Review

Development of the Human Hypothalamus*

D. F. Swaab¹

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The hypothalamus has been claimed to be involved in a great number of physiological functions in development, such as sexual differentiation (gender, sexual orientation) and birth, as well as in various developmental disorders including mental retardation, sudden infant death syndrome (SIDS), Kallman's syndrome and Prader-Willi syndrome. In this review a number of hypothalamic nuclei have therefore been discussed with respect to their development in health and disease.

The suprachiasmatic nucleus (SCN) is the clock of the brain and shows circadian and seasonal fluctuations in vasopressin-expressing cell numbers. The SCN also seems to be involved in reproduction, adding interest to the sex differences in shape of the vasopressin-containing SCN sub-nucleus and in its VIP cell number. In addition, differences in relation to sexual orientation can be seen in this perspective. The vasopressin and VIP neurons of the SCN develop mainly postnatally, but as premature children may have circadian temperature rhythms, a different SCN cell type is probably more mature at birth.

The sexually dimorphic nucleus (SDN, intermediate nucleus, INAH-1) is twice as large in young male adults as in young females. At the moment of birth only 20% of the SDN cell number is present. From birth until two to four years of age cell numbers increase equally rapidly in both sexes. After this age cell numbers start to decrease in girls, creating the sex difference. The size of the SDN does not show any relationship to sexual orientation in men.

The large neurosecretory cells of the *supraoptic (SON)* and *paraventricular nucleus (PVN)* project to the neurohypophysis, where they release vasopressin and oxytocin into the blood circulation. In the fetus these hormones play an active role in the birth process. Fetal oxytocin may initiate or accelerate the course of labor. Fetal vasopressin plays a role in the adaptation to stress—caused by the birth process—by redistribution of the fetal blood flow.

Corticotropin-releasing hormone (CRH) neurons of the PVN play a central role in stress response. Thus fetal CRH neurons may play a role in the timing of the moment of birth. Recently, alterations have been described in peptidergic, aminergic and cholinergic transmitters in the hypothalamus in SIDS. Future research will have to establish whether these changes are part of the course of SIDS. A large proportion of the SON and PVN neurons also produce tyrosine hydroxylase (TH). In neonates the majority of TH-immunoreactive neurons colocalizes vasopressin, while in the adult the majority of TH-positive neurons colocalizes oxytocin. TH-expression might be a sign of hyperactivation, for example from perinatal hypoxia.

Oxytocin neurons also project to the brain stem. These neurons have an inhibitory effect on eating. Interestingly, in the Prader-Willi syndrome, characterized for example by insatiable hunger, we have found that the number of oxytocin-expressing neurons is about half the normal value.

It can be concluded that the various hypothalamic nuclei are involved in a great number of functions and show clear and differential changes in development with respect to sexual differentiation, birth and a number of diseases. I believe that only a small proportion of such changes has at present been revealed.

KEY WORDS: Human hypothalamus; suprachiasmatic nucleus; sexually dimorphic nucleus; supraoptic nucleus; paraventricular nucleus; vasopressin; oxytocin; development.

¹ Graduate School Neurosciences Amsterdam, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, ZO, The

Netherlands, Tel: +31 (0)20 5665500, Fax: +31 (0)20 6961006

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Fig. 1. Chiasmatic and tuberal region of the hypothalamus in the human adult. The diagram shows the main landmarks and nuclear gray matter that are encountered in coronal sections and are traced antero-posteriorly (a–h). The space between sections is 800 μ m; ac, anterior commissure; an, accessory neurosecretory nucleus; cm, corpus mammillare; cu, cuneate nucleus; db, nucleus of the diagonal band; dm, dorsomedial nucleus; fm, fasciculus mammillo-thalamicus; fo, fornix; hg, hypothalamic gray; if, infundibular nucleus; in, intermediate nucleus; oc, optic chiasm; ot, optic tract; pe, periventricular nucleus; ph, posterior hypothalamic nucleus; pm, posteromedial nucleus; pv, paraventricular nucleus; rc, retrochiasmatic nucleus; sc, suprachiasmatic nucleus; so, supraoptic nucleus; st, nucleus of the stria terminalis; su, subthalamic nucleus; th, thalamus; tl, lateral tuberal nucleus; tm, tuberomammillary nucleus; un, unicate nucleus; vm, ventromedial nucleus. Modified from Braak and Braak (102), with permission.

Some 55 years ago Morgan (1) investigated the hypothalamus in 16 institutional cases of mental deficiency. Pathologic involvement of the third ventricle region was evident in all but 2 cases. The tuberomammillary nucleus was the only cell group in the hypothalamus which did not show a marked reduction in cell density, which led Morgan to conclude "... that the hypothalamus plays an important role in the etiology of mental deficiency ...", an idea that has never been followed up in more recent research. This brain structure is, however, known to be involved in a wide range of functions in the developing, adult and aging subject, as well as in various diseases of different etiologies. Alterations in hypothalamic structures and functions are thought to be operative in diseases such as sudden infant death syndrome (SIDS), Kallmann's syndrome, anorexia nervosa, bulimia, depression, Cushing's disease, diabetes insipidus, Prader-Willi syndrome, polycystic ovaries syndrome and the malignant neuroleptic syndrome, as well as in disturbances in sleep and temperature regu-

lation. The hypothalamus is affected in neurodegenerative diseases and this might be the reason for particular symptoms, e.g. in Alzheimer's, Parkinson's, and Huntington's disease, and in multiple sclerosis. In addition, attention has recently been paid to, for example, the relationship between the structural development of the human hypothalamus and gender and sexual orientation (2).

The hypothalamus has a number of unique properties that make it very suitable for fundamental neurobiological research. In the first place it contains, in addition to conventional neurons, neuroendocrine cells whose activity can be monitored by the measurement of plasma levels of hormones secreted by these cells. Moreover, the hypothalamic nuclei can well be delineated (Fig. 1), which makes it possible to monitor the basic developmental processes—cell formation, migration, differentiation, sexual differentiation and cell death—in a quantitative way by means of morphometry. The neurotransmitter, neuromodulator or neurohormonal content of many of the hypothalamic nuclei is currently well known. The various hypothalamic nuclei and cell types have specific functions: the suprachiasmatic nucleus is the hypothalamic clock, the vasopressin neurons of the supraoptic and paraventricular nuclei are involved in antidiuresis, and the corticotropin-releasing hormone neurons of the paraventricular nucleus are pivotal in the stress response. All these properties make the hypothalamus an extremely suitable brain area for the study of structure-function relationships. Nevertheless, only relatively few neuroscientists are involved in the study of the hypothalamus, and a neuropathology of the hypothalamus is practically non-existent—a fact that may well be explained by the complexity of this structure.

In the present review a number of hypothalamic nuclei are therefore discussed in relation to their development in health and disease.

Suprachiasmatic Nucleus (SCN)

The suprachiasmatic nucleus (SCN) is a small structure (0.25 mm³; 3) considered to be the major circadian pacemaker of the mammalian brain and coordinating hormonal and behavioral circadian rhythms (4). In conventionally thionine-stained sections the human SCN cannot be recognized with certainty and therefore immunocytochemical labeling of the nucleus, for example with anti-vasopressin, is necessary (5). The shape of the human SCN is sexually dimorphic, being more elongated in women and more spherical in men, but the vasopressin cell number and volume are similar in both sexes (3). Neurons that are immunoreactive for vaso-

Fig. 2. Development of the human suprachiasmatic nucleus (SCN) of the hypothalamus, log-log scale. The period of term (38–42 weeks of gestation) is indicated by the vertical bar. Note that total cell number is low at the moment of birth (13% of the cell number found in adulthood). There is no difference in the developmental course of the SCN in boys and girls. Cell numbers around 1 to 1.5 years postnatally are more than twice the amount of adult cell numbers. From Swaab et al. (5) with permission.

pressin, vasoactive intestinal polypeptide (VIP), neuropeptide-Y (NPY) and neurotensin are present in the SCN in a specific anatomical organization (6,7). Typical for the human SCN, as compared to monkeys and other animals, are the very large population of neurotensin cells, and also the large population of NPY neurons obscuring a geniculohypothalamic tract—if such a tract is present in the human brain at all (7).

Recent observations have revealed a marked seasonal variation in the number of cells expressing vasopressin in the human SCN in relation to variations in photoperiod. Values were twice as high in autumn as in summer (8) and similar circadian fluctuations were observed in the SCN of young adults but they were absent after the age of 50 (9).

Morphometric analysis of the SCN in 10 homosexual men revealed that the volume of this nucleus was 1.7 times as large as that of a reference group of 18 presumably heterosexual male subjects, and that it contained 2.1 times as many vasopressin-producing cells (10). It might be that the programmed postnatal cell death, usually occurring from 13 to 16 months from birth onwards (see below) is limited in homosexual men. It is not yet clear what the functional implications of this finding might be, although there are various indications that the SCN is involved in aspects of sexual behavior and reproduction (11).

SCN Development and Circadian Rhythms

Various circadian rhythms of the fetus disappear immediately after birth, but reemerge in the neonate and

continue to develop over a period of several weeks to months postnatally. Therefore it is generally believed that the fetal rhythms are predominantly driven by the mother (12). This idea was reinforced by the observation that postnatal development of various overt rhythms, for example in N-acetyltransferase and sleep/wakefulness patterns, is paralleled by a strong increase in the number of vasopressin-expressing neurons in the SCN (5). On the other hand, the fetal SCN itself already shows metabolic circadian changes in the squirrel monkey (13). Moreover, recent observations have shown that temperature rhythms are already present in some 50% of human prematures (14). In addition, melatonin receptors are apparent in the SCN area as early as the 18th week of gestation (13). We must therefore conclude that the fetal SCN, although immature, already shows endogenous circadian rhythms, and that while most fetal rhythms are driven by the mother, some overt circadian rhythms (for example temperature rhythms) may be present as early as the premature period. These observations suggest the involvement of a SCN cell type that is already mature well before birth. Vasopressin neurons do not mature so early; at birth the SCN contains some 13% of the vasopressin-expressing neurons and 20% of the total cell number found in adulthood (Fig. 2). Subsequently, cell numbers rise to maximum values around one to two years postnatally, after which they decrease gradually to some 50% of these numbers in adulthood (5). From animal experiments it is known that vasoactive intestinal polypeptide (VIP) neurons in the SCN develop well before the vasopressin neurons (15,16). In order to assess the course of maturation of the VIP neurons in the human SCN, the number of VIP-expressing neurons was determined by immunocytochemistry and morphometry in 43 subjects from mid-gestation up to 30 years of age (17; Fig. 3). VIP and vasopressin neurons were first observed at 31 weeks of gestation in the ventrolateral part of the SCN. From three months postnatally onwards, VIP-positive neurons were observed in some subjects in the centromedial part of the SCN, but the centromedial type of VIP staining became a constant finding only from about 20 years of age. Some VIP neurons stained in the ventrolateral SCN in a few subjects, but their number and nuclear diameter were small. Postnatally, the number of VIP neurons increased gradually until at around three years of age adult values were reached (17; Fig. 3). After the age of 10 a clear sex difference in the number of VIP neurons was found, males having on average twice as many VIP neurons in the SCN as females (17; Fig. 4). In the adult the number of VIP cells in the SCN amounted to only 35% of those previously found to contain vasopressin. The data do not indicate a par-

Fig. 3. Development of vasoactive intestinal polypeptide (VIP)-expressing neurons of the human suprachiasmatic nucleus (SCN) of the hypothalamus. Log-log scale. The period of term (38–42 weeks of gestation) is indicated by the vertical bar. Note that until the end of term VIP cell numbers are low, and the majority of subjects do not show any VIP staining at all. After term there is a gradual increase in VIP neuron numbers and after 10 years of age the values for males are clearly greater than those of females (from Swaab et al. (17) with permission).

Fig. 4. VIP cell numbers in the SCN of males of 10–30 years of age are twice as large as those in females. The bars indicate the standard error of the mean (from Swaab et al. (17) with permission).

ticular role for VIP neurons in those rhythms that are already present in early development, for example of the temperature rhythm in prematures of around 30 weeks gestational age. However, our observations in this and earlier studies, as well as animal studies, do suggest a possible role for VIP neurons in the SCN in sexually

dimorphic functions such as reproduction and sexual behavior.

Sexually Dimorphic Nucleus (SDN, Intermediate Nucleus, INAH-1)

The sexually dimorphic nucleus of the preoptic area (SDN) was first described in the rat brain by Gorski et al. (18). Due to differences in perinatal steroid levels, the SDN in the male rat is three to eight times larger than in the female rat (19). On the basis of lesion experiments in rats it was found that the SDN seems to be involved in aspects of male sexual behavior—mounting, intromission and ejaculation (20,21). However, the effects of lesions on sexual behavior are only slight, so it might well be that the major functions of the SDN are still unknown at present.

The SDN in the young adult human brain is twice as large in males (0.20 mm³) as in females (0.10 mm³) and contains twice as many cells (22). The SDN is located between the supraoptic and paraventricular nucleus, at the same rostro-caudal level as the suprachiasmatic nucleus. The SDN is identical to the “intermediate nucleus” described by Braak and Braak (23), and to the INAH-1 of Allen et al. (24). In the human brain sexual dimorphism is not present at birth. At that moment, cell numbers are similar in boys and girls and the SDN contains no more than some 20% of the cell number found around two to four years of age. From birth up to this age, cell numbers increase equally rapidly in both sexes (Fig. 5). A major question in this respect is where the cells come from that are formed after birth, as the matrix layer around the third ventricle has disappeared by about 25–28 weeks of gestation (25).

A sex difference does not occur until about the fourth year after birth, when cell numbers start to decrease in girls, whereas in males the cell numbers in the SDN remain stable until approximately 50 years of age, when they rapidly decrease. In females a second phase of marked cell loss sets in after the age of 70 (Fig. 5; 26,27). The sharp decrease in cell numbers in the SDN later in life might be related to the dramatic hormonal changes which accompany both male and female senescence (27), and to the decrease in male sexual activity (28). It is not clear whether the hormonal changes are cause or effect of the cell loss observed in this nucleus. Cell numbers in the SDN of Alzheimer’s disease patients were found to be within the normal range for age and sex (26).

A prominent theory is that sexual orientation develops as a result of an interaction between the developing brain and sex hormones (29,30). According to

Fig. 5. Developmental and sexual differentiation of the human sexually dimorphic nucleus (SDN) of the preoptic area of the hypothalamus, log-log scale. Note that at the moment of birth the SDN is equally small in boys ($\blacktriangle$) and girls ($\circ$) and contains about 20% of the cell number found at 2–4 years of age. Cell numbers reach a peak value around 2–4 years post-natally, after which a sexual differentiation occurs in the SDN due to a decrease in cell number in the SDN of women, whereas the cell number in men remains approximately unchanged up to the age of 50. In women, after a period of relative stability, cell number decreases again after the age of about 60, dropping to values which are only 10–15% of the cell number found at two years postnatally. Note that in men the reduction in cell number in senescence is less dramatic. The largest difference in cell number between men and women is found around 30 years and in people older than 80, whereas the sexual dimorphism in the SDN cell number is least around the age of 60. The SDN cell number in homosexual men ($\blacksquare$) does not differ from that in the male reference group. The curves are quintic polynomial functions fitted to the original data for males (full line) and females (dashed line). Adapted from Swaab and Hofman (26) with permission).

Dörner's hypothesis, male homosexuals have a female differentiation of the hypothalamus. This theory was not supported by our data on the SDN in homosexual men. Neither the SDN volume nor the cell number of homosexual men who died of AIDS differed from that of the male reference groups in the same age range, nor from that of heterosexuals also suffering from AIDS (10,26). The fact that no difference in SDN cell number was observed between homo- and heterosexual men who had died of AIDS refutes the general formulation of Dörner's hypothesis that male homosexuals have "a female hypothalamus".

Supraoptic (SON) and Paraventricular Nucleus (PVN)

The large neurosecretory cells of the hypothalamic supraoptic and paraventricular nucleus (SON and PVN) produce the neuropeptides vasopressin and oxytocin which are released into the blood circulation in the neurohypophysis. Vasopressin acts as an anti-diuretic hormone on the kidney and in women oxytocin is involved

in labor and lactation. Parvocellular vasopressin neurons of the PVN project into the brain (e.g. 31) and influence central processes. Oxytocin has central effects on, for example, food intake (see below), affiliation, and maternal and reproductive behaviors (32,33). In males it may be involved in sexual arousal and ejaculation (34). In addition to the neurohypophysial peptides, magnocellular oxytocin and vasopressin-containing cells of the SON and PVN co-express tyrosine hydroxylase, suggesting the possibility of dopamine production (35,36). Clusters of magnocellular neurosecretory neurons containing oxytocin or vasopressin are also found in the hypothalamic gray between these nuclei. These ectopic clusters, which tend to be arranged around blood vessels, are referred to as accessory nuclei (37).

The SON is subdivided into three parts. The largest part, the dorsolateral SON, has a volume of 3 mm³ (38) and contains 53,000 neurons, 90% of which contain vasopressin and 10% oxytocin (39). The dorsomedial and ventromedial SON together contain some 23,000 neurons. The entire SON thus contains 76,000 neurons (40).

Fig. 6. Vasopressin (light bars) and oxytocin (dark bars) cell numbers in the PVN of premature (26–37 weeks) and mature (37–42 weeks) fetuses and in adults. Adult numbers were already present around 26 weeks gestation (from Goudsmit et al. (103).

The PVN has a volume of 6 mm³ (38) and was estimated to consist of about 56,000 neurons (40), of which some 25,000 contain oxytocin and 21,000 express vasopressin (41–43).

Familial hypothalamic diabetes insipidus is transmitted as an autosomal dominant gene. Affected individuals have low or undetectable levels of circulating vasopressin and suffer from polydipsia and polyuria, but they respond to substitution therapy with exogenous vasopressin or analogues. Urine production may amount to as much as 20 liters per day. Members of a Dutch family suffering from this disease appeared to have a point mutation in one allele of the affected family members, based upon a G to T transversion within the neurophysin-encoding exon B (44). In a Japanese diabetes insipidus family a G to A transition has been described in the same exon (45). Some of the few postmortem histological observations in other families with hereditary hypothalamic diabetes insipidus point to severe neuronal death in the SON and PVN in the case of familial hypothalamic diabetes insipidus (46–48) suggesting that the mutated product might be toxic to the neurosecretory cell. Cell death later in development would also explain an interesting developmental feature, in that we observed that diabetes insipidus in an affected individual only started around nine years of age. More data from age-related studies and more postmortem observations are needed to establish this effect definitively.

The Fetal SON, PVN, and Birth

Fetal neurohypophyseal hormones play an active role in the birth process. Fetal oxytocin may initiate (49)

or accelerate (50,51) the course of labor. Fetal vasopressin levels in cord blood are much higher after normal delivery than in any other stage of life (52,53). Fetal vasopressin is one of the stress hormones that plays a role in the adaptation of the fetus to the stress of labor, for example by the redistribution of the fetal blood flow with a marked reduction in the flow to gastrointestinal and peripheral circulations and an increase in the flow to essential organs, such as brain, pituitary, heart and adrenals (54,55). These neuropeptides are already present early in fetal life. Vasopressin and oxytocin have been found as early as 11 and 14 weeks of gestational age respectively (56–62). Vasopressin-mRNA was found from 21 weeks of gestation onwards (63). An increase in vasopressin and oxytocin levels in pituitary and brain during the development of the fetus has been described (56–58,60). Because premature children are more prone to the stress of birth, we investigated the development of oxytocin and vasopressin neurons in the human fetal hypothalamo-neurohypophyseal system (HNS). For this purpose the oxytocin- and vasopressin-expressing neurons were stained immunocytochemically, and their number was determined morphometrically in the SON and PVN between 26 and 42 weeks of gestational age and compared to data obtained in adulthood by our group (Fig. 6; 41,43).

From the youngest fetus onwards of our study, that is from 26 weeks gestational age, adult vasopressin and oxytocin cell numbers were found in both SON and PVN (Fig. 6). This is in agreement with Dörner and Staudt's (25) estimation that the hypothalamic nuclei are already formed around 25 weeks of gestation.

That the fetal HNS is far from mature, even at term, is apparent, for example from the neuronal densities which are still decreasing. Rinne et al. (64) found a gradual increase in nuclear volume in the SON and PVN during fetal development, although he did not distinguish between oxytocin and vasopressin neurons in that study. As judged from the strongly increasing nuclear size of the oxytocin neurons during the last part of gestation, these neurons seem to become gradually strongly activated towards term. This should however be confirmed by other measures of activity, such as in-situ hybridization for oxytocin-mRNA. Nevertheless, it seems quite possible that less mature OXT neurons in premature children would be, at least partly, responsible for the increased incidence of obstetrical problems. This idea is reinforced by at least two observations. Firstly, human anencephalics do not have a neurohypophysis and have an impaired oxytocin and vasopressin release (65,66). In anencephalics expulsion takes twice as long and birth of the placenta even three times longer (50).

The second observation is derived from children suffering from Prader-Willi syndrome, who have considerable obstetrical problems (67). We recently found that they have only 58% of the normal number of oxytocin neurons, whereas the number of vasopressin neurons was normal (68).

Immaturity at term seems to affect to a lesser degree vasopressin neurons; these neurons are already further advanced in the developmental process as has also been reported for other species, and the AVP levels in fetal cord are extremely high after delivery (53). This adaptive response (55) has been said to be induced by stress (52), hypoxemia, acidemia (69,70) or by rises in intracranial pressure associated with delivery. Perinatal hypoxia stimulates the AVP neurons in particular, as became apparent from their co-expression of tyrosine-hydroxylase (71). The importance of neuroendocrine adaptive responses may be deduced from the fact that two-thirds of anencephalic children die during the course of labor (72). These children do not have a functional neurohypophysis (66).

It is clear from our studies that, if disturbances in the birth process are due to immaturity or disorders in fetal OXT or AVP neurons, these are caused by disturbances in their function rather than by differences in number, since this number has already reached the adult level around mid-gestation.

CRH and Birth

In contrast to the SON, the PVN not only contains magnocellular vasopressin and oxytocin neurons but also parvicellular ones that project to central brain regions or to the median eminence. Examples of the latter type of neurons are the corticotropin-releasing hormone (CRH) neurons that play a central role in stress response. In the human PVN they are not located in a well-defined subnucleus as they are in the rat, but are spread all over the PVN except for the most rostral part where they are absent. Another property of CRH neurons in the PVN is that they may co-express vasopressin when activated. This occurs in, for example the process of aging (73) and in Alzheimer's disease (74).

The idea that fetal CRH neurons might play an essential role in the initiation of labor is mainly based upon observations in sheep and cattle. Increased gestation length was found in Guernsey cattle that had a fetal pituitary aplasia (75) and in cyclopic sheep fetuses. The latter disorder was caused by the toxin *Veratrum Californicum*, and the pituitary was either absent or situated in an abnormal place (76). These observations were followed by classical experiments in sheep involving ad-

renalectomy, fetal hypophysectomy, and administration of ACTH or glucocorticoids. It became apparent from these experiments that the fetal hypothalamo-pituitary-adrenal system induced labor in sheep (77–80). In a recent crucial experiment, McDonald and Nathanielsz (81) showed that stereotactic bilateral destruction of the fetal sheep PVN prolonged gestation and thus fetal CRH may trigger off labor.

Our observations on 147 human anencephalics showed, however, that the human fetal brain does not so much determine the mean gestation time but rather rules the precise timing of the moment of birth. This normally takes place between 38 and 42 weeks of gestation, and anencephalics without hydramnios that are born spontaneously have a normal mean gestation length (39.7 weeks). It was remarkable however that about one-third of these anencephalics was born prematurely and one-third postmaturely. The normal mechanism that terminates gestation at around 40 weeks appeared to be completely lost (72). The finding that corticosteroid administration in the human fetus initiates labor in postmature pregnancies supports the possibility that in humans the hypothalamo-pituitary-adrenal axis is involved in the timing mechanism of birth. However, corticosteroid administration does not induce labor at term. Moreover, a tendency towards prolongation of pregnancy length was reported following betamethasone treatment of premature labor (for references see 82). These are arguments in favor of the notion that the CRH-pituitary-adrenal axis plays the part of "interval timer" in human labor (83). Fetal human hypothalamic CRH neurons should thus be studied in relation to pre- and postmature labor, the causes of which are largely unknown.

Colocalization of Tyrosine-Hydroxylase (TH) with OXT and AVP in Development

Previous immunohistochemical studies indicated that in the adult human PVN and SON a large proportion of neurons contains the catecholamine-synthesizing enzyme tyrosine hydroxylase (TH) (84,85). In the developing human PVN and SON TH-immunoreactive (IR) perikarya appear in the late gestational period. A large number of TH-IR neurons was found in full-term neonates, while only few were evident in the premature ones (36). Among full-term infants there is a large variation in the number and distribution of TH-IR perikarya, especially in the SON (36). Since a large number of TH-IR neurons were observed in full-term neonates who died of delivery-related asphyxia or hypoxia, the question was raised as to whether the presence of a larger

number of TH-IR perikarya in these cases represents a primary developmental phenomenon or a secondary phenomenon related to the activation of the vasopressinergic systems during the process of labor (36,55). The first step in studying this subject was to investigate whether the TH-IR neurons in the neurosecretory nuclei of the human neonate also synthesize vasopressin.

In the adult human PVN and SON Li et al. (84) estimated that the TH-IR neurons represent 38% and 41% respectively of the total neuronal population, and that the majority of these neurons is magnocellular. The same authors applied a double immunofluorescent method to show that only a subclass of the TH-IR perikarya in the adult PVN and SON also contains oxytocin, whereas no colocalization with vasopressin was observed. They showed however that in the SON the majority (77%) of the TH-IR perikarya is concentrated in the lateral subdivision of this nucleus where, as previously reported (37,39), more than 90% of the neurons contain VP. On the other hand they reported that the combined number of TH and vasopressin-IR neurons is higher than the number of Nissl-stained perikarya, and they therefore suggested that some cells may contain both TH and VP, but the double immunofluorescent method they applied was not sensitive enough to demonstrate that (84). We therefore investigated the colocalization of TH, not only with oxytocin but also with vasopressin, in the adult and neonatal PVN and SON. Adjacent paraffin sections were incubated simultaneously with two antibodies, a polyclonal against TH and a monoclonal against OXT or VP, and stained by a double peroxidase-antiperoxidase/alkaline phosphatase method.

We observed that TH-IR perikarya in the human PVN and SON were also positive for oxytocin or vasopressin. A clear difference between the neonates and adult cases of our sample was observed in the proportion of TH-IR neurons that colocalize oxytocin or vasopressin. In the neonates the majority of the TH-IR perikarya was also stained for vasopressin, while only few TH-IR neurons were also positive for oxytocin. The opposite was observed in the adults, where the majority of the double-stained TH-IR neurons colocalizes oxytocin while only few TH-IR perikarya appear to contain vasopressin. Our study establishes the colocalization of TH with either oxytocin or vasopressin in the adult and neonatal PVN and SON, and indicates that antemortem factors such as perinatal hypoxia might increase TH-immunoreactivity of the VP neurons in man (71).

Oxytocin, Food Intake, and Prader-Willi Syndrome

Animal experiments have shown that the parvocellular oxytocin neurons of the hypothalamic PVN are cru-

cial for the regulation of food intake. These oxytocin neurons project to brain stem nuclei, for example the nucleus of the solitary tract (NST) and the dorsal motor nucleus of the nervus vagus (DMN) (86–88). Small lesions in the PVN produce overeating and obesity (89), and stimulation of the medial parvocellular subdivision of the PVN elicits significant increases in gastric acid secretion (90), suggesting that the PVN usually has an inhibitory effect on eating and body weight. Central administration of oxytocin or oxytocin agonists inhibits food intake and gastric motility, whereas these effects are prevented by an OXT receptor antagonist (90–94).

We recently investigated whether a disorder of the PVN, more particularly its putative satiety neurons—the oxytocin neurons—might be the basis of the insatiable hunger and obesity in the most common type of human genetic obesity, the Prader-(Labhart)Willi syndrome (PWS) (95). Apart from gross obesity and problems during the process of birth (67), this syndrome is characterized by diminished fetal motor activity, severe infantile hypotonia, mental retardation, hypogonadism and hypogenitalism (96). The last two features were of particular interest for our study because oxytocin neurons are also thought to be crucial in various aspects of sexual behavior, including sexual arousal, orgasm, sexual satiety and other aspects of sociosexual interaction (32,34,97–99).

We conducted a morphometric study of the size of the PVN by conventional staining with thionin and immunocytochemical staining for oxytocin and vasopressin, of five PWS patients (two males and three females), varying in age between 22 and 64 years, and 26 controls (14 males and 12 females) without primary neurological or psychiatric diseases. The thionin-stained volume of the PVN was 28% smaller in PWS patients and the total cell number 38% lower. Following immunocytochemistry the immunoreactivities for oxytocin and vasopressin were decreased in PWS patients, although the variation within the groups was high. A large and highly significant decrease (42%) in the number of oxytocin-expressing neurons was found in all five PWS patients. The volume of the PVN containing the OXT-expressing neurons was decreased by 54% in PWS. The number of vasopressin-expressing neurons in the PVN was not altered significantly. The finding that the PVN was greatly decreased in volume and cell number in PWS indicates a developmental hypothalamic disorder. OXT neurons of the PVN are good candidates for a physiological role in ingestive behavior as “satiety neurons”, including in the human brain.

The Hypothalamus in Sudden Infant Death Syndrome (SIDS)

An interesting recent observation is that the density of LHRH-containing fibres in the PVN and periventricular nucleus was dramatically decreased in cases of sudden infant death syndrome (100). Another study (101) had shown that in SIDS in the hypothalamus tryptophan content is increased, serotonin content decreased and binding increased, monoamine oxidase-A activity was increased and choline-acetyl transferase activity was decreased.

Future research will have to determine whether these changes are a part of the cause of SIDS or a result of brain dysfunctions elsewhere leading to SIDS.

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