

Neuromedin U in the Paraventricular and Arcuate Hypothalamic Nuclei Increases Non-Exercise Activity Thermogenesis

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Brain neuromedin U (NMU) has been associated with the regulation of both energy intake and expenditure. We hypothesized that NMU induces changes in spontaneous physical activity and nonexercise activity thermogenesis (NEAT) through its actions on hypothalamic nuclei. We applied increasing doses of NMU directly to the paraventricular (PVN) and arcuate hypothalamic nuclei using chronic unilateral guide cannulae. In both nuclei, NMU significantly and dose-dependently increased physical activity and NEAT. Moreover, NMU increased physical activity and NEAT during the first hour of the dark phase, indicating that the reduction of sleep is unlikely to account for the increased physical activity seen with NMU treatment. As a positive control, we demonstrated that paraventricular NMU also significantly decreased food intake, as well as body weight. These data demonstrate that NMU is positively associated with NEAT through its actions in the PVN and arcuate nucleus. In co-ordination with its suppressive effects on feeding, the NEAT-activating effects of NMU make it a potential candidate in the combat of obesity.

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The rise in obesity is paralleled by a decrease in the physical activity levels in the population (1–3). A sedentary lifestyle can have a significant contribution to weight gain and poor health (1, 4–7). Energy expended through physical activity is the most variable component of energy expenditure and, in the majority of individuals, is composed primarily of nonexercise activity thermogenesis (NEAT) (8–10). Lean and obese individuals show differing amounts of daily NEAT, and individuals with high NEAT also show a heightened ability to ward off weight gain in the face of increased caloric intake (11, 12). Therefore, the energy expenditure associated with nonexercise activity can have a substantial impact on energy balance and weight gain.

Total daily energy expenditure is comprised of basal metabolic rate, thermic effect of food, and the energy expenditure of activity (EEA). In humans, EEA is divided into the energy expenditure of exercise and NEAT. In animals, NEAT is all of the energy expenditure of activity. Growing evidence indicates that levels of physical activity and NEAT are centrally regulated (13, 14). Moreover, many of the same neural and hormonal factors that regulate energy intake also modulate energy expenditure, often through altering NEAT (14–17). One such peptide is neuromedin

U (NMU) (18). Neuromedin U not only decreases energy intake, but also increases energy expenditure and grooming behaviour (18–26). The present study aimed to address the hypothesis that NMU increases physical activity and NEAT through its actions on the paraventricular nucleus (PVN) and arcuate nucleus using site-specific hypothalamic microinjections. The PVN contains NMU-2 receptors (NMU2R) (21, 27, 28), is an important region in NMU-induced feeding (22), and responds to NMU stimulation (29, 30). We microinjected progressive doses of NMU into the PVN and measured changes in physical activity and energy expenditure. The arcuate nucleus, in addition to containing NMU-positive cells, is also a putative target of the effects of NMU on feeding (22, 28). We therefore examined how local application of NMU into the arcuate nucleus alters NEAT in rats.

Materials and methods

Nineteen adult male Sprague-Dawley rats (Harlan; Indianapolis, IN, USA) were used in these studies. Animals were singly housed on a 12 : 12 h light/dark cycle (lights on 05.00 hours CST) and had access to water and food *ad lib* (Laboratory Rodent Diet 5001, PMI Nutrition International;

St Louis, MO, USA). The protocol was approved by the Mayo Foundation Institutional Animal Care and Use Committee.

Stereotaxic surgery

Each rat was stereotaxically fitted with a chronic unilateral guide cannulae aimed at the PVN, as described previously (13, 31). All cannulation supplies (guide cannulae, dummy cannulae, and microinjection needles) came from Plastics One (Roanoke, VA, USA). Each animal was anaesthetized with sodium pentobarbitone (Nembutal; 50 mg/kg), shaved, cleaned, and implanted with a guide cannulae using the following coordinates for the PVN: AP -1.3 from bregma; ML 0.5 from bregma; DV -7.0 (for rats less than 290 g) to -7.3 (for rats over 290 g) from skull. The following coordinates were used for the arcuate nucleus: AP -2.4 from bregma; ML 2.0 from bregma; DV -6.2 from dura mater at a 10° angle from vertical. The microinjection needles were fit to extend 1 mm (PVN) or 3 mm (arcuate nucleus) beyond the guide cannulae. After surgery, dummy cannulae were screwed into the guide cannulae, and each animal received buprenex, as they did on subsequent postsurgical days as deemed necessary for pain relief (up to 3 days).

Microinjection of NMU

Each rat was removed from its acclimation cage and gently restrained. The dummy cannulae was removed and the microinjection needle, attached to a 2- μ l Hamilton syringe, was inserted into the guide cannulae. The rat was microinjected with NMU or vehicle (artificial cerebrospinal fluid; aCSF) over 30 s; the microinjection needle was allowed to remain in place for an additional 30 s. After the needle was removed and the dummy cannulae replaced, the measurement began. Unilateral microinjections were used in the studies described here. Unilateral injections are reliably sufficient to induce changes in behaviours (31, 32). It is possible that we underestimated the potential effects of NMU on physical activity and energy expenditure by using unilateral instead of bilateral microinjections.

Measurements of physical activity and energy expenditure

Each rat was acclimatized to the testing room and chamber for at least 24 h prior to NEAT measurement and weighed just prior to measurement. We measured physical activity and energy expenditure using two Columbus Instruments small animal, open-circuit, indirect calorimeters (Columbus, OH, USA), which are able to measure minute-to-minute energy expenditure in two rats concurrently. Physical activity was measured simultaneously using Opto-M Varimex Minor activity monitors (Columbus Instruments). These devices contain 45 collimated infrared activity sensors that detect horizontal and vertical movement, as well as ambulatory movements (excluding repetitive signals from a single infrared beam). Before measurements commenced, each calorimeter was calibrated using a primary gas standard. Physical activity and energy expenditure data were collected simultaneously once every minute, excluding the first 4 min of the measurement as well as 4 min after every 30 1-min samples thereafter for the collection of reference data. Data from the first 20 min after microinjection were not included in the analyses due to the characteristic hyperactivity induced by the injection procedure. For each NEAT measurement, each rat was videotaped using a time-lapse videorecorder and, if necessary for night viewing, a light with an ultraviolet filter.

Determination of injection site

At the conclusion of each study, the microinjection site was determined. Each rat was given a terminal injection of sodium pentobarbitone (Nembutal). India ink (500 nl) was microinjected through the guide cannulae.

Brains were removed and fixed in 10% buffered formalin (Fisher Scientific, Minneapolis, MN, USA) for 2 days, then allowed to sink in 30% sucrose dissolved in buffered formalin for 2–3 days. Brains were then sectioned at 50 μ m using a Leica cryostat (Leica Microsystems, Wetzlar, Germany) and mounted onto Fisher Superfrost Plus slides. The slides were stained with cresyl violet and coverslipped using DPX. Each brain section containing the area of interest (i.e. PVN or arcuate nucleus) was examined by two investigators using a microscope equipped with a calibrated reticle. If the injection site was within 250 μ m of the brain region targeted, then the data from that animal were used in the final analysis. The final animal numbers used in the analyses were seven (PVN NEAT), seven (arcuate NEAT), six (dark phase PVN microinjections), and six (PVN feeding). The microinjections into the PVN were not specific to either the magnocellular or parvocellular region.

NMU in the PVN: effects on NEAT

In the first study, we hypothesized that NMU acts in the PVN to increase NEAT in rats. To address this hypothesis, we injected rats with progressive doses of NMU (Phoenix Pharmaceuticals; Belmont, CA, USA; 0, 0.125, 0.25, 0.5, and 1.0 nmol in 500 nl aCSF). Rats were measured in pairs (one rat was randomly assigned to one of the two calorimeters), and each pair of rats received a different dose of NMU on a given day, resulting in a different order of doses on five contiguous days for each pair of rats. Each measurement lasted 2 h, and all measurements were made during the light phase of the cycle (excluding the first 15 min and last 30 min of the light phase). A given rat was measured at the same time each day. After the microinjection, the rat was gently placed in the calorimetry chamber (without food or water), the chamber was sealed, and the measurement was started. Room air was pumped through the chamber at 3 l/min, with the sample flow set at 0.7 l/min. Energy expenditure was calculated from the measurements of O_2 consumption and CO_2 production. After the end of the test, the rat was returned to its acclimation chamber and the calorimeter chamber was cleaned in preparation for the next measurement. Data from the first hour (excluding the first 20 min) of the measurement as well as the entire 2-h test were averaged to yield mean oxygen consumption (VO_2), respiratory quotient (RQ, or the ratio of CO_2 produced to O_2 consumed), thermogenesis, physical activity (horizontal, vertical, ambulatory, total, as well as 'stationary activity', or the horizontal counts minus the ambulatory counts, to capture repetitive, stereotypic movement), and the percent time ambulatory (the percent of 1-min bins showing at least one ambulatory count).

NMU in the arcuate nucleus: effects on NEAT

We hypothesized that the arcuate nucleus may also be responsive to the actions of NMU on physical activity and NEAT. To test this hypothesis, we microinjected rats with progressive doses of NMU, as described above, and measured changes in physical activity NEAT. Separate sets of animals were used for arcuate and PVN injections.

NMU in the PVN: time course

We tested the hypothesis that NMU increases NEAT through the prevention of sleep by microinjecting NMU during the active phase of the daily cycle. Rats ($n = 6$) were given 1.0 nmol NMU immediately before the start of the dark phase (mean = 10 min before lights-off at 18.00 h), and the first 20 min of data collection were discarded. Physical activity and NEAT were measured as described above, and the measurement continued throughout the dark phase (18.00 h to 06.00 h).

NMU in the PVN: effects on feeding

As a positive control for NMU, we tested the hypothesis that NMU acts in the PVN to decrease feeding after an 18-h fast in these rats. Rats were acclimated to the testing room for 24 h. Food was removed from the acclimation chambers 18 h before the start of the feeding measurement. At the start of the light phase, each rat ($n = 6$) was weighed and microinjected with either NMU (1 nmol in 500 nl vehicle) or vehicle (500 nl aCSF). The rats were then placed back into the clean acclimation chamber with premeasured food. Food intake, including spillage, was measured 1, 2, 4, 8, and 24 h after the start of the measurement; body weight was measured at 2, 4, 8, and 24 h. The second feeding test was started on the fifth day after the first feeding measurement to allow the rats to fully recover from the initial food deprivation. At this time, each rat was injected with either NMU or vehicle, whichever it had not yet received. The cumulative food intake and body weight changes were calculated after vehicle and NMU treatment.

Statistical analysis

For the NEAT response after increasing doses of NMU, the data were analysed using one-way repeated-measures ANOVAs, with the dose as the within-subjects independent variable and the metabolic or physical activity data as the dependent variables. Fisher's post-hoc least significant difference was used to analyse differences between NMU doses when the ANOVA showed a significant main effect ($P < 0.05$). To analyse percent time active, the data were transformed (arcsine square-root transformation) to normalize the distribution before ANOVA analysis. To determine the time course of NMU-induced increases in physical activity and NEAT during the dark phase of the cycle, hourly data were averaged and analysed using a two-way analysis of variance, with hour and treatment (NMU or vehicle) as the independent variables. For the feeding test, cumulative feeding data and body weight data were analysed using a two-way repeated-measures ANOVA, with hour of test and treatment (NMU or vehicle) as the independent variables and cumulative food intake or body weight gain from baseline as the dependent variables. Paired t-tests were used to probe differences between the cumulative feeding and body weight responses to NMU compared to vehicle at

each time point after injection. Finally, for the group of rats receiving cannulae aimed at the arcuate nuclei, data from those with accurate cannulae placements were compared with those with inaccurate placement using a two-way mixed ANOVA, with cannulae placement (hit or miss) as the between-subject independent variable and dose of NMU as the within-subject independent variable. One-way ANOVAs were used to probe main effects and determine if those with inaccurate cannulae placement showed a significant effect of NMU dose on activity and metabolic variables.

Results

NMU in the PVN: effects on NEAT

We addressed the hypothesis that NMU acts on the PVN to increase physical activity and NEAT by microinjecting NMU directly into the PVN and measuring the resulting changes in physical activity and NEAT. Local application of NMU to the PVN significantly increased physical activity (horizontal, ambulatory, and total beam break counts, as well as percent time active) and metabolic variables (oxygen consumption and thermogenesis) in both the first hour and the entire 2-h measurement, compared to vehicle treatment (Fig. 1). Stationary activity was also significantly increased after 0.25, 0.5, and 1.0 nmol NMU. The effect of NMU on physical activity and thermogenesis was dose-dependent, peaking at 0.5 nmol. Respiratory quotient was not significantly altered by intra-PVN treatment with NMU. The number of vertical beam breaks was also not significantly increased after NMU.

NMU in the arcuate nucleus: effects on NEAT

To address the hypothesis that NMU acts through the arcuate nucleus to increase physical activity and NEAT, we microinjected NMU directly into the arcuate nucleus and measured the resulting

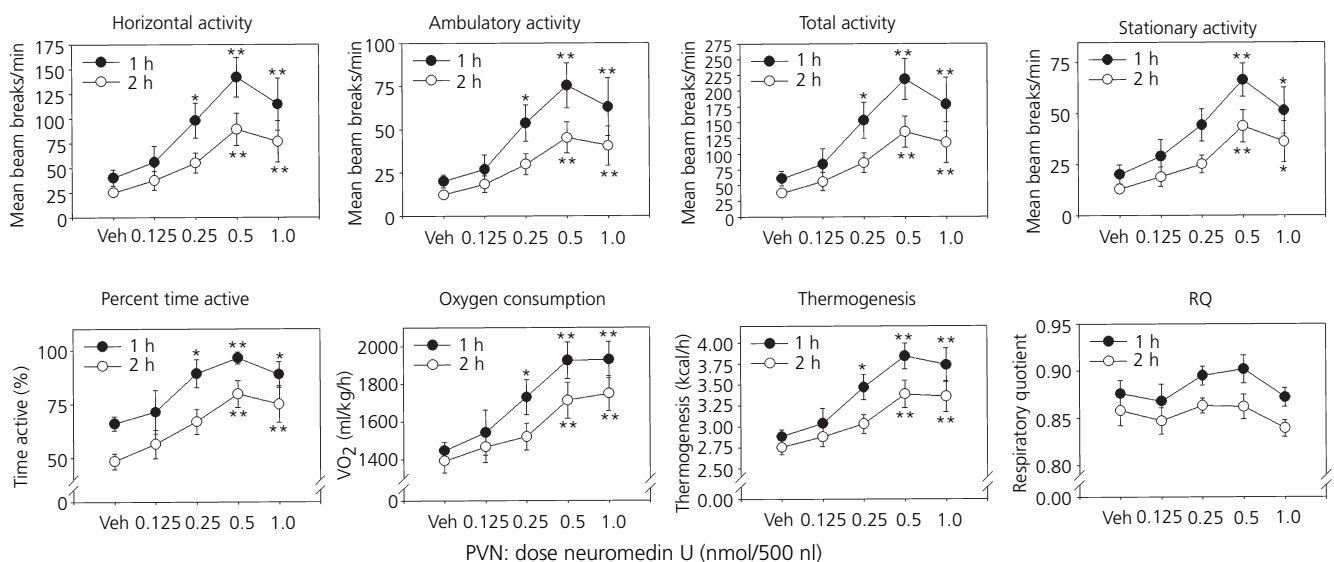


Fig. 1. Neuromedin U (NMU) applied locally to the paraventricular hypothalamic nucleus (PVN) dose-dependently increased physical activity and energy expenditure in rats in both the first hour and first two hours after microinjection. VO_2 , Oxygen consumption; RQ, respiratory quotient. *Significantly different from vehicle; **significantly different from vehicle and 0.125 nmol; $P < 0.05$ ($n = 7$).

changes in physical activity and NEAT. Intra-arcuate microinjection of NMU significantly increased physical activity (horizontal, ambulatory, total beam break counts, and stationary activity, as well as percent time active) and metabolic variables (oxygen consumption, RQ, and thermogenesis) in both the first hour and the entire 2-h measurement, compared to vehicle treatment (Fig. 2). As with PVN treatment, there was no significant effect of NMU on vertical (rearing) activity. The effect of NMU on physical activity and thermogenesis was dose-dependent, increasing through each dose of NMU, with a maximum effect at 1.0 nmol.

NMU in the PVN: time course

We determined the time course of NMU-induced increases in NEAT and also determined if NMU increased NEAT through the prevention of sleep by microinjecting NMU into the PVN and measuring physical activity and NEAT throughout the dark (active) phase of the cycle. Oxygen consumption, thermogenesis, and physical activity (horizontal, vertical, ambulatory, and total activity counts) showed significant interactions. Neuromedin U significantly increased physical activity, oxygen consumption, and thermogenesis compared to vehicle, but only during the first hour of the 12-h dark phase (18.00–19.00 h), immediately after NMU treatment (Fig. 3). Oxygen consumption and thermogenesis showed a reversal of this pattern later in the dark phase: from 01.00–02.00 h, rats given NMU showed a small but significant decrease in energy expenditure compared to vehicle treatment (Fig. 3). Finally, microinjection of 1 nmol NMU did not induce a greater magnitude of change in activity or thermogenesis (compared to vehicle) when given during the daytime compared to the early dark phase (change in oxygen consumption: light phase = 481 ml/kg/h, dark phase = 531 ml/kg/h; change in thermogenesis: light phase = 0.85 kcal/h, dark phase =

1.17 kcal/h; change in horizontal activity: light phase = 74 counts, dark phase = 83 counts; change in ambulatory activity: light phase = 43 counts, dark phase = 50 counts; change in total activity, light phase = 117 counts, dark phase = 135 counts).

NMU in the PVN: effects on feeding

As a positive control, we determined whether NMU acts through the PVN to decrease food intake. Microinjection of NMU into the PVN after an 18-h fast significantly decreased food intake at 1, 2, and 24 h after treatment (Fig. 4). Body weight was significantly decreased by NMU, compared to vehicle treatment, at 2 h after microinjection.

Accuracy of cannulae placement

The placements of the microinjection needles with respect to the PVN and arcuate nuclei are shown in Fig. 5. For the PVN microinjections, only one animal had a misplaced cannula (i.e. greater than 250 μm from the PVN). For arcuate microinjections, on the other hand, four animals had misplaced cannulae ('misses'). In one of these animals, the needle hit the third ventricle (3V). This animal showed sharp increases in 1-h activity and metabolic variables compared to those with accurate arcuate cannulae placements ('hits'): 3V = 77% increase in VO_2 , 68% increase in thermogenesis, 551% increase in horizontal activity, 556% increase in ambulatory activity; hits = 48% increase in VO_2 , 53% increase in thermogenesis, 299% increase in horizontal activity, 387% increase in ambulatory activity). For the 1-h time point, the data from the three remaining misses were analysed compared to the hits (VO_2 ; thermogenesis; RER; and horizontal, ambulatory, and total activity counts were analysed). The two-way ANOVA showed significant main effects

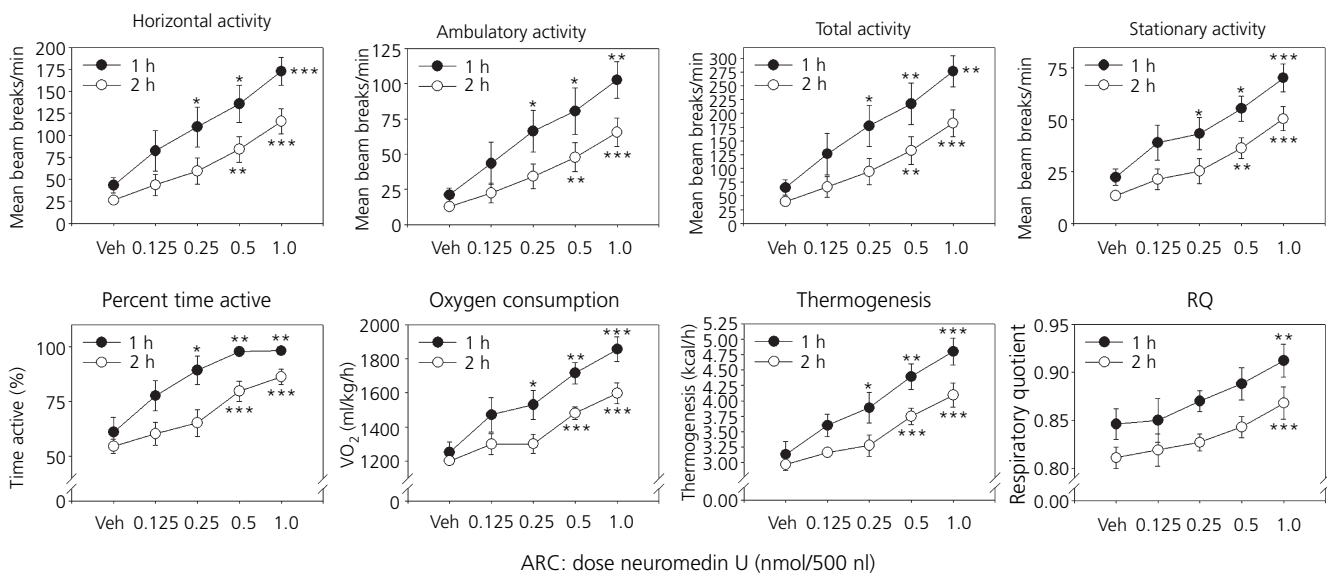


Fig. 2. Neuromedin U (NMU) applied locally to the arcuate nucleus dose-dependently increased physical activity and energy expenditure in rats in both the first and first two hours after microinjection. VO_2 , Oxygen consumption; RQ, respiratory quotient. *Significantly different from vehicle; **significantly different from vehicle and 0.125 nmol; ***significantly different from vehicle, 0.125 nmol, and 0.25 nmol; $P < 0.05$ ($n = 7$).

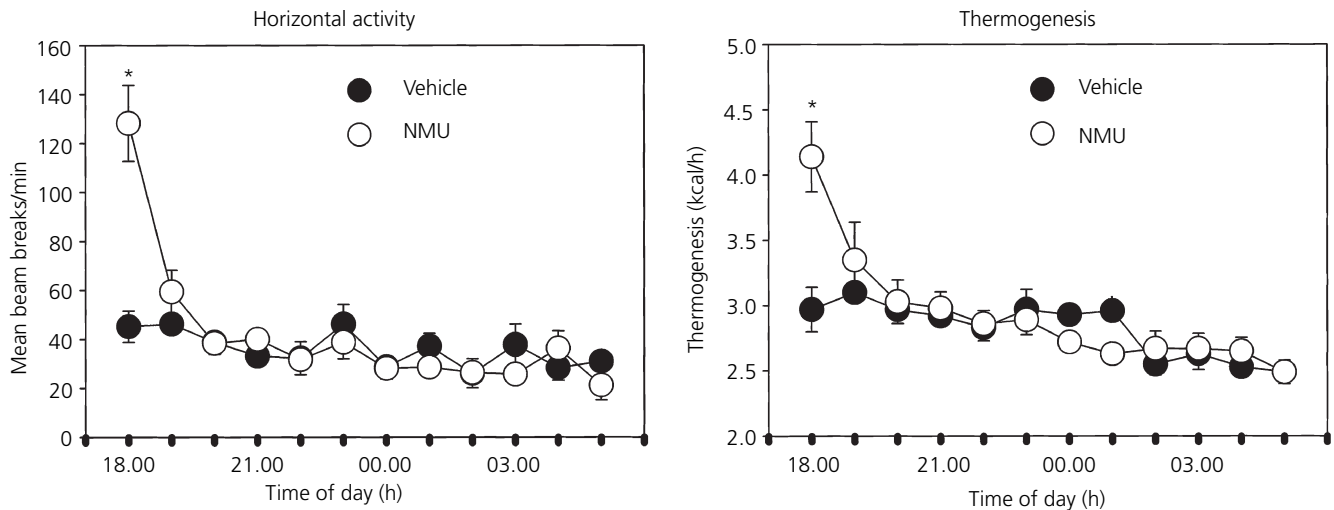


Fig. 3. Microinjection of 1 nmol neuromedin U (NMU) into the paraventricular hypothalamic nucleus (PVN) induces significant increases in nonexercise activity thermogenesis in the early dark (activity) phase. Animals showed significantly more horizontal activity and greater thermogenesis after NMU compared to vehicle treatment. *Significantly different from vehicle, $P < 0.05$ ($n = 6$).

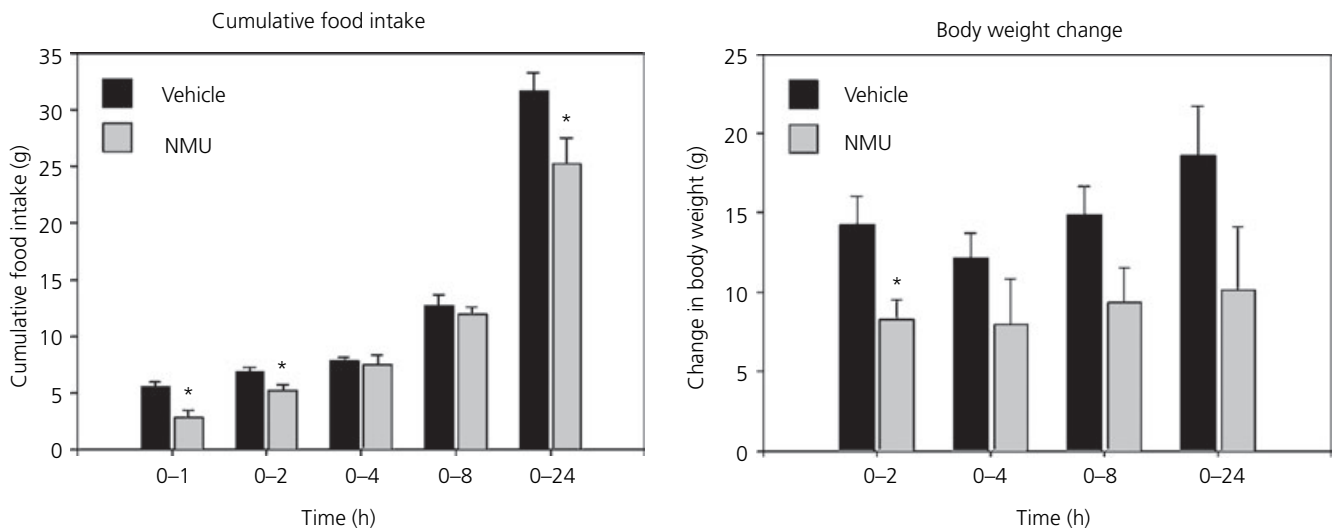
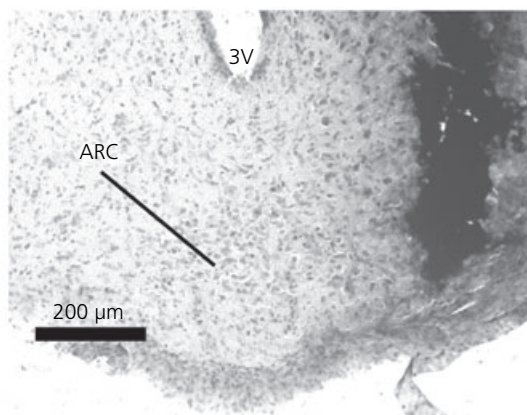
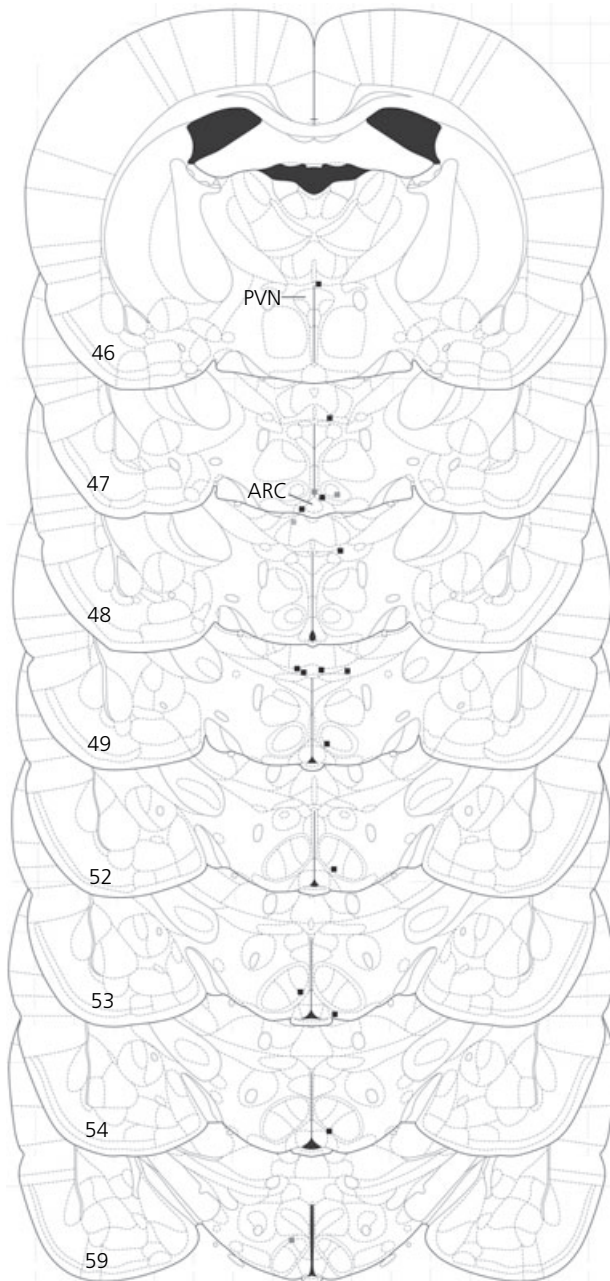


Fig. 4. Local application of Neuromedin U (NMU) to the paraventricular nucleus of the hypothalamus (PVN) significantly decreased 1-, 2- and 24-h food intake (in g) after 18 h of food deprivation. The change in body weight (at 2 h) was also significantly lower in NMU-treated rats compared to vehicle-treated rats. *Significantly different from vehicle, $P < 0.05$ ($n = 6$).

of group (accurate versus inaccurate cannulae placement). Further analyses demonstrated that there were no significant effects of NMU dose on any of these dependent variables in animals with inaccurate cannulae placements. Furthermore, NMU-induced effects on metabolic variables (VO_2 and thermogenesis) were significantly greater in the animals with hits compared to misses at 0.25, 0.5, and 1.0 nmol NMU. A similar effect was seen on activity (horizontal, ambulatory, and total activity) at 0.5 and 1.0 nmol NMU, where animals with accurate cannulae placements showed significantly more NMU-induced activity compared to those with inaccurate placements.

Discussion

The present studies are the first to demonstrate that NMU acts in the PVN and arcuate nucleus to increase NEAT in rats. This increase in thermogenesis is correlated with a concurrent increase in spontaneous locomotor activity. Stationary as well as locomotor (ambulatory) indices of activity were increased in response to NMU. In the PVN, NMU showed the maximum effect at 0.5 nmol whereas in the arcuate nucleus, NMU showed maximal effectiveness at increasing physical activity and energy expenditure at 1.0 nmol. These findings are similar to those showing increases in behaviours



such as grooming and locomotion after comparable doses of NMU in the PVN (22, 33). Our data further demonstrate that the energy expenditure of activity is significantly increased by site-specific application of NMU in the PVN and arcuate nucleus. Although NMU transgenic mice (34) show no alterations in oxygen consumption, thermogenesis, or physical activity compared to controls, 24-h measurements of these parameters were not carried out [group differences in physical activity seen over 24 h may not be detectable using measurements of shorter durations (31)]. In addition to the effects of NMU effects on physical activity and energy expenditure, the inhibitory effect of NMU in the PVN on feeding shown here is similar to that previously reported (22). Taken together with previous data (18, 22, 33), these findings imply that NMU acts in the PVN and arcuate nucleus to promote negative energy balance (20): to decrease body weight through decreasing food intake, as well as increasing energy expenditure at least partially through increasing NEAT.

Although the PVN clearly contains NMU2R (21, 27, 28), the mechanism of NMU action in the arcuate nucleus is less clear. In mice, NMU2R are distributed sparsely, at most, in the arcuate and adjacent areas (28). Despite this, it has been demonstrated that NMU in the arcuate nucleus induces changes in physical activity and food intake in rats (22). The arcuate nucleus is one of several hypothalamic regions that show increased Fos expression after i.c.v. injection of NMU (25). No changes in feeding are seen when NMU is injected into regions adjacent to the arcuate nucleus such as the ventromedial or posterior dorsomedial hypothalamus (22). It is also possible that NMU acts via the dense distribution of NMU2R in the ependymal lining of the third ventricle (21, 28) to alter food intake and physical activity, though the possible mechanisms of this effect are unknown.

Neuromedin U could be one mechanism through which leptin acts to decrease body weight through decreasing feeding (22, 23). Neuromedin U release from hypothalamic explants increases after leptin treatment (22), and blocking the central actions of NMU attenuates the ability of leptin to decrease food intake (23). Because both leptin (15) and NMU (present data) increase physical activity and NEAT, it is possible that leptin acts through NMU to increase NEAT as well. Neuromedin U may also act through other separate pathways to influence energy intake and metabolism (18). For example, CCK, another satiety signal implicated in changes in locomotor activity (35), activates brain NMU

Fig. 5. Placements of microinjections sites in rats with guide cannulae aimed at the paraventricular (PVN) and arcuate (ARC) hypothalamic nuclei. Black squares represent accurate cannulae placements (PVN, $n = 7$; ARC, $n = 7$) and grey squares represent inaccurate placements (PVN, $n = 1$; ARC, $n = 4$, one not shown). Injection sites are positioned according to coronal drawings of the rat brain atlas, with the atlas figure numbers on the left side of each section (41). One inaccurate injection site, aimed at the arcuate, is not represented (located rostral to plate 46). (All microinjections were unilateral on the left side of the brain; lateralizations were altered on the drawings for clarity of presentation.) The photomicrograph illustrates a coronal section containing the rostral pole of the arcuate nucleus and a microinjection site (see brain section 47) marked by ink. 3V, Third ventricle.

cells (24). Similar to NMU, CCK is implicated in the response to anxiety (36).

Our data imply that NMU affects feeding and NEAT at least partially through its actions on the PVN and arcuate nucleus. Both of these nuclei consist of several subregions with different characteristic cell types and neuropeptides that may differentially affect physical activity. Within the PVN, NMU is likely to increase physical activity through its actions on corticotrophin-releasing factor (CRF). Similar to CRF, NMU may be involved in the stress response or, alternatively, NEAT induction and the stress response may utilize overlapping neural circuits. Central NMU increases the activation of CRF-immunopositive (CRF+) cells in the PVN (29). Central treatment with a CRF antagonist abolishes NMU-induced increases in locomotor activity (37), and the ability of NMU to increase locomotor activity, body temperature, and oxygen consumption, as well as decrease food intake, is not seen in CRF-deficient mice (37, 38). It has been suggested that NMU participates in the brain circuit regulating the stress response: following NMU administration, the changes in physical activities seen are similar to those seen during a stress response (i.e. grooming) (22, 26, 33, 37). In addition, central NMU increases stress hormone levels and increases Fos expression in brain areas associated with the stress response (22, 25, 29, 30, 33). A putative mechanism for the ability of CRF to increase NEAT is the activation of PVN CRF+ neurones projecting to the locus coeruleus (39), an area important in arousal and the sleep-wake cycle. To test this hypothesis, microinjections of NMU restricted to the parvocellular PVN, or microinjections of CRF antagonists in the target regions (e.g. locus coeruleus) are needed.

Studies investigating the effects of NMU on feeding behaviour and activity have noted that NMU prevents rats from sleeping (22, 33). It is plausible that NMU treatment during the light (sleep) phase of the cycle decreases sleep and therefore increases the amount of physical activity by default. To test this hypothesis, we gave rats NMU or vehicle injections at the start of the dark (active) phase of the cycle, when rats are normally awake and active. Neuromedin U increased physical activity and NEAT during the first hour after microinjection. This implies that NMU in the PVN acts as a short-term signal to increase NEAT. If the ability of NMU to increase physical activity was due to the suppression of sleep, we would expect NMU to be more effective at increasing NEAT during the daytime (sleep phase) than the night (active phase). We found that the magnitude of the effect of 1 nmol NMU on NEAT during the dark (active) phase was not decreased compared to the light (inactive) phase. Therefore, the prevention of sleep alone cannot account for the increased NEAT shown in animals receiving central injections of NMU, and it is likely that NMU acts on hypothalamic circuits to regulate levels of physical activity rather than sleep alone.

We have demonstrated that NMU increases NEAT when applied directly to the PVN or arcuate nucleus. Because of the sensitivity of NMU neurones throughout the brain to leptin and CCK (22, 24), and the ability of intra-PVN NMU to increase NEAT demonstrated in the present study, one might speculate that one role of NMU is to increase NEAT during conditions of caloric excess. Due in part to its actions on NEAT, NMU is an attractive target for the treatment of obesity (40).

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References

- Hu FB, Li TY, Colditz GA, Willett WC, Manson JE. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA* 2003; **289**: 1785–1791.
- Bassett DR, Schneider PL, Huntington GE. Physical activity in an Old Order Amish community. *Med Sci Sports Exerc* 2004; **36**: 79–85.
- Wyatt HR, Peters JC, Reed GW, Barry M, Hill JO. A Colorado statewide survey of walking and its relation to excessive weight. *Med Sci Sports Exerc* 2005; **37**: 724–730.
- Weinsier RL, Hunter GR, Heini AF, Goran MI, Sell SM. The etiology of obesity: relative contribution of metabolic factors, diet, and physical activity. *Am J Med* 1998; **105**: 145–150.
- Brown DW, Brown DR, Heath GW, Balluz L, Giles WH, Ford ES, Mokdad AH. Associations between physical activity dose and health-related quality of life. *Med Sci Sports Exerc* 2004; **36**: 890–896.
- Hu FB, Willett WC, Li T, Stampfer MJ, Colditz GA, Manson JE. Adiposity as compared with physical activity in predicting mortality among women. *Obstet Gynecol Surv* 2005; **60**: 311–312.
- Kesaniemi YK, Danforth E Jr, Jensen MD, Kopelman PG, Lefebvre P, Reeder BA. Dose–response issues concerning physical activity and health: an evidence-based symposium. *Med Sci Sports Exerc* 2001; **33**: S351–S358.
- Levine J, Melanson EL, Westerterp KR, Hill JO. Measurement of the components of nonexercise activity thermogenesis. *Am J Physiol Endocrinol Metab* 2001; **281**: E670–E675.
- Levine JA, Schleusner SJ, Jensen MD. Energy expenditure of nonexercise activity. *Am J Clin Nutr* 2000; **72**: 1451–1454.
- Levine JA. Nonexercise activity thermogenesis (NEAT). environment and biology. *Am J Physiol Endocrinol Metab* 2004; **286**: E675–685.
- Levine JA, Lanningham-Foster LM, McCrady SK, Krizan AC, Olson LR, Kane PH, Jensen MD, Clark MM. Interindividual variation in posture allocation: possible role in human obesity. *Science* 2005; **307**: 584–586.
- Levine JA, Eberhardt NL, Jensen MD. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 1999; **283**: 212–214.
- Kiwaki K, Kotz CM, Wang C, Lanningham-Foster L, Levine JA. Orexin A (hypocretin 1) injected into hypothalamic paraventricular nucleus and spontaneous physical activity in rats. *Am J Physiol Endocrinol Metab* 2004; **286**: E551–E559.
- Castaneda TR, Jurgens H, Wiedmer P, Pfluger P, Diano S, Horvath TL, Tang-Christensen M, Tschop MH. Obesity and the neuroendocrine control of energy homeostasis: the role of spontaneous locomotor activity. *J Nutr* 2005; **135**: 1314–1319.
- Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995; **269**: 540–543.
- Marsh DJ, Weingarh DT, Novi DE, Chen HY, Trumbauer ME, Chen AS, Guan XM, Jiang MM, Feng Y, Camacho RE, Shen Z, Frazier EG, Yu H, Metzger JM, Kuca SJ, Shearman LP, Gopal-Truter S, MacNeil DJ, Strack AM, MacIntyre DE, Van der Ploeg LH, Qian S. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic

- and have altered metabolism. *Proc Natl Acad Sci USA* 2002; **99**: 3240–3245.
- 17 Tang-Christensen M, Vrang N, Ortmann S, Bidlingmaier M, Horvath TL, Tschop M. Central administration of ghrelin and agouti-related protein (83–132) increases food intake and decreases spontaneous locomotor activity in rats. *Endocrinology* 2004; **145**: 4645–4652.
 - 18 Hanada R, Teranishi H, Pearson JT, Kurokawa M, Hosoda H, Fukushima N, Fukue Y, Serino R, Fujihara H, Ueta Y, Ikawa M, Okabe M, Murakami N, Shirai M, Yoshimatsu H, Kangawa K, Kojima M. Neuromedin U has a novel anorexigenic effect independent of the leptin signaling pathway. *Nat Med* 2004; **10**: 1067–1073.
 - 19 Niimi M, Muraio K, Taminato T. Central administration of neuromedin U activates neurons in ventrobasal hypothalamus and brainstem. *Endocrine* 2001; **16**: 201–206.
 - 20 Nakazato M, Hanada R, Murakami N, Date Y, Mondal MS, Kojima M, Yoshimatsu H, Kangawa K, Matsukura S. Central effects of neuromedin U in the regulation of energy homeostasis. *Biochem Biophys Res Commun* 2000; **277**: 191–194.
 - 21 Howard AD, Wang R, Pong SS, Mellin TN, Strack A, Guan XM, Zeng Z, Williams DL Jr, Feighner SD, Nunes CN, Murphy B, Stair JN, Yu H, Jiang Q, Clements MK, Tan CP, McKee KK, Hreniuk DL, McDonald TP, Lynch KR, Evans JF, Austin CP, Caskey CT, Van der Ploeg LH, Liu Q. Identification of receptors for neuromedin U and its role in feeding. *Nature* 2000; **406**: 70–74.
 - 22 Wren AM, Small CJ, Abbott CR, Jethwa PH, Kennedy AR, Murphy KG, Stanley SA, Zollner AN, Ghatei MA, Bloom SR. Hypothalamic actions of neuromedin U. *Endocrinology* 2002; **143**: 4227–4234.
 - 23 Jethwa PH, Small CJ, Smith KL, Seth A, Darch SJ, Abbott CR, Murphy KG, Todd JF, Ghatei MA, Bloom SR. Neuromedin U has a physiological role in the regulation of food intake and partially mediates the effects of leptin. *Am J Physiol Endocrinol Metab* 2005; **289**: E301–E305.
 - 24 Ivanov TR, Le Rouzic P, Stanley PJ, Ling WY, Parello R, Luckman SM. Neuromedin U neurones in the rat nucleus of the tractus solitarius are catecholaminergic and respond to peripheral cholecystokinin. *J Neuroendocrinol* 2004; **16**: 612–619.
 - 25 Ivanov TR, Lawrence CB, Stanley PJ, Luckman SM. Evaluation of neuromedin U actions in energy homeostasis and pituitary function. *Endocrinology* 2002; **143**: 3813–3821.
 - 26 Gartlon J, Szekeres P, Pullen M, Sarau HM, Aiyar N, Shabon U, Michalovich D, Stepelwski K, Ellis C, Elshourbagy N, Duxon M, Ashmeade TE, Harrison DC, Murdock P, Wilson S, Ennaceur A, Atkins A, Heidbreder C, Hagan JJ, Hunter AJ, Jones DN. Localisation of NMU1R and NMU2R in human and rat central nervous system and effects of neuromedin-U following central administration in rats. *Psychopharmacology (Berl)* 2004; **177**: 1–14.
 - 27 Guan XM, Yu H, Jiang Q, Van Der Ploeg LH, Liu Q. Distribution of neuromedin U receptor subtype 2 mRNA in the rat brain. *Brain Res Gene Expr Patt* 2001; **1**: 1–4.
 - 28 Graham ES, Turnbull Y, Fotheringham P, Nilaweera K, Mercer JG, Morgan PJ, Barrett P. Neuromedin U and neuromedin U receptor-2 expression in the mouse and rat hypothalamus: effects of nutritional status. *J Neurochem* 2003; **87**: 1165–1173.
 - 29 Yokota M, Ozaki Y, Sakamoto F, Yamada S, Saito J, Fujihara H, Ueta Y. Fos expression in CRF-containing neurons in the rat paraventricular nucleus after central administration of neuromedin U. *Stress* 2004; **7**: 109–112.
 - 30 Ozaki Y, Onaka T, Nakazato M, Saito J, Kanemoto K, Matsumoto T, Ueta Y. Centrally administered neuromedin U activates neurosecretion and induction of c-fos messenger ribonucleic acid in the paraventricular and supraoptic nuclei of rat. *Endocrinology* 2002; **143**: 4320–4329.
 - 31 Novak CM, Kotz CM, Levine JA. Central orexin sensitivity, physical activity, and obesity in diet-induced obese and diet-resistant rats. *Am J Physiol Endocrinol Metab* 2005; **290**: E396–E403.
 - 32 Novak CM, Albers HE. Novel phase-shifting effects of GABAA receptor activation in the suprachiasmatic nucleus of a diurnal rodent. *Am J Physiol Regul Integr Comp Physiol* 2004; **286**: R820–R825.
 - 33 Thompson EL, Murphy KG, Todd JF, Martin NM, Small CJ, Ghatei MA, Bloom SR. Chronic administration of NMU into the paraventricular nucleus stimulates the HPA axis but does not influence food intake or body weight. *Biochem Biophys Res Commun* 2004; **323**: 65–71.
 - 34 Kowalski TJ, Spar BD, Markowitz L, Maguire M, Golovko A, Yang S, Farley C, Cook JA, Tetzloff G, Hoos L, Del Vecchio RA, Kazdoba TM, McCool MF, Hwa JJ, Hyde LA, Davis H, Vassileva G, Hedrick JA, Gustafson EL. Transgenic overexpression of neuromedin U promotes leanness and hypophagia in mice. *J Endocrinol* 2005; **185**: 151–164.
 - 35 Weiland TJ, Voudouris NJ, Kent S. The role of CCK2 receptors in energy homeostasis: insights from the CCK2 receptor-deficient mouse. *Physiol Behav* 2004; **82**: 471–476.
 - 36 Wang H, Wong PT, Spiess J, Zhu YZ. Cholecystokinin-2 (CCK2) receptor-mediated anxiety-like behaviors in rats. *Neurosci Biobehav Rev* 2005; **29**: 1361–1373.
 - 37 Hanada R, Nakazato M, Murakami N, Sakihara S, Yoshimatsu H, Toshinai K, Hanada T, Suda T, Kangawa K, Matsukura S, Sakata T. A role for neuromedin U in stress response. *Biochem Biophys Res Commun* 2001; **289**: 225–228.
 - 38 Hanada T, Date Y, Shimbara T, Sakihara S, Murakami N, Hayashi Y, Kanai Y, Suda T, Kangawa K, Nakazato M. Central actions of neuromedin U via corticotropin-releasing hormone. *Biochem Biophys Res Commun* 2003; **311**: 954–958.
 - 39 Reyes BA, Valentino RJ, Xu G, Van Bockstaele EJ. Hypothalamic projections to locus coeruleus neurons in rat brain. *Eur J Neurosci* 2005; **22**: 93–106.
 - 40 Daggrell SA. Neuromedin U – a new target in obesity. *Expert Opin Ther Targets* 2005; **9**: 875–877.
 - 41 Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*, 5th edn. Amsterdam: Elsevier, 2005.