Central Administration of Neuropeptide Y Induces Wakefulness in Rats

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Neuropeptide Y (NPY) is a well-characterized neuromodulator in the central nervous system, primarily implicated in the regulation of feeding. NPY, orexins and ghrelin form a hypothalamic food intake regulatory circuit. Orexin and ghrelin are also implicated in sleep-wake regulation. In the present experiments, we studied the sleep-modulating effects of central administration of NPY in rats. Rats received intracerebroventricular injection of physiological saline or three different doses of NPY (0.4 µg, 2 µg and 10 µg in a volume of 4 µl) at light onset. Another group of rats received bilateral microinjection of saline or 2 µg NPY into the lateral hypothalamus in a volume of 0.2 µl. Sleep-wake activity and motor activity were recorded for 23 hours. Food intake after the control and treatment injections was also measured on separate days.

Intracerebroventricular and lateral hypothalamic administration of NPY suppressed non rapid-eye-movement sleep and rapid-eye-movement sleep in rats during the first hour after the injection and also induced changes in EEG delta power spectra. Neuropeptide Y stimulated food intake in the first hour after both routes of administration. Data are consistent with the hypothesis that NPY has a role in the integration of feeding, metabolism and sleep regulation.

Keywords: food intake, lateral hypothalamus, EEG, FFT, slow-wave activity
INTRODUCTION

Neuropeptide Y (NPY) is widely distributed in high concentrations in the central nervous system and acts as a neurohormone and neuromodulator. The main source of NPY in the brain is the hypothalamus, particularly the arcuate nucleus (ARC), dorsomedial nucleus (DMN), paraventricular nucleus (PVN), suprachiasmatic nucleus (SCN) and the brainstem (2). NPY is implicated in the regulation of several physiological processes, such as food intake (24; 38), hormone secretions (13; 23; 42), circadian rhythms (1), thermoregulation (19) and blood pressure (7).

NPY is part of the widely studied hypothalamic food intake regulatory circuit which also involves orexin, ghrelin, agouti-related peptide and melanin concentrating hormone. All these neuropeptides stimulate food intake when injected into the cerebral ventricle or into various hypothalamic nuclei (9; 20; 34-36; 46). The NPY receptor family includes at least 6 subtypes from which the Y1 and Y5 are implicated in the regulation of food intake. Both receptors are present in the PVN, ARC, medial preoptic area, SCN, supraoptic nucleus and in the lateral hypothalamus (LH) (44). The NPY Y1 receptor may be involved in mediating behavioral effects other than feeding since mice lacking NPY Y1 receptor show reduced locomotor activity (33).

NPY-immunoreactive neurons, originating from the ARC, innervate orexinergic cells in the LH (16). Orexin-immunoreactive axon terminals from the LH end on NPYergic neurons in the ARC (16; 28). Ghrelin is known to act through NPYergic pathways in the
ARC (22; 37) to stimulate feeding. Orexin is known to play important role in maintaining wakefulness (36). Orexin stimulates wakefulness when injected into the cerebral ventricles (14) or into the PVN, DMH and LH (8). The lack of orexin and/or orexin receptors is linked to narcolepsy (26). Ghrelin also inhibits sleep in rats when injected into the cerebral ventricle (41) or various hypothalamic nuclei (Szentirmai and Krueger, unpublished observations).

Little is known concerning the potential role of NPY in sleep regulation. In one study, visual inspection of the electroencephalograms (EEG) suggested that NPY induces a reduction in desynchronized EEG activity and, an increase in synchronized and mixed activity in rats (49). Ehlers at al. (11), found that high doses of NPY decrease EEG power of all frequencies in rats, but does not influence sleep onset or the amount of non-rapid-eye-movement sleep (NREMS). In humans, repeated intravenous injection of NPY was reported to promote sleep and reduce sleep latency when given to young normal male subjects (3). The same research group in a more recent study which was done in older male and female patients with depression using age-matched controls found no change in sleep time, only shortened sleep latency after repeated intravenous administration of NPY (15). The aim of our experiments was to study sleep and EEG responses to centrally injected NPY in rats.
METHODS

Animals.
Male Sprague-Dawley rats, weighing 275-300 g at the time of surgeries, were used. Rats were housed individually in Plexiglas cages in temperature-controlled (23 ± 1°C) environmental chambers at a 12:12 h light-dark cycle (light on at 9:00 am). Water and food were available ad libitum. Institutional guidelines for the care and use of research animals were followed and approved by the Institutional Animal Care and Use Committees.

Surgery.
Rats were anesthetized by intraperitoneal injection of a ketamine and xylazine mixture (87 and 13 mg/kg, respectively). Stainless steel screw electrodes for EEG recordings were placed over the frontal (1.5 mm anterior and 1.5 mm lateral to the bregma) and parietal cortices (4 mm posterior and 2 mm lateral to the bregma) and electromyographic (EMG) electrodes were implanted into the dorsal neck muscle. Stereotaxic equipment was used to insert an intracerebroventricular (icv) cannula (Plastics One, 22 G) into the right lateral cerebral ventricle [coordinates of the tip of the guide cannula: 0.80 mm posterior and 1.5 mm lateral to the bregma, and 4.0 mm ventral from the surface of the skull, according to the rat brain atlas by Paxinos and Watson (32)] or microinjection cannulae (26 G) bilaterally into the lateral hypothalamus [coordinates of the tip of the guide cannula: 2.1 mm posterior, 2 mm lateral, and 7.8 mm ventral]. The size of the injector cannulae were 30 G for the icv cannula and 33 G for the microinjection cannulae:
both extended 0.5 mm beyond the tip of the guide. The guide cannulae and the screws were fixed with dental cement to the skull.

*Verification of cannula placement.*

The location of the icv cannula was determined by the gravity method (sudden drop in pressure) during implantation and the drinking response to icv injection of angiotensin (Bachem, California Inc., Torrance, Ca) tested 3-4 days after surgery and also after the end of the experiments. To verify the location of the microinjection cannulae in the LH, 0.2 µl 5 % horseradish peroxidase was injected into the cannulae at the end of the experiment. Rats, anesthetized with Isoflurane, were perfused with saline and 4% paraformaldehyde. Brains were removed and kept at 4°C in paraformaldehyde until further examinations. The peroxidase–H₂O₂ reaction was visualized by diamonobenzidine in 100 µm thick neutral red-stained coronal brain sections. The spread of the injections was less than 1 mm as indicated by the enzyme reaction. The injection sites were localized with reference to the rat brain atlas (32) (Figure 1).

*Sleep-wake recording.*

After surgery, a 7-10 day recovery period followed, and then the rats were connected to the recording cable and habituated to the experimental conditions for an additional 5 days. The recording cables were attached to commutators. Cables from the commutators were connected to amplifiers. The digitized (128 Hz sampling rate) signals of the EEG and EMG were collected by computers. The EEG was filtered below 0.1 Hz and above 40 Hz. EMG activity served the purpose of aiding in determining the vigilance state of
the animals. The states of vigilance were determined off-line in 10-s epochs by using the conventional criteria as NREMS [high-amplitude EEG waves, lack of body movement, predominant EEG power in the delta range (0.5-4.0 Hz)]; REMS (highly regular low amplitude EEG, dominance of theta activity with corresponding high FFT theta (4.5-8 Hz) power, general lack of body movements with occasional twitches); and wakefulness (less regular low amplitude EEG, the lack of the visible theta dominance, frequent body movements). The amount of time spent in each vigilance state was calculated in 1-h time blocks. Power density values were calculated for each vigilance state by fast-Fourier transformation (FFT) for consecutive 10-s epochs in the frequency range of 0.5–16.0 Hz for 0.5-Hz bands. In addition, EEG power values for the 0.5- to 4-Hz delta range during NREMS were integrated and used to characterize NREM sleep intensity, also known as EEG slow-wave activity (SWA). Those epochs that contained EEG artifacts were excluded from the FFT analyses.

**Experimental procedures.**

In experiment 1, rats were icv injected with NPY (Bachem California Inc., Torrance, Ca) or pyrogen-free isotonic NaCl (PFS) 10-15 min before light onset. The three doses of NPY were of 0.4 μg (n = 8), 2 μg (n = 9), and 10 μg (n = 8) injected in a volume of 4 μl. In each group, two conditions, a baseline day when 4 μl of PFS was administered and an experimental day when NPY was injected, were used. The order of the baseline and experimental days was randomly chosen. Some of the rats were injected with more than one dose of NPY; at least one week separated the injections. These rats were not selected based on previous responses to NPY. In experiment 2, another group of rats (n = 8) with
bilateral intrahypothalamic cannulae received 2 μg NPY/injection on each side in a volume of 0.2 μl on the experimental day and equal volumes of PFS on the control day. Microinjections took place over a 1-min period and the microinjection cannulae were left in place for one additional minute. The rats were adapted to the experimental procedures for at least 7 days prior to the experiments; by the time of the recording, the injection procedures did not cause any visible stress or discomfort to the rats. Each rat was recorded beginning at 9:00 am for 23 hours starting immediately after injections.

*Measurement of food intake.*

Food intake was measured in each group of rats 4 days after the sleep studies. The experimental procedures were similar to those above. Immediately after injection, animals were returned to their home cages where known amount of chow had been placed. Food pellets were reweighed 1 h after injection. Results are expressed as g food intake/kg body weight ± SEM.

*Statistics.*

Two-way analysis of variance (ANOVA) for repeated measures was performed on sleep and power spectra data (factors: treatment and time effect or treatment and frequency effect, respectively). Those hours, during which a rat did not have at least 5 min NREMS were excluded from the SWA analysis, resulting in missing data points. Therefore, instead of repeated measures ANOVA, two-way ANOVA was performed on SWA. Time spent in sleep and SWA data were analyzed in 1-h time blocks for hours 1, 2 and 3 and on 3-h time blocks for hours 4-23 of the recording period between the baseline day
and the experimental days in each group. Average power spectra values during each vigilance state were analyzed in the first 3 hours after injections. When ANOVA indicated significant effects, the Student-Newman-Keuls test (SNK-test) was used for post-hoc analysis to identify which group and treatment differed from the other groups and treatments. The episode numbers and the average episode duration of NREMS and rapid-eye-movement sleep (REMS) and the effects of NPY on food intake in the first hour after the injection were analyzed by paired t-test. When at least the half of the rats did not have a REMS episode in that hour statistical analysis on average REMS episode duration was not performed. An $\alpha$-level of $p < 0.05$ was considered to be significant.
RESULTS

1. Effects of NPY icv injection on sleep.

Intracerebroventricular injection of NPY elicited decreases in NREMS and REMS in the first hour after injection (Figure 2). The lowest dose, 0.4 µg NPY had statistically significant effect on NREMS as indicated by ANOVA (Table 1) which was confined to the third hour after the injection (SNK-test, Figure 2); the biological significance of this isolated difference in NREMS between the baseline and experimental day is questionable. There was no significant effect on the total episode number and episode duration of NREMS and REMS and on EEG SWA after the 0.4 µg dose of NPY (Table 2 and Figure 2). The detailed analysis of EEG power spectrum in the first three hours revealed significant decreases in the NREMS power spectrum in the 0.5-4.5 Hz frequency band; wake and REMS EEG were not affected (Figure 3). There was no significant change in the food intake of the rats in response to 0.4 µg NPY (Figure 4).

Administration of 2 µg NPY had significant effects on both NREMS and REMS as indicated by ANOVA (Table 1). The effects were confined to the first hour; NREMS decreased from a baseline of 26.6 ± 2.2 min to 12.6 ± 2.3 min after NPY treatment. REMS virtually disappeared in hour one on the test day. The reduced time spent in sleep may have resulted from a significant decrease in the average duration of NREMS episodes and a significant decrease in the number of REMS episodes (Table 2). There was a tendency toward decreased NREMS episode number, but statistical analyses did
not show significant differences. The EEG SWA did not change significantly. Detailed analysis of the EEG showed a significant increase in EEG power spectrum in the 4-7 Hz frequency band during wake and REMS (Figure 3). Two µg NPY significantly increased the food intake of the rats (Figure 4).

The 10 µg NPY injection was also followed by a significant decrease in both NREMS and REMS amount as indicated by ANOVA (Table 1). Post hoc analyses showed significant suppression in NREMS in hour 1. The NREMS decrease may be due to the significant decrease in the number of NREMS episodes; the changes in average NREMS episode duration were not significant. In hour 1, on the baseline day, rats had already minimal amount of REMS, and on the NPY day they had no REMS at all. Injection of 10 µg NPY did not change the EEG SWA. The EEG power spectra did not showed any significant difference in any vigilance state (Figure 3). The highest dose of NPY significantly increased the food intake of the rats (Figure 4).

2. Effects of NPY lateral hypothalamic injection on sleep.

The effects of NPY lateral hypothalamic injections on sleep and feeding were similar to those observed after icv treatment (Figure 1). Time spent in NREMS and REMS was significantly decreased in hour 1 and 3, respectively (Table 1). NREMS episode number significantly decreased in hour 1 and there was a tendency toward decrease in average NREMS episode duration, as well (Table 2). EEG SWA increased in response to the injection starting from hour 3, however, post hoc analyses did not show significance in
any particular hour. The detailed analyses of EEG power spectra showed a slight, but statistically significant increase the EEG power spectrum in the 6-7.5 Hz frequency band during wake (Figure 2). Food intake was significantly enhanced by lateral hypothalamic injection of NPY (Figure 4).
DISCUSSION

Intracerebroventricular and lateral hypothalamic administration of NPY suppressed NREMS and REMS in rats when injected at light onset. In addition, it also stimulated food intake in the first hour after both routes of administration. NPY is primarily implicated in feeding regulation. Our findings confirm previous studies that central injection of NPY increases food intake in rats (24; 39).

Previous reports concerning NPY’s sleep-modulating effect did not yield consistent results. In rats, icv injection of NPY three hours after light onset failed to change the amount of time spent in slow-wave sleep (11): the differences in the results, between that study and current one may be due to the different time of injection. In humans, repeated intravenous bolus injections of NPY during the dark period promoted NREMS and had no effect on sleep EEG spectra in normal young male subjects (3). The same research group carried out a more recent study in older male and female patients with depression (15). NPY infusion caused the shortening of NREMS and REMS latencies, but did not affect the time spent in stage 2 sleep, slow wave sleep, REMS or total sleep time. There was no significant difference in the responsiveness to NPY between the depressed and control groups. In our experiments, when NPY was injected at light onset, the sleep suppressive effects were robust; both NREMS and REMS significantly decreased in the first hour of the light period, REMS practically disappeared. The decrease in NREMS amount in the first hour after the injection is clearly reflected in the decreased total number of NREMS episodes; nevertheless, there was a tendency towards decreased
average duration of NREMS episodes, as well. After NPY injection REMS completely
disappeared in the first hour of the light period, however the amount of REMS on the
baseline day was also relatively low.

The mechanism through which NPY promotes wakefulness is unknown. NPY-
immunoreactive cell bodies are present in the ARC, PVN, SCN, DMH and LH; nuclei
implicated in feeding and sleep-wake regulation. NPY Y1 and Y5 receptors, which are
mainly involved in the food intake stimulatory activity of NPY, are also present in these
hypothalamic nuclei (44). NPY-containing axon terminals innervate orexinergic neurons
in the LH. Intracerebroventricular injection of NPY increases c-fos immunoreactivity in
the ARC, PVN (25) and in the lateral hypothalamic orexinergic neurons (6). Besides
stimulating feeding, orexins promote wakefulness and locomotor activity (36), therefore
it is also possible that NPY’s stimulatory action on orexinergic cells in the LH mediates
the wake-promoting effect of NPY. This notion is supported by our observation that
NREMS decreased in response to lateral hypothalamic injection of NPY. A reciprocal
relationship exists between NPY and orexinergic neurons. Intracerebroventricular
administration of orexins stimulates NPY expression in the ARC (27). Orexinergic
neuron terminals originating from the lateral hypothalamus form synapses on NPY-
immunoreactive cells in the ARC and also have close contact with NPYergic cells in the
PVN (16). Orexin receptor immunoreactivity is present on NPY neurons in the ARC (4).
The feeding stimulatory effect of orexin may be mediated, at least partly, by NPY, since
orexin-induced feeding is inhibited by pretreatment with NPY receptor antagonists (17;
47). Conversely, orexin antiserum significantly attenuates the feeding response to NPY
In addition to orexinergic neurons, NPY activates other mechanisms in the hypothalamus known to be involved in promoting arousal. For example, NPY stimulates CRH release and increases CRH gene expression in the PVN (40). CRH is known to inhibit sleep (10; 31). Therefore, CRH is another candidate for mediating the wakefulness stimulating effect of NPY.

The sleep-suppressing and food intake-promoting activities of NPY in rats are very similar to those observed after central injection of ghrelin. Intracerebroventricular administration of ghrelin decreases NREMS and REMS dose-dependently and increases food intake of rats in h 1 after the injection (41). Ghrelin and NPY are the part of the hypothalamic food intake regulatory circuit. Both peptides strongly promote feeding behavior in rats (5) and we found that both suppress sleep. Sleep and feeding are mutually exclusive behaviors therefore any increase in feeding might result in less sleep time. In our previous study, the wakefulness-enhancing activity of ghrelin was not a direct consequence of its food intake-promoting activity because ghrelin also induced wakefulness in rats that had no access to food. In the present study, the sleep effect of NPY in food deprived rats was not examined, nevertheless it is possible that NPY, similarly to ghrelin, suppresses sleep not exclusively because it enhances a competitive behavior such as feeding. There is growing body of evidence suggesting that NPY may mediate the food intake-promoting activity of ghrelin. Ghrelin receptors are located on NPY neurons in the ARC (43). Icv injection of ghrelin induces c-fos (29) and NPY mRNA expression in the ARC (37) and stimulates electrophysiological activity in NPY neurons (22). Ghrelin stimulates the synthesis and release of NPY in the ARC (45).
Ghrelin-induced feeding is decreased by pretreatment with NPY antibodies or receptor antagonists (29).

In addition to its sleep modulating effect, NPY also induced changes in EEG power spectra. The 0.4 µg dose significantly decreased EEG power spectrum in the 0.5-4.5 Hz frequency range during NREMS, while the 2 µg dose induced an increase in the power spectra during wake and REMS in the 4-7 Hz frequency band in the first three hours following the injections. In contrast, previous studies found that icv injection of NPY induced the slowing of high frequency theta activity simultaneously with the speeding up of low frequency theta waves in cortex, hippocampus and amygdala in rats (11). Two studies from the same research group found increased synchronized and mixed EEG activity after central administration of NPY in rats (12; 49). The differences between the results of the studies above and ours may be due to the different experimental conditions. In our experiment, we recorded the EEG from the beginning of the light onset, starting immediately after the injection. In the above experiments NPY was injected 2 hours after light onset, and the EEG recording started 20 minutes after the injection and lasted for about 2 hours during which the animals were removed from their home cages. The timing of NPY injection could be a crucial factor, since NPY is known to modulate the activity of the suprachiasmatic nucleus (48).

In conclusion, current findings suggest that NPY elicits prompt increase in wakefulness and feeding when given before the rest phase of the day. The food intake-enhancing and the wakefulness-promoting effects of NPY are in the same dose range since the lowest
dose that did not induced significant food intake also failed to cause biologically relevant
difference in sleep. Increased eating activity accompanied by high percentage of time
spent awake are characteristics of the first and second hours of the active period of rats
under normal conditions (“dark onset syndrome”). Orexins, NPY and ghrelin are parts of
the hypothalamic food intake regulatory circuit. It is possible, that the activation of the
same circuit results in wakefulness, which is independent, at least in part, from the food
intake-inducing actions. Additional evidence supporting the role of NPY in sleep
regulation include that chronic REMS deprivation increases NPY expression in the rat
hypothalamus (21) and NPY-like immunoreactivity shows a diurnal rhythm in the SCN
and ARC, with significant peak prior to onset of the dark period (18). We hypothesize
that increasing hypothalamic NPY levels may be responsible, at least in part, for
triggering behavioral changes characteristic of the dark onset syndrome.
ACKNOWLEDGEMENTS

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Figure legends

Figure 1. Representation of the microinjection sites in the lateralhypothalamus (Paxinos and Watson, 2005). Each rat received bilateral microinjections. Values on the panels denote distances from bregma.

Figure 2. The effects of intracerebroventricular (icv) and lateral hypothalamic administration of neuropeptide Y (NPY) (●) and pyrogen-free physiological saline (PFS) (○) on sleep and slow-wave activity (SWA). The amounts of non-rapid-eye-movement sleep (NREMS), rapid-eye-movement sleep (REMS) and EEG slow-wave activity (SWA) were calculated in 1-h time blocks for the first 3 h after the injection and in 3-h time blocks for the remaining part of the recording period. Error bars: standard error. Asterisks denote significant differences between baseline and experimental day (p < 0.05, Student-Neumann-Keuls test).

Figure 3. The EEG power spectra of wake, NREMS and REMS in the first 3-h time block after icv and lateral hypothalamic administration of NPY and PFS. See legends to Fig. 1 for details.

Figure 4. The effects of icv and lateral hypothalamic (LH) administration of NPY and PFS on food intake in the first h after injection. Error bars: standard error. Asterisks denote significant differences between baseline day and experimental day (p < 0.05, paired t-test).
Figure 1.
Figure 2.

- **0.4 μg NPY**
- **2 μg NPY**
- **10 μg NPY**
- **LH 2 μg NPY**

<table>
<thead>
<tr>
<th>Time After Injection (h)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
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<tr>
<td>% of Recording Time</td>
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</table>

*Statistically significant differences indicated by asterisks.*
Figure 3.
Figure 4.
Table 1. The effects of icv and lateral hypothalamic administration of neuropeptide Y (NPY) on non-rapid-eye-movement sleep (NREMS), rapid-eye-movement sleep (REMS) amount, EEG slow-wave activity (SWA) and EEG power spectra of vigilance states: statistical results. p < 0.05: significant difference between baseline and treatment condition. n.s.: non-significant difference between baseline and treatment condition.

<table>
<thead>
<tr>
<th></th>
<th>0.4 μg NPY</th>
<th>2 μg NPY</th>
<th>10 μg NPY</th>
<th>LH 2 μg NPY</th>
</tr>
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<tr>
<td></td>
<td>Treatment</td>
<td>Interaction</td>
<td>Treatment</td>
<td>Interaction</td>
</tr>
<tr>
<td>NREMS amount</td>
<td>F(1,7) = 2.287 n.s.</td>
<td>F(9,63) = 3.402 p &lt; 0.05</td>
<td>F(1,8) = 1.864 n.s.</td>
<td>F(9,72) = 4.735 p &lt; 0.05</td>
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<tr>
<td>REMS amount</td>
<td>F(1,7) = 2.088 n.s.</td>
<td>F(9,63) = 0.539 n.s.</td>
<td>F(1,8) = 9.885 p &lt; 0.05</td>
<td>F(9,72) = 2.483</td>
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<td>SWA</td>
<td>F(1,140) = 1.939 n.s.</td>
<td>F(9,140) = 1.835 n.s.</td>
<td>F(1,156) = 3.271 n.s.</td>
<td>F(9,156) = 1.263 n.s.</td>
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<tr>
<td>WAKE Power Spectra</td>
<td>F(1,7) = 0.188 n.s.</td>
<td>F(31,217) = 0.523 n.s.</td>
<td>F(1,8) = 4.885 n.s.</td>
<td>F(31,248) = 7.304 p &lt; 0.05</td>
</tr>
<tr>
<td>NREMS Power Spectra</td>
<td>F(1,7) = 3.917 n.s.</td>
<td>F(31,217) = 6.426 p &lt; 0.05</td>
<td>F(1,8) = 0.143 n.s.</td>
<td>F(31,248) = 0.619 n.s.</td>
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<td>REMS Power Spectra</td>
<td>F(1,7) = 1.442 n.s.</td>
<td>F(31,217) = 1.038 n.s.</td>
<td>F(1,8) = 6.555 p &lt; 0.05</td>
<td>F(31,248) = 6.576 p &lt; 0.05</td>
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</tbody>
</table>
Table 2. The total NREMS and REMS episode number and the average NREMS and REMS episode duration after icv and lateral hypothalamic administration of NPY in the first hour after the injections. N/A: not available. See legends to Table. 1 for details.

<table>
<thead>
<tr>
<th></th>
<th>NREMS Episode Number</th>
<th>Average NREMS Episode Duration (min)</th>
<th>REMS Episode Number</th>
<th>Average REMS Episode Duration (min)</th>
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<tr>
<td>Baseline</td>
<td>6.8 ± 0.8</td>
<td>4.4 ± 0.5</td>
<td>0.6 ± 0.3</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>0.4 µg NPY</td>
<td>8.4 ± 1.1</td>
<td>4.4 ± 0.6</td>
<td>1.3 ± 0.4</td>
<td>1.9 ± 0.4</td>
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<tr>
<td>Baseline</td>
<td>7.2 ± 0.7</td>
<td>4.0 ± 0.5</td>
<td>1.5 ± 0.4</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>2 µg NPY</td>
<td>5.2 ± 1.1</td>
<td>2.5 ± 0.4*</td>
<td>0.1 ± 0.1*</td>
<td>N/A</td>
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<tr>
<td>Baseline</td>
<td>8.1 ± 0.6</td>
<td>3.1 ± 0.3</td>
<td>0.4 ± 0.3</td>
<td>N/A</td>
</tr>
<tr>
<td>10 µg NPY</td>
<td>4.4 ± 0.9 *</td>
<td>2.6 ± 0.8</td>
<td>0.0 ± 0.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Baseline</td>
<td>6.9 ± 0.9</td>
<td>4.5 ± 0.8</td>
<td>0.5 ± 0.3</td>
<td>N/A</td>
</tr>
<tr>
<td>LH 2 µg NPY</td>
<td>3.8 ± 1.3 *</td>
<td>2.4 ± 0.7</td>
<td>0.2 ± 0.2</td>
<td>N/A</td>
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