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CHANGES IN CEREBROSPINAL FLUID LEVELS OF VASOPRESSIN AND OXYTOCIN OF THE RAT DURING VARIOUS LIGHT–DARK REGIMES

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Levels of arginine-vasopressin (AVP) and oxytocin (OXT) in cerebrospinal fluid (CSF) of rats were determined at various times of the day and the night under normal and changed light–dark conditions.

During a regular daily 14 h light and 10 h dark cycle (lights on 06.00 h, off 20.00 h), AVP in CSF reached a peak at 13.00 h, while the lowest levels were found at 19.00 h. Reversal of the normal light–dark cycle into a 14 h dark and 10 h light cycle (lights on 20.00 h, off 06.00 h) did not change the normal AVP rhythm. These lighting conditions elevated the OXT levels in the CSF as compared to those found during a normal light–dark regime, but again no differences were observed between the OXT levels determined at the various time-points. A shift of 6 h of the light–dark cycle (lights on 12.00 h, lights off 02.00 h), completely disrupted the AVP rhythm. However, after 3 weeks of adaptation to this new light–dark regime a high level of AVP in CSF was again observed at 13.00 h, and a low level at 19.00 h.

The present data suggest that changes in the normal light–dark conditions affect the levels of neurohypophyseal peptides in the CSF. The results are of interest because of reported changes in memory function induced by alterations in the light–dark cycle.

Many processes in the mammalian body are controlled in a rhythmic manner. The secretion of hormones is thought to occur as a rhythmic phenomenon [8, 19]. However, there is no evidence for a consistent daily change of neurohypophyseal hormones in the peripheral circulation. An organized rhythm of arginine-8-vasopressin (AVP) was not observed in the blood of the cat [13], and neither was a daily rhythm in oxytocin (OXT) levels detected in the blood of the rhesus monkey [12]. However, circadian rhythms of AVP and OXT in the cerebrospinal fluid (CSF) of various species have been described. In the rat, cat and rhesus monkey, rhythms of AVP have been demonstrated [2, 12, 13, 16]. A circadian rhythm of OXT exists in the monkey, whereas CSF levels of this peptide in the cat do not appear to be organized in a rhythmic way [2].

Rats display a repetitive daily variation in retention performance of a passive avoidance test [9, 15]. Furthermore, changes in the normal light–dark cycle

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influence the ability of rats to learn a new task. A phase shift in the circadian rhythm in rats shortly after passive avoidance training caused an impaired performance on retention tests [17].

AVP and OXT affect memory processes, whereas AVP facilitates both storage and retrieval of information [1, 5, 14, 18], OXT has an opposite effect and possesses amnesic properties [3]. Because of the opposite effects of AVP and OXT on memory processes it was deemed of interest to study AVP and OXT levels in the CSF of rats at various times under normal and changed light–dark conditions. The results of such experiments could contribute to the understanding of the changes in behavior and memory function induced by altering light–dark conditions.

Male rats of an inbred Wistar strain, weighing 200–220 g at the onset of the experiments, were used. A cannulation technique for repeated sampling of CSF from the cisterna magna in freely moving rats was used, with slight modification [4,

AVP and OXT levels in CSF were determined at different time-points during the day or night at 01.00, 07.00, 13.00 and 19.00 h. During these experiments the animals were maintained in a 14 h light and 10 h dark schedule of illumination (lights on at 06.00 h and off at 20.00 h). CSF samples were taken on different days and the experiments were scheduled in such a way that a period of at least 18 h elapsed between different removals of CSF from the same animal. This procedure was chosen to exclude possible dilution effects. In a separate series of experiments the normal 14 h light and 10 h dark cycle was changed into a 14 h dark and 10 h light cycle (lights on 20.00 and off at 06.00 h). Similar times for withdrawal of CSF and experimental conditions were chosen as described above. In another group of experiments the normal light–dark cycle was shifted by a 6 h period. During these experiments the lights were on from 12.00 to 02.00 h and off from 02.00 to 12.00 h. CSF was collected with an interval of at least 18 h between sampling, at 01.00, 07.00, 13.00 and 19.00 h, immediately after the rats were subjected to this new light–dark cycle as well as 3 weeks after the animals had been submitted to this cycle. During this latter experiment the animals were operated in the third week, in order to create the optimal conditions for the withdrawal of CSF. AVP and OXT were determined in the various CSF samples by radioimmunoassay (RIA) after their extraction with activated Vycor glass powder. The details of the extraction and assay procedures have been described elsewhere [6, 7, 11]. The antiserum used for the determination of AVP was highly specific for AVP; the cross-reactivity with arginine-vasotocin (AVT) was 12.4% and with OXT less than 0.1%. The antiserum for OXT was highly specific for OXT; the cross-reactivity with AVP was less than 0.3% and with AVT approximately 1.5%. CSF samples were pooled from 2 animals and stored at −20°C until assaying.

Differences between groups were assessed using paired Student's *t*-test or analysis of variance (ANOVA) as appropriate. A P value of < 0.05 was considered as significant.

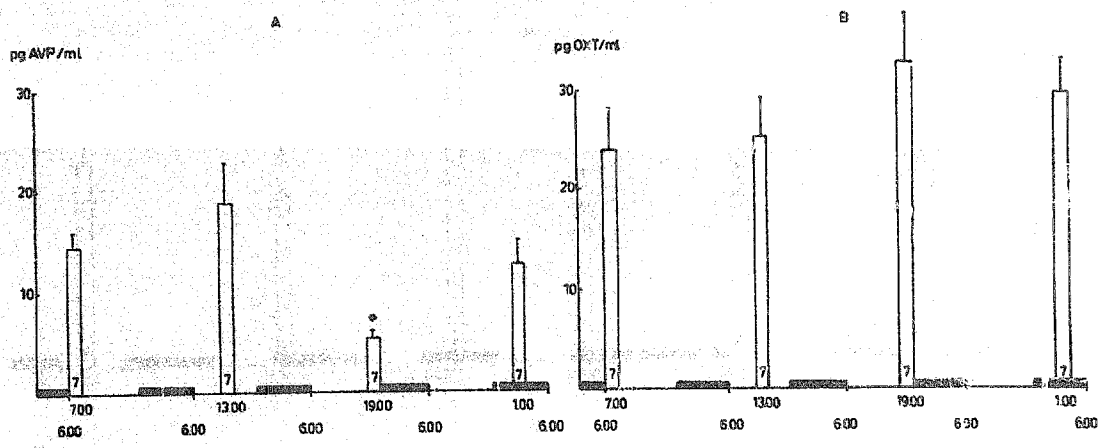

Fig. 1. Concentrations (pg/ml) of AVP (A) and of OXT (B) in CSF at various time-points of the day or night during a regular 14 h light and 10 h dark cycle of illumination (lights on at 06.00 h and off at 20.00 h). The black horizontal bars represent the periods during which the lights were off, while the vertical bars depict the mean ± S.E.M. The number in the bar refers to the number of samples. $^{*}P < 0.02$, 13.00 versus 19.00 h.

AVP concentrations in the CSF differed at various times of the day during a normal 14 h light and 10 h dark cycle (Fig. 1). The highest levels (18.5 ± 2.5 pg/ml means ± S.E.M.) of AVP were measured at 13.00 h, when the lights were on for 7 h, whereas at 19.00 h, 1 h before the lights were switched off, CSF AVP levels were at their lowest (5.5 ± 0.8 pg/ml). In the dark period (sampling-time 01.00 h) and 1 h after the lights had been switched on (07.00 h), AVP levels in the CSF were around 13.0 pg/ml. In contrast to AVP levels, OXT concentrations in the CSF did not differ at the various time-points of the day or night (Fig. 1). The OXT levels ranged between 23.1 ± 5.8 and 34.0 ± 6.2 pg/ml CSF.

In rats that were subjected to a 14 h dark and 10 h light cycle the peak level of AVP in CSF remained at 13.00 h, when the lights were off for 7 h, whereas at 19.00 h, 1 h before the lights were switched on, the lowest AVP levels were observed (Fig. 2). Placing rats in a reversed light–dark schedule of illumination resulted in increased levels of OXT in CSF as compared to the levels found during a normal light–dark cycle (df (1,58) = 7.03; $P < 0.01$), but again no peak or trough was observed (Fig. 2).

When the lights were on from 12.00 to 02.00 h, a shift of 6 h from the normal light–dark cycle, there was no difference between the AVP levels measured at the various time-points (Fig. 3A). After 3 weeks during which the rats were placed in this new light–dark cycle, AVP levels in CSF were comparable to those found under normal light conditions: highest at 13.00 h, 1 h after the lights were switched on, and lowest at 19.00 h, 7 h after the lights were on (Fig. 3B).

Under normal light and dark conditions AVP concentrations in CSF exhibit a daily rhythm, whereas such a rhythm was not demonstrated for OXT. Circadian

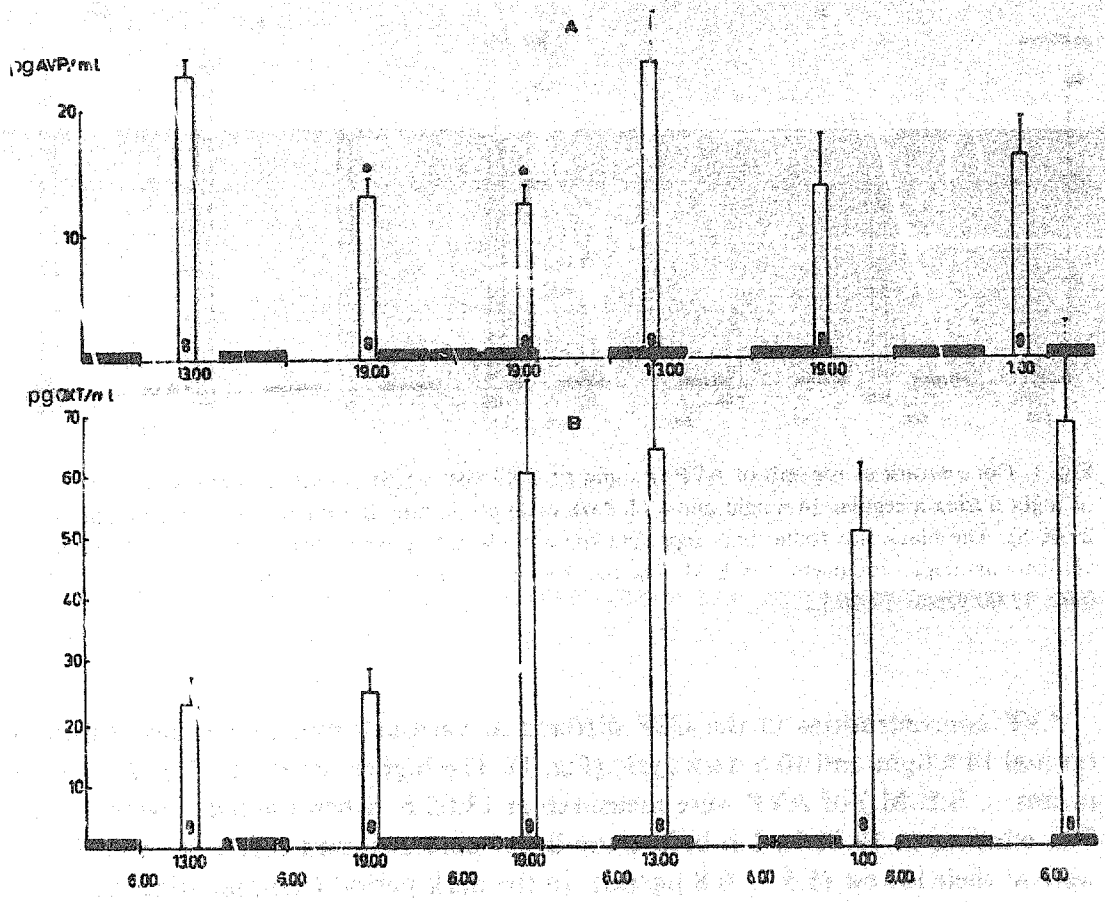

Fig. 2. Concentrations (pg/ml) of AVP (A) and of OXT (B) in CSF at various time-points of the day or night during a 10 h light and 14 h dark schedule of illumination (lights on at 20.00 h and off at 06.00 h). The black horizontal bars represent the periods during which the lights were switched off, while the vertical bars depict the mean ± S.E.M. The number in the bar refers to the number of samples. $^{*}P < 0.05$, 13.00 versus 19.00 h.

rhythms for AVP in CSF have been found in the rat, cat, sheep and monkey [2, 12, 13, 16]. The highest levels of AVP in CSF were found during daylight hours, whereas at the end of the light period and during the dark period these levels were diminished. These circadian rhythms were not affected by changes in the light–dark cycle. In the cat the CSF AVP rhythm persisted during 3 days of continuous light [13]. These data suggest that the AVP rhythm in the CSF is generated endogenously. The present findings from experiments in which the normal light–dark schedule was changed into a 14 h dark and 10 h light cycle supports this hypothesis. However, the results from the experiments in which the normal cycle was shifted by 6 h revealed that this alteration in the light–dark schedule induced a disturbance in the pattern of AVP levels in the CSF as compared to the data obtained under normal light–dark conditions.

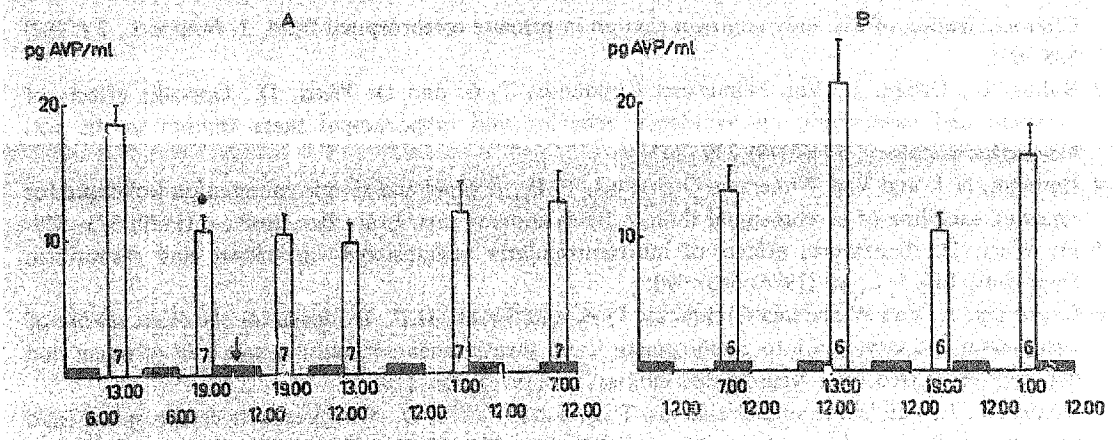

Fig. 3. Concentrations (pg/ml) of AVP and of OXT in CSF at different time-points of the day or night during a changed 14 h light and 10 h dark schedule of illumination (lights on at 12.00 h and off at 02.00 h). The left part of the figure (A) represents the values as measured immediately after the rats were subjected to the new light–dark cycle, while the right part of the figure (B) reflects the values 3 weeks after the animals had been submitted to this new schedule. The arrow indicates the time-point when the lighting conditions were changed. The black horizontal bars represent the periods during which the lights were off, while the vertical bars depict the mean ± S.E.M. The number in the bar refers to the number of samples. $^{*}P < 0.05$, 13.00 versus 19.00 h.

The existence of a circadian rhythm of OXT in CSF seems to depend upon the species. In the rhesus monkey a daily rhythm in OXT concentrations in CSF was observed, which persisted during constant light and dark conditions [2, 12]. In the cat [2], the guinea pig [10] and the rat (present study) rhythms for OXT in the CSF have not been found. A shift of 12 h in the light–dark conditions in the rhesus monkey resulted in a resynchronization of the rhythm to the new lighting schedule within 3–4 days. In the present study reversal of the normal light–dark cycle resulted in an increased OXT level in the CSF. It appears that the OXT levels are more easily influenced by alterations of the lighting conditions than the AVP levels of CSF.

The changes in neurohypophyseal hormone levels in CSF observed after altering the regular light–dark regime are of interest with respect to the disturbed behavior and memory function occurring under these conditions [9, 17]. Since the levels of these hormones in the brain play an important role in processes related to behavior and memory [5, 18], and as the levels of AVP and OXT in the CSF may reflect their concentrations in various brain regions, the observed change in the ratio of these hormones in the CSF may play a role in the disturbances in behavior following changes in the light–dark schedule.

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