CHAPTER 26

Suprachiasmatic nucleus in aging, Alzheimer’s disease, transsexuality and Prader–Willi syndrome

D. F. Swaab\(^a\), B. Roozendaal\(^a\), R. Ravid\(^a\), D. N. Velis\(^b\),
L. Gooren\(^c\) and R. S. Williams\(^d\)

\(^a\)Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, \(^b\)Department of Neurosurgery, Academic
Medical Center, University of Amsterdam, Amsterdam, \(^c\)Department of Endocrinology, Free University, Amsterdam.
The Netherlands and \(^d\)Neuropathology Laboratory, Shriver Center, Walton, MA 02154, USA

Introduction

Disorganization of circadian rhythmicity is a hallmark of aging (Ingram et al., 1982; Miles and Dement, 1980; Van Gool and Mirmiran, 1986a,b). Circadian sleep/wake rhythms alter considerably in senescence, and even more seriously in Alzheimer’s disease (Prinz et al., 1982). Such sleep disturbances, accompanied by sundowning (state of confusion associated with onset of dusk) and nocturnal wandering, comprise significant aspects in Alzheimer’s disease (Miller and Bartus, 1982). The increase both in time spent in wakefulness during the night, and naps in daytime, characteristic of many of the elderly (Prinz et al., 1982) is a symptom of disruption of circadian sleep rhythms similar to that found following experimental suprachiasmatic nucleus (SCN) lesions (Eastman et al., 1984). It is interesting in this respect that disruption of circadian rhythms may not only lead to disappearance of the diurnal rhythm of urine excretion and incontinence (Minamisawa, 1980), but also to cognitive disturbances (Fekete et al., 1985; Van Gool and Mirmiran, 1986a). Since the SCN is considered to be the endogenous clock of the mammalian brain, which coordinates hormonal and behavioral circadian rhythms, the question was raised whether alteration in the human SCN might be the morphological basis for the observed circadian disturbances. A combination of immunocytochemical and morphometric techniques was applied (Swaab et al., 1985) to test this hypothesis. The study is extended here to include findings on human brains: 32 non-demented control subjects and 9 patients suffering from Alzheimer’s disease. Pronounced changes in the SCN in senescence and Alzheimer’s disease were indeed revealed in the present study.

The human SCN in senescence and Alzheimer’s disease

Although earlier studies questioned the existence of the SCN in the human brain (cf. Lydic et al., 1980), immunocytochemical staining with antibodies against arginine-vasopressin (AVP) turned out to be a good marker for this nucleus in the same way as we have described earlier in the rat (Swaab et al., 1975). That rat and human SCN are not only homologous with regard to peptide content but the SCN in the human brain is also innervated by a retinohypothalamic tract (Sadun et al., 1984). In a case where the SCN region was selectively destroyed by a metastasis, circadian fluctuations in temperature disappeared (Schwartz et al., 1986), which illustrates the
Fig. 1. Number of AVP cells (left panel) and total number of cells in the SCN (right panel). Note the low values in the 81- to 100-yr-old group and in the Alzheimer patients (DEM) that were 78 ± 5 yr of age. The vertical lines denote the SEM. The extremely high values for the two transsexual subjects (T1 and T2) and one case of Prader–Willi syndrome (P) are not included in the group means and are only given as individual values.

Functional homology with the rat SCN. In our human material, 16–17% of the total SCN cell number was stained with anti-AVP in the age groups of 0–80 yr. In the oldest age group and in the Alzheimer patients the proportion of AVP cells was about 13%. AVP cells and fibers were visible throughout the SCN (Swaab et al., 1985). This enabled us to apply morphometric techniques in order to follow age-related changes in volume and cell number of the human SCN.

A marked decrease in SCN volume, AVP cell number and total SCN cell number was found in 80- to 100-yr-old patients as compared with the younger age groups. In 9 Alzheimer patients (mean age 78 ± 5 yr), corresponding SCN changes were even more pronounced than those observed during normal aging (Swaab et al., 1985; for the extended series of observations see Fig. 1.). It became clear from partial lesions in the rat SCN that the size of the SCN is crucial for the expression of its pacemaker properties (Pickard and Turek, 1983; Van den Pol and Powley, 1979). The observed decrease in SCN volume and cell number in senescence and in Alzheimer’s disease therefore suggests a causal relationship between age-related changes in the SCN and disturbances of circadian rhythmicity, such as sleep/wake patterns. Since more frequent and prolonged awakenings and shorter sleep periods were already found in 50- to 60-yr-old subjects (Webb, 1982), whereas SCN cell loss was only present from the age of 80 onwards (Fig. 1) cell loss is
probably a rather late phenomenon in SCN dys-
function.

The SCN in the senile rat

Although numerous studies have shown that the
circadian organization deteriorates progressively in
senescence, in the rat also, Peng et al. (1980),
using conventionally stained material, found no
decrease in overall SCN cell number in senescent
rats. This result was confirmed recently by us in
a morphometric study comparing young
(7–8 mth) with old (32–33 mth) Brown Norway
(BNBiRij) rats (Roozendaal et al., 1987). We
determined subsequently whether a well-defined
population of neurons, i.e. vasopressinergic
(AVP) cells, might show changes with aging.
Immunocytochemical staining with anti-AVP
revealed a decrease of 31% (p < 0.007) in the
number of anti-AVP stained SCN neurons. The
cell diameter increased significantly (p < 0.001)
in the remaining AVP cells. In the supraoptic
nucleus (SON) and paraventricular nucleus
(PVN), cell size has turned out to be a good
parameter for peptide production (Fliers et al.,
1985), although this relationship has not yet been
investigated in the SCN. The increased cell
diameter may be a compensatory mechanism for
cell loss. There appeared to be no statistically
significant differences in cell numbers between
rats housed in standard cages and those housed
in an enriched environment in either age group,
which is in accordance with the lack of effect of an
enriched environment upon the circadian
organization of sleep/wakefulness patterns in old
rats (Van Gool and Mirmiran, 1986b). Changes in
SCN vasopressin neurons seem to go together
with the circadian disturbances in senescence, not
only in man (Swaab et al., 1985), but also in the
rat. The question whether the loss of AVP cells in
the rat SCN is due (1) to a reduced staining
intensity in senescence, whereas the neurons as
such remain intact, or (2) to a selective loss of
these neurons that is either masked because of the
relatively small proportion (4.3–3.1%) of the

AVP-cell population in the rat SCN or compen-
sated for by an increase in the number of glial
cells, cannot be solved at present.

Cerebrospinal fluid (CSF) does not have a
circadian message

Decreased (Raskind et al., 1986; Sørensen et al.,
1983; Sundquist et al., 1983) as well as increased
AVP levels (Tsui et al., 1981) in human
cerebrospinal fluid (CSF) have been reported in
Alzheimer’s disease. Although these observations
make unequivocal conclusions on the possible
relationship between SCN and CSF-AVP
changes in Alzheimer’s disease impossible to
draw at present, animal experiments certainly
point to the existence of a relationship between
the SCN and CSF levels of AVP. The circadian
activity of the SCN is reflected in diurnal AVP
levels in the cerebrospinal fluid of various animals
(Reppert et al., 1983). This raises the question
whether circadian sleep/wake patterns are regu-
lated by the SCN via AVP as a hormonal
messenger and the CSF as transport medium to
brain sleep/wake centers. However, two recent
observations described below make such a role
for CSF-AVP very improbable. In the first place
AVP implants in the CSF do not result in
circadian sleep/wake changes, although the pept-
id was found to enter the brain (Fig. 2) and,
secondly, there are no clear circadian AVP pat-
tterns measurable in human CSF, even in cases
where normal circadian patterns of rectal temper-
ature and plasma cortisol are present (Figs. 3 and
4).

Accurel polypropylene tubing loaded with
vasopressin, implanted into the rat lateral ven-
tricle, showed AVP to be bound in the brain in
two distinct patterns (Fig. 2): (a) in perineuronal
structures and dots between cells, in the lateral
septum (dorso-rostral part) striatum, cingulate
cortex, granular cells of the dentate gyrus of the
hippocampus, hippocampal pyramidal cells of
CA1 and CA3 and around cerebellar Purkinje
cells; and (b) in the cytoplasm of neuronal cell
bodies in the lateral and medial septum, striatum, cingulate cortex, bed nucleus of the stria terminalis, organum vasculosum of the laminae terminalis and locus coeruleus. A variety of controls proved that no aspecific uptake was involved in the procedure. The distribution of AVP binding sites was partly coincident with known sites of AVP fiber innervation, and agreed largely with data obtained by autoradiographic techniques for $[^3H]$AVP-binding (Ravid et al., 1986).

Subsequently, this procedure was applied to rats whose sleep/wake patterns were followed.
Continuously increased CSF levels of AVP up to 300 pg/ml were found following Accurel implants. The CSF levels no longer showed any circadian fluctuation. The endogeneous diurnal CSF-AVP rhythm involving a few pg/ml was thus masked. The circadian sleep/wake patterns of these animals appeared, however, not to be disturbed; the pattern of wakefulness, quiet sleep and REM-sleep over the day/night period remained fully intact (Kruisbrink et al., 1987). These observations show that the circadian CSF-AVP levels in the rat do not transfer the diurnal message from the SCN to the rest of the brain. This message will instead be transferred from the SCN into other brain areas by the SCN efferents (cf. Hoorneman and Buijs, 1982), e.g. to the periventricular nucleus. Interestingly enough, the periventricular

patient remained in the Intensive Care Unit. Records of patient's neurologic status included the Glasgow Coma Scale score (Teasdale et al., 1974). Rectal temperatures were charted 4 times a day and total plasma cortisol levels were determined according to Farmer and Pierce (1974), using a Corning Immo Phase kit, twice on two consecutive days in the period during which CSF samples were drawn. There is a well defined circadian rhythm in cortisol levels in man (Mattingly and Tyler, 1965). Short-term perioperative administration of dexamethasone does not appear to cause adrenal insufficiency (Nelson, 1979). Methods: Patient monitoring took place in the Intensive Care Unit. Room lighting was switched off from 23.00 h to 06.00 hrs. Approximately 10 ml of fresh CSF was drawn from the Cordis external ventricular drains at 09.00, 12.00, 21.00 and 24.00 h. for at least three consecutive 24-h periods. All CSF samples were obtained and kept in polyethylene tubes in ice and were immediately centrifuged for 5 min. The supernatant was kept at −20 °C until measurement of AVP and OXT by means of radioimmunoassay according to Dogterom et al. (1977), except for the extraction before the OXT assay on CSF of patient R.H. and before the AVP assays on CSF of patient E.B., which was performed by Seppak C 18 (La Rochelle et al., 1980) instead of Vycor. In the two lower panels of Figs. 3 and 4, △ indicates values below detection levels and ▽ high values beyond the scale, which are mentioned separately.

Fig. 3. Patient E.B., a 24-yr-old woman, was admitted because of an intracerebroventricular tumor, situated at the sella media on the left and immediately anterior of the pineal gland. The tumor had caused an obstruction hydrocephalus. She had not been known to suffer from any degenerative neurologic condition and, prior to the onset of hydrocephalus, she had functioned normally as a primary school teacher. The tumor, a grade II astrocytoma, was extirpated by way of a left-side fronto-temporal craniotomy under dexamethasone treatment and general anesthesia. A Cordis external ventricular drain was placed in the anterior horn of the right lateral ventricle. The patient was fully conscious and without any neurologic deficit from the evening of the day of operation onwards. From days one up to and including four postoperatively fresh CSF samples were obtained through the drain while the
zone was found to be innervated in those cases of SCN transplants that induced reappearance of rhythmicity in SCN-lesioned rats (Sawaki et al., 1984). Thus, AVP derived from the SCN acts as a neurotransmitter rather than as a hormone. CSF-AVP may, of course, reflect the circadian SCN activity, because AVP that is released from this nucleus (or from another AVP-producing nucleus, e.g. the bed nucleus of the stria terminalis that may be driven in a circadian way by the SCN) into several brain areas, is subsequently removed by the CSF. However, CSF-AVP does not seem to carry information on the diurnal state for the rest of the brain.

Lack of CSF-AVP rhythm in human subjects

A second argument against the possible importance of CSF-AVP levels for circadian rhythms is presented by the lack of a diurnal pattern of AVP in the human CSF. Unlike what was seen in various other species, no circadian rhythm in CSF-AVP was observed in 5 patients with normal pressure hydrocephalus and 5 with ventricular enlargement due to cerebral atrophy (Sørensen et al., 1985). The lack of a clear circadian CSF-AVP pattern was confirmed in other studies (Sørensen et al., 1987; Kuboyama et al., 1987). In order to investigate whether the CSF of subjects not known to suffer from a degenerative neurologic disorder would show any CSF-AVP or CSF-oxytocin (OXT) periodicity we investigated CSF samples serially drawn from two patients treated for acute onset hydrocephalus due to obstruction of the cerebral aqueduct. A brief presentation of their case histories is given in the legends of Figs. 3 and 4. The results of rectal temperature fluctuations, cortisol, CSF-AVP and CSF-OXT levels are plotted as a function of the 24-h cycle in Fig. 3 for patient E.B. and Fig. 4 for patient R.H. Both subjects demonstrated a normal diurnal rectal temperature rhythm as well as a normal cortisol rhythm. There was, however, no clear rhythmic pattern for either ventricular CSF-AVP or CSF-OXT levels in either of the two subjects.

Fig. 4. Patient R.H., a 67-yr-old man, presented with a hematoma of the right cerebellar hemisphere following rupture of a small arteriovenous malformation of the right cerebello-pontine angle, as demonstrated after Seldinger angiography of the right vertebral artery. The intracerebellar bleed was exacerbated by use of oral anticoagulants, prescribed after a myocardial infarction at the age of 62 yr. He was not known to suffer from any degenerative neurologic condition. Prior to the rupture of the A-V anomaly he had functioned normally. Two days after the bleed, he developed hydrocephalus on the basis of compression of the top of the fourth cerebral ventricle, shown on computed tomography of the posterior cranial fossa, and obtained a Cordis external ventricular drain in the anterior horn of the right lateral ventricle. From days four up to and including nine post-bleed fresh CSF samples were obtained through this drain. For further details see Fig. 3.
The low levels of AVP in human CSF and the lack of circadian fluctuations might be explained by species differences in AVP-innervation of the brain. The limbic system in the human brain is hardly innervated whereas it is one of the main targets for AVP fibers in the rat. On the other hand, the locus coeruleus is even more densely innervated in the human brain than in the rat brain (Fliers et al., 1986).

Other possible functions of the SCN

Various observations indicate that the SCN might be involved in the process of reproduction, in which circadian rhythms play an important part. AVP-CSF rhythm is inversely related to the rhythm of lordosis in female rats. Since AVP antagonists facilitate this behavior, whereas AVP administration inhibits it, SCN-derived AVP has been suggested to influence rhythms in lordosis behavior (Södersten et al., 1983; 1985). The recent observation of Caldwell et al., 1986 that AVP increased lordosis in female rats indicates that this AVP effect needs further study. Coronal knife cuts placed posterior to the SCN eliminated the estrous cycle of rats, but had no effect on feeding, drinking or activity rhythms (Nunez and Casati, 1979). Lesions of the SCN eliminated phasic LH release in ovariectomized rats, but left basal LH levels unaffected (Gray et al., 1978). Such lesions result in failure of the rat to ovulate. The anovulatory animals showed, moreover, a high level of sexual receptivity (Brown-Grant and Raisman, 1977), and were in persistent behavioral oestrus (Raisman and Brown-Grant, 1977). Moreover, DG-LVP treatment delayed the disappearance of ejaculatory and intromission patterns following castration of male Wistar rats (Bohus, 1977).

Although the overall picture of SCN involvement in sexual behavior is not yet clear, the observations described above certainly suggest that the SCN may play an important role in reproduction and sexual behavior. In this respect some of our rather accidental findings concerning the SCN of two transsexuals and one case of a Prader–Willi syndrome may be of interest.

The SCN in transsexism and Prader–Willi syndrome

An extremely large SCN was found in brains of two male-to-female transsexuals, 44 and 50 years of age. The number of AVP cells as well as the total cell number of SCN cells was twice as large as that of the age-matched controls (Fig. 1). The estrogens given to both, and Androcur given to one of these patients do not seem to explain these observations. In the first place, the control subjects we studied showed no sex difference in the number of AVP cells or total cell number in the SCN; the same holds true for the lack of any change in the menopause (Swaab et al., 1985 and Fig. 1), so that the levels of sex hormones in adulthood do not seem to be very critical for the SCN data. Secondly, the SCN of a 46-yr-old woman suffering from a virilizing tumor, resulting in high levels of androstenedione and testosterone, had a normal number of AVP cells and total cell number in the SCN (Swaab et al., 1985). On the other hand, a recent observation indicates that the presence of a high number of SCN cells might be related to a deficiency in sex hormone levels during development. A 30-yr-old woman suffering from Prader–Willi syndrome appeared to have similar, extremely high numbers of AVP and total cells in the SCN. This syndrome, consisting of hypotonia, hypogonadism, hypometria and obesity, is characterized by a congenital lack of LHRH (Bray et al., 1983). The possibility that the extremely large SCN in transsexuals and Prader–Willi syndrome is indeed due to lack of LHRH and consequently to a lack of sex hormones during development is currently under investigation. Interesting circumstantial evidence for our observations comes from Södersten et al. (1981) who showed that treatment of rat neonates with an anti-estrogen enhanced the daily rhythmicity in mounting and lordosis behavior. In addition, Prader–Willi patients reveal striking disturb-
ances in sleep/wakefulness patterns. They had excessive daytime sleepiness (Vela-Bueno et al., 1984) that might indicate alterations in SCN function. No information is available at present on changes of sleep patterns in transsexuals.

Conclusions and summary

The human suprachiasmatic nucleus (SCN) shows a clear cell loss in senescence, which is even more pronounced in Alzheimer's disease. SCN changes seem to be the morphological substrate for the disrupted circadian rhythms which have been reported in these conditions. In addition, such changes might be a mechanism in the process of aging and Alzheimer's disease, since disruption of circadian rhythms leads to cognitive disturbances (cf. Van Gool and Mirmiran, 1986a). At present, neither the cause of the SCN degeneration nor a possible therapeutic intervention is known. Concerning the first point we are currently searching for a change in SCN innervation. Concerning the second point, we made one negative finding. The enriched environment that was applied as a non-specific way of activating old rats appeared to improve the sleep patterns to some extent but had no effect on circadian sleep/wake patterns or on the diminished number of vasopressin (AVP) neurons in the senescent rat SCN. It is clear, however, that the drugs that are so often given to senescent patients and in Alzheimer's disease in order to control their disturbed sleep patterns will not be effective and may even have disturbing effects (Swaab and Fliers, 1986). For example, the frequently used benzodiazepines may induce confusion, daytime sleepiness or may impair breathing (Guilleminault and Silvestri, 1982).

The circadian pattern of the SCN is not transmitted to the rest of the brain by the cerebrospinal fluid (CSF)-AVP acting as a hormone. This became clear from Accurel-vasopressin implants into the lateral ventricle that caused high constant CSF levels of AVP, thus masking the endogenous circadian rhythm of these levels. Although AVP was found to enter the brain this way and bind to neurons in a number of specific areas, the circadian sleep/wake patterns remained undisturbed. Since, in addition, diurnal rhythms in human CSF-AVP do not seem to exist in the human brain, there seems to be no essential circadian message conveyed by AVP via the CFS route. Consequently, AVP derived from the SCN is instead communicating its circadian information to the rest of the brain as a neurotransmitter.

The SCN is not only involved in circadian rhythms, but may play a role in reproductive processes and sexual behavior as well. In this connection our finding of extremely large SCN cell numbers in two male to female transsexuals and one patient with Prader–Willi syndrome may be of interest. It is suggested by these observations that a lack of sex hormones during a certain stage of brain development induced a large SCN. This possibility is currently under investigation.

The technology used on human post-mortem brain material, i.e. a combination of immunocytochemistry and morphometry, may open new vistas in neuropathology. Cell density — the parameter so far used most often in neuropathology — did not evince any alteration in aging, Alzheimer's disease, transsexuals or in the case of Prader–Willi syndrome, although great changes were observed in the number of AVP neurons and total SCN cell numbers. This means that, on one hand, total cell numbers of brain structures should be determined instead of cell densities, and on the other hand, that major changes in the human brain, in the case of psychopathologies, may have been missed thus far.

Acknowledgements

We are grateful to Mrs. W. Chen-Pelt and T. Eikelboom for secretarial help, to J.J. Van Heerikhuize for performing the radioimmunoassays and to G. Van der Meulen for the photography. This investigation was partly supported by the John Douglas French Foundation for Alzheimer's disease (Los Angeles, USA).
human brain material was obtained from the Brain Bank in the Netherlands Institute for Brain Research, Amsterdam.

Reference


