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Intracerebral oxytocin modulates sleep–wake behaviour in male rats

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Abstract

Oxytocin released within the brain under basal conditions and in response to stress is differentially involved in the regulation of the hypothalamo-pituitary-adrenal (HPA) axis. Because the HPA axis plays an important role in the regulation of wakefulness, central oxytocin may modulate sleep–wake behaviour. In the present vehicle-controlled study, we assessed the influence of a selective oxytocin receptor antagonist (des-Gly-NH_2-$d(CH_2)_5$ [Tyr(Me)^2,Thr^4] OVT; 0.75 μg/5 μl) or of synthetic oxytocin (0.1 μg and 1 μg/5 μl), infused into the lateral ventricle (icv), on the sleep pattern in male Wistar rats ($n = 7$). Compared to vehicle, the oxytocin antagonist slightly but persistently increased wakefulness at the expense of all sleep states. This finding indicates that endogenous brain oxytocin promotes sleep. However, acute icv infusion of oxytocin delayed sleep onset latency, which resulted in a transient reduction of non-REMS and REMS, and augmented high-frequency activity in the electroencephalogram (EEG) within non-REMS. These observations agree with previous reports that icv oxytocin induces a state of arousal. Based on these findings, we postulate that oxytocin has a dual mechanism of action in dependence of the physiological state. Under basal, stress-free conditions, endogenous oxytocin may promote sleep. Conversely, the high brain levels of oxytocin after central oxytocin infusion may reflect a condition of stress accompanied by behavioural arousal and, possibly via an excitatory action on the CRH system, increase vigilance.

Keywords: Oxytocin; Oxytocin receptor antagonist; Sleep; EEG spectral analysis; Rat

1. Introduction

Oxytocin is a reproductive hormone which facilitates parturition and suckling in the female mammal and is also secreted into the blood, among other stimuli, during osmotic challenge, physical stress and orgasm in both sexes. In addition to its presence in and release from neurohypophysial terminals, oxytocin immunoreactive fibers are also found within the brain, for instance, in hypothalamic and extrahypothalamic limbic brain regions. Within the septum, the amygdala and the hypothalamic supraoptic and paraventricular nuclei, for example, it is released in response to various emotional, physical and pharmacological challenges (for review, see Refs. [1,2]).

We recently demonstrated that in both female and male rats, intracerebral oxytocin is significantly involved in the regulation of the hypothalamo-pituitary-adrenal (HPA) axis, which constitutes the main neuroendocrine stress system [3,4]. Administration of a selective oxytocin receptor antagonist into the rat lateral cerebral ventricle (icv) revealed an inhibitory effect of brain oxytocin on both basal as well as stress-induced activity of the HPA axis, that is, on the secretion of corticotropin (ACTH) and, consequently, corticosterone into the blood. Under stress-free and basal conditions, this inhibitory effect is at least partly mediated by the paraventricular nucleus [4], in which neurons synthesizing corticotropin-releasing hormone (CRH) are localized. In contrast, under conditions of stress, oxytocin exerts both inhibitory and excitatory effects on the HPA axis responsiveness in dependence on the brain region [3–5]. Although a direct, receptor-mediated action of oxytocin on CRH neuronal activity has not been shown until now, it is very likely that oxytocin regulates CRH functions [5]. In addition to its role as the main regulator of the HPA axis when released into the portal blood, CRH within the brain has also been shown to regulate immunological processes, mood and sleep. With respect to the latter, high activity of the brain

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CRH system, as under conditions of stress, has been implicated in the impairment of sleep quality in both human and animal studies [6]. Thus, administration of CRH reduces non-rapid eye movement sleep (non-REMS) and EEG-slow wave activity (SWA) in rats [7] and in rabbits [8], and decreases slow wave sleep in humans [9], whereas administration of CRH receptor antagonists reduces wakefulness [10,11]. Furthermore, cortisol infusions during the night powerfully promote slow wave sleep in man, most likely as a result of cortisol-suppressed CRH activity [12,13]. Impaired sleep quality is one of the diagnostic markers in patients suffering from depressive disorders, a psychiatric disease which is further characterized by a pathologically elevated activity of the brain CRH system [14–16]. Based on the finding that brain oxytocin regulates the activity of the HPA axis [3–5,17], brain oxytocin may have an impact on sleep–wake behaviour.

The aim of the present placebo-controlled experiment was to investigate the involvement of the brain oxytocin system in sleep mechanisms by assessing sleep–wake patterns in male rats after icv infusions of two doses of oxytocin and of a selective oxytocin receptor antagonist.

The results have been reported in abstract form [18].

2. Materials and methods

2.1. Animals

The present experiment was approved by the local commission for animal welfare and performed in accordance with the European Communities Council Directive of 1986 (86/609/EEC). Under deep halothane (Hoechst, Frankfurt am Main, Germany) anaesthesia, seven adult male Wistar rats (Charles River Laboratories, Sulzfeld, Germany), weighing between 240 and 260 g, were implanted with electrodes to record the electroencephalogram (EEG) and electromyogram (EMG) as described in detail elsewhere [19]. Additionally, an epidural thermistor (Betatherm Ireland, Galway, Ireland, type 10K3MCD1) was inserted to record brain temperature (Tbr), and a guide cannula (21 ga) was placed above the lateral ventricle for icv injections (0.6 mm caudal to bregma, 1.6 mm lateral to midline, 1.8 mm beneath the surface of the skull; [20]) and closed by a stylette. The animals were housed individually in a ventilated, sound-attenuated Faraday room under a 12-h light/12-h dark cycle (lights on from 8:30 AM) at a regulated ambient temperature of 20–22 °C. Food and water were available ad libitum. After at least 10 days of recovery, the rats were connected to recording cables.

2.2. Experimental protocol

After 4 days to adapt to the recording conditions, each rat was subjected to three sets of experiments, which were run in random order and separated by at least 3 days. Each experiment consisted of two consecutive days. On both of these days, at light-onset, an icv infusion cannula (25 ga), which was connected to a 10-μl microsyringe, was lowered into the guide cannula and fixed in place with a piece of silicone tubing. The rats were left undisturbed for the next hour, and then icv infusions were delivered without additional disturbances. On the first day, vehicle (Ringer's solution, pH 7.4, 5 μl) was injected and on the second day, either a selective oxytocin receptor antagonist (des-Gly-$NH_2d(CH_2)_5$ [Tyr(Me)^2,Thr^4] OVT; 0.75 μg/5 μl; pH 7.4; Dr. M. Manning, Toledo, OH, USA) or synthetic oxytocin in two different doses (0.1 or 1 μg/5 μl oxytocin; Syntocin, Bachem, Germany). The infusion cannula was left in place until the end of the recording period.

2.3. Recording

EEG, EMG and Tbr were continuously recorded during the first 6 h after each injection. The EEG and EMG signals were amplified and filtered as described earlier [19]. EEG, the rectified and integrated EMG and Tbr were digitized with a sampling rate of 64 Hz. An offline program displayed 10-s epochs of EEG and EMG on screen for the visual scoring of the vigilance states wakefulness, non-REMS, pre-REMS and REMS (for scoring criteria, see Ref. [21]). Pre-REMS, also known as intermediate state, usually precedes REMS and is characterized by high-amplitude spindle-like EEG signals on a background of theta (6–9 Hz) activity. The EEG was subjected to an online fast Fourier transform routine. A power spectrum was computed for 2-s windows in 0.5-Hz bins for the frequencies between 0.5 and 4.5 Hz and in 1-Hz bins for the frequencies between 5 and 25 Hz. Power spectra were averaged over 10-s epochs.

2.4. Data analysis and statistics

For each 6-h recording period, the latency to non-REMS and REMS (arbitrarily defined as the 20th 10-s epoch of non-REMS and the 3rd epoch of REMS) and the total number and average duration of the non-REMS and REMS episodes were determined. Total time spent in each vigilance state and average Tbr were computed per 2-h interval. The visually scored sleep parameters and Tbr data of the three vehicle conditions were pooled together. As absolute Tbr values differed between the rats, the data were expressed as deviation from the 6-h vehicle mean (2-h value − 6-h vehicle). For each 2-h interval, average EEG power densities within non-REMS were computed. Because of large interindividual differences, EEG power densities were normalized by expressing them as percentage of the average power density in the same frequency band within non-REMS during the corresponding 6-h vehicle period and were then log-transformed. Differences in the sleep architecture variables between the vehicle and other treatments were tested by means of two-sided, paired *t*-tests. Analysis of the other parameters was performed by

a two-factorial analysis of variance (ANOVA) with a repeated measures design (Greenhouse–Geisser correction) with treatment (vehicle versus another treatment) and time (three 2-h intervals) as within-subjects factors. Where appropriate, ANOVA was followed by two-sided, paired *t*-tests.

3. Results

3.1. Brain temperature

Analysis of Tbr after icv infusion of vehicle and the lower dose of oxytocin yielded a significant interaction

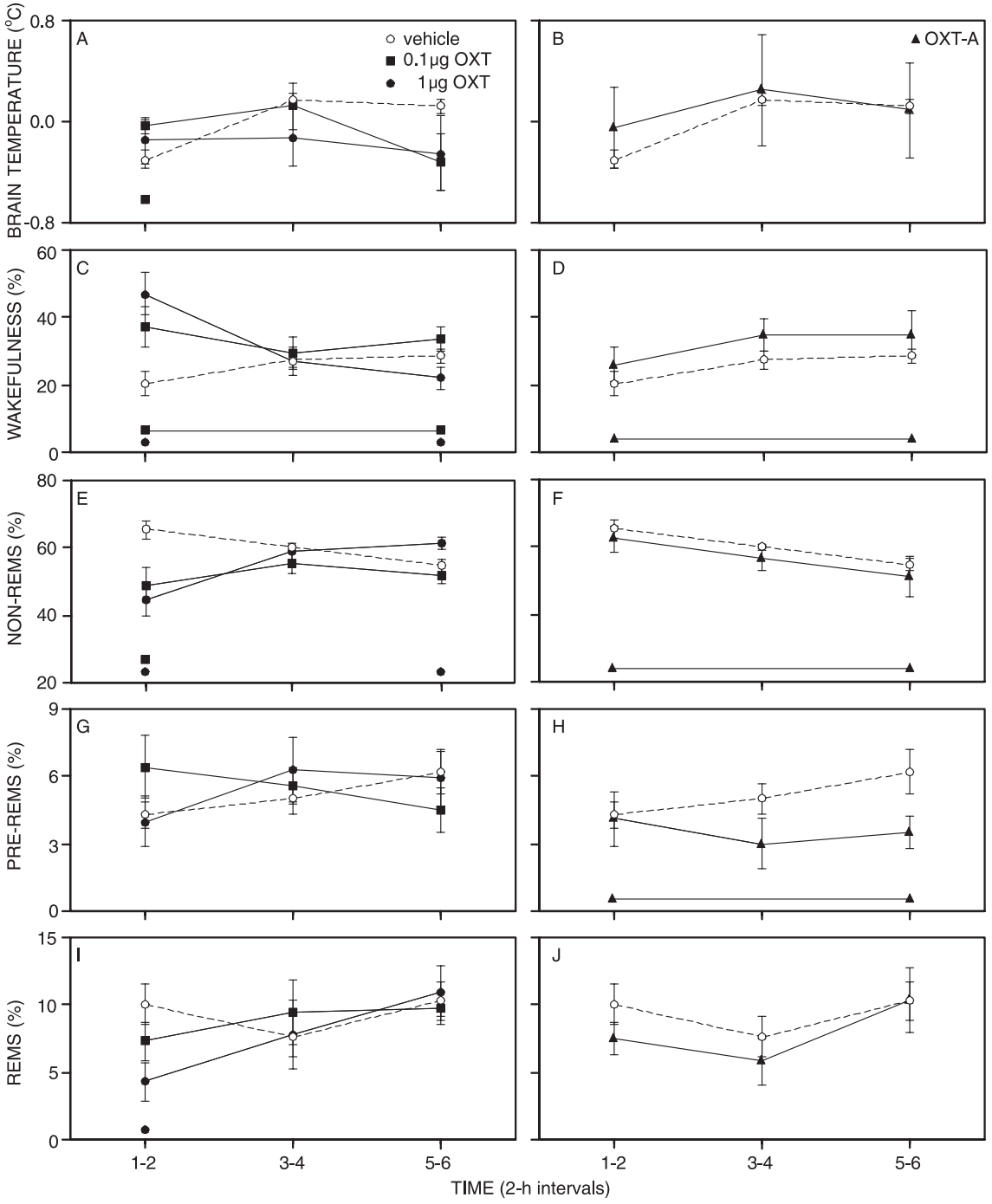

Fig. 1. Time course of brain temperature (A and B) and of percentage of time spent in wakefulness (C and D), non-REMS (E and F), pre-REMS (G and H) and REMS (I and J) during the first 6 h after icv infusion of vehicle, 0.1 or 1 μg oxytocin (OXT, left graphs) and of vehicle or the oxytocin receptor antagonist (OXT-A, right graphs). Curves connect mean values ± S.E.M. ($n = 7$). Brain temperature is expressed as deviation from the vehicle mean. Symbols connected by a line at the bottom of the graphs refer to a significant main effect of the respective treatment (ANOVA, $P < 0.05$) and isolated symbols to a significant difference in the 2-h value between the respective treatment and vehicle (two-sided paired *t*-test, $P < 0.05$).

Table 1
Influence of oxytocin (0.1 and 1 μg, icv) and an oxytocin receptor antagonist (0.75 μg/5 μl, icv) on sleep latency and on the number and duration of sleep episodes in rats

	Vehicle	Oxytocin		
		0.1 μg	1 μg	Antagonist
Non-REMS				
Latency (min)	8.7 ± 6.3	28.8 ± 27.1*	37.3 ± 22.2*	9.6 ± 8.8
Episode number	95.6 ± 20.6	95.9 ± 29.2	84.4 ± 24.4	93.0 ± 22.5
Episode duration (min)	2.64 ± 0.62	2.42 ± 0.62	2.84 ± 0.81	2.54 ± 0.55
REMS				
Latency (min)	21.4 ± 12.2	41.6 ± 34.8	85.0 ± 72.2*	38.9 ± 38.9
Episode number	26.2 ± 8.0	28.7 ± 8.1	22.6 ± 9.8	24.4 ± 11.3
Episode duration (min)	1.27 ± 0.27	1.09 ± 0.26	1.15 ± 0.30	1.12 ± 0.33

Mean values ± S.D. ($n=7$). Significant differences between vehicle and another treatment are indicated by * ($P<0.05$, two-sided paired t-test).

between the factors treatment and time ($F_{2,12}=9.6$, $P<0.01$), indicating that oxytocin affected the temporal evolution of Tbr. Compared with vehicle, oxytocin significantly increased Tbr during the first 2-h interval, whereas it tendentiously decreased Tbr during the last interval ($P<0.1$; Fig. 1A).

ANOVA did not reveal any significant effect either of the higher dose of oxytocin or of the oxytocin receptor antagonist (Fig. 1A and B).

3.2. Vigilance states

ANOVA performed on the time spent in each vigilance state after infusion of vehicle and 0.1 μg oxytocin found a significant effect of treatment for wakefulness ($F_{1,6}=31.7$, $P<0.01$). Oxytocin increased overall wakefulness from 25.5 ± 7.3% during vehicle to 33.6 ± 8.4%, which was particularly due to a greater amount of wakefulness during the first 2-h interval (Fig. 1C). For non-REMS, a significant effect of treatment and of treatment by time emerged ($F_{1,6}=18.2$, $P<0.01$ and $F_{2,12}=5.6$, $P<0.05$, respectively). Oxytocin decreased overall non-REMS from 60.0 ± 4.2% to 52.1 ± 6.8%, and this was entirely caused by reductions during the first 2-h interval (Fig. 1E). Neither pre-REMS nor REMS was significantly affected by the lower dose of oxytocin (Fig. 1G and I).

For the higher dose of oxytocin, ANOVA found significant effects of treatment and of treatment by time for wakefulness ($F_{1,6}=11.1$, $P<0.05$ and $F_{2,12}=48.5$, $P<0.0001$, respectively) and for non-REMS ($F_{1,6}=9.0$, $P<0.05$ and $F_{2,12}=35.1$, $P<0.0001$, respectively). Oxytocin increased overall wakefulness to 32.0 ± 10.4% and decreased non-REMS to 54.9 ± 5.8%. Pairwise comparisons per 2-h interval showed that wakefulness was significantly and markedly promoted and non-REMS was inhibited during the first 2-h interval (Fig. 1C and E). In contrast, wakefulness was significantly reduced and non-REMS increased during the last 2-h interval. For pre-REMS, no significant effect was found, whereas for REMS, a significant treatment by time effect emerged ($F_{2,12}=5.6$, $P<0.05$), which was due to a transient suppression of this state by oxytocin (Fig. 1I).

Analysis of the vehicle and the oxytocin receptor antagonist data yielded a significant treatment effect for wakefulness ($F_{1,6}=27.8$, $P<0.01$), non-REMS ($F_{1,6}=6.6$, $P<0.05$) and pre-REMS ($F_{1,6}=14.0$, $P<0.01$). Oxytocin antagonist increased overall wakefulness to 32.0 ± 8.9% and decreased overall non-REMS to 56.6 ± 6.4% and reduced pre-REMS from 5.2 ± 1.8% during vehicle to 3.5 ± 2.2%. The effects on wakefulness and non-REMS were present already during the first 2-h interval and sustained throughout the recording period (Fig. 1D and F), whereas decreases in pre-REMS occurred with a delay of

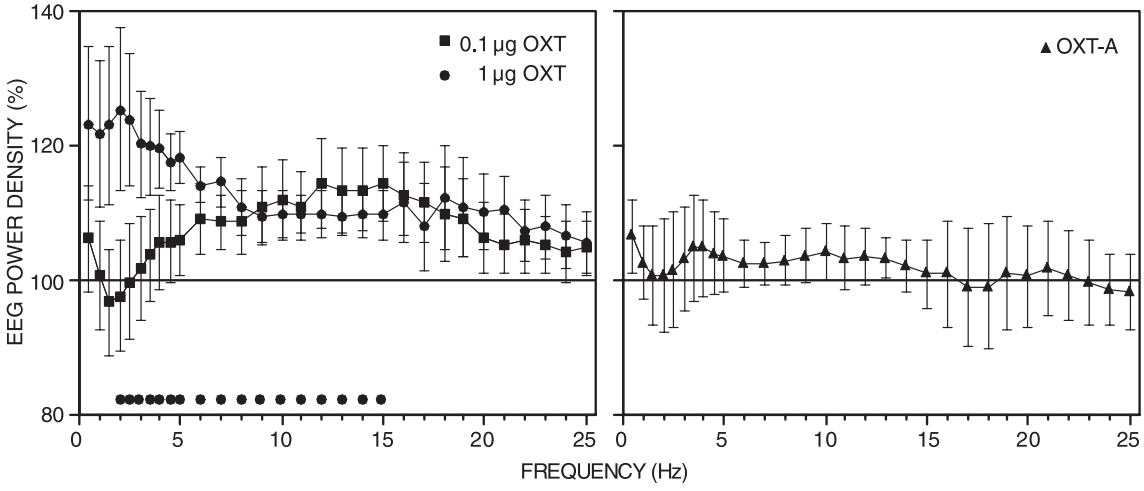

Fig. 2. EEG power densities within non-REMS during the first 6 h after icv infusion of 0.1 and 1 μg oxytocin (OXT, left graph) or of the oxytocin receptor antagonist (OXT-A, right graph). Curves connect mean values ± S.E.M. ($n=7$). For plotting purposes, the data are expressed as percentage of the corresponding 6-h vehicle value. Filled circles at the bottom of the left graph denote frequency bands for which the ANOVA yielded a significant main effect ($P<0.05$) of 1 μg oxytocin.

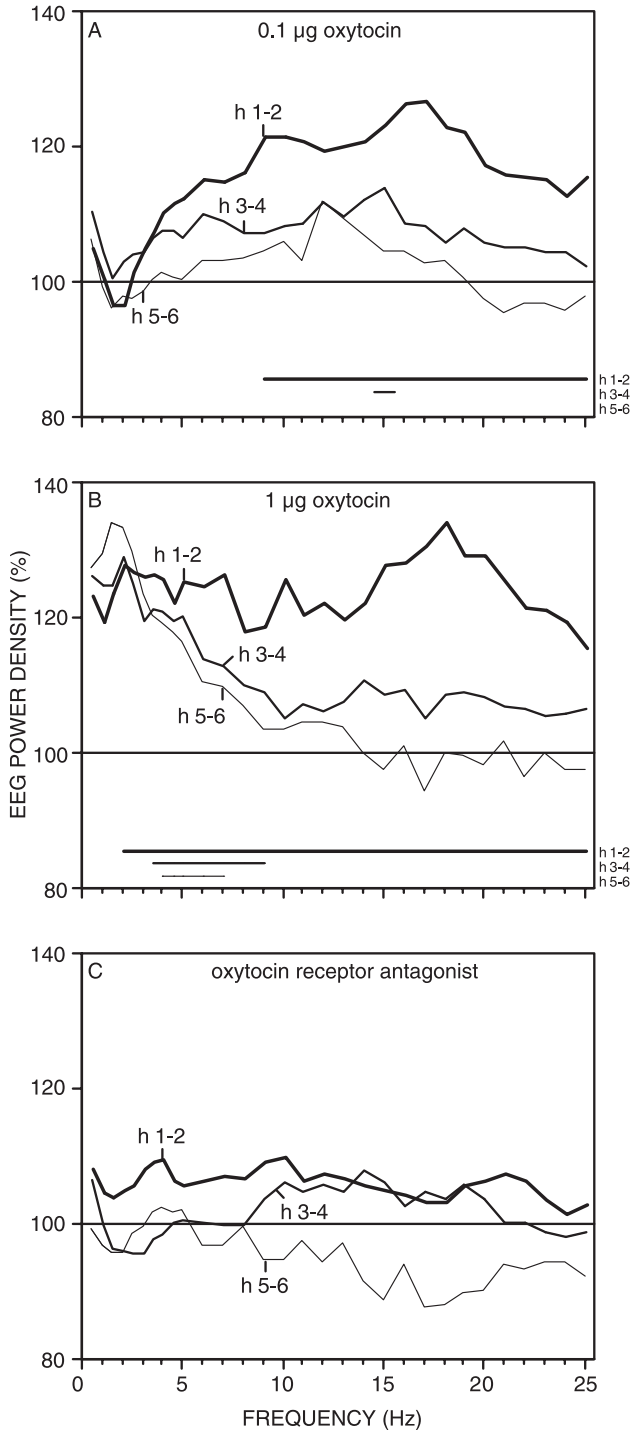

Fig. 3. EEG power densities within non-REMS during the first three 2-h intervals after icv infusion of 0.1 µg oxytocin (A), 1 µg oxytocin (B) or the oxytocin receptor antagonist (C). Curves connect mean values ± S.E.M. ($n = 7$). For plotting purposes, the data are expressed as percentage of the corresponding 2-h vehicle value. Dots at the bottom of the graphs refer to frequency bands. Lines through the dots indicate frequencies for which significant differences from vehicle were found ($P < 0.05$, two-sided paired t-test).

about 2 h (Fig. 1H). Oxytocin antagonist treatment exerted no major effect on time spent in REMS (Fig. 1J).

3.3. Sleep architecture

Compared to vehicle, the lower dose of oxytocin significantly increased the latency to non-REMS (Table 1). The higher dose of oxytocin significantly prolonged the latency to non-REMS as well as to REMS. Oxytocin receptor antagonist did not significantly affect any of the sleep architecture parameters.

3.4. EEG power densities within non-REMS

Analysis of the EEG power densities within non-REMS after icv infusion of vehicle and the lower dose of oxytocin found no significant effect of the factor treatment. As can be seen in Fig. 2, EEG activity over the 6-h recording period after oxytocin injection deviated little from that of vehicle. However, for most frequency bands above 8 Hz, a significant ($P < 0.05$) treatment by time effect emerged. Pairwise comparisons between corresponding 2-h intervals showed that 0.1 µg oxytocin initially augmented high-frequency activity (Fig. 3A).

For 1 µg of oxytocin, ANOVA yielded a significant effect of treatment for the frequency bands from 2 up to 15 Hz and of treatment by time for all frequencies above 9 Hz. This dose produced a general enhancement of EEG power density (Fig. 2) that was most pronounced during the first 2-h interval and gradually declined thereafter (Fig. 3B).

ANOVA performed on the data of the oxytocin antagonist found no significant effect of treatment (Fig. 2) but of treatment by time for all frequency bands between 13 and 22 Hz. Compared with vehicle, oxytocin antagonist tended to attenuate EEG activity in the respective frequency region during the last 2-h interval (Fig. 3C).

4. Discussion

The results of the present study indicate that oxytocin within the brain is significantly involved in the modulation of sleep–wake behaviour in male rats. Acute administration of synthetic oxytocin into the lateral cerebral ventricle initially increased wakefulness and thus delayed sleep onset latency which reflects increased arousal of the animal. In contrast, administration of the oxytocin receptor antagonist reveals a hypnotic effect of endogenous oxytocin in that the antagonist reduced non-REMS and pre-REMS. We therefore postulate a dual mechanism of action of brain oxytocin in dependence on the physiological state: under basal conditions, endogenous oxytocin may stimulate physiological sleep, whereas under conditions of stress and increased brain oxytocin levels, it is involved in the behavioural arousal accompanied by impaired sleep quality.

In agreement with the literature (for review, see Ref. [22]), both doses of oxytocin tended to produce a small, short-lasting increase of Tbr. Although its biological significance is questionable, this moderate increase may be secondary to an arousal response, because Tbr in rats largely depends on behaviour [23]. Administration of oxytocin into the cerebral ventricle transiently promoted wakefulness, thereby delaying the occurrence of non-REMS and REMS in a dose-related manner, and enhanced high-frequency activity in the EEG within non-REMS. The observation that exogenous oxytocin evokes wakefulness is in accordance with a previous report on the influence of chronic icv oxytocin infusion in rats [24]. In the present study, the highest dosage of oxytocin additionally elevated low-frequency activity in the EEG within non-REMS and increased time spent in non-REMS during the last 2-h interval. As extended periods of wakefulness are known to increase non-REMS and to enhance slow frequency components in non-REMS-specific EEG [25,26], these effects may partially represent recovery effects from sleep loss.

The effect of icv infusion of synthetic oxytocin on sleep–wake behaviour may mimic intracerebral release of endogenous oxytocin under conditions of stress. Oxytocin is not only released into blood, but also within various brain regions, including the paraventricular nucleus, during exposure to various stressors, such as swimming, shaker stress, social or maternal defeat in both male and female rats [27–30] (for review, see Refs. [1,2]). Such intracerebrally released oxytocin was found to be involved not only in the regulation of the behavioural stress response [3,17], but also in the maintenance/potentiation of the neuroendocrine response to a given stressor. Indeed, blockade of the receptor-mediated action of locally released oxytocin within the paraventricular nucleus during swimming significantly attenuates the stress-induced secretion of ACTH into blood, which reflects a local excitatory action of oxytocin on the reactivity of the HPA axis during stress [5]. However, this effect seems to be restricted to the paraventricular nucleus and may thus be mediated by regulating the activity of CRH neurons and consequently the HPA axis activity [5]. With respect to the present results, a comparable high bolus of icv synthetic oxytocin may reflect such a stress condition, resulting not only in behavioural arousal (see below), but also in an activated brain CRH system. Sleep–wake behaviour is susceptible to perturbation, that is, it is altered in response to stressors, and represents the summated output of multiple brain systems. In this context, again, a particular role of brain CRH in the regulation of wakefulness and sleep has to be emphasized [6]. Therefore, the postulated activation of CRH neurons by acute intracerebral oxytocin administration may thus result in a prolonged sleep onset latency and consequently in a reduction of sleep time as seen in Table 1 and Fig. 1.

A variety of studies performed in the 1980s demonstrated that the neurohypophysial neuropeptide oxytocin exerts behavioural effects, in particular to induce a state of arousal when administered into the brain ventricular system. In mice, pronounced hyperactivity, especially foraging and squeaking, was observed after oxytocin treatment [31]. Similarly, in rats, excessive grooming [32], increased locomotor activity and rearing in the open field test [33] have been described to be induced by oxytocin. In a detailed study performed by Drago et al. [34], oxytocin, in a range between 0.1 and 10 μg, increased the grooming score 4- to 9-fold, respectively, in both male and female rats. With respect to the time course of the effect of acute oxytocin, Drago et al. [34] reported increased behavioural activation for 45 min, which nicely fits our finding of a non-REMS latency of 37.3 min after 1 μg oxytocin versus 8.7 min after vehicle administration. Although to a lesser extent than the related neuropeptide vasopressin, oxytocin was also found to induce barrel rotations in rats and mice [31,35]. Fits of barrel rotations were also observed in some rats of the present study immediately after infusion of the higher dose of oxytocin (data not shown).

Treatment with the oxytocin antagonist under stress-free conditions—which was guaranteed by the method used for substance application—revealed the functional significance of endogenous brain oxytocin in the regulation of sleep under basal conditions. The antagonist also promoted wakefulness, although in a different fashion than synthetic oxytocin. Without affecting sleep onset latency, it slightly but persistently increased waking time more or less at the expense of all sleep states. This observation suggests that endogenous oxytocin exerts mild hypnotic properties. We could recently show a tonic inhibitory action of brain oxytocin on HPA axis activity in both male and female rats under undisturbed conditions [3,4]. This inhibitory effect is at least partly mediated by its action within the paraventricular nuclei [4]. Further, conditions associated with a high basal activity of the brain oxytocin system, like lactation [3,36–38] or chronic icv administration of synthetic oxytocin [17], are characterized by increased calmness and reduced stress responsiveness. It is therefore very likely that the hypnotic effect of central oxytocin is mediated by the brain CRH system, as reduced synthetic activity of CRH neurons under basal conditions has been found after chronic oxytocin treatment [39] and, as mentioned in the introduction, CRH system activity is negatively correlated with sleep quality.

Based on these findings, one may hypothesize a dual mechanism of action of oxytocin. Under basal, undisturbed conditions, that is, in a safe environment, brain oxytocin may exert a sleep-promoting action as revealed by the reduction in sleep time after application of the oxytocin receptor antagonist. In contrast, after its release within the hypothalamus, in particular within the paraventricular nucleus, in response to stressful events [27–30], oxytocin may facilitate the stress response of the

organism to return to homeostasis and thus induce a state of arousal and consequently delay sleep onset latency.

Interestingly, brain oxytocin reportedly modulates the activity of neurons located in the dorsal motor nucleus of the vagus nerve and thus regulates autonomic functions [40] and was also found to mediate stress-induced tachycardia, whereas it did not affect cardiovascular parameters under basal conditions [41]. These findings further support our hypothesis of a dual mechanism of action.

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