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Effect of basal forebrain neuropeptide Y administration on sleep and spontaneous behavior in freely moving rats

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Abstract

Neuropeptide Y (NPY) is present both in local neurons as well as in fibers in the basal forebrain (BF), an area that plays an important role in the regulation of cortical activation. In our previous experiments in anaesthetized rats, significant EEG changes were found after NPY injections to BF. EEG delta power increased while power in theta, alpha, and beta range decreased. The aim of the present experiments was to determine whether NPY infusion to BF can modulate sleep and behavior in freely moving rats.

In this study, microinjections were made into the BF. Saline was injected to the control side, while either saline or one of two doses of NPY (0.5 μl, 300–500 pmol) to the treated side. EEG as well as behavioral changes were recorded.

Behavioral elements after the NPY injections changed in a characteristic fashion in time and three consecutive phases were defined. In phase I (half hour 2), activated behavioral items (moving, rearing, grooming) appeared frequently. In phase II (half hours 3 and 4) activity decreased, while motionless state increased. Reappearance of activity was seen in phase III (half hours 5 and 6).

NPY injections caused sleep–wake changes. The three phases described for behavioral changes were also reflected in the sleep data. During phase I, lower NPY dose increased wakefulness and decreased deep sleep. Reduced behavioral activity seen in phase II was partially reflected in the sleep. In this phase, wakefulness tended to increase in the third half hour, while decreased in the 4th half hour. Deep sleep and total slow wave sleep non-significantly decreased in the third and increased in the 4th half hour. In most cases, wakefulness was elevated again during Phase III, while sleep decreased. Length of single sleep–wake epochs did not change after NPY injections.

Our results suggest a role for NPY in the integration of sleep and behavioral stages via the BF.
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Keywords: Neuropeptide Y; Cortical activation; Basal forebrain; Freely moving rats; Spontaneous behavior; Sleep; EEG

1. Introduction

Neuropeptide Y (NPY) is one of the most abundant and widely distributed neuropeptide in the mammalian central and peripheral nervous system [26]. It is involved in several physiological functions such as feeding [3], memory [27], regulation of blood pressure [16], circadian rhythms [41], and possibly in the regulation of sleep–wake stages. NPY has anxiolytic-like action in various animal anxiety models [10,14,40], but other behavioral changes, like reduction motor activity [15,20], induction of catalepsy [20], increase in searching behavior [18], and reduction in rearing activity [39] has been also reported after intracerebroventricular (icv) administration.

NPY is present in the basal forebrain (BF), an area that plays an important role in the regulation of cortical activation (for a recent review, see [44]). The BF contains a heterogeneous population of cholinergic and non-cholinergic (GABAergic, peptidergic, and glutamatergic) corticopetal neurons as well as various types of interneurons containing different peptides, including NPY and somatostatin [44].

The role of NPY containing BF neurons in the modulation of sleep–wake states and cortical EEG is not fully understood. NPY was found to colocalize with GABA in many forebrain neurons [1]. Single NPY neurons can innervate several cholinergic corticopetal neurons in the horizontal limb of the diagonal band [37,43] and in the substantia innominata

Abbreviations: BF, basal forebrain; EEG, electroencephalogram; NPY, neuropeptide Y

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[43] with symmetrical synapses [43] that are assumed to be inhibitory [28]. In anesthetized rats, NPY containing BF neurons represent a subpopulation of S-cells (slow-wave-active cells) which are silent during spontaneous or tail pinch-induced cortical activation, but have a higher firing rate during episodes of cortical slow waves [9]. These neurons have been suggested to inhibit cortically projecting cholinergic neurons [9]. In our previous experiments, significant changes were found in the ipsilateral fronto-parietal EEG after NPY injections into the BF of urethane-anaesthetized rats [38]. EEG delta power increased, while power in higher frequency ranges (theta, alpha, and beta) decreased. These observations suggest that basal forebrain NPY might have a role in the regulation of cortical activation. The aim of the present experiments was to examine whether NPY infusion into the BF of freely moving rats can modulate sleep–wake stages and behavior.

2. Methods

2.1. Surgical procedure

Adult male Wistar rats ($n = 9$, 265–295 g) were used in the experiments. The animals were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg ip, Phylaxia-Sanofi), and placed into a stereotaxic frame (David-Kopf) with bregma and lambda at the same horizontal plane [25]. To record EEG activity, 0.8 mm stainless steel screws (Fine Science Tools, USA) were placed into burr holes over the frontal (Br 2.0; L2.0) and parietal cortices (Br-4.5; L2.0) on both sides. An additional screw over the cerebellum served for grounding purposes. To monitor EMG activity, a pair of Teflon-insulated stainless steel wires (diameter: 250 μm; California Fine Wire, CA, USA) were inserted into the neck musculature close to the caudal surface of the skull. Electrode leads were soldered to a miniature female connector. Stainless steel guide cannulas (C313G/SPC, 22 gauge, Plastic One) with fitted dummy stylus (C313DC, 28 gauge, Plastics One) cut 1 mm longer than the guide was lowered into BF on both sides in 10° angle to enable intraparenchymal injections. The cannulas and the connector were fixed to the bone with cranioplastic cement (Plastic One). Following surgery, animals were returned to their home cages. Experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and with the guidelines set forth in the US Public Health Service manual "Humane Care and Use of Laboratory Animals" and the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Guide). All efforts were made to minimize animal suffering and the number of animals used.

2.2. Electrophysiological recording

Rats were housed in individual cages located a sound-attenuated room throughout the whole experiment. The cages were prepared from clear Plexiglas cylinders (height: 330 mm, diameter: 300 mm). The whole setup was similar to that described by Bertram et al. [2]. Water and standard laboratory chow was given ad libitum. The rats were exposed to a 12-h light/12-h dark cycle (lights on at 09:30 h).

Recording sessions started after an at least 2-week long recovery period following surgery. All recordings were carried out in the home cages between 10:00 a.m. and 8:30 a.m. on the next day (22.5 h). Rats were connected to the recording system through flexible flat cables attached to fixed swivels (Plastic One or Litton) above the home cages. The animals were left connected for the whole experiment, except for brief periods of drug treatments.

EEG was measured between the frontal and parietal electrode pairs on both sides (FPL-fronto-parietal left, FPR-fronto-parietal right) through home-designed headstages based on the TLC2264I (Texas Instruments, USA) amplifiers built into the male connector [32]. EEG and EMG signals were amplified, filtered (0.3–100 Hz) and continuously digitalized by a pair of analog-to-digital (A/D) converters (UAM-3216) installed in an IBM-compatible computer and controlled by a custom-written software. Sampling rate was set to 102.4 Hz to yield 512 data points per 5 s recording time to facilitate Fast Fourier Transformation (FFT). All EEG and EMG data obtained during the recording sessions were stored on hard disk for off-line analysis.

2.3. Behavioral recording

Behavior was recorded in the first four hours of the sessions by commercial video equipments. Videotapes were analyzed off-line by observers blind to the treatment received by the animals. Using custom-written software, they scored behavioral items into one of the following categories: quiet (absence of locomotion or movement of body parts), moving (locomotion or movement of body parts), grooming, rearing, drinking, and eating. Time spent with the different behavioral items was summarized for consecutive half hours.

2.4. Treatments

Rats were placed into small, open boxes during injections and were gently restrained to prevent escaping if needed. Dummy cannulas were replaced by 28 gauge internal cannulas (C313I/SPC, Plastics One) connected through polyethylene tubing to microprocessor controlled syringe pumps (IITC Inc., CA, USA) holding two microsyringes (Hamilton, 25 μl). Pressure injections (volume 0.5 μl, speed 0.25 μl/min) were carried out bilaterally. Cannulas were left in place for an additional 2 min following injection. Rats were habituated to the injection procedure before treatments started. We did not observe visible signs of stress response during the injection procedures.

NPY (Sigma–Aldrich, Schnelldorf, Germany) was dissolved in sterile physiological saline and administered in a dose of either 300 or 500 pmol/0.5 μl. The left side always received saline, while to the right side either saline (control) or one of the two NPY doses was injected. Each rat received all three treatments in a randomized order. Treatments were separated by at least 2 days.

2.5. Data analysis

To separate sleep stages, power spectra were constructed for consecutive 5-s epochs from the EEG signals using a custom-made software. From the spectra, integrated power of the following frequency bands was calculated: low delta (0.5–2 Hz), high delta (2–4 Hz), total delta (0.5–4 Hz), theta (4–10 Hz), alpha (10–16 Hz), beta (16–30 Hz), and gamma (30–48 Hz). In addition, ratio of theta and total delta bands were also determined. EMG power was integrated between 5 and 48 Hz. All these values were stored and used in the next steps of the analysis. EEG recordings were 22.5 h long, but only the first 4 h were evaluated.

Sleep stages were scored with the help of an interactive, semiautomatic computer program using EEG data from the control (FPL, saline-injected) side. Epochs containing artefacts were marked and excluded from further analysis. PS (paradoxical sleep) epochs were also marked manually, by inspecting the calculated power values, the theta/delta ratio and the original EEG/EMG recordings. Only PS epochs longer than 30 s were included in the analysis.

EEG slow wave content (delta power, 0.5–4 Hz) is closely and inversely related to the level of cortical arousal [29], thus this parameter was used for an approximate discrimination of active (AW) and quiet (QW) wakefulness, light (LS) and deep (DS) sleep, as described earlier [6,8]. Briefly, delta power histograms were constructed from the control recording taken after saline treatment, excluding PS epochs. Five-second periods with delta power values falling into the uppermost quarter (above 75% percentile) of the histogram were categorized as DS, while periods with power values falling into the third, second and first quarter of the histogram were identified as LS, QW, and AW, respectively. Delta power values limiting the four quarters of the histogram were then used as criteria for scoring all the other recordings. Total wakefulness (tWAKE; AW and QW together) as well as total slow wave sleep (tSWS; LS and DS together) were also calculated.

2.6. Statistical analysis

Behavioral and sleep data were analyzed in consecutive 30-min long periods. The first half hour was left out, as rats needed time to calm down following the injection procedure. Time spent with a given behavioral element was

summarized for consecutive half hours and expressed as percent of the period. Sleep–wake stages were similarly summarized, but in this case the number of epochs for the different sleep–wake states was also calculated. Statistical significance of the observed changes was checked with two-way mixed-design ANOVA (split-plot) with time as the first, and NPY dose as the second factor. All tests were two-tailed and $p < 0.05$ was accepted as the lowest limit of significant difference.

2.7. Histology

When all treatments were completed, animals were deeply anesthetized with urethane (1.2 g/kg, ip). To assess the spread of injected peptides in the BF and to verify the cannula locations, horseradish peroxidase (HRP; Sigma–Aldrich, Schnelldorf, Germany) was injected (5% solution, 0.5 μl) through the implanted cannulas. Rats were transcardially perfused with 150 ml of 0.9% saline followed by 400 ml of 4% paraformaldehyde in PBS (pH 7.4) immediately after the HRP injection to prevent uptake and cellular transport. Brains were removed and cryoprotected in the same fixative containing 30% sucrose until equilibration. Coronal sections (50 μm) were cut through the area of interest with a freezing microtome.

The peroxidase reaction was visualized with 3,3′-diaminobenzidine (DAB; Sigma–Aldrich, Schnelldorf, Germany) as the chromogen. The spread of HRP was found to be about 1000 μm from the injection site. Sections were counterstained with gallocyanine (Sigma, Germany), dehydrated, and coverslipped with DepEx (Serva, Heidelberg, Germany). Injection sites were located based on the stereotaxic atlas of Paxinos and Watson [25].

3. Results

3.1. Behavioral changes

Rats tolerated handling and administration of drugs without intensive struggling or vocalization. However, the injection procedure caused a transient, non-specific behavioral activation gradually wearing down during the first half hour after the injection. This period was excluded from behavioral as well as from sleep analysis. Following this period, the motionless state dominated the behavior of rats, representing in average at least 60% of total recording time. However, moderate differences in the proportion of the quiet state in the control as well as in all other recording sessions enabled the definition of three consecutive phases (Fig. 1). Phase I (half hour 2) was characterized by a relatively high amount of activated behavioral items (moving, rearing, grooming) probably still due to the aftereffects of injection. In phase II (half hours 3 and 4) activity sharply decreased, sleep increased. This period was followed by the reappearance of activity in phase III (half hours 5 and 6). In general, NPY caused only mild to moderate changes in behavior that often remained below the $p < 0.05$ significance level.

Phase I. Both NPY doses significantly increased rearing, eating and drinking activity compared to saline control (Fig. 1). The lower dose significantly increased grooming too. However, the dominant behavior was quiet state and moving, occupying about 60% and 15% of the total time, respectively (Fig. 1A and B).

Phase II. Moving, rearing, grooming, and eating strongly decreased in half hour 3 and remained low in half hour 4 as well. At the same time, quiet, motionless state increased after saline as well as NPY treatments. As rats spent most of the time motionless and other behavioral elements were only sporadic, very few significant changes were seen. NPY treatment tended to decrease movements and increase quiet state. This effect was more pronounced with the lower dose and reached significant levels in half hour 4. Drinking occurred less frequently after any

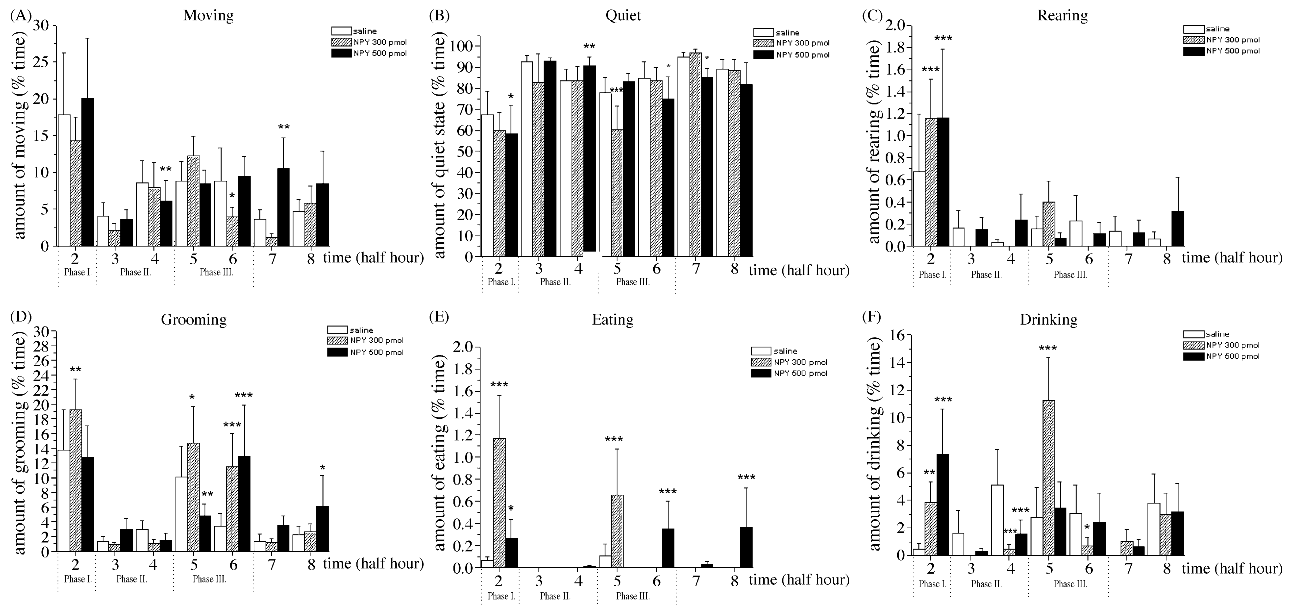

Fig. 1. Behavioral effects of NPY injected to the BF ($n = 8$). (A) Moving, (B) quiet, (C) rearing, (D) grooming, (E) eating and (F) drinking. The proportion of the quiet state changed in a characteristic fashion in the control as well as in all other recordings enabling the definition of three consecutive phases (phase I: half hour 2, phase II: half hours 3 and 4, phase III: half hours 5 and 6). NPY and saline injections were made at the beginning of the light period and behavior was videotaped for 4 h. The first half hours after the injections were excluded from the analysis. After the excluded period, values scored as a given behavioral element were summarized from the consecutive 30 min long periods and expressed as percent of the period. Asterisk (*) indicates significant deviation from the corresponding control (saline) value after the NPY injections. Significance was tested with two-way mixed-design ANOVA (split-plot). Significance levels: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Data are expressed as mean and S.E.M.

of the NPY doses compared to saline injection (Fig. 1F). This difference might have reflected some rebound effect, as during Phase I, treatment groups drank more frequently than controls.

Phase III. Following the behavioral inactivity in half hours 3 and 4, rats became more active again, regardless of the treatment. The lower NPY dose significantly decreased time spent motionless and increased grooming, eating and drinking in half hour 5. Grooming was still high in half hour 6 (Fig. 1D). The higher dose caused less significant changes in behavior, though it strongly increased grooming and eating in half hour 6.

3.2. Sleep changes

NPY injections into the basal forebrain caused no abnormal EEG activity and only moderate changes in the sleep–wake states (Figs. 2 and 3). The three consecutive phases described during the behavioral analysis were partly reflected in the relative proportion of sleep–wake stages as well.

Phase I. Half hour 2 following treatment was characterized by behavioral activation. The lower dose of NPY increased the amount of wakefulness (Fig. 2C) and decreased deep sleep (Fig. 3D). Similar, but much weaker effects were seen after the higher dose. In this case, changes were not statistically significant. The amount of light sleep and paradoxical sleep remained unchanged (Fig. 3A and B).

During phase II, behavioral activation was strongly reduced. However, this decrease of activity was not so evident in the sleep–wake data. Waking (AW, QW, tWAKE) showed moderate, non-significant elevation in the third half hour in case of both doses, while decrease was seen in the 4th half hour (Fig. 2). DS and tSWS non-significantly decreased in the third and increased in the 4th half hour (Fig. 3C and D). Higher dose significantly increased PS in the 4th half hour (Fig. 3A).

In most cases, AW, QW and tWAKE level was elevated during phase III, while sleep values (LS, DS, PS, tSWS) decreased (Fig. 3). However, the higher NPY dose significantly increased DS and tSWS in the 5th half hour (Fig. 3C and D). After phase III, sleep decreased and wakefulness increased until the end of the light phase regardless of the treatment.

The observed changes in the total amount of time spent in different sleep stages resulted from the alteration of the number of episodes (data not shown). Thus, NPY injections had no influence on the length of single sleep–wake epochs.

3.3. Histological results

As Fig. 4 depicts injection sites were located in the ventral part of globus pallidus, substantia innominata, ventral pallidum and magnocellular preoptic nucleus, BF regions that were found to contain large numbers of cholinergic cells by

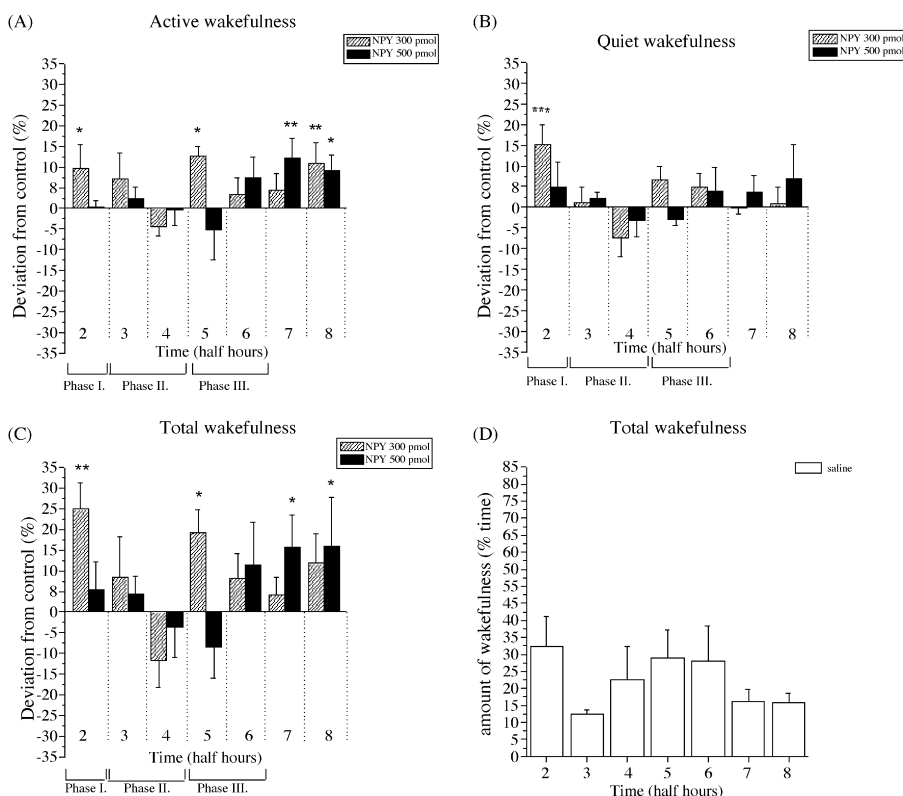

Fig. 2. Effects of NPY injected to the BF on wakefulness ($n = 6$). The figure shows % deviation from the control and S.E.M. (A) Active wakefulness, (B) quiet wakefulness, (C) total wakefulness (active and quiet wakefulness together), (D) absolute total wakefulness values after control (saline) injections. NPY and saline injections were made at the beginning of the light period. The first half hour after the injections were excluded from the analysis. After the excluded period, data were analyzed in 30 min long blocks. Asterisk (*) indicates significant deviation from the corresponding control (saline) value after the NPY injections. Significance was tested with two-way mixed-design ANOVA (split-plot). Significance levels: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

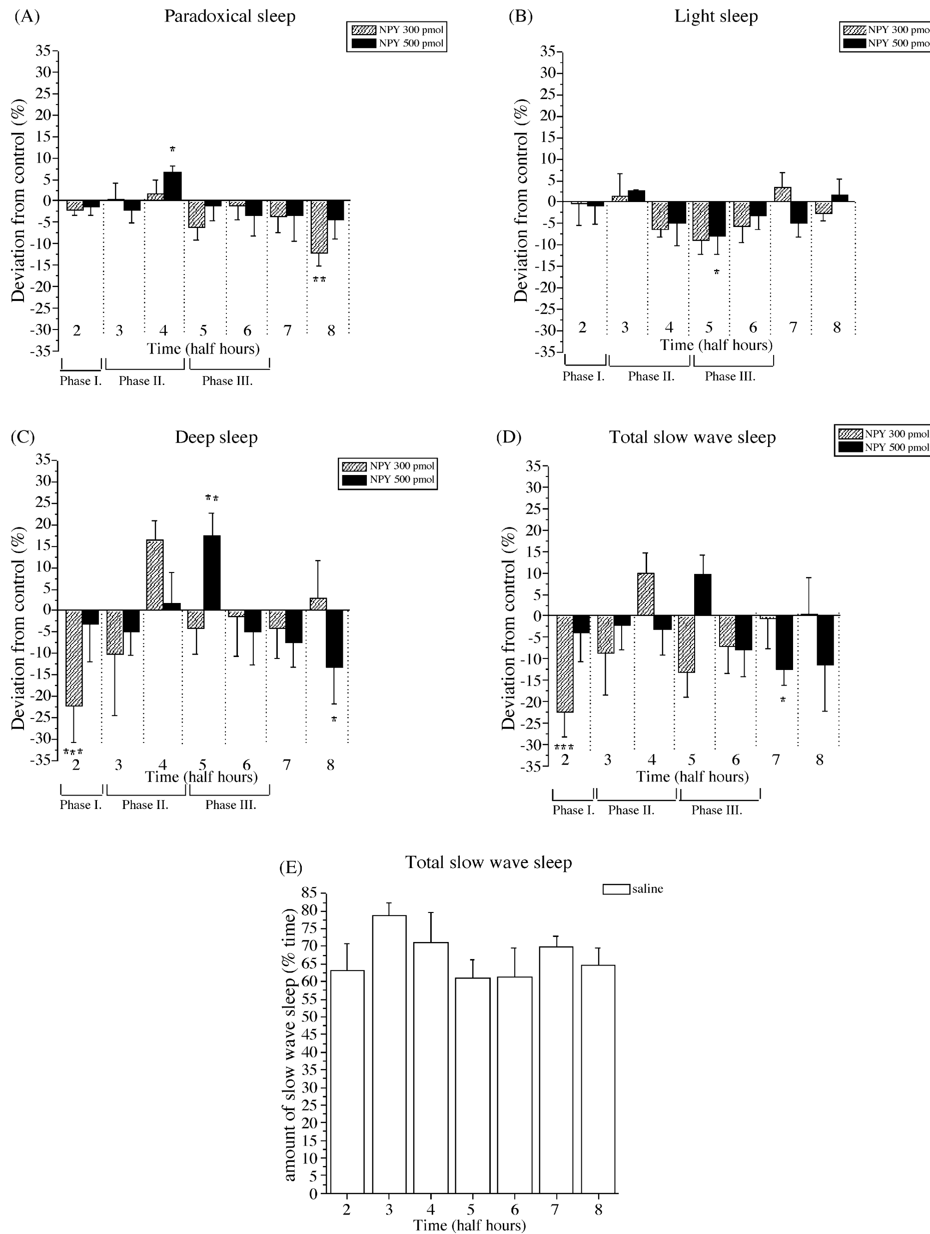

Fig. 3. Effects of NPY injected to the BF on sleep ($n = 6$). The figure shows % deviation from the control and S.E.M. (A) Paradoxical sleep, (B) light sleep, (C) deep sleep, (D) total slow wave sleep (light and deep sleep together), (E) absolute total slow wave sleep values after control (saline) injections. NPY and saline injections were made at the beginning of the light period. The first half hour after the injections were excluded from the analysis. After the excluded period, data were analyzed in 30 min long blocks. Blank asterisk (☆) indicates significant deviation from the corresponding control (saline) value after the injection of the lower NPY dose (300 pmol/0.5 μl). Asterisk (*) indicates significant deviation from the corresponding control (saline) value after the NPY injections. Significance was tested with two-way mixed-design ANOVA (split-plot). Significance levels: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

previous anatomical studies [24,25]. There were no systematic differences in NPY effects depending on the exact site of the injection (data not shown). Fig. 5 shows a photomicrograph of a representative cannula location in the BF.

4. Discussion

These data represent the first examination of sleep and behavioral effects of NPY injected to the BF in freely moving rats. In our previous experiments in urethane-anaesthetized rats, NPY injection to BF increased delta power and decreased power in higher frequency ranges (theta, alpha and beta) in the ipsilateral EEG [38]. We attributed these changes to a local inhibitory effect of NPY on corticopetal neurons, and expected a decrease in wakefulness and an increase in sleep following BF NPY injections in freely moving rats. In contrast, changes were more complex, but a tendency toward more wakefulness and more frequent occurance of behavioral elements related to the waking state were observed, especially following the lower dose.

NPY was found to influence spontaneous behavior in several studies after icv or intraparenchymal administration into different brain regions. NPY effects clearly depended on the site

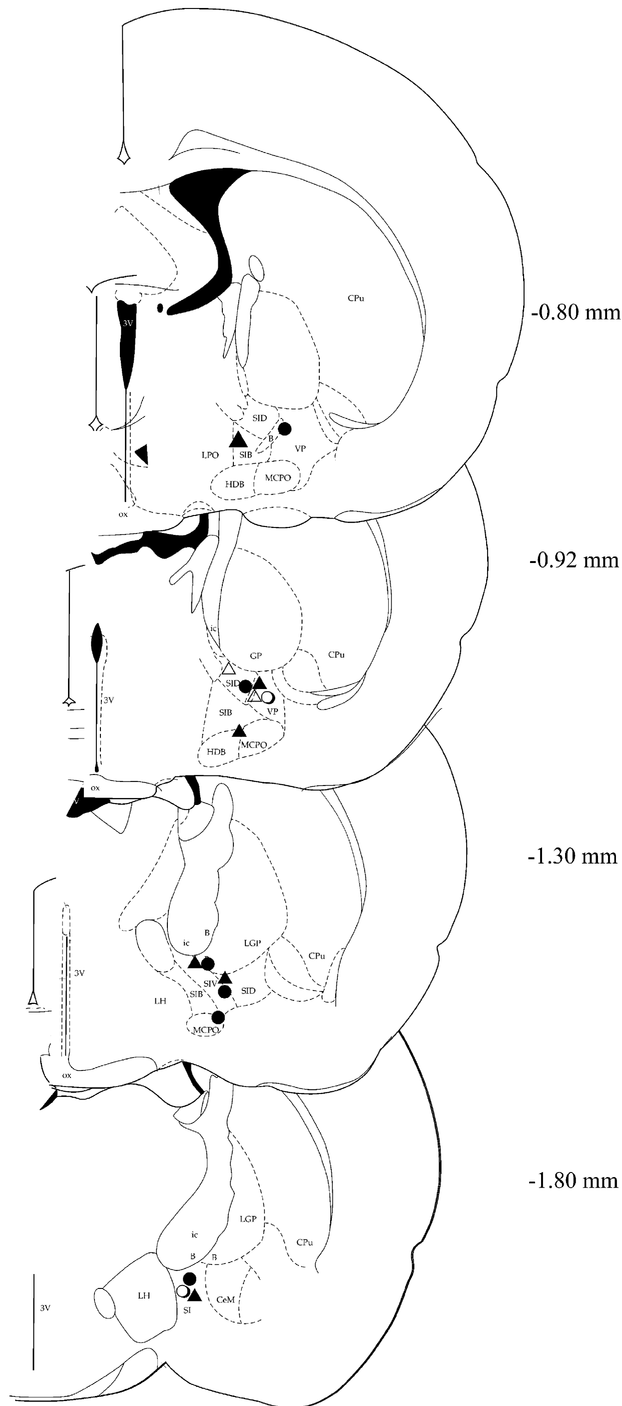

Fig. 4. Cannula locations in the basal forebrain. For the sake of clarity, every injection sites are drawn to one side. Frontal sections are derived from stereotaxic atlas of Paxinos and Watson [25]. Numbers on the right site indicate distance from Bregma. Legends: (▲) NPY injection sites in rats included both in sleep and behavioral analysis (six sites), (●) saline injection sites in rats included both in sleep and behavioral analysis (six sites), (△) NPY injection sites in rats included only in the behavioral analysis (two sites), (◯) saline injection sites in rats included only in the behavioral analysis (two sites). Abbreviations: 3V, third ventricle; B, nucleus basalis Meynert; CeM, central amygdala, medial; CPu, caudate putamen; GP, globus pallidus; HDB, horizontal limb of diagonal band; ic, internal capsule; LGP, lateral globus pallidus; LH, lateral hypothalamus; LPO, lateral preoptic area; MCPO, magnocellular preoptic nucleus; ox, optic chiasm; SIB, substantia innominata; basal part; SID, substantia innominata, dorsal part; SIV, substantia innominata, ventral part; VP, ventral pallidum.

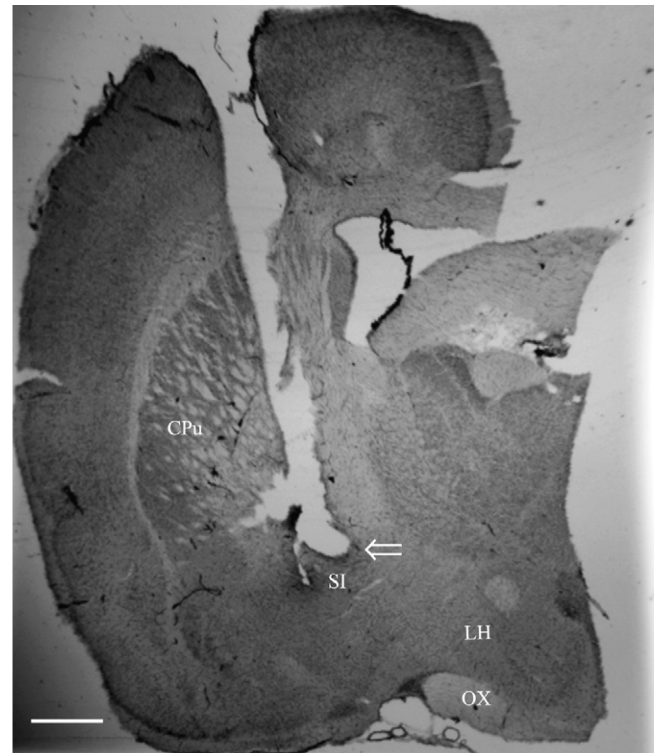

Fig. 5. Photomicrograph showing a representative cannula location in the basal forebrain. Arrowhead marks the tip of the guide cannula in the substantia innominata. Abbreviations: CPu, caudate putamen; LH, lateral hypothalamus; ox, optic chiasm; SI, substantia innominata. Calibration bar: 1 mm.

of administration. For example, icv NPY administration significantly decreased motor activity both in an open field and in the home cage [14,20], increased muscle tone and induced catalepsy in a dose dependent manner [20]. In contrast, both exploratory and locomotor activity increased after intracortical NPY administration [33], but no changes were found in locomotion and feeding when NPY was injected into the central nucleus of the amygdala [22]. Our results suggest that BF NPY injections caused global changes in cortical activity and arousal, but this aroused state was not accompanied by increased locomotion.

NPY was found to increase feeding after icv injection [18]. Feeding as well as drinking increased when NPY was injected into the paraventricular nucleus [34]. We found significant increment in time spent with eating at both NPY doses (Fig. 1E). However, the exact weight of consumed food pellets as well as the volume of consumed water was not measured. NPY injections influenced drinking in a complex pattern. Strong drinking response was seen shortly after the injections (phase I) and toward the end of behavioral recordings (phase III, Fig. 1F), but significant decrease was detected in phase II. In our experiments, both NPY doses increased grooming. However, time spent with grooming was found unchanged following icv administration [18] and following injection into the paraventricular nucleus [34]. Rearing activity significantly increased after the injection of NPY (Fig. 1C) in phase I in our experiments. However, icv injection was found to reduce rearing activity [39] while

NPY injections to the paraventricular nucleus did not change its occurance [34].

Previous studies, in which sleep effects of NPY were examined, yielded conflicting results. In one study, icv NPY administration in freely moving rats caused no significant difference in time spent in slow wave sleep [10]. However, a recent study reported sleep suppression [36]. As NPY is abundant in structures surrounding the third ventricle, this route of administration is not appropriate to reveal the complex nature of NPY effects on sleep regulation. Local NPY injections to different structures caused various effects. Administration to the paraventricular nucleus did not influence sleep [34], while lateral hypothalamic infusion suppressed both non rapid-eye-movement- and rapid-eye-movement sleep [36]. In our experiments, NPY was injected into the BF and we found significant sleep–wake changes. It seems to be likely, that similar to the behavioral effects, sleep effects of NPY strongly depend on the site of administration. As BF is an important brain region involved in the regulation of cortical activation and sleep–wake stages [21,7,44] our results suggest a role for NPY in sleep–wake regulation via the BF. However, BF is an anatomically very complex area with many different cell types. To precisely localize the site of action, selective blockade of the NPY effect on these different components would be needed. This would be very difficult to accomplish, as NPY receptors are not differentially distributed in this area.

We tested NPY effects in two doses: 300 and 500 pmol/rat. The two doses caused different, often opposing changes both in sleep and behavioral parameters. In our preliminary experiments, a separate set of rats were injected with a low NPY dose (100 pmol/rat) (data not shown). This dose caused similar behavioral and sleep changes as the 300 pmol/rat dose in the present experiment. It is known from the literature that NPY can elicit opposite physiological changes depending on the dose. When administered in the lateral hypothalamus, NPY produced a biphasic effect: hyperthermia at low doses (30–50 pmol) and hypothermia at high doses (200–400 pmol) [19]. However, the exact mechanism of this phenomenon is unknown. Our sleep as well as behavioral data show similar biphasic changes and it is possible that similar mechanism can explain the previous findings and our results.

One of the most important questions arising about the NPY effects in the BF is that what kind of cell groups are mediating the effects. HRP injected into the same area of the BF diffused for a distance of about 1 mm (see Section 2). Because of the distance between the injection sites and the third ventricle exceeded the distance of NPY diffusion (2.5–3 mm versus 1 mm), it is unlikely that NPY could reach the lumen of the ventricle. Thus, the changes in the EEG are probably due to local actions of NPY.

Different parts of the BF contain rather heterogeneous cell populations [42,43]. Although the number of the NPY containing BF cells are relatively low [44], these cells might play an important role in the regulation of activity of different BF cell groups. Injection of different neuropeptides to the BF was found to cause sleep and EEG changes. These effects were often attributed to changes in the activity of BF cholinergic neurons evoked by the injected peptide. This mechanism was proposed for neurotensin [4], somatostatin [12] and NPY [38]. NPY containing terminals were shown to form symmetrical synapses with cholinergic neurons [43] and were assumed to be inhibitory [28]. Somatostatin containing cells were also found to synapse with cholinergic projection neurons [44]. Administration of the somatostatin analog octreotide into lateral sites of the lateral preoptic area and lateral hypothalamus stimulated sleep, perhaps via inhibiting cholinergic neurons [12]. However, the exact interaction between the activity of BF cholinergic and NPY-containing cells are unknown in vitro as well as in vivo.

Firing of BF neurons was found to be closely associated with the sleep–wake stages (for a recent review, see [7]). Even without direct evidences (i.e. histochemical identification of the recorded neuron), it was suggested that the activity of the cholinergic BF cells show strong variations during the sleep–wake cycle [5]. It may be hypothesized that firing of NPY-containing cells also show correlation with the different vigilance levels. Thus, NPY cells may influence cholinergic BF cells to a different extent during the different sleep–wake stages. However, there is no direct information to date about changes in NPY or NPY-mRNA levels or in the intensity of NPY transmission in the BF across the sleep–wake cycle. Cholinergic projection neurons represent important synaptic targets for BF NPY neurons as well as for other peptidergic BF interneurons. Previous studies have shown that at least half of the cortically projecting basal forebrain neurons are GABAergic [31]. However, there are no data on NPY neurons innervating corticopetal GABAergic BF cells. If this were the case, then NPY could influence cortical activity through GABAergic cells in addition to cholinergic neurons. The area where NPY was injected contains not only cholinergic and GABAergic corticopetal neurons, but supposedly glutamatergic cells as well, some of which might be corticopetal projection neurons [17]. Thus, further studies are needed to determine the exact targets of NPY through which the observed sleep effects were induced.

EEG effects of NPY injected to BF seem to depend on the actual state of the BF neural circuits. In urethane-anaesthetized rats, NPY had no effect on cortical EEG in very deep anesthesia (our unpublished observations). When anesthesia was more superficial, clear EEG effect was observed [38]. In anesthetized animals, activity of BF circuits is depressed to an extent depending on the depth of anesthesia. Thus, NPY seems to cause EEG changes only when the activity of BF circuits is considerably high. In freely moving rats, BF activity is considerably higher compared to urethane-anaesthesia. In contrast to our expectations, BF injection of NPY increased wakefulness and waking behaviors especially in the lower dose. In earlier reports, NPY was found to modulate the release of several neurotransmitters [13,23], among others GABA, through presynaptic mechanisms [35]. On the other hand, BF cholinergic neurons have been shown to be under GABAergic control [45] that was more pronounced during activated states [11,30]. Thus, disinhibition of cholinergic cells might explain the increase of waking behaviors, while the weaker effect following the higher dose and the increase of slow activity in the anesthetized preparation might be attributed to an opposite, direct effect on cholinergic cells.

Conflict of interest

No conflict of interest was declared.

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