Original Article
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Ghrelin Receptor Stimulation of the Lateral Parabrachial Nucleus in Rats Increases Food Intake but not Food Motivation

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Objective: The lateral parabrachial nucleus (lPBN) in the brainstem has emerged as a key area involved in feeding control that is targeted by several circulating anorexigenic hormones. Here, the objective was to determine whether the lPBN is also a relevant site for the orexigenic hormone ghrelin, inspired by studies in mice and rats showing that there is an abundance of ghrelin receptors in this area.

Methods: This study first explored whether iPBN cells respond to ghrelin involving Fos mapping and electrophysiological studies in rats. Next, rats were injected acutely with ghrelin, a ghrelin receptor antagonist, or vehicle into the lPBN to investigate feeding-linked behaviors.

Results: Curiously, ghrelin injection (intracerebroventricular or intravenous) increased Fos protein expression in the lPBN yet the predominant electrophysiological response was inhibitory. Intra-lPBN ghrelin injection increased chow or high-fat diet intake, whereas the antagonist decreased chow intake only. In a choice paradigm, intra-lPBN ghrelin increased intake of chow but not lard or sucrose. Intra-lPBN ghrelin did not alter progressive ratio lever pressing for sucrose or conditioned place preference for chocolate.

Conclusions: The lPBN is a novel locus from which ghrelin can alter consummatory behaviors (food intake and choice) but not appetitive behaviors (food reward and motivation).

Obesity (2020) **28**, 1503-1511.

Study Importance

What is already known?

- The orexigenic stomach-derived hormone ghrelin affects a wide range of feeding-linked behaviors in many different brain areas.
- The lateral parabrachial nucleus (lPBN) in the brainstem is a key area involved in feeding control targeted by several circulating anorexigenic hormones.

What does this study add?

- We identified the lPBN as a novel locus from which ghrelin can alter consummatory behaviors such as food intake and food choice.
- lPBN ghrelin receptor activation did not affect food reward or the motivation to feed.

How might these results change the direction of research?

- Ghrelin signaling research can be extended to the lPBN such as exploring the neurochemical identity of ghrelin-responsive neurons in the lPBN.

Introduction

Ghrelin is an important orexigenic hormone (1). It was isolated from the stomach and identified as the first endogenous ligand for the growth hormone secretagogue receptor 1A (GHSR-1A) (2,3). In humans, ghrelin is released before meals and it appears to function as a circulating hunger hormone, causing meal initiation and increasing food intake (4). Studies in rodents have elucidated the role for ghrelin in a wide diversity of food-linked behaviors that include food choice (5,6), food reward (7), food motivation (8-10), and food anticipation (11).

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Received: 30 January 2020; **Accepted:** 25 April 2020; **Published online 6 July 2020. doi:10.1002/oby.22875**

A great deal is known about the pathways engaged by ghrelin for its behavioral effects. The central ghrelin signaling system is extensive, as GHSR-1A is expressed in many forebrain and brainstem areas of importance for feeding control (3,12,13). Two especially well-studied targets for ghrelin include the orexigenic agouti-related peptide (AgRP) neurons of the hypothalamic arcuate nucleus (ARC) that coexpress neuropeptide Y and gamma-aminobutyric acid (GABA) (14,15) and the midbrain dopamine neurons located in the ventral tegmental area (VTA) that confer reward (8-10).

Here, we explore the lateral parabrachial nucleus (lPBN) as a potential new target for ghrelin's behavioral effects. The lPBN is an area of relevance in the control of food intake (16) and food reward (17), and it appears to be of importance for conditioned taste aversion (18). It is located in the pons of the brainstem and relays gustatory and visceral sensory information from the body to higher cortical areas. It has close connections to both homeostatic (19) and reward (20) feeding systems.

Many circulating appetite-regulating hormones act at the level of the lPBN to alter feeding behavior. For instance, activation of glucagon-like peptide 1 (GLP-1) receptors in the lPBN reduces food intake and food motivation for sucrose, and conversely, antagonism of GLP-1 receptors leads to increased food intake (21). Leptin receptor activation in the lPBN also leads to a decrease in food intake, without affecting motivation for sucrose or conditioned place preference (CPP) for a palatable food (22). Furthermore, melanocortin receptor activation of the lPBN also leads to a decrease in feeding (23), whereas cannabinoid (24) and μ-opioid (25) receptor activation increases feeding. Blockade of GABA-ergic signaling by AgRP neurons from the ARC to the lPBN or blockade of GABA receptors in the lPBN in mice promote anorexia and starvation (26). The question arises as to whether ghrelin signaling in the lPBN would also impact feeding behaviors. GHSR-1A is expressed in abundance in the lPBN in both rats and mice (12) although, to our knowledge, almost nothing is known about the behavioral consequences of ghrelin action at this site. In particular, we sought to explore whether ghrelin signaling at the level of the lPBN impacts food consumption and food choice as well as whether it alters food motivation and food reward in rats.

Methods

Animals

Six different experimental studies were performed in male Sprague Dawley rats (7 weeks old; 180-220 g body weight [BWt]; Charles River, Sulzfeld, Germany). Rats were single-housed 1 week after arrival and maintained in nonbarrier conditions in a 12/12-hour light/dark cycle at 20°C to 22°C and 50% humidity. They had ad libitum access to standard chow (Harlan Labs, Indianapolis, Indiana; #2016; 22% protein, 66% carbohydrate, 12% fat by energy, 3.00 kcal/g) and water. Ethical permissions from the local animal welfare committees were obtained: Institute of Experimental Biomedicine (University of Gothenburg, Sweden; #156-12 for study 6 and #45-2014 for studies 1-5) and Institute of Experimental Medicine (Budapest, Hungary; #XIV-I-001/2326-4/2012) in accordance with legal requirements of the European Community.

Intracranial catheter surgery

Intracerebroventricular cannulation. For studies 1 and 6, rats were implanted with a unilateral intracerebroventricular (i.c.v.) cannula targeting the lateral ventricle with coordinates −0.9 mm posterior to bregma, ±1.6 mm lateral to the midline, and −2.5 mm ventral to the skull (27). Cannula were implanted and their position verified by a dipsogenic response (online Supporting Information Supplement 1).

Intra-lPBN cannulation. For studies 3, 4, and 5, rats were implanted with a unilateral guide cannula (online Supporting Information Supplement 1) for subsequent targeting of the lPBN using coordinates −9.5 mm posterior to bregma, ±2.0 mm lateral to the midline, and −6.5 mm ventral to the skull (23,28). Correct cannula placement was verified post mortem by injecting 0.5 μL of India ink (representative injection site shown in Supporting Information Figure S1). Only rats with correct cannula placement were included in the data analysis.

Study 1: Effect of intravenous or i.c.v. ghrelin on Fos expression in lPBN

Preparation for immunohistochemistry. This study had two parts. Twenty-four rats were implanted with jugular vein catheters for an intravenous (i.v.) delivery of ghrelin (method according to Hewson and Dickson (29)), and sixteen rats were implanted with a guide cannula into the lateral ventricle for i.c.v. ghrelin delivery. In both parts, rats were allocated into two groups balanced by BWt. On the experimental day, rats with jugular vein catheters received an i.v. injection of either vehicle (0.2 mL of saline; $n=7$) or ghrelin (20 μg in 0.2 mL; #1465, Tocris, Bristol, UK; $n=8$) (29). Rats with a lateral ventricle cannula received an i.c.v. injection of either vehicle (2 μL of artificial cerebrospinal fluid [aCSF]; $n=9$) or ghrelin (2 μg in 2 μL of aCSF [1]; $n=7$). Ninety minutes after injection, the rats were deeply anesthetized with 75 mg/kg of Ketaminol vet (Intervet, Boxmeer, the Netherlands) and 10 mg/kg of Rompun vet (Bayer, Leverkusen, Germany) and perfusion fixed. Brains were prepared for subsequent immunocytochemistry (online Supporting Information Supplement 2).

Analysis. Images of the area postrema (AP; control area) (30) and lPBN were acquired using a fluorescent microscope (Axio Imager.Z2; Zeiss, Oberkochen, Germany). Regions of interest were identified with reference to a brain atlas (27). The number of Fos-positive neurons per brain section was counted manually using the multipoint tool in the ImageJ software (NIH, Bethesda, Maryland). For each brain, one AP-containing and one to two lPBN-containing sections were counted blind.

Study 2: Responsiveness of lPBN neurons to ghrelin and JMV2959

Brain slice preparation. Rats were anesthetized using isoflurane inhalation. Brains were removed rapidly and immersed in ice-cold sodium-free solution (online Supporting Information Supplement 3) oxygenated with 95% O_2 and 5% CO_2. Acute 300-μm-thick coronal slices containing the lPBN were then prepared with a VT-1000S vibratome (Leica GmBH, Wetzlar, Germany) in the sodium-free solution. The slices were bisected along the midline and transferred into aCSF (Supporting Information Supplement 3) saturated with O_2/CO_2 and kept for 1 hour to equilibrate. Electrophysiological recordings were carried out at 33°C, in oxygenated aCSF. Axopatch 200B patch clamp amplifier, Digidata-1322A data acquisition system, and pCLAMP 9.2 software (Molecular Devices Co., Sunnyvale, California) were used for recording. Cells were visualized with a BX51WI-IR-DIC microscope (Olympus Co., Tokyo, Japan).

Loose-patch clamp electrophysiology. Loose-patch clamp measurements to record action currents were carried out as described earlier (31) with slight modifications (see online Supporting Information Supplement 3). The lPBN was identified under microscopic control, and large fusiform

cells of this area (32) were chosen for recordings. Many of these fusiform cells are known to express calcitonin gene-related peptide (CGRP) (33). Measurements were carried out with an initial control recording (4 minutes). Ghrelin (4μM) (34) was added to the aCSF by a single bolus into the recording chamber (recorded for 11 minutes). When the ghrelin receptor antagonist JMV2959 (10μM; #AEZS-123, Aeterna Zentaris GmBH, Frankfurt, Germany) (34) was used, it was added to the aCSF 5 minutes before the second addition of ghrelin. Each neuron served as its own control.

Analysis. Each experimental group contained 14 recorded cells from six to seven rats. Event detection was performed using the Clampfit module of the PClamp 10.4 software (Molecular Devices). Change in firing rate upon ghrelin administration was expressed as percentage ratio of the firing rates of the ghrelin-treated (11 minute) and control (4 minute) periods of the recording.

Study 3: Effect of intra-lPBN ghrelin and JMV2959 on food intake

Intra-lPBN cannulated rats were either fed chow ($n=15$) or a high-fat diet (HFD; 20% protein, 20% carbohydrate, 60% fat by energy, 5.24 kcal/g; $n=12$; #D12492, Research Diets, New Brunswick, New Jersey) for 2 weeks before the injections commenced. All injections were made in a counterbalanced manner with at least 48 hours in between injections. Ghrelin (0.5 μg or 1 μg in 0.5 μL) (35) versus vehicle injections were made in free-fed rats, and JMV2959 (1 μg or 2 μg in 0.5 μL) (8) versus vehicle injections were made in overnight-fasted rats. The doses selected for intra-lPBN injection were based on those used previously for other parenchymal targets such as the VTA (8). On injection days, the food was measured pre injection and then at 1 hour, 2 hours, 3 hours, and 24 hours post injection. All injections were performed in the early light phase.

Study 4: Effect of intra-lPBN ghrelin and JMV2959 on food choice

Eighteen rats with an intra-lPBN cannula were acclimatized to a free-choice diet for 2 weeks prior to the injection schedule. The free-choice feeding paradigm consisted of chow, sucrose pellets (#1811254; TestDiet, St. Louis, Missouri), and lard (saturated animal fat; Dragsbæk, Thisted, Denmark). First, ghrelin (0.5 μg or 1 μg in 0.5 μL) versus vehicle injections were given to free-fed rats in a counterbalanced manner with >48 hours between the three injections. The animals were allowed to recover for >48 hours. Again, using a counterbalanced design, with >48 hours between injections, we delivered either JMV2959 (2 μg in 0.5 μL) or vehicle, this time to overnight-fasted rats. On injection days, the food was measured pre injection and then at 3 hours, 6 hours, and 24 hours post injection. All injections were performed in the early light phase.

Study 5: Effect of intra-lPBN ghrelin and JMV2959 on food motivation and reward

Progressive ratio operant conditioning. Nineteen rats with an intra-lPBN cannula underwent sucrose-induced progressive ratio (PR) operant conditioning training (see online Supporting Information Supplement 4 and Skibicka et al. (8)) and testing in rat conditioning chambers (Med-Associates Inc., St Albans, Vermont) to investigate food motivation for 45-mg sucrose pellets (#1811251; TestDiet). The injection experiment commenced after a total of 3 weeks of operant training. All injections were made 10 minutes prior to starting a PR session. First, ghrelin (0.5 μg or 1 μg in 0.5 μL) versus vehicle injections were made in free-fed rats, using a counterbalanced design with at least 48 hours between each injection. The animals were allowed to recover for >48 hours. We then tested the effects of JMV2959 (2 μg in 0.5 μL) versus vehicle injection, this time to overnight-fasted rats, again using a counterbalanced design and with >48 hours between injections. All injections were performed in the early or mid-light phase.

CPP. The same rats ($n=18$) also underwent a CPP test (see online Supporting Information Supplement 5 and Egecioglu et al. (7)). All parts of the test were performed in fed rats. First, the initial chamber preference was tested during a 20-minute pretest in the CPP apparatus (Med-Associates). The conditioning procedure was performed using chocolate pellets and a biased design. On the day after the last conditioning session, rats were allocated to two groups according to initial preference and BWt and then injected with either ghrelin (1 μg in 0.5 μL; $n=10$) or vehicle (aCSF; $n=9$) 10 minutes prior to being placed in the CPP apparatus for a 20-minute test session. The time spent in each compartment during the pretest and the test was registered and processed by Med-PC IV software (version 4.2; Med-Associates).

Study 6: Effect of i.c.v. ghrelin and JMV2959 on mRNA expression in lPBN

This effect is described in online Supporting Information Supplement 6.

Statistical analysis

Statistical analysis for studies 1 and 3-6 was performed using SPSS Statistics (version 22; IBM Corp., Armonk, New York). Statistical analysis for study 2 was performed using Prism (GraphPad Software, Inc., San Diego, California). In study 6, the mRNA expression was normalized to the respective vehicle group before further analysis. Data were checked for normal distribution and heterogeneity before being analyzed by either two-tailed *t* tests (when comparing two groups) or one-way ANOVA (when comparing three groups). Post hoc and planned comparisons were assessed by Dunnett test (for dose-response curves in study 3 and 4). No more than one outlier per group and data set was excluded by Grubb's tests. Data are presented as mean (SEM). Statistical significance was considered as $P<0.05$.

Results

Study 1: Fos expression in lPBN is increased after i.v. or i.c.v. ghrelin

Both i.v. and i.c.v. injection of ghrelin induced an increase in the number of Fos-positive cells detected in the lPBN: from 12.3 (3.1) to 58.3 (12.9) for the i.v. injections (4.7-fold increase; $P=0.009$; Figure 1A) and from 22.8 (10.9) to 110.7 (8.8) for the i.c.v. injections (4.9-fold increase; $P=0.001$; Figure 1B). In the area postrema, ghrelin injection caused an increase in the number of Fos-positive cells detected: from 15.8 (5.9) to 126.2 (26.4) for the i.v. injections (8.0-fold increase; $P<0.001$) and from 4.6 (2.4) to 38.1 (7.4) (8.2-fold increase; $P=0.001$) for the i.c.v. injections (data not shown).

Study 2: Effects of ghrelin and JMV2959 on loose-patch clamp recordings from cells in lPBN

The baseline firing rate was 1.25 (0.32) Hz (Figure 2A). When ghrelin (4μM) was applied to the recorded neurons, it decreased the firing rate

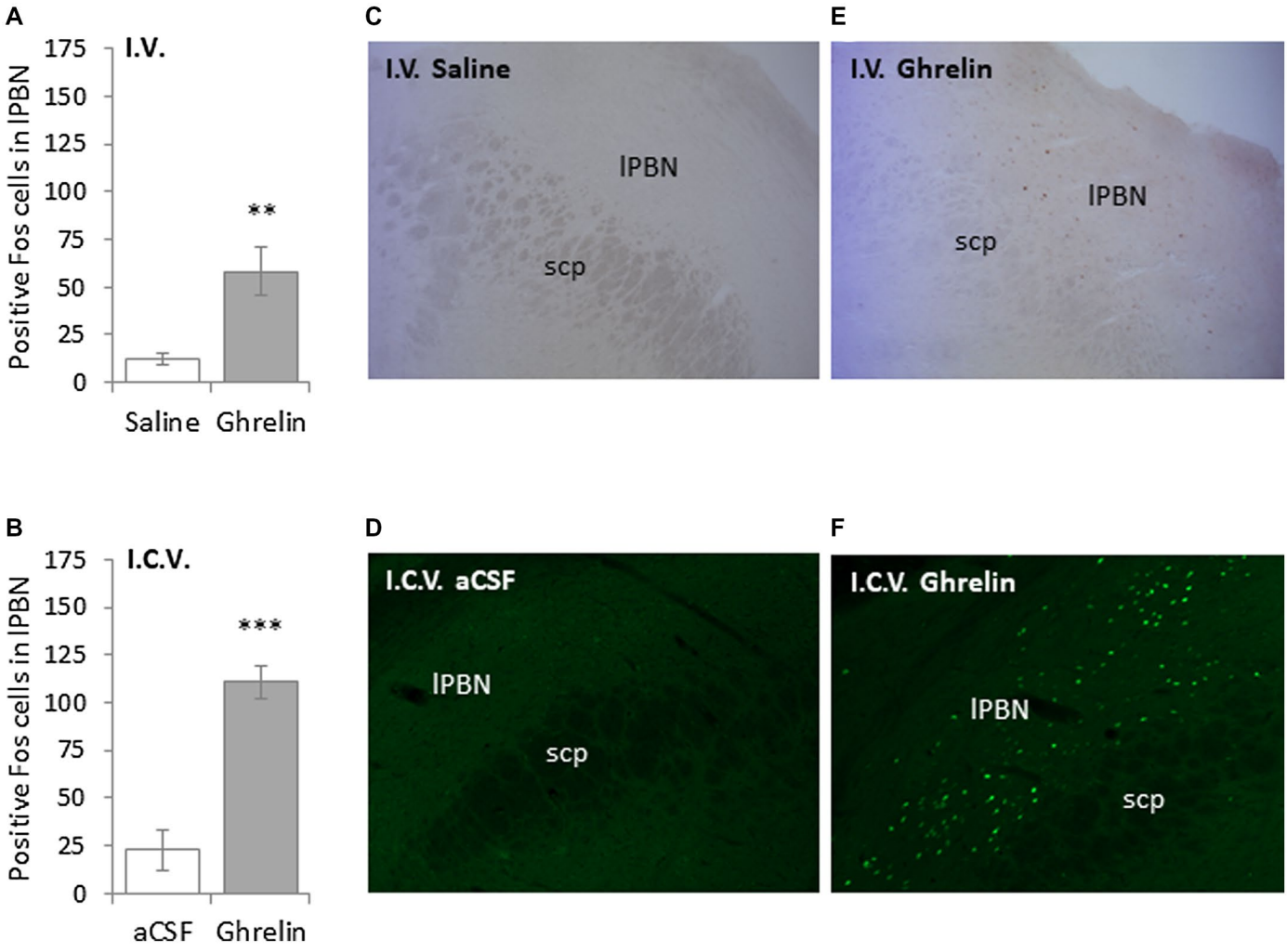

Figure 1 Fos expression after i.v. or i.c.v. ghrelin into the lPBN. Both (**A**) i.v. injection of ghrelin (20 μg) and (**B**) i.c.v. injection of ghrelin (2 μg) induced a positive Fos cell response in the lPBN after 90 minutes. Vehicle for i.v. injection was saline, and vehicle for i.c.v. injection was aCSF. Representative photomicrographs of the lPBN after (**C**) i.v. and (**D**) i.c.v. vehicle injection without Fos expression and after (**E**) i.v. and (**F**) i.c.v. ghrelin injection showing Fos-positive cells. Data analyzed by two-tailed *t* tests and presented as mean ± SEM, $^{**}P<0.01$ and $^{***}P<0.001$, $n=6$-9 rats per group in each area. aCSF, artificial cerebrospinal fluid; i.c.v., intracerebroventricular; i.v., intravenous; lPBN, lateral parabrachial nucleus; scp, superior cerebellar peduncle.

significantly (42.17% [12.88%] of the control; $P<0.01$; Figure 2A-2C) in 6 out of 14 neurons. The effect of ghrelin could be washed out in these cells. Repetitive application of ghrelin also resulted in a diminished firing rate (Figure 2A). In a second study, a total of 14 ghrelin-inhibited cells were identified. After washout, we retested these cells with ghrelin, this time in the presence of JMV2959 (Figure 2B-2C). Application of JMV2959 alone did not affect the firing rate. Overall, adding ghrelin resulted in a significant decrease in the firing rate, which could be abolished by pretreatment of the neurons with JMV2959 (Figure 2C).

Study 3: Intra-lPBN ghrelin increases chow and HFD intake

Injection of the higher ghrelin dose (1 μg) in fed rats led to a trend toward increased chow diet at 1 hour ($P=0.082$) and a significant increase in the intake of chow diet at 2 hours ($P=0.002$) and 3 hours ($P=0.019$, Figure 3A). The lower ghrelin dose (0.5 μg) showed a trend toward increased chow diet intake only at 2 hours ($P=0.062$, Figure 3A). The intake of HFD was significantly increased with both the lower ghrelin dose (1 hour, $P=0.018$; 2 hours, $P=0.015$; 3 hours, $P=0.072$) and the higher ghrelin dose (1 hour, $P=0.028$; 2 hours, $P=0.005$; 3 hours, $P=0.001$; Figure 3B). Injection of JMV2959 in overnight-fasted rats significantly decreased the 1-hour intake of chow diet with the higher dose ($P=0.028$, Figure 3C). There was no effect of JMV2959 on HFD intake (Figure 3D). The effects on food intake by ghrelin or JMV2959 injection were gone at 24 hours post injection (Figure 3E-3H).

Study 4: Intra-lPBN ghrelin increases chow but not lard or sucrose intake in a food choice paradigm

During baseline feeding, rats had an almost equal preference for the three offered foods (31.3% lard, 32.4% chow, and 36.2% chow on day 21, data not shown). The effects of ghrelin to increase energy

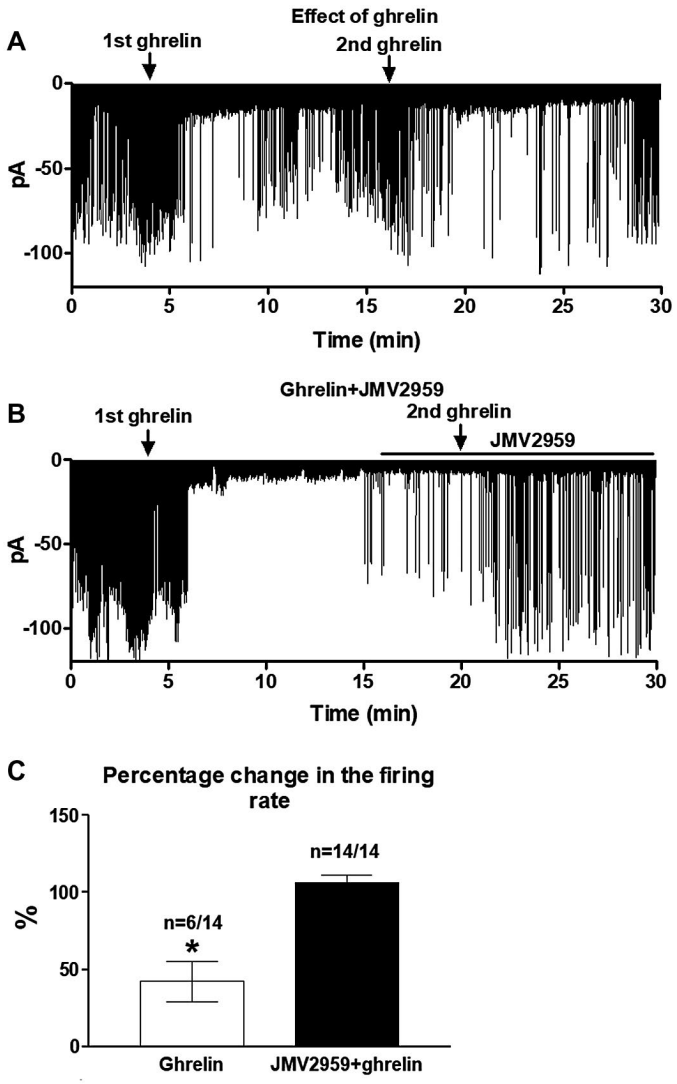

Figure 2 Representative loose-patch clamp recordings of action currents in a large fusiform neuron of the lPBN. (**A**) Application of ghrelin (4μM) in the extracellular solution decreased the firing rate in the first 15 minutes. Following washout, repetitive administration of ghrelin again diminished the firing rate in the second 15 minutes. (**B**) Addition of the ghrelin receptor antagonist JMV2959 (10μM) blocked the effect of the second administration of ghrelin. (**C**) Bar graph shows a significant effect of ghrelin on the firing rate in these neurons, which was blocked by the antagonist. Arrow shows application of ghrelin, whereas horizontal bar represents period of adding JMV2959. *$P<0.05$. n = number of the neurons involved in the analyses/all of the neurons measured. lPBN, lateral parabrachial nucleus.

intake were not evenly distributed between these foods, as previously shown (6). Intra-lPBN injection of the higher ghrelin concentration (1 μg) induced a marked significant increase only in chow intake relative to vehicle administration at all three time points. At 3 hours post injection, chow intake was 5.4 (0.8) kcal with ghrelin compared with 1.9 (0.6) kcal with vehicle, a more than twofold increase ($P=0.002$, Figure 4A). At 6 hours post injection, there was still more than a twofold increase, by which time chow intake was 6.3 (0.8) kcal for ghrelin treatment compared with 2.4 (0.6) kcal for vehicle treatment ($P=0.001$, Figure 4B). The orexigenic effect on chow was still present at 24 hours with 34.1 (2.1) kcal for ghrelin treatment compared with 25.1 (1.7) kcal for vehicle treatment ($P=0.003$, Figure 4C). There was also a trend toward increased chow intake with the lower ghrelin concentration (0.5 μg) at 3 hours after injection with 3.9 (0.7) kcal compared with vehicle ($P=0.093$, Figure 4A) and a significant increase at 24 hours after injection with 31.3 (1.9) kcal compared with vehicle ($P=0.049$, Figure 4C). Total cumulative energy intake was not significantly increased by intra-lPBN ghrelin relative to vehicle at any measured time point. JMV2959 did not have an effect on food choice (Figure 4D-4E).

Study 5: Intra-lPBN ghrelin does not affect food motivation or reward

During PR lever pressing for sucrose, injection of ghrelin in fed rats did not lead to a significant increase of active lever presses (Figure 5A), earned pellets (Figure 5B), or the response ratio (Figure 5C). Injection of JMV2959 in overnight-fasted rats did not lead to a decrease of active lever presses (Figure 5D), earned pellets (Figure 5E), or the response ratio (Figure 5F). In the CPP study part, a place preference could be conditioned with chocolate in both vehicle- ($P=0.037$) and ghrelin-injected rats ($P=0.048$; Figure 6A). However, there was no difference in the preference shift between vehicle- and ghrelin-injected rats (Figure 6B).

Discussion

Inspired by the fact that the lPBN is one of the few brain areas with a very high level of expression of receptors for the orexigenic hormone ghrelin (12), the present study was designed to explore this nucleus as a potential target for its effects on feeding control. We demonstrated that the neural activity of lPBN neurons is regulated by ghrelin, since both peripheral and central delivery of ghrelin caused an increase in the number of cells detected that express Fos protein in this region. Patch clamp recordings from large fusiform lPBN neurons demonstrated that ghrelin is able to alter their activity, intriguingly involving an inhibitory response in a subpopulation of these cells. Moreover, we demonstrated a functional role for GHSR-1A activation in the lPBN for the control of food intake. Direct activation of lPBN GHSR-1A by ghrelin injection increased the intake of both chow and HFD, while lPBN delivery of a GHSR-1A antagonist decreased chow intake. In addition, intra-lPBN ghrelin increased the intake of chow but not lard or sucrose pellets when rats were on a free-choice feeding paradigm. Intra-lPBN delivery of ghrelin had no effect on behaviors linked to food reward/motivation. Thus, the emerging role of ghrelin at the level of the lPBN appears to be more related to the consumption of food rather than motivation for it.

Our functional mapping studies involving the detection of Fos protein after peripheral or central ghrelin injection indicate that the lPBN forms part of the neurocircuitry engaged by circulating ghrelin. These effects could involve, at least in part, a direct action of ghrelin in the lPBN (since GHSR-1A is expressed there (12)) and since we demonstrated changes in activity of lPBN cells recorded in a slice preparation, in which all but closely adjacent inputs were severed. At first glance, it may seem somewhat of a contradiction that ghrelin increases the expression of Fos protein (which is often linked to neuronal activation) and also causes neuronal inhibition in the patch clamp recordings. It may be that this particular ghrelin-responsive cell population expresses Fos protein when inhibited. The coupling of Fos expression with neuronal inhibition has been demonstrated in other contexts; melanocortin 4 receptor agonists, for example, were shown to induce Fos protein in

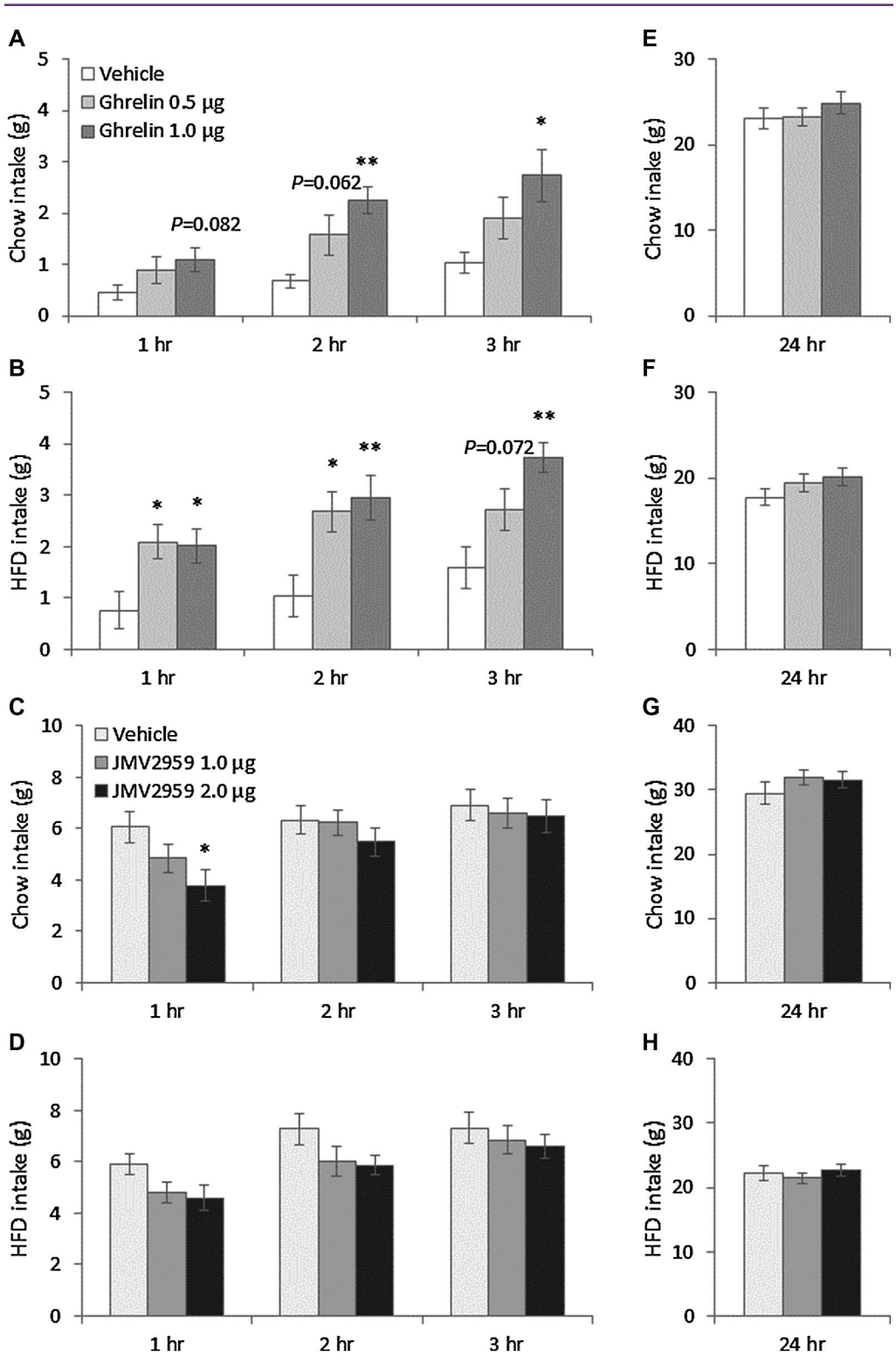

Figure 3 Effect of intra-lPBN ghrelin or ghrelin receptor antagonist (JMV2959) on food intake. Injection of ghrelin in fed rats led to an increase of both (**A**) chow diet and (**B**) HFD within 3 hours. Injection of JMV2959 in overnight fasted rats decreased the intake of (**C**) chow diet within 1 hour but did not affect intake of (**D**) HFD. (**E**-**H**) Effects on food intake were gone at 24 hours post injection. Data analyzed by one-way ANOVA followed by Dunnett post hoc tests and presented as mean ± SEM, $^{*}P < 0.05$ and $^{**}P < 0.01$, $n = 15$ rats on chow diet and $n = 12$ rats on HFD. HFD, high-fat diet; lPBN, lateral parabrachial nucleus.

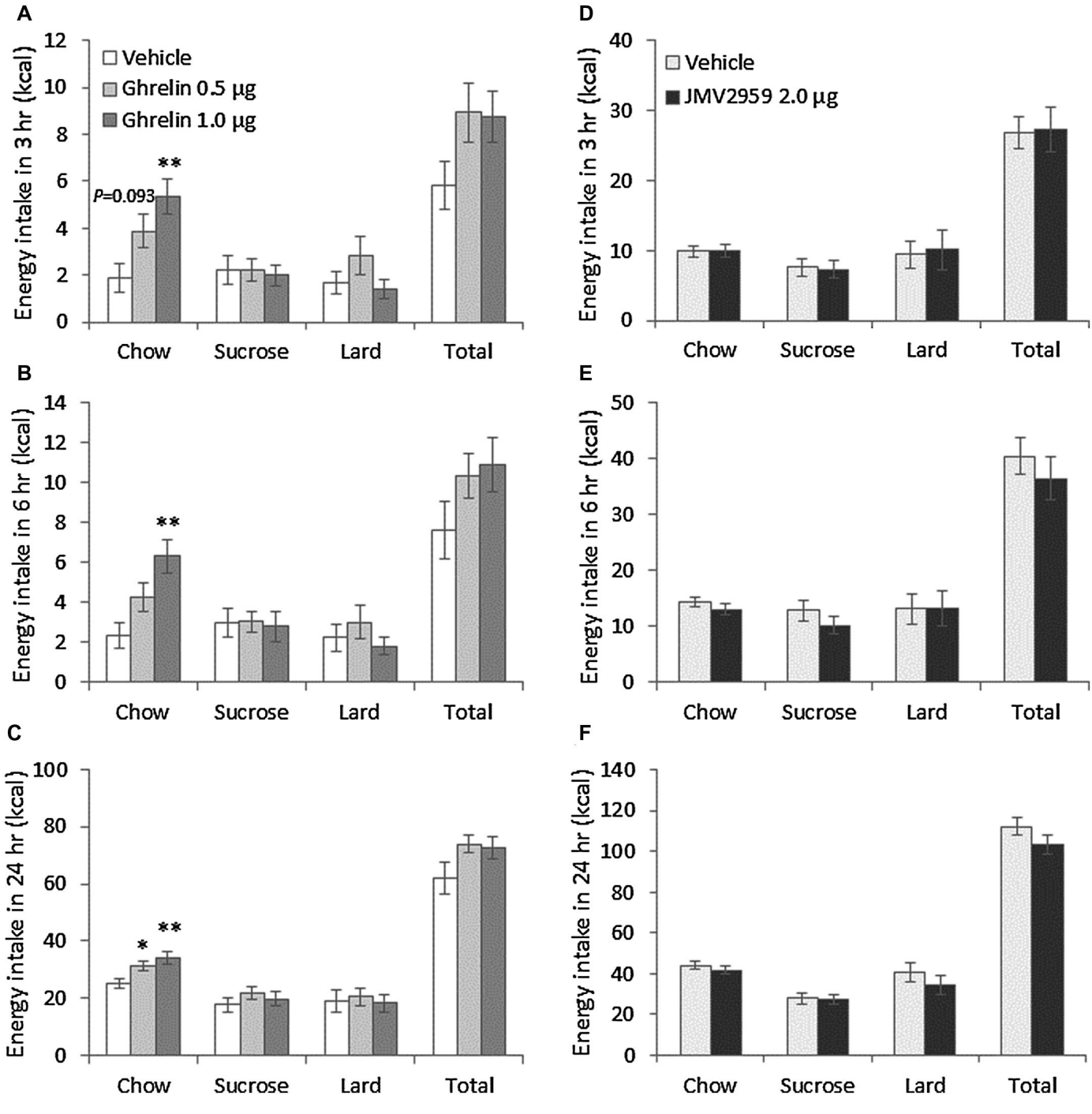

Figure 4 Effect of intra-lPBN ghrelin or ghrelin receptor antagonist (JMV2959) on food choice. Rats were fed a free-choice diet consisting of chow, sucrose pellets, and lard. Injection of ghrelin in fed rats led to an increase of chow intake at (**A**) 3 hours, (**B**) 6 hours, and (**C**) 24 hours but not of sucrose, lard, or the total energy intake. (**D**-**F**) Injection of JMV2959 in overnight-fasted rats did not have an effect on food choice at any of the investigated time points. Data analyzed by either one-way ANOVA followed by Dunnett post hoc tests (ghrelin injections) or two-tailed *t* tests (JMV2959 injections) and presented as mean ± SEM, $^{*}P < 0.05$ and $^{**}P < 0.01$, $n = 18$ rats. lPBN, lateral parabrachial nucleus.

oxytocin neurons in the supraoptic nucleus while profoundly inhibiting their electrical activity and release of oxytocin (36). Alternatively, it may be that the cells expressing Fos in response to ghrelin in this region are a different population from those recorded (i.e., the large fusiform neurons, many of which likely contain CGRP (33)).

Given that CGRP neurons in the lPBN are anorexigenic (26,37), it makes physiological sense that ghrelin would inhibit them. It may be that ghrelin promotes GABAergic signaling in the lPBN (which is known to promote feeding), potentially driven from the ARC AgRP neurons that are GABAergic (26). Our attempts to identify genes

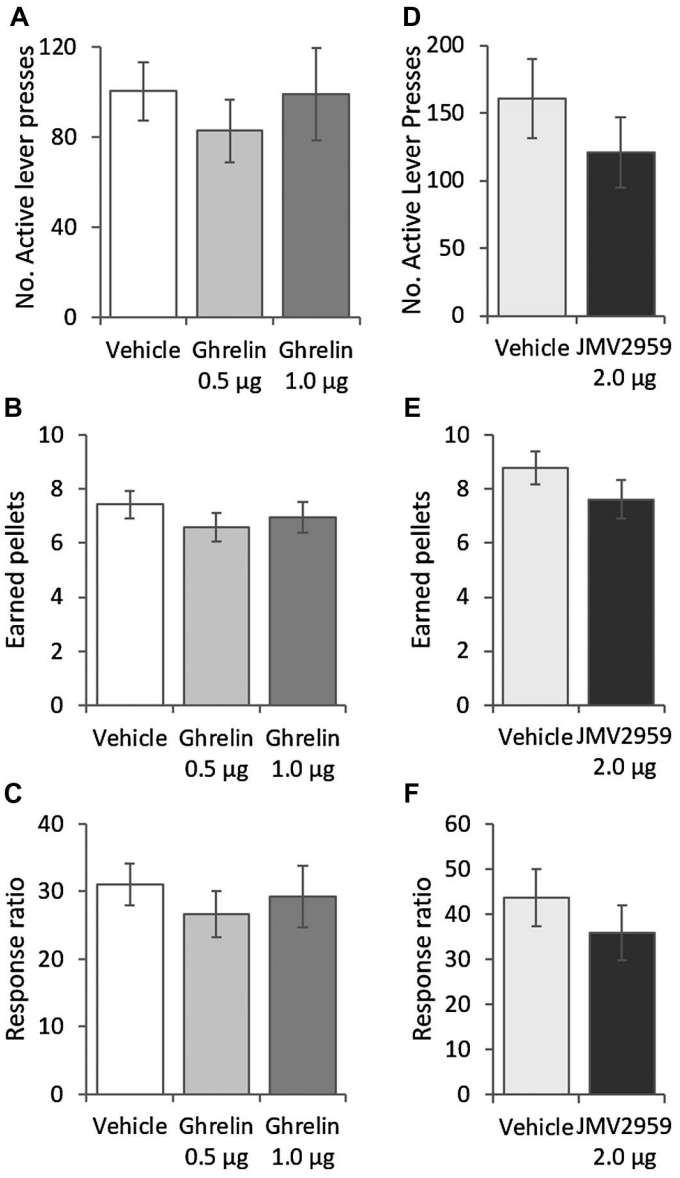

Figure 5 Effect of intra-lPBN ghrelin or ghrelin receptor antagonist (JMV2959) on food motivation. Rats were trained to lever press for sucrose pellets in a progressive ratio paradigm to assess food motivation. Injection of ghrelin in fed rats did not lead to an increase of (**A**) active lever presses, (**B**) earned pellets, or (**C**) response ratio. Injection of JMV2959 in overnight-fasted rats did not lead to a decrease of (**D**) active lever presses, (**E**) earned pellets, or (**F**) response ratio. Data analyzed by either one-way ANOVA (ghrelin injections) or two-tailed *t* tests (JMV2959 injections) and presented as mean ± SEM, $n = 19$ rats. lPBN, lateral parabrachial nucleus.

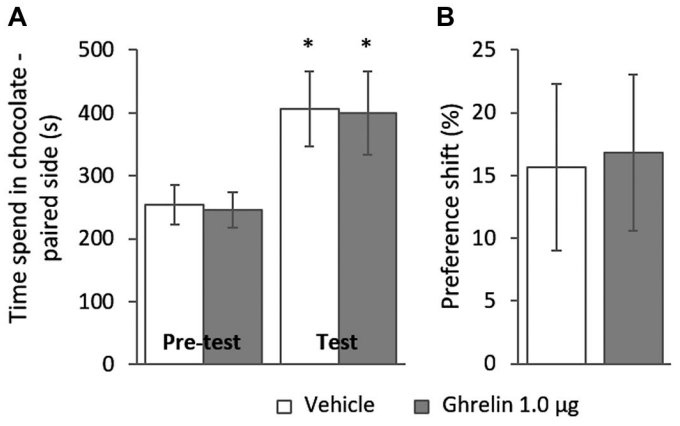

Figure 6 Effect of intra-lPBN ghrelin on food reward. Rats were conditioned with chocolate in a conditioned place preference paradigm to assess food reward. (**A**) Place preference could be conditioned with chocolate in both vehicle- and ghrelin-injected rats. (**B**) However, there was no difference in the preference shift between vehicle- and ghrelin-injected rats. Data analyzed by two-tailed *t* tests and presented as mean ± SEM, $^*P < 0.05$, $n = 18$ rats. lPBN, lateral parabrachial nucleus.

regulated by ghrelin in the lPBN (see online Supporting Information Supplement 6 and Supporting Information Figure S2) did not reveal any obvious candidates, however.

One of the best-described effects of ghrelin is its acute effect to increase the amount of food consumed within the first few hours of injection. Indeed, ghrelin has been shown to drive a feeding response when injected into many discrete brain areas linked to feeding control, such as the ARC (38), the hypothalamic paraventricular (39) and supramammillary nuclei (40), the dorsal vagal complex (41), the VTA (35), the nucleus accumbens (35), and the amygdala (34). Here, we report that the lPBN is also a brain area of relevance for ghrelin's orexigenic effects since ghrelin injected directly into the lPBN induced an acute orexigenic response, including for foods with differing palatability (chow and HFD).

The change in dietary food choice with intra-lPBN injections of ghrelin was, to some extent, similar to that reported previously for intra-VTA injections of ghrelin when both chow and lard (but not sucrose) intake increased at 3 hours and 6 hours post injection, leading to an increase in total energy intake (6). Prior to injecting ghrelin and its receptor antagonist into the lPBN, rats had similar preferences for the three foods offered as a choice. When ghrelin was administered, the effects to increased energy intake were no longer evenly distributed between these three offered foods; the intake of chow was more than double at the same time points, but lard and sucrose intake was unaffected. Thus, it appears that the intake of a palatable food is increased by intra-lPBN ghrelin only when these foods are fed without a choice (as shown with HFD). It is somewhat surprising that only chow intake was increased in a choice situation, since the lPBN is involved in processing taste information and the hedonic valuation of food (42).

There are indications that the lPBN is involved in food intake and/or food reward as evidenced by the fact that hormones such as GLP-1 (21) and neurochemicals such as endocannabinoids (24), GABA (43), glutamate (16), and melanocortin (23) alter food intake and/or motivation when delivered to this site. The effects of peripherally and centrally administered ghrelin on food motivation are well described (8,10,44). The primary target for these effects of ghrelin, however, is believed to be the VTA (7,8). In the present study, surprisingly, we did not find evidence that ghrelin action at the level of the lPBN is able to drive food-motivated behaviors (as shown by PR operant responding for sucrose) or to heighten food reward (since it did not alter the ability of chocolate to condition a place preference). It may be that ghrelin's

effects on reward/motivation could have differed if we had used more nutritive foods such as those used previously in a food cue setting (45). It should be noted, however, that ghrelin's effects on motivation for regular chow (which, arguably, is more nutritive) were not as marked as those for sucrose (44). Thus, ghrelin receptor signaling in the lPBN contributes to food intake without affecting food reward or the motivation to feed, likely engaging different subpopulations of cells from those important for food motivation.

In summary, our data show that a subpopulation of lPBN neurons expresses a functional GHSR-1A. We have shown, for the first time, that ghrelin signaling affects feeding behavior at the level of the lPBN. Collectively, the results suggest that lPBN ghrelin receptor activation increases food intake without affecting the motivation to feed. Thus, we identify the lPBN as a novel substrate from which ghrelin can alter consummatory behaviors such as food intake and food choice but not appetitive behaviors linked to food reward and motivation.O

Funding agencies: This research was supported by the EC FP7 project "Nudge-it" (grant agreement number 607310), the Swedish Research Council for Medicine (Vetenskapsrådet, grant number 2016-02195; 2019-01051), Hjärnfonden (FO2017-0180; FO2018-0262; FO2019-0086), NovoNordisk Fonden (NNF17OC0027206; NNF19OC0056694), National Research, Development and Innovation Office (K128278), and Avtal om Läkarutbildning och Forskning (ALFGBG-723681). The funding bodies did not have a role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Disclosure: The authors declared no conflict of interest.

Author contributions: The work was undertaken in the research group of SLD, who headed the project. TB, ZL, KPS, and SLD designed the research studies; TB, MVLM, CEE, HV, UB, and IF performed the research; MNA performed exploratory studies; TB, MVLM, CEE, HV, and IF analyzed the data; TB, MLM, and SLD wrote the manuscript.

Supporting information: Additional Supporting Information may be found in the online version of this article.

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