing sleep.
- Ipsilateral LH D-AP5 or muscimol halts AcbSh feeding without affecting sleep.
- Bilateral, but not ipsilateral, LH inhibition has non-specific behavioral effects.
- Results solidify an ipsilateral, feeding-specific connection between AcbSh and LH.

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ABSTRACT

The nucleus accumbens shell (AcbSh) and lateral hypothalamus (LH) are linked in the control of food intake. Pharmacological inhibition of the LH may block AcbSh-elicited feeding, but the behavioral phenotype associated with this feeding suppression is unknown. To examine this phenotype, adult male Sprague-Dawley rats were implanted with three cannulas – one unilaterally in the AcbSh and two bilaterally in the LH – to allow for central drug injections. The AcbSh received injections of the AMPA receptor antagonist DNQX or the $GABA_A$ receptor agonist muscimol, while the LH received injections of the NMDA receptor antagonist D-AP5 or muscimol. Eating, drinking, grooming, locomotion, quiescence, and sleeping behaviors were measured every minute for 60 min post-injection. From these observational data, feeding bout durations, feeding frequency, and latency to feed were determined. AcbSh muscimol or DNQX increased food intake by increasing feeding bout durations and frequency and decreasing latency to feed. D-AP5 or muscimol, injected into the LH bilaterally or ipsilateral to the AcbSh injection, reversed these AcbSh-mediated effects. Though bilateral LH D-AP5 or muscimol injections blocked feeding responses, they also hastened onset of sleep. In contrast, ipsilateral LH D-AP5 or muscimol injections suppressed AcbSh-mediated feeding behaviors without substantially altering sleeping or other behaviors. These results suggest bilateral LH inhibition via NMDA receptor blockade or $GABA_A$ receptor activation produces behavioral effects that might indirectly suppress feeding, but ipsilateral LH inhibition through these receptors suppresses AcbSh AMPA and $GABA_A$ receptor-mediated feeding specifically. This evidence strengthens the concept of a feeding-specific association between these regions.

1. Introduction

The prevalence of obesity in developed nations has led to much research that investigates the neural circuitry governing food intake. A notable intake-controlling brain region is the lateral hypothalamus (LH). Early studies showed that LH lesions produce hypophagia, while electrical stimulation of the LH elicits eating [1,2]. Studies from our lab implicate the LH as a key brain region through which glutamate and gamma-aminobutyric acid (GABA) regulate feeding. Specifically, activation of *N*-methyl-D-aspartate receptors (NMDARs) or blockade of GABA subtype A receptors ($GABA_A$Rs) will induce feeding, and will do so with behavioral specificity [3–5]. Mapping studies show that the LH is a primary site for these effects [6,7]. Also, LH administration of the NMDAR antagonist D-AP5 or the $GABA_A$R agonist muscimol will suppress drug-elicited, nocturnal, and deprivation-induced feeding in rats [4,8]. Food intake mediated through the LH is thought to be regulated in part by the balance between synaptic glutamate and GABA

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levels in this region [9]. Thus, glutamate and GABA act within the LH to coordinate the initiation and cessation of appetitive responses.

Although initially regarded as a regulator of reward, substantial research also implicates the nucleus accumbens shell subregion (AcbSh) in controlling food intake [10–12], with glutamate and GABA also mediating feeding through this region. Within the AcbSh, blockade of 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid receptors (AMPARs) with DNQX or activation of $GABA_A$Rs with muscimol induces food intake, an effect not mediated by surrounding brain regions [10,12]. Further data demonstrates that the induced food intake is specific to eating and not due to behavioral hyperactivity [11,12]. Additionally, action potential activity ceases in certain AcbSh neurons prior to initiation of sucrose intake [13], further solidifying the AcbSh's role in natural feeding processes.

Much research links the AcbSh and the LH in the control of eating, especially studies in which bilateral inhibition of the LH with D-AP5 or muscimol blocks AcbSh DNQX- or muscimol-elicited feeding [10,14,15]. Also, unilateral AcbSh muscimol induces c-fos expression in the ipsilateral but not contralateral LH [16]. Retrograde tract tracer infusion into the LH labels ipsilateral AcbSh neurons, suggesting that a connection between the nuclei may be direct and ipsilateral [17]. Most importantly, unilateral AcbSh muscimol-elicited eating can be suppressed by ipsilateral LH lesions [18] or by ipsilateral LH $GABA_A$R activation or NMDAR blockade [15], an effect not shared by such receptor modulation in the contralateral LH. These studies suggest that the AcbSh requires normal LH functioning to produce feeding behaviors, and that these nuclei may have a feeding-specific relationship.

However, the LH controls functions in addition to feeding. Studies using bilateral lesions have shown behavioral deficits aside from hypophagia. For example, bilateral LH lesions may produce adipsia, hypolocomotion, increased sedation, and neglect of or aversion to stimuli [19,20]. It has not been determined whether or not acute bilateral LH inhibition (via drug application) impacts behaviors other than feeding, though we did incidentally observe a decrease in overall activity in our prior study [15]. This issue is important when interpreting past studies that used bilateral LH inhibition to suppress natural, deprivation, or drug-induced food intake, as this suppression might have been an artifact of an indirect behavioral effect. These non-specific behavioral effects are not seen from unilateral LH lesions [18,21], but it is unclear if unilateral LH inhibition produces such non-specific effects, especially on AcbSh-mediated feeding. We hypothesize that bilateral LH inhibition with D-AP5 or muscimol halts AcbSh-mediated feeding non-specifically, whereas ipsilateral LH inhibition with D-AP5 or muscimol will halt this feeding specifically. Using behavioral assays, we assessed the behavioral phenotypes resulting from bilateral or unilateral LH D-AP5 or muscimol on normal and AcbSh DNQX- or muscimol-induced behaviors.

2. Methods

2.1. Subjects and housing

Forty adult male Sprague-Dawley rats received surgery for the experiments described in this paper. However, only the data from 29 rats were used, as the data from the remaining 11 were not included due to mistargeted guide cannulas. These subjects, weighing 350–450 g at the time of surgery, were individually housed and tested in wire mesh cages in a temperature-controlled vivarium at 21 °C with a 12 h light-dark schedule, with lights on at 9 AM. These subjects were maintained and tested on a sweetened milk-mash diet, which was presented to the animals in plastic food bowls and consisted of 39.3% Purina rat chow powder, 31.4% sugar, and 29.3% evaporated milk. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of California—Riverside.

Table 1
Injection paradigms.

Exp	*N*	Injection sites and drugs used	
		AcbSh	LH
1	6/8	DNQX (1.25 μg)	D-AP5 (2 μg bilaterally)
2	6/8	Muscimol (100 ng)	Muscimol (100 ng bilaterally)
3	8/12	DNQX (1.25 μg)	D-AP5 (2 μg unilaterally)
4	9/12	Muscimol (100 ng)	Muscimol (100 ng unilaterally)

Drugs were injected unilaterally into the AcbSh and either bilaterally or unilaterally injected into the LH. All drug doses are per hemisphere. Vehicle was a DMSO/aCSF mixture for DNQX and aCSF for all other drugs. N represents the number of subjects whose data was used out of the total number monitored in each experiment (as some were excluded due to mistargeted cannulas). All subjects in each experiment were tested with all drug combinations.

2.2. Surgical implantation of guide cannulas

Subjects under pentobarbital anesthesia (50 mg/kg body weight, i.p.) were stereotaxically implanted with three 18 mm length, 26 ga stainless steel guide cannulas, one directed at the AcbSh unilaterally (0.9 mm lateral, 2.3 mm anterior, and 5.8 mm ventral to bregma) and two into the LH bilaterally (±1.8 mm lateral, 2.9 mm posterior, and 8.2 mm ventral to bregma). Cold cure dental acrylic, anchored to the skull by stainless steel screws, secured the cannulas in place. A plastic guard was embedded in the dental cement to protect the cannulas, and 33 ga stainless steel obturators were inserted into the cannulas to maintain patency. Central injection tests commenced seven days after surgery to ensure full recovery. Prior to central injection tests, animals were handled multiple times and mock-injected to adapt them to testing procedures.

2.3. Central injection technique

A drug solution or vehicle was injected into the brain tissue in 0.3 μL volumes via 33 ga injectors protruding 1 mm beyond the end of the guide cannula. Vehicle consisted of artificial cerebrospinal fluid (aCSF), containing 147 mM Na^+, 154 mM Cl^-, 3 mM K^+, 1.2 mM Ca^{2+}, and 0.9 mM Mg^{2+}. The $GABA_A$R agonist muscimol (100 ng), the AMPAR antagonist DNQX (1.25 μg), and the NMDAR antagonist D-AP5 (2 μg) were used in this study; doses are per brain hemisphere. DNQX was dissolved in a 1:1 mixture of dimethylsulfoxide (DMSO) and aCSF. Drugs and dosages are based on our prior study [15], except that the dose of muscimol in the LH was increased to more effectively suppress AcbSh-elicited eating. Drug doses, site combinations, and number of subjects in each experiment are given in Table 1.

2.4. Experimental design for behavioral assessments

Subjects received fresh mash diet at the start of the light cycle to maximize satiety and injections commenced 1 h later. For Experiments 1 and 2, rats received vehicle or drug unilaterally into the AcbSh, then vehicle or drug bilaterally into the LH. In Experiments 3 and 4, AcbSh injections were the same, but LH injections utilized either vehicle on both sides, drug in the ipsilateral hemisphere and vehicle contralaterally, or vehicle ipsilaterally and drug contralaterally. To simplify graphical representation of the behavioral results in Experiments 3 and 4, groups receiving vehicle in the AcbSh and drug in either the ipsilateral or contralateral LH were merged into an AcbSh vehicle–unilateral LH drug group after determining that there were no significant behavioral differences between these treatments. Subjects were tested in every condition

in counterbalanced order over multiple days, each separated by a rest day without injections. Food intake was measured immediately prior to injections and 1 h post-injection. Subjects were only used in one experiment before sacrifice.

Behavioral observations lasted 70 min, starting 10 min prior to injection to assess baselines and ending 60 min post-injection to assess drug effects. Subjects were observed in their home cages pre- and post-injection by a trained rater that was unaware of which rats received which treatments. Each subject was monitored for 7 s/min in Experiments 1 and 2, and 5 s/min in Experiments 3 and 4. These time lengths were determined by dividing 60 s by the number of subjects in each experiment; Experiments 1 and 2 had 8 subjects each, while Experiments 3 and 4 had 12 subjects each (Table 1). During the 5–7 s monitoring period, only the single dominant behavior observed (the behavior the rat spent the most time engaging in) was recorded for each rat. The same rater was used for a given experiment across all experimental days to avoid inter-rater discrepancies in the observations. Minute-by-minute counts for individual behaviors were combined into 5 min bins.

Behaviors were classified as follows: eating—consuming food directly from the bowl or scooping food into paws and eating from them; drinking—licking water spout; grooming—licking body fur, scratching at the head using paws, or cleaning the tail; locomotion—any form of ambulation around the cage, including rearing; sleeping—inactive with eyes closed and curled into species-typical sleeping posture; quiescence—inactive but visible wakefulness, eyes open, alert, not in sleeping posture.

Feeding characteristics such as average bout duration, maximum bout duration, number of bouts, and latency to eat were also assessed. A feeding bout was defined as the interval over which a subject was continually observed to be eating. Bout characteristics were derived from the observed times at which eating commenced and stopped for each rat. For each subject, an average of the durations of multiple feeding bouts defined average bout duration, while the duration of the largest observed feeding bout defined maximum bout duration. These values were averaged across subjects within each treatment.

2.5. Nissl stain histology

Cannula placement was determined after completion of the behavioral tests. Subjects were anesthetized by overdose of sodium pentobarbital (400 mg/kg body weight, i.p.) and transcardially perfused with a 10% formalin solution. Brains were post-fixed in this solution for a minimum of two days, frozen with dry ice, sliced on a microtome into 100 μm thick coronal sections, and mounted onto glass microscope slides. Once dried, brain slices were stained with cresyl violet and coverslipped with Permount (Fisher Scientific). The Nissl stained sections were then studied to assess correct placement of guide cannulas. The sections were compared against templates made from a brain atlas [22]. Subjects possessing any cannula located more than 0.5 mm outside the LH or AcbSh were excluded. This distance was determined using a reverse-projection microscope and printed brain atlas sheets scaled to the size of the Nissl-stained slice projections. A rat required all cannulas to correctly target the AcbSh and the LH to be included in this study.

2.6. Statistical tests

Differences within each behavior between treatment groups over time were assessed for each experiment using a two-way repeated measures ANOVA with a $p < 0.05$ criterion for significance. When a significant main effect of treatment or a significant treatment by time interaction was found, Tukey's pairwise comparisons were conducted to localize the specific differences. Food intake characteristics (intake amount, feeding bout durations, latency to eat, and number of feeding bouts) were assessed using a one-way repeated measures ANOVA. Significant ANOVA results were followed by Fisher's pairwise comparisons to localize the specific differences.

3. Results

3.1. Treatment effects on feeding characteristics

AcbSh injections of DNQX or muscimol produced significant food intake in all experiments (Fig. 1A–D). Significant differences were detected between groups in all experiments (Exp. 1: $F(3,15) = 8.7$, $p < 0.001$; Exp. 2: $F(3,15) = 8.7$, $p < 0.001$; Exp. 3: $F(4,28) = 3.3$, $p < 0.05$; Exp. 4: $F(4,32) = 6.6$, $p < 0.001$). Bilateral (Fig. 1A) or ipsilateral (Fig. 1C) LH D-AP5 injection abolished AcbSh-mediated feeding, as did bilateral (Fig. 1B) or ipsilateral (Fig. 1D) LH muscimol.

In Experiments 1 and 2, ANOVA tests revealed significant effects of treatment on average feeding bout duration, maximum feeding bout duration, number of feeding bouts, and latency to eat (Table 2). In Experiment 1, AcbSh DNQX significantly increased all bout characteristics and decreased latency relative to control. Concurrent bilateral LH D-AP5 injection blocked these changes. In Experiment 2, AcbSh muscimol treatment significantly increased bout characteristics but did not significantly alter latency. Concurrent bilateral LH muscimol treatment abolished the bout characteristic increases, and increased latency to eat relative to AcbSh muscimol alone.

AVOVA showed significant effects of treatment on average bout duration, number of bouts and latency to eat in Experiment 3, and on average bout duration, maximum bout duration, number of bouts, and latency to eat in Experiment 4 (Table 2). In Experiment 3, AcbSh DNQX alone and AcbSh DNQX with concurrent contralateral LH D-AP5 significantly increased average bout duration and number of bouts while decreasing latency to eat. Ipsilateral LH D-AP5, given concurrently with AcbSh DNQX, significantly abolished these effects. In Experiment 4, AcbSh muscimol alone significantly increased maximum bout duration and number of bouts while decreasing latency. AcbSh muscimol with contralateral LH muscimol increased both average and maximum bout duration but did not significantly affect number of bouts or latency. Ipsilateral LH muscimol, injected with AcbSh muscimol, significantly decreased average bout duration relative to AcbSh muscimol with contralateral LH muscimol and also decreased maximum bout duration relative to both other AcbSh muscimol groups. However, ipsilateral LH muscimol treatment did not significantly alter other AcbSh muscimol-mediated feeding changes.

3.2. Bilateral LH inhibition interferes with feeding and other behaviors

In Experiment 1 (Fig. 2), main effects of treatment were significant for eating ($F(3,195) = 8.9$, $p < 0.001$), grooming ($F(3,195) = 3.4$, $p < 0.05$), sleeping ($F(3,195) = 13.8$, $p < 0.001$), and quiescence ($F(3,195) = 8.427$, $p < 0.01$), but not for locomotion or drinking (no drinking was observed). Time was significant for all behaviors (except drinking; not shown), and interaction of treatment by time was significant for eating, grooming, and sleeping, but not for locomotion or quiescence. As shown in Fig. 2A, AcbSh DNQX paired with bilateral LH vehicle produced feeding that was sustained from 5 to 20 min post-injection. Subsequently, a marked increase in sleeping was observed in this group (Fig. 2E). Importantly, co-treatment with bilateral LH D-AP5 suppressed this elicited feeding, but also accelerated the onset of sleep. Specifically, this treatment significantly increased sleep versus control from 20 to 40 min post-injection while significantly decreasing quiescence from 25 to

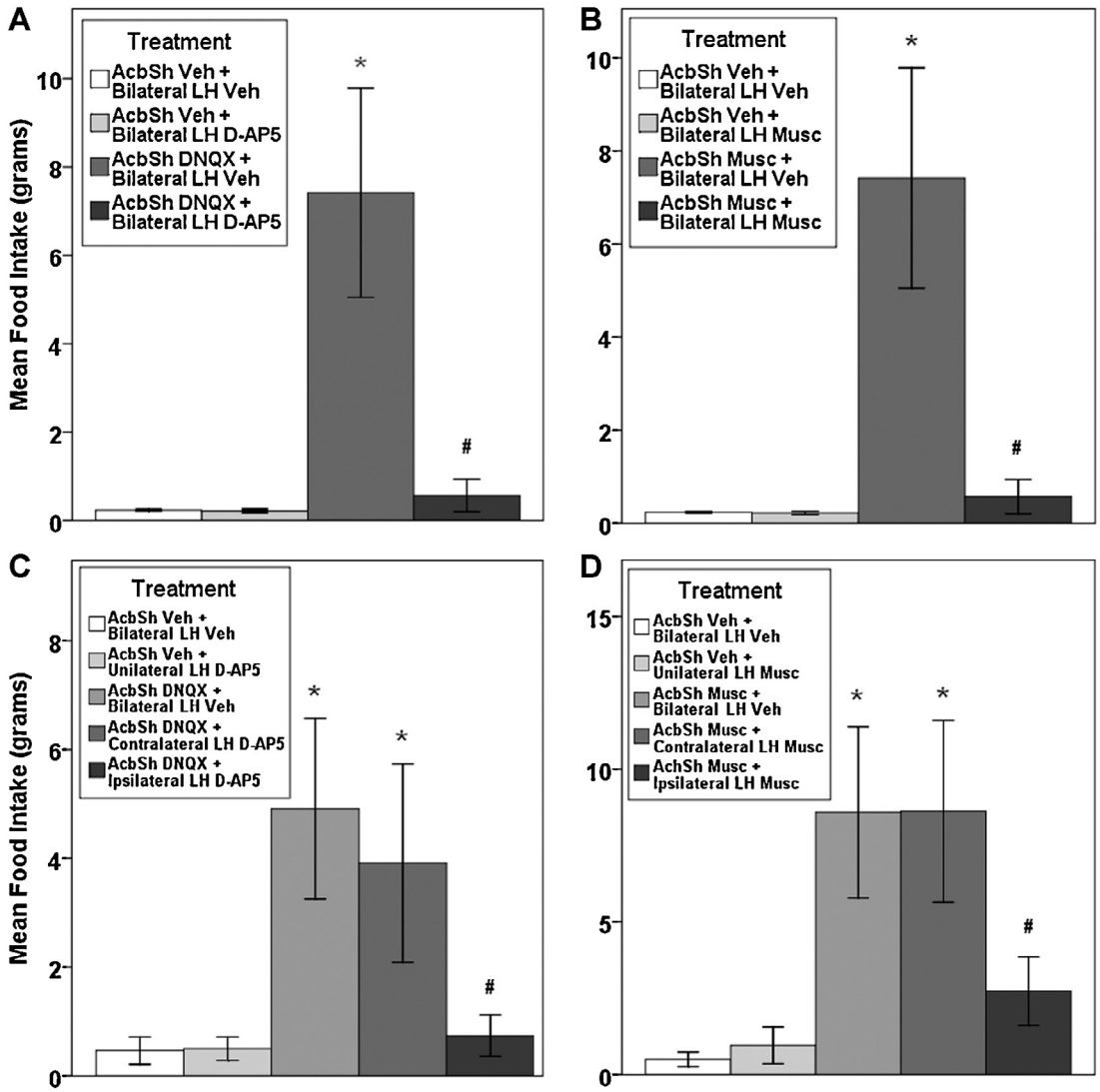

Fig. 1. Mean food intake through the duration of the monitoring period for Experiment 1 (A), 2 (B), 3 (C), and 4 (D). Error bars are ±1 S.E.M. *denotes a significant increase in intake versus control group while # indicates a significant suppression of elicited intake.

30 min post-injection. Both groups receiving D-AP5 bilaterally into the LH exhibited suppressed grooming relative to control from 20 to 25 min post injection (Fig. 2C). AcbSh vehicle with bilateral LH D-AP5 resulted in a substantial suppression of sleep versus control from 55 to 60 min (Fig. 2E), at which point quiescence was substantially increased versus both AcbSh DNQX groups (Fig. 2F). In summary, AcbSh DNQX induced feeding, while bilateral LH D-AP5 treatment suppressed AcbSh DNQX-induced feeding but also altered other behaviors.

In Experiment 2 (Fig. 3), main effects of treatment were significant for eating ($F(3,195) = 5.6$, $p < 0.01$), grooming ($F(3,195) = 5.5$, $p < 0.01$), locomotion ($F(3,195) = 3.7$, $p < 0.05$), and sleeping ($F(3,195) = 11.1$, $p < 0.001$), but not in drinking or quiescence. ANOVA tests on all behaviors except drinking revealed a significant effect of time (not shown). Significant interaction of treatment by time were seen in locomotion ($F(39,195) = 1.5$, $p < 0.05$) and quiescence ($F(39,195) = 1.4$, $p < 0.05$) only. Experiment 2 showed similarities to Experiment 1 in feeding and sleeping. AcbSh muscimol alone increased feeding from 15 to 20 min post-injection (Fig. 3A). This treatment also increased locomotion at 60 min post-injection (Fig. 3D) and did not significantly change sleep versus control. Co-treatment with bilateral LH muscimol blocked the elicited feeding, substantially decreased locomotion from 10 to 20 min, decreased grooming from 30 to 35 min (Fig. 3C), and increased sleeping from 15 to 25 min post-injection (Fig. 3E). From 30 to 60 min post-injection, both groups receiving muscimol bilaterally to the LH exhibited increased sleeping relative to the AcbSh muscimol-LH vehicle group. Overall, AcbSh muscimol induced feeding and locomotive behaviors, while co-treatment suppressed this feeding and locomotion but also altered other behaviors.

3.3. Ipsilateral LH inhibition suppresses feeding specifically

Fewer behavioral effects were seen in Experiment 3 (Fig. 4) compared to prior experiments. Main effects of treatment were significant only for grooming ($F(4,364) = 4.0$, $p < 0.05$) and quiescence ($F(4,364) = 2.7$, $p < 0.05$). Time had a significant main effect on all behaviors except drinking (not shown). The only significant interaction effect was on eating ($F(13,364) = 1.4$, $p < 0.05$). Both AcbSh DNQX alone and with contralateral D-AP5 increased eating relative to control at 10–15 min post-injection (Fig. 4A). Notably, ipsilateral LH D-AP5 blocked this AcbSh DNQX-elicited feeding. AcbSh DNQX alone also increased grooming behaviors at 25 and 40 min post-injection (Fig. 4C). Unilateral D-AP5, with AcbSh vehicle, elevated quiescence transiently at 35–40 min post-injection relative

Table 2
Food intake characteristics.

Treatment	Average bout duration (min)	Maximum bout duration (min)	No. of bouts	Latency to eat (min)	No. of subjects observed feeding
Experiment 1					
Across all treatments (ANOVA results)	$F(3,15) = 4.9$, $p < 0.05$	$F(3,15) = 8.6$, $p < 0.001$	$F(3,15) = 5.5$, $p < 0.01$	$F(3,15) = 6.3$, $p < 0.01$	
AcbSh Veh, Bilateral LH Veh	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	60 ± 0.0	0/6
AcbSh Veh, Bilateral LH D-AP5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	60 ± 0.0	0/6
AcbSh DNQX, Bilateral LH Veh	3.5 ± 1.5*	5.6 ± 1.8*	1.8 ± 0.7*	22.8 ± 11.8*	4/6
AcbSh DNQX, Bilateral LH D-AP5	0.4 ± 0.4**	0.5 ± 0.5**	0.3 ± 0.3**	51 ± 9.0**	1/6
Experiment 2					
Across all treatments (ANOVA results)	$F(3,15) = 4.7$, $p < 0.05$	$F(3,15) = 4.7$, $p < 0.05$	$F(3,15) = 6.8$, $p < 0.01$	$F(3,15) = 3.9$, $p < 0.05$	
AcbSh Veh, Bilateral LH Veh	0.5 ± 0.3	0.6 ± 0.4	0.6 ± 0.4	43.5 ± 10.7	2/6
AcbSh Veh, Bilateral LH Musc	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	60.0 ± 0.0	0/6
AcbSh Musc, Bilateral LH Veh	2.9 ± 1.1*	3.6 ± 1.4*	2.8 ± 0.8*	25.3 ± 10.5	5/6
AcbSh Musc, Bilateral LH Musc	0.0 ± 0.0**	0.0 ± 0.0**	0.0 ± 0.0**	60 ± 0.0**	0/6
Experiment 3					
Across all treatments (ANOVA results)	$F(4,28) = 3.0$, $p < 0.05$	$F(4,28) = 2.1$, $p = 0.10$	$F(4,28) = 3.8$, $p < 0.05$	$F(4,28) = 3.1$, $p < 0.05$	
AcbSh Veh, Bilateral LH Veh	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	54.1 ± 5.8	1/8
AcbSh Veh, Unilateral LH D-AP5	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	51.1 ± 6.2	2/8
AcbSh DNQX, Bilateral LH Veh	2.0 ± 0.7*	3.0 ± 1.3	1.2 ± 0.3*	30.6 ± 8.7*	6/8
AcbSh DNQX, Contralateral LH D-AP5	2.3 ± 1.1*	3.6 ± 2.1	1.0 ± 0.3*	28.8 ± 9.5*	5/8
AcbSh DNQX, Ipsilateral LH D-AP5	0.1 ± 0.1**	0.1 ± 0.1	0.2 ± 0.2**	55.8 ± 4.2**	1/8
Experiment 4					
Across all treatments (ANOVA results)	$F(4,32) = 2.8$, $p < 0.05$	$F(4,32) = 3.3$, $p < 0.05$	$F(4,32) = 3.3$, $p < 0.05$	$F(4,32) = 4.8$, $p < 0.01$	
AcbSh Veh, Bilateral LH Veh	0.4 ± 0.3	0.4 ± 0.3	0.2 ± 0.1	50.7 ± 6.2	2/9
AcbSh Veh, Unilateral LH Musc	0.7 ± 0.5	1.0 ± 0.6	0.4 ± 0.3	54.7 ± 3.5	2/9
AcbSh Musc, Bilateral LH Veh	6.4 ± 3.3	8.8 ± 4.3*	2.1 ± 0.7*	19.8 ± 7.9*	7/9
AcbSh Musc, Contralateral LH Musc	8.9 ± 4.3*	12.0 ± 5.4*	1.1 ± 0.4	34.3 ± 9.9	5/9
AcbSh Musc, Ipsilateral LH Musc	2.2 ± 1.2**	2.7 ± 1.4**	1.1 ± 0.4	32.0 ± 9.0	7/9

Excluding ANOVA results in the initial line for each experiment, the feeding bout durations, number of bouts, and latency to eat are mean values (plus or minus S.E.M.) across all subjects, including those that did not eat. Subjects observed eating is given as a ratio of total subjects in the experiment.

* A significant difference versus control.

** Significant difference between feeding–eliciting and feeding-suppressive treatments.

to AcbSh DNQX alone (Fig. 4F). However, no differences in sleeping were noted across treatments (Fig. 4E).

Comparable effects were observed in Experiment 4 (Fig. 5). Main effects of treatment were significant for eating ($F(4,416) = 4.1$, $p < 0.01$), grooming ($F(4,416) = 7.9$, $p < 0.001$), and locomotion ($F(4,416) = 4.0$, $p < 0.01$) only. A significant main effect of time occurred in all behaviors except drinking (not shown). Significant interaction effects occurred for eating ($F(52,416) = 2.3$, $p < 0.001$) and grooming ($F(52,416) = 2.3$, $p < 0.001$) only. As shown in Fig. 5A, feeding was equivalently increased by AcbSh muscimol alone or when paired with contralateral LH muscimol from 5 to 30 min post-injection. Although ipsilateral LH muscimol did not suppress AcbSh muscimol-elicited eating at 10 min, this treatment did prevent eating at all other times. Ipsilateral LH muscimol significantly suppressed AcbSh muscimol-elicited feeding compared to contralateral LH muscimol at 20 min post-injection. All treatment groups exhibited significantly less grooming than control at 10 and 50 min post injection (Fig. 5C). At 5 min post-injection, subjects given AcbSh muscimol alone exhibited less locomotion than AcbSh vehicle–unilateral LH muscimol and AcbSh muscimol–ipsilateral LH muscimol (Fig. 5D). The AcbSh muscimol–contralateral LH muscimol group had suppressed locomotion versus AcbSh vehicle unilateral LH muscimol at 20–25 min and versus control at 20 min, which coincides with increases in eating and sleeping. Again, no effect of treatment was noted on sleeping patterns (Fig. 5E).

3.4. Histological verification

Of the 40 rats used for these experiments, 29 rats possessed cannulas correctly targeting both the AcbSh unilaterally and the LH bilaterally The distribution of identified injection sites for these 29 subjects is shown in Fig. 6, with an example of a Nissl-stained pair of sections in Fig. 7. As shown, the injection sites were within or proximal to the AcbSh and LH. The 11 additional rats used in these experiments possessed mistargeted cannulas, and their data were omitted from this study.

4. Discussion

Our findings replicated previous studies showing that AcbSh injection of DNQX or muscimol elicits feeding that can be blocked by concurrent LH injection of D-AP5 or muscimol [10,12,15]. Further, while ipsilateral LH D-AP5 or muscimol was highly effective in suppressing feeding elicited by AcbSh DNQX or muscimol, contralateral LH administration of D-AP5 or muscimol was ineffective, as we have shown previously [15]. We expand upon these studies with new findings suggesting that AcbSh-elicited feeding can be suppressed in a behaviorally specific manner by ipsilateral LH administration of D-AP5 or muscimol. While ipsilateral LH injection of D-AP5 or muscimol strongly suppresses AcbSh-mediated feeding, they produced little or no effects on other behaviors. In contrast, bilateral LH injection of these drugs markedly increased sleeping. Specifically, subjects receiving bilateral LH D-AP5 or muscimol began sleeping only minutes post-injection during the time that AcbSh DNQX- or muscimol-elicited eating would have occurred. This behavioral evidence suggests that the suppression of the AcbSh-elicited eating by presumed bilateral suppression of LH neural activity may have been an artifact of the elicited sleeping response. In contrast, our data also suggest that ipsilateral LH inhibition suppressed feeding in a behaviorally specific manner. These experiments provide further evidence for an association between these brain regions as neural substrates in feeding control. However, we also demonstrate that bilateral LH inhibition can alter other behaviors, suggesting caution in interpreting findings that use bilateral manipulations to suppress food intake or other appetitive behaviors.

An intriguing finding is that bilateral LH injections elicited sleep, while unilateral LH injections did not. This contrast suggests that sustained LH activity in a single hemisphere may be

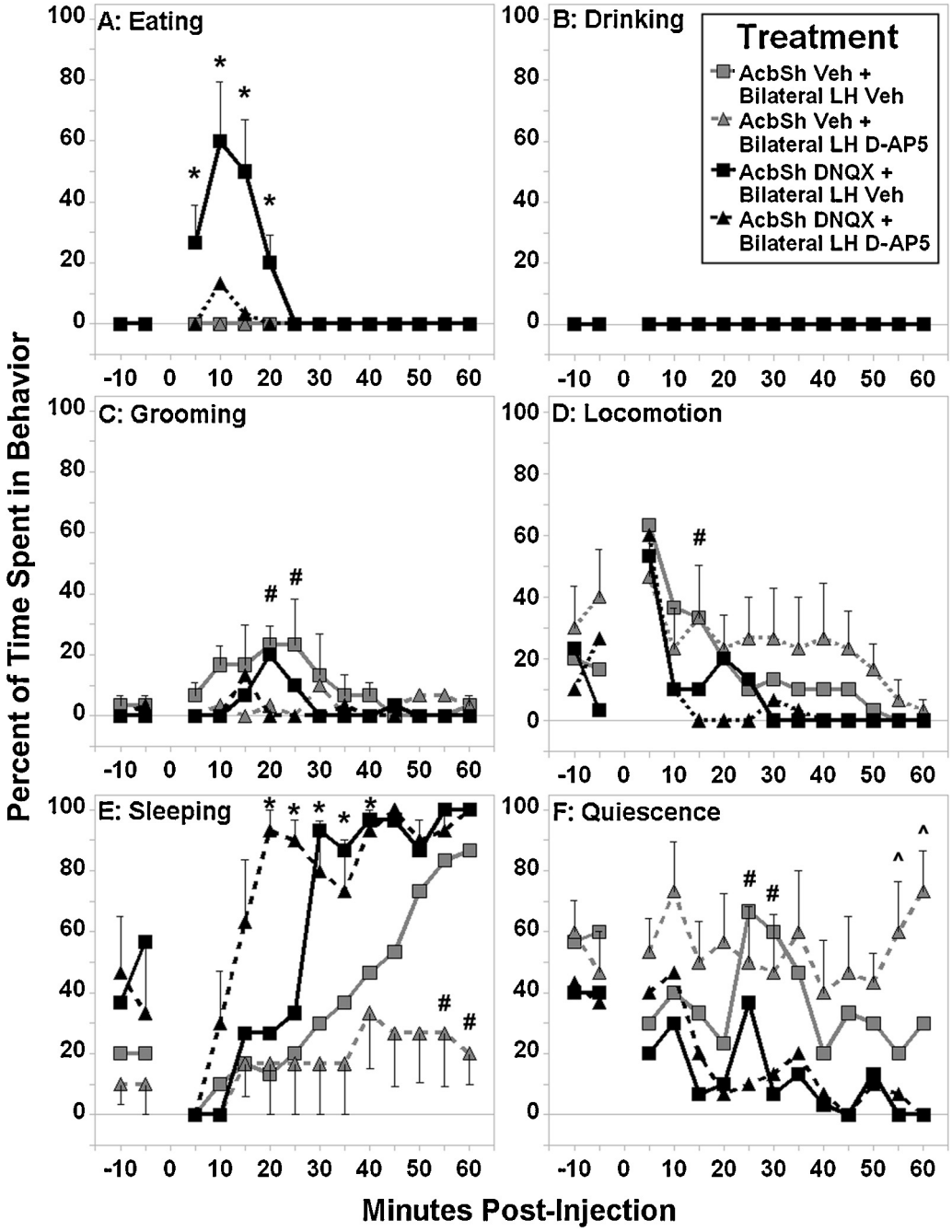

Fig. 2. Animal behaviors throughout the monitoring period in Experiment 1. Behavioral activity in each panel is represented as a percentage the total time spent in a particular behavior within each 5 min interval, averaged across all subjects. AcbSh DNQX elicited eating soon after injection, while bilateral LH D-AP5 both halted this feeding and increased sleep. Gray lines represent AcbSh vehicle treatments while black lines represent AcbSh DNQX treatments. Squares and solid lines represent treatments that received bilateral LH vehicle, whereas triangles and dashed lines represent treatments with bilateral LH D-AP5. Error bars are ±1 S.E.M. Error bars are colored and solid/dashed in the same manner as the line they are associated with. *denotes significant increase and # indicates significant decrease in a behavior versus control. ^indicates a significant difference between treatment groups other than AcbSh vehicle + LH vehicle.

sufficient to maintain wakefulness. Neurons that contain the orexins, arousal-associated neurotransmitter peptides, are likely involved as they are located exclusively in or near the LH [23,24]. Manipulations that activate these neurons promote wakefulness, while those that inhibit these neurons promote sleep [25–27]. In some cases, bilateral ablation or bilateral inhibition of orexin neurons is required to significantly affect sleep [28,29]. Our bilateral LH drug injections may have inhibited these orexin neurons sufficiently to cause sleep, whereas the unilateral injections were apparently insufficient. If feeding were interrupted by sleep onset in our ipsilateral–contralateral paradigm, both the ipsilateral and contralateral LH drug injection would have been equally effective in suppressing or reducing feeding. However, only ipsilateral LH injection suppressed feeding, while contralateral injection did not. Thus, we propose that the feeding-suppressive effects of ipsilateral LH drug injections acted specifically on feeding and not arousal.

It should be noted that bilateral LH D-AP5 with AcbSh vehicle did not induce sleep but instead promoted locomotion (Fig. 2D) or quiescence (Fig. 2F). This was an unexpected effect, considering that bilateral LH muscimol resulted in a clear increase in sleep (Fig. 3E) regardless of AcbSh treatment. Moreover, this effect of LH D-AP5 was not seen when injected ipsilateral or contralateral to AcbSh

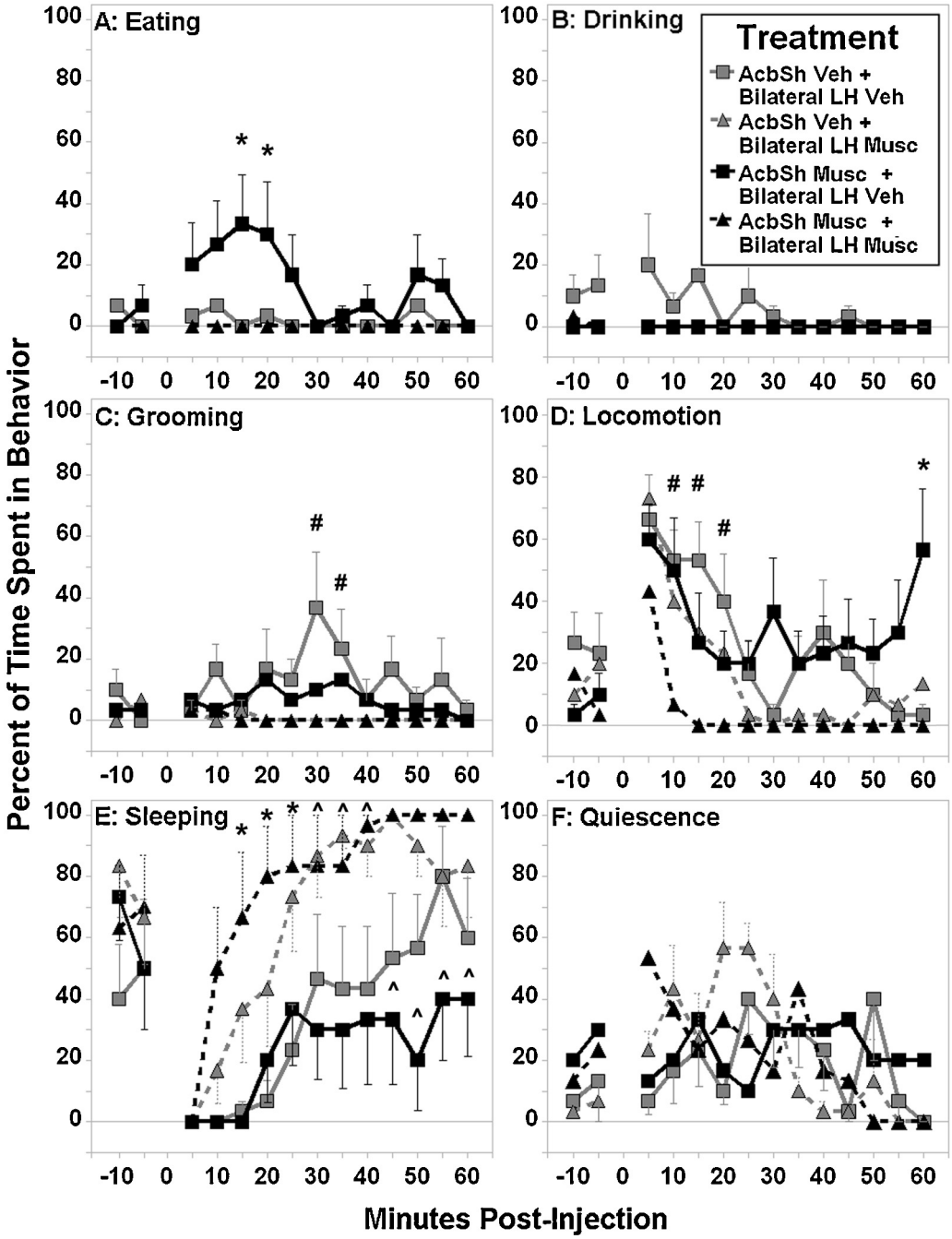

Fig. 3. Behaviors exhibited in Experiment 2. Conventions are similar to Fig. 1, except that muscimol was used instead of DNQX or D-AP5. AcbSh muscimol increased feeding within 15 min after injection, while bilateral LH muscimol abolished this feeding and hastened onset of sleep. Conventions for this graph are the same as in Fig. 2.

vehicle. These results suggest that bilateral LH NMDAR antagonism may be associated with an alternate, yet-to-be-defined behavioral phenotype that may act on a different circuit than those influenced by $GABA_AR$ activation.

Analysis of feeding characteristics reinforced prior findings and revealed more specific data on how feeding bouts were affected. Earlier findings demonstrated that AcbSh DNQX or muscimol decreased latency to feed while increasing feeding duration, leading to increased intake [10,12]. We replicated these findings and add new data showing increased meal frequency from these AcbSh drug injections. The latency data revealed that feeding onset was later than that noted in prior studies [11,12]; this may be due to our use of unilateral instead of bilateral AcbSh drug injections. The increases in bout size and frequency and decrease in latency caused by AcbSh DNQX or muscimol mimic those seen from LH neuropeptide Y (NPY) injection [30]. Marín Bivens et al. proposed that this NPY effect is mediated by brain mechanisms mediating hunger, as these patterns also mimic those exhibited by diabetic or fasted rats. It is possible that AcbSh-mediated eating utilizes similar mechanisms.

We previously demonstrated that bilateral or ipsilateral LH D-AP5 injection suppresses AcbSh DNQX-elicited intake, and bilateral or ipsilateral LH muscimol suppresses AcbSh muscimol-mediated intake [15]. Our current results show that the LH manipulations suppressed AcbSh-mediated eating by consistently lowering bout duration and number of bouts while raising latency to eat. It should be noted that in treatments where no animals ate, the latency was capped at the maximum of 60 min. Thus, latency differences

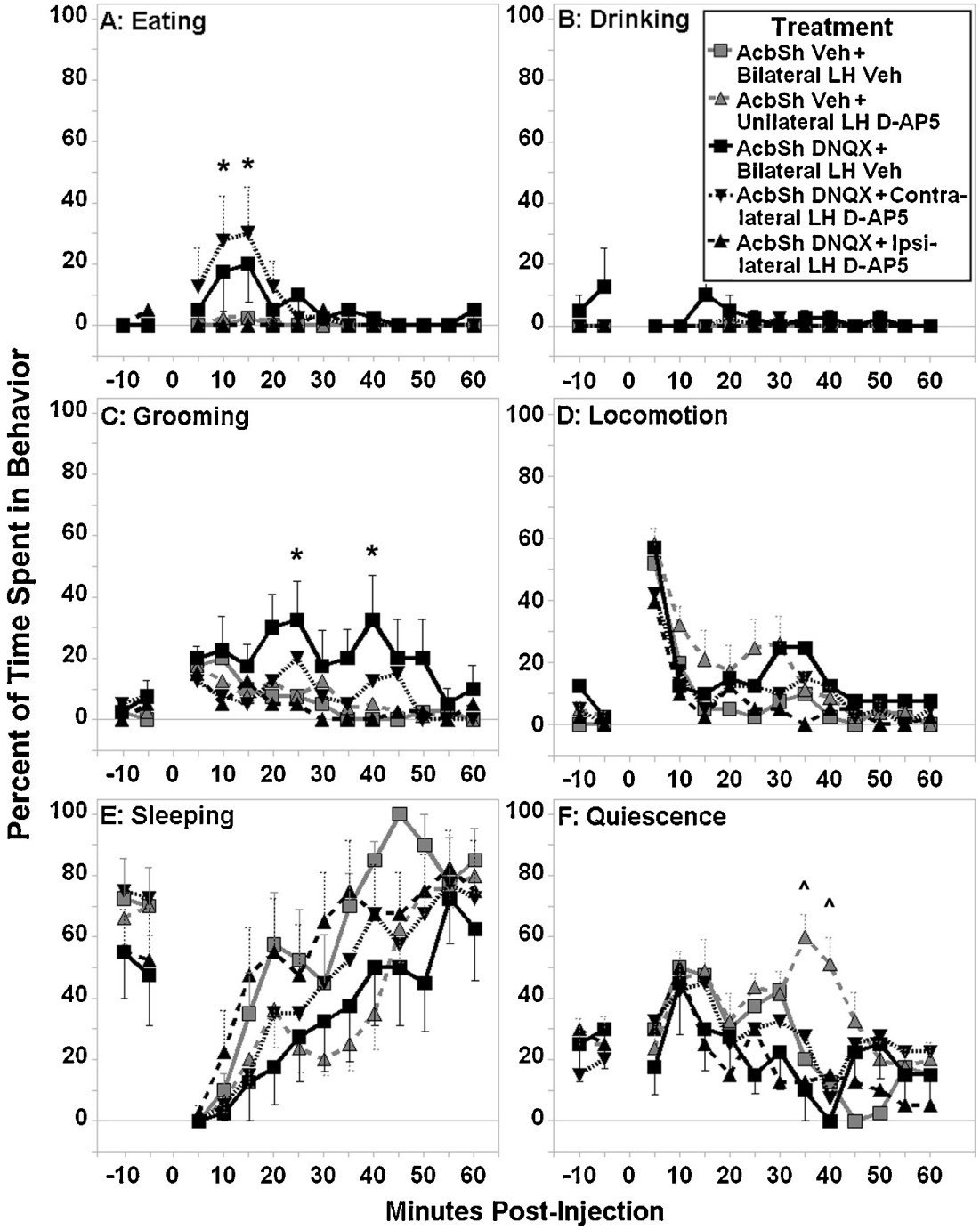

Fig. 4. Behavioral activity in Experiment 3. Conventions are similar to Fig. 1, though treatments differ (see legend). A fifth line, consisting of black inverted triangles and a dense dotted line, was added to represent the AcbSh DNQX–contralateral LH D-AP5 treatment. AcbSh DNQX increased feeding within 10 min post-injection, and contralateral LH D-AP5 co-treatment failed to suppress this feeding. Ipsilateral LH D-AP5 did suppress the feeding without altering sleep onset. Graph conventions are the same as those used in prior graphs.

between groups that fed and groups that did not feed may be larger than represented here. Also worth noting is that bilateral LH injections were stronger in their suppression of feeding characteristics than ipsilateral-only LH injections. This difference in effectiveness, based on our behavioral assays, may be due to both suppression of arousal and ingestive circuitry for bilateral injections, while ipsilateral LH injections primarily suppress the AcbSh-activated ingestive circuits.

The ipsilateral LH manipulations used in this study to suppress AcbSh-mediated feeding may operate by reducing the drive to eat. Considering that the AcbSh projects directly to the LH [17], AcbSh projections are primarily GABAergic [31], and that inhibition of the LH via $GABA_A$Rs suppresses nocturnal, deprivation-induced, and AcbSh elicited feeding [4,7,10], the evidence supports the proposal that feeding occurs due to increases in appetite when the LH is released from AcbSh inhibition [32]. This possibility is supported by evidence showing that pauses in AcbSh neuron firing precede the onset of sucrose fluid intake [13]. However, AcbSh manipulations that increase bout duration and frequency while decreasing latency, and the ipsilateral LH manipulations that reverse these effects, may also act on motivation-controlling circuits [33]. Our ipsilateral LH drug injections may reduce the rewarding qualities

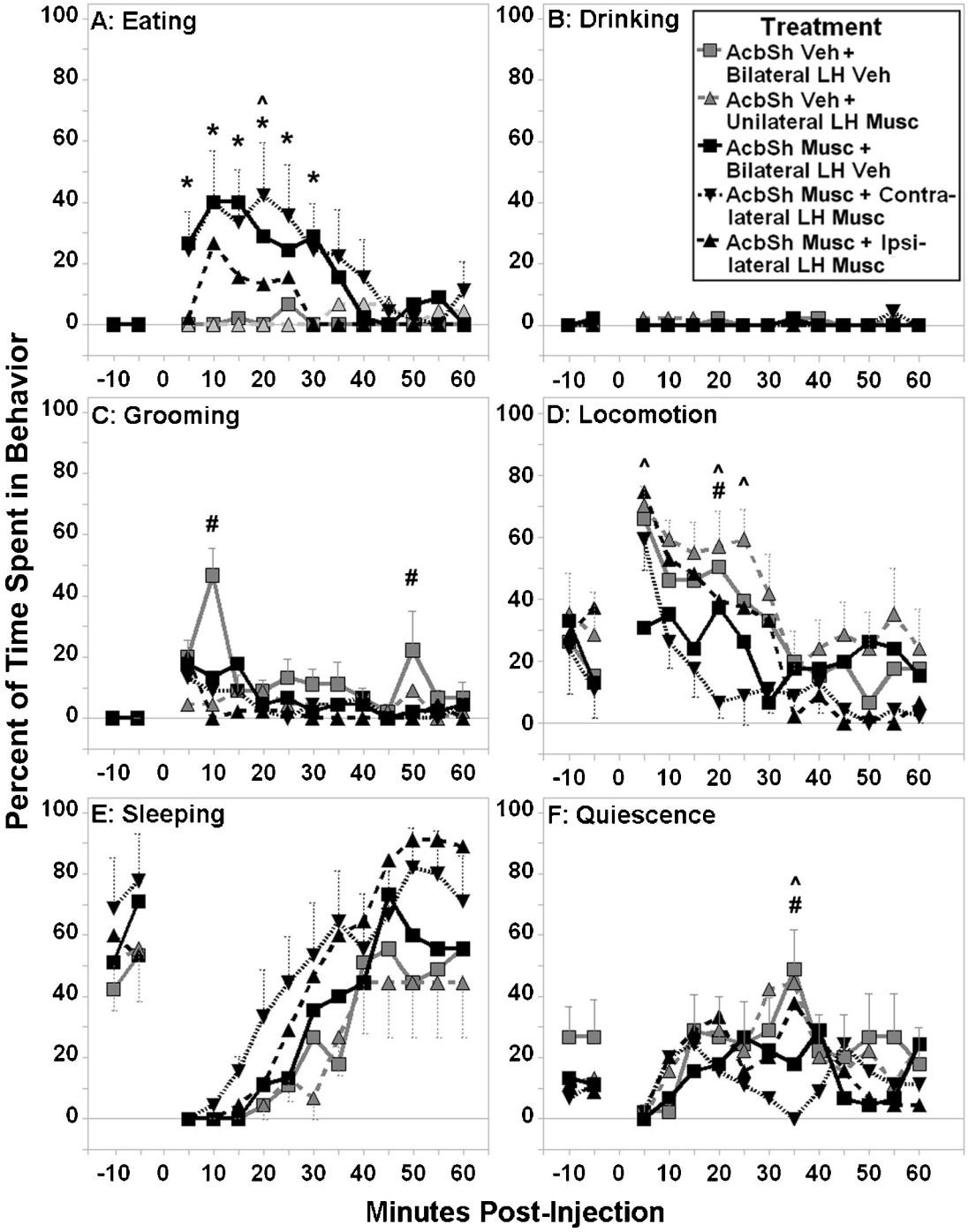

Fig. 5. Behavioral changes in Experiment 4. Conventions are similar to Fig. 3, except that muscimol replaces both DNQX and D-AP5. AcbSh muscimol increased feeding soon after injection. Concurrent contralateral LH muscimol failed to suppress this feeding. Ipsilateral LH muscimol did partially suppress AcbSh muscimol-elicited eating without compromising wakefulness. Graph conventions are the same as those used in prior graphs.

of food that AcbSh drug injections have been shown to promote [11,34]. One LH substrate through which these motivational effects may be mediated is orexin neurons, which are not only associated with arousal but also feeding and reward. Interesting evidence shows that AcbSh inhibition with muscimol activates orexin-containing neurons in the lateral perifornical LH [35]. Food reward and drug reward cues preferentially activate this lateral orexin neuron group [36]. Also, the AcbSh has been shown to project preferentially to orexin neurons in lateral subregion [37]. Considering this evidence, it seems that AcbSh connections to the LH may regulate both motivation and appetite through lateral perifornical orexin neurons, and inhibition of the LH may counteract such effects through these orexin neurons.

It is unclear whether the intake-controlling communication between the AcbSh and the LH is direct, indirect, or both. Growing evidence implicates the ventral pallidum (VP) as a possible functional and anatomical intermediate. Unilateral AcbSh muscimol induces c-fos expression in the ipsilateral VP and the ipsilateral LH [16]. Also, unilateral AcbSh muscimol-elicited feeding is suppressed by ipsilateral VP or LH lesions, and feeding elicited by VP administration of the $GABA_AR$ antagonist bicuculline is blocked by ipsilateral LH lesions [18,38]. Anatomical evidence shows AcbSh

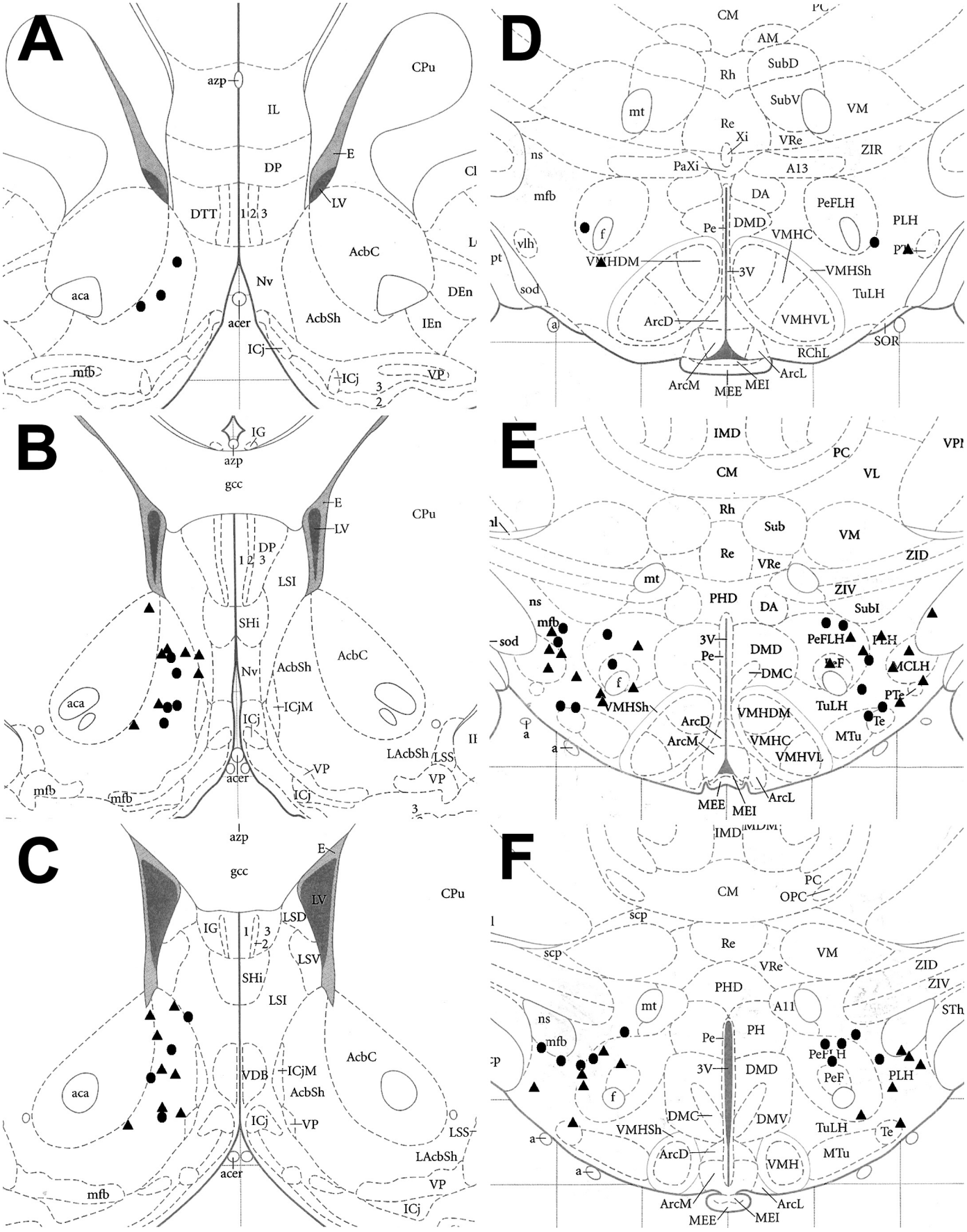

Fig. 6. Schematics representing brain regions containing the AcbSh (A–C) and LH (D–F). Symbols represent injection sites for each cannula in each subject. Circles designate sites in animals used in Experiments 1 and 2, while triangles designate sites for animals used in Experiments 3 and 4. Images were modified from a brain atlas [22].

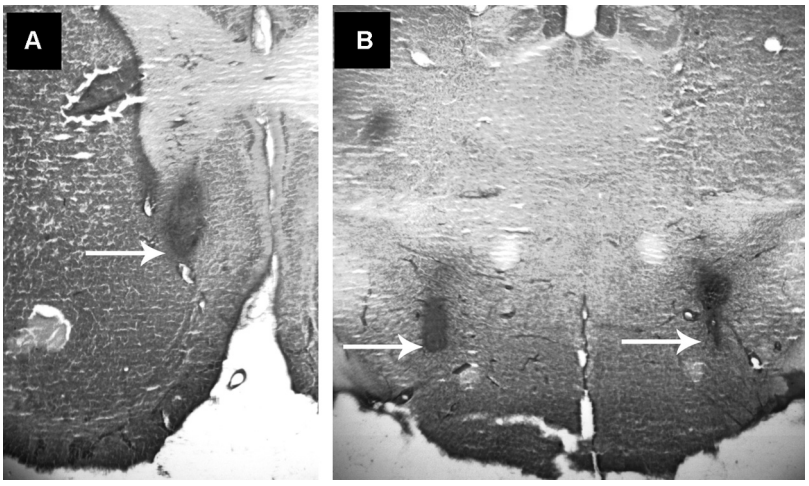

Fig. 7. Photomicrographs of brain slices containing cannula tracts terminating in the AcbSh (A) and the LH (B). Arrows indicate sites of injection.

projections to the VP that may secrete GABA [31,39]. The same VP subregion that receives AcbSh projections also appears to send projections to the LH [17,40,41]. Despite this evidence, it is still unclear whether or to what extent AcbSh-mediated feeding relies on the VP to relay signals to the LH. Retrograde labeling identified the tuberal LH, the LH locus regulating NMDAR and $GABA_A$R-mediated feeding [6,7], as the site that produced the most retrograde labeling in the AcbSh, whereas tracer injections into the anterior or posterior LH labeled far fewer AcbSh cells [17]. However, the VP appears to send equal amounts of projections throughout the anterior-posterior extent of the LH [17,40,41]. Anatomical evidence suggests that the VP may utilize different feeding or non-feeding circuitry through the LH in comparison to that used by the AcbSh, and AcbSh anatomical and LH functional evidence suggests that the direct connection between the AcbSh and the LH regulates feeding.

We have previously proposed that the balance in the synaptic release of glutamate and GABA within the LH is important in regulating feeding behavior [9], and a similar balance has been suggested for the AcbSh's role in food intake [32]. Thus, in these experiments, we used drugs that act on ionotropic glutamate and GABA receptors. Although other neurotransmitter receptors play a role in AcbSh-LH food intake control, we selected AcbSh AMPARs and $GABA_A$Rs in addition to LH NMDARs and $GABA_A$Rs because of the consistent feeding-specific effects produced by modulating these receptors [4,8,10,11,14,15], and our current results reflect this effect. Within the AcbSh and the LH, these receptors are likely the targets through which the balance of glutamate and GABA regulates food intake specifically.

Increases or decreases in specific behaviors between treatment groups are likely directly caused by specific drug treatments. However, indirect effects can also occur a treatment can cause one behavior to increase, thereby interrupting and decreasing competing behaviors. For example, AcbSh inhibition has been shown to increase locomotion in the absence of food [11], but eating behavior likely prevented this increased locomotion (Fig. 5A and D, AcbSh muscimol–contralateral LH muscimol treatment). Also, one behavior caused by a treatment directly can lead to an increase in a subsequent behavior. An example of this phenomenon is when food intake leads to post-prandial somnolence [42], as seen in Experiment 1 (Fig. 2A and E). Despite the drug treatments having indirect effects, behaviors observed immediately after injection such significant elicited eating or significant elicited sleep were likely caused directly by the drug injections and were not caused by other behaviors.

Past studies using bilateral LH inhibition had suggested that the LH bilaterally governed elicited, nocturnal, or deprivation-induced food intake specifically [4,8,10,12]. However, our data suggest that bilateral LH inhibition may have suppressed feeding through non-specific behavioral mechanisms in these and other studies. Further, we suggest that certain types of elicited feeding, such as AcbSh-induced intake, can be controlled unilaterally/ipsilaterally through the LH in a specific manner. This evidence indicates that the AcbSh and the LH communicate using glutamate and GABA to specifically regulate feeding through an ipsilateral connection. Further study may yet demonstrate this connection as a clinical target to treat obesity or other eating disorders.

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