3 Relation between maturation of neurotransmitter systems in the human brain and psychosocial disorders

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INTRODUCTION

Developmental hypotheses and psychosocial disorders

An increasing number of psychosocial disorders have been associated with developmental risk factors. This includes: mental retardation and behavioral abnormalities stemming from hypoxic perinatal brain damage (Benveniste et al., 1984; Bosley et al., 1983), dyslexia (Kemper, 1984), disturbed language development, sleep disorders, schizophrenia, autism, sudden-infant death syndrome (SIDS), hyperkinetic syndrome, depression and anxiety (for references, see Swaab et al., 1988). Left handedness, gender identity and sexual preference are also thought to be determined during early development although they are expressed much later (Dörner, 1979, 1986; Gladue et al., 1984; Ellis et al., 1988). It is important to note that developmental sequelae are often multifactorial and that the same outcomes may have different origins. Sexual differentiation, for instance, may be influenced not only by hormones and other neuroactive compounds (Dörner, 1979; Swaab & Mirmiran, 1986; Rudeen et al., 1986; Zimmerberg & Reuter, 1989), but also by stress (Anderson et al., 1985; Ellis et al., 1988; Dörner, 1979, 1986). This makes the distinction between biological and environmental explanations of developmental disorders obsolete (e.g. Swaab & Hofman, 1988). It is also the case that similar situations or compounds may lead to different developmental sequelae. Maternal stress may lead to differences in sexual orientation of the offspring (Ellis et al., 1988), but also to neurological dysfunction, developmental delays and behavior disturbances (Stott, 1973; Meijer, 1985). Diethylstilbestrol (DES) administration to pregnant women may lead to an increased frequency of bisexuality or homosexuality in their daughters (Ehrhardt et al., 1988) and to more frequent depression and anxiety in sons and daughters (Vessey et al., 1983; Meyer-Bahlburg & Ehrhardt, 1987).
Psychosocial disorders, brain structures and transmitters

Some of the developmental disturbances mentioned above are postulated to be related to alterations in particular brain structures or certain transmitter systems during brain development. Thus, some sleep disturbances in children have been related to clonidine taken by the mother during pregnancy and affecting the noradrenergic system of the child (Huisjes et al., 1986). In SIDS a dramatic disappearance of luteinizing hormone releasing hormone (LHRH) fibres has been found in the mediobasal periventricular and paraventricular nuclei (Najimi et al., 1989). Smoking during pregnancy may affect the school performance of the child (Butler & Goldstein, 1973; Abel, 1980). In brains of schizophrenic patients, disorganized hippocampal pyramid cells (Conrad & Scheibel, 1987) and heterotopic displacement of nerve cells in the entorhinal cortex (Jakob & Beckmann, 1986) have been described, suggesting the presence of factors causing around mid-gestation a disturbance of neuronal migration. In addition, schizophrenia has been associated with alterations in the dopaminergic innervation of the prefrontal cortex and other limbic brain areas (Weinberger, 1988).

Autism is a developmental disorder that results in severe deficits in social, communicative and cognitive functioning. In his initial report, Kanner (1943) emphasized the apparent biological origin of this disorder as reflected by its onset during the first two years of postnatal life. Others have considered it to be a psychological disorder resulting from environmental factors (see Sanua, 1986). The study of Courchesne et al. (1988), however, showed that this condition involves abnormal neuroanatomical development of the cerebellum. The authors obtained magnetic resonance scans of the cerebella of 18 patients with autism and without other medical disorders. By using planimetry the neo-cerebellar vermal lobules VI and VII were found to be significantly smaller than those of controls. The small size of the cerebella appeared to be the result of developmental hypoplasia rather than shrinkage after full development since there was no sign of sulcal widening. It is not clear whether the cerebellar abnormality directly or indirectly impairs cognitive functions or occurs concomitantly with damage to other neural sites. Since autism has been observed in association with, for example, congenital rubella and with the preconceptional exposure of the parents to chemicals (Coleman, 1979), autism can at present be regarded as a developmental neurobiological disorder which is probably caused by a number of different factors (e.g. chemical, viral, genetic) before birth. The detection of these factors clearly demands a multidisciplinary approach in the following decades.

Hypoxic, hypoglycaemic and ischaemic encephalopathy leading to
cerebral palsy, mental retardation or epilepsy is presently thought to be mediated by an increased release and extracellular concentration of excitatory amino acids, such as glutamate (Benveniste et al., 1984; Bosley et al., 1983). One should note, however, that brain injuries related to birth are generally overdiagnosed. Many of them may, in fact, rather be due to prenatal brain damage. Prenatal brain damage often predisposes to difficult birth, which leads to the false impression that the birth itself is responsible for disorders such as mental retardation (Chaney et al., 1986). This can be understood since the fetal brain is playing an active role in the process of labour, e.g. by accelerating its course (Swaab et al., 1977).

Gilles de la Tourette’s syndrome is thought to be due to abnormal dopamine function as indicated by the therapeutic effect of haloperidol, to abnormal noradrenaline metabolism by the effect of clonidine, and to serotonin on the basis of reduced levels of cerebrospinal fluid (CSF) metabolites. In addition, alterations in encephalin, dynorphim and substance-P have been reported in this condition (Haber et al., 1986). The biochemistry of this disease is, however, far from clear (Messiha, 1988) and obviously needs systematic investigation of postmortem brain tissue of these patients by modern neurobiological techniques.

All these observations certainly need to be replicated and followed up further by studying in a systematic way other brain systems and structures in patients with these conditions. Although the data obtained to date suggest that certain neurotransmitter systems and brain structures are affected during human brain development, it is certainly not yet clear how particular psychosocial disorders may result from the reported alterations.

Syndrome specificity and the functional implications of the structural changes still have to be shown. However, for the time being, the reported structural changes, e.g. in schizophrenia and autism, can be considered as an important index for the period in which the disorder arose, even if the lesions do not at present explain the psychosocial disorders.

**Neurotransmitters**

Neurotransmitters (i.e. acetylcholine, amines, amino acids and peptides) play a key role in the investigation of the development of psychosocial disorders. Thus, firstly, they may constitute the substrate of such disorders (see above). Secondly, they may mediate risk factors and brain damage. Clonidine causes sleep disturbances, probably by acting on the noradrenergic system. Perinatal hypoxia causes brain damage by stimulating the release of excitatory amino acids. Thirdly, they may mediate therapeutic effects. As examples, the effect of haloperidol or clonidine
on Gilles de la Tourette's syndrome may be mentioned, as well as the attempts to prevent perinatal brain damage due to hypoxia by NMDA blockers.

For research in brain ontogeny and the development of brain disorders, neurotransmitters are, moreover, important as markers for the developmental stage of brain structures or systems, and as markers for the delineation of brain structures or systems that are otherwise not visible. As an example, one may mention the human suprachiasmatic nucleus that is not visible in conventionally stained sections, but that can clearly be delineated following staining of one of its main peptide neurotransmitters, i.e. vasopressin (Swaab et al., 1985).

**DEVELOPMENT OF TRANSMITTER SYSTEMS IN THE HUMAN BRAIN**

There are various major neurotransmitter systems such as cholinergic, aminergic, amino acid containing and peptidergic systems. From all four systems, examples can be given that show their early presence during development in the human fetal brain.

**Cholinergic systems**

Kostovic (1986), using acetylcholinesterase (AChE) activity as a histochemical marker for the developing human cholinergic system, observed activity in the nucleus basalis complex anlage as early as 9 weeks of gestation. Basal forebrain fibres are sent from there to the anlage of the neocortex and the limbic cortex by the end of the second trimester. Biochemical measurements (Brooksbank et al., 1978) revealed the presence of AChE in the earliest fetuses studied (i.e. 18 weeks of gestation) and an increase in AChE activity in the cerebral cortex with neonatal development to a level higher than that found in the adult. The cerebellum was much richer in AChE than the cerebral cortex and there was a modest developmental increase, the highest concentrations being found in the adult cerebellum. In addition, an increase was found in choline-acetyltransferase (CAT) activity from 18 weeks of gestation towards term, in both the cerebral cortex and the forebrain. The activity had not reached adult levels at birth (Brooksbank et al., 1978). Muscarinic binding sites were present in the brainstem and midbrain of the human fetus at 12 weeks of gestation (Schlumpf & Lichtensteiger, 1987). Muscarinic receptor development (i.e. quinuclidinyl benzilate (QNB) binding sites concentration) appears in the corpus striatum at 16 weeks of gestation (Ravikumar & Sastry, 1985) and reached adult levels at term. In contrast, cerebellar maximal CAT activity was attained at 26–42 weeks of gestation and the highest concentration of QNB
binding was reached at 18–22 weeks of gestation (Brooksbank et al., 1978). The sequence observed in the development of QNB and CAT, viz. postsynaptic receptor development preceding the neurotransmitter enzyme or synapse formation, is a phenomenon that has been reported in other systems (in the GABAergic system: see below, for other examples see Brooksbank et al., 1978). This phenomenon also implies that brain areas might be affected during development by exogenous neurotransmitter-like substances well before such a structure is innervated by the endogenous neurotransmitter.

Aminergic systems

The distribution of structures containing catecholamine (CA) and indolamine (IA) in human fetuses has been described by means of the Falck–Hillarp method from 7 weeks of gestation onwards (Nobin & Björklund, 1973; Olson et al., 1973). At 3–4 months of gestation, four CA- and three IA-containing cell groups were present and major axonal pathways were already visible. CA-containing varicose fibres occurred in the hypothalamus, basal ganglia, septum and olfactory regions. By means of tyrosine hydroxylase immunoreactivity, also the locus coeruleus and substantia nigra showed reactivity by 9–10 weeks of gestation (Pearson et al., 1980). Varicose IA fibres were observed only in the rostral pons. The cerebral and cerebellar cortices had no or only very few CA-containing fibres. The overall stage of development of the monoamine neuron systems in these 3–4 months old human fetuses seemed to be comparable with that of the rat during the first or second week after birth. The concentrations of dopamine noradrenaline and serotonin in the human fetal brain are rather low with the exception of a high hypothalamic dopamine level (Hyypä, 1972). The CA in the lower brain stem of the human infant has also been described by immunocytochemical techniques (Robert et al., 1984).

Amino acid containing systems

The development of the γ-aminobutyric acid (GABA) system has been studied biochemically in the human cerebral cortex and cerebellum by estimating glutamate decarboxylase (GAD) and binding for muscimol. GAD activity in the cerebral cortex at term was only 20% of the adult value that was reached after 60 weeks gestational age. The concentration of muscimol binding sites rose more rapidly than GAD activity. In the cerebellum, however, GAD-specific activity reached approximately 40% of its adult level at term and muscimol binding only 10%, and was still increasing at 60 weeks gestational age (Brooksbank et al., 1981). Benzodiazepine binding sites have already been observed in the
brainstem and midbrain of the human fetus at 12 weeks of gestation (Schlumpf & Lichtensteiger, 1987), whereas glutamate, NMDA and quisqualate binding sites were found in the hippocampus at mid-gestation (Represa et al., 1989). The concentration of taurine in the human fetal brain is higher than in the adult brain, and decreases with the course of gestation between 7 and 25 cm crown/rump length (Sturman & Gaull, 1975).

**Peptidergic systems**

Neuropeptides are also present in different brain structures during early human development. Fetal hypothalamic and cortical tissue contained LHRH at 4½–6 weeks of pregnancy (Aksel & Tyrey, 1977; Siler-Khodr & Khodr, 1978; Winters et al., 1974) and LHRH-producing cells were stained at 9–10 weeks of gestation (Bugnon et al., 1977a, Paulin et al., 1977). Thyroid releasing hormone (TRH) was also assayed in the fetal brain as early as 4–5 weeks of pregnancy (Winters et al., 1974) and in the fetal hypothalamus and cortex from 8 weeks of gestation (Aubert, 1979). The presence of TRH in the cerebellum of an anencephalic child (Winters et al., 1974) suggests extrahypothalamic production sites for this material. Somatostatin has been determined in the fetal hypothalamus and in the fetal cerebral cortex at mid-gestation (Paulin et al., 1976; Bugnon et al., 1977b), while α-endorphin staining appeared in the infundibular region from the 11th week of development onwards (Bloch et al., 1978). Substance-P is present at 8 weeks of gestation in the spinal cord and hypothalamus (Paulin et al., 1986). Already in 1953, Dicker & Tyler showed, by means of bio-assays, the occurrence of vasopressin and oxytocin in the human fetal neurohypophysis from 70 days of gestation onwards. Others (Burford & Robinson, 1982; Paulin et al., 1978; Fellman et al., 1979; Visser & Swaab, 1979; Schubert et al., 1981; Khan-Dawood & Dawood, 1984) showed the presence of the same peptides from 11–14 weeks of gestation in the pituitary and hypothalamus, and their exponential increase in the neurohypophysis towards birth. Neurophysins were found from 12–13 weeks onwards. In addition, we observed extrahypothalamic fibres containing neurohypophysial hormones in human fetuses from 17 weeks of gestation (Swaab & Ter Borg, 1981).

**THE RELATIONSHIP BETWEEN NEUROTRANSMITTER DEVELOPMENT AND DISTURBED BRAIN DEVELOPMENT**

Animal experiments have shown that perturbations of the development of each of the major neurotransmitter classes, i.e. acetylcholine, amines,
amino acids or peptides, may result in subtle alterations in brain and behavior. This led to the development of a relatively new field of research, 'behavior' or 'functional teratology' (for review, see Swaab et al., 1988). The developing brain is, in this respect, certainly not only affected by neurotransmitter-like substances. In fact, all those chemical compounds that are capable of influencing the adult brain may also affect brain development (Swaab, 1980). This has been established now, e.g. for sex hormones, corticosteroids, thyroid hormones, neurotransmitters, stimulating compounds (alcohol, nicotine, caffeine, marihuana), anaesthetics and metals.

Since neurotransmitter systems may be the substrate of psychosocial disorders, and may mediate effects of factors influencing brain development and of therapeutic effects, information on their pattern of development may be useful. Excitatory amino acids, for instance, are thought to be involved in hypoxic/ischaemic encephalopathy (Benveniste et al., 1984; Bosley et al., 1983). It will be useful, therefore, to obtain more information on their regional developmental pattern in the human brain. The currently available information on neurotransmitter development in the human brain is, however, only of limited value in this connection, due to a number of factors:

(1) Neuronal systems themselves may already be affected during development before neurotransmitters are expressed, i.e. by factors influencing cell acquisition, cell death, migration, the formation of neurites and synapses or receptor setting. These processes might be disturbed by either alcohol (Swaab et al., 1988) or benzodiazepines (Laegreid et al., 1989).

(2) There has been considerable emphasis on researching when a neurotransmitter can be detected for the first time. It is questionable, however, whether such a line of investigation is very meaningful. Firstly, neurotransmitters are found earlier in development as techniques become more sensitive. Neurotransmitters can even be detected in fertilized egg cells and in the initial phases of neural tube formation (Buznikov et al., 1972; Ignarro & Shideman, 1968). Secondly, such data do not provide the necessary information for estimating when in development a particular system is sensitive to a particular (disturbing) developmental factor, since brain areas may express receptors well before they are innervated by neurotransmitter systems (see above). Consequently, the period during development that a particular neurotransmitter system appears in a certain structure might differ considerably in time with the sensitive phase of that brain area for neurotransmitter-like substances. Important information may, therefore, come from receptor
studies. The transient increase in density of NMDA binding sites in the fetal human hippocampus around 23–40 weeks of gestation (Represa et al., 1989) may well indicate a sensitive period for excitatory amino acids and thus for hypoxia. Also the concentration of muscarinic receptors in fetuses is higher than in adults (Ravikumar & Sastry, 1985). One should, in addition, note that the tissue content of neurotransmitters during development does not hold any information on their release or turnover.

(3) Overall biochemical data on neurotransmitter systems do not reveal information on the marked regional differences in rate of development. The hypothalamic magnocellular vasopressin neurons of the supraoptic and paraventricular nucleus (SON and PVN) have been visualized already at 11 weeks of gestation (Fellman et al., 1979) whereas the majority of the neurons of the nearby suprachiasmatic nucleus (SCN) express vasopressin only after birth (Swaab et al., 1990). ‘Molecular anatomical’ techniques, such as immunocytochemistry and in situ hybridization, are thus preferable.

(4) There are various problems that counteract studies relating developmental sequelae to the causative factors. In the first place, the interval between the time when such factors act on the developing brain and the occurrence of symptoms might be long, extending over years up to decades. In addition, the symptoms might not be specific to the causal factors, or the factors might contribute to multicausal disease entities (Swaab et al., 1988). Longitudinal studies are difficult but essential in the field of functional teratology.

(5) One additional major point of concern for all the data on human brain development is whether or not the measurements are indeed representative for normal development, and not influenced by the disease from which the subject died, the administered medicines, the agonal state, or postmortem interval (Swaab & Uylings, 1988). Stable markers of the neurotransmitter systems that show only limited change during a reasonable postmortem interval should be chosen preferentially to follow the development, whereas the clinical state of the subject and the general neuropathology should be extensively documented by a well-organized brain bank (Swaab et al., 1989). In this way, valuable correlations may be established between a change in transmitter development and psychosocial disorders. The causality of such correlations, however, has to be established in animal experiments. Such experimental observations, selecting the right stage of development, are often not available at present.
(6) A final point concerns the way neuropathologists generally screen the brain. Studying brain structures by using one or even a few sections per area can give only an impression of cell density, which is a very poor way of studying cell numbers. Even a factor of 2 more or fewer neurons per structure may pass unnoticed (Swaab & Uylings, 1987). Major changes in the human brain might, therefore, have been missed so far.

ESTIMATION OF THE PERIOD THAT DEVELOPMENTAL SEQUELAE MIGHT HAVE TAKEN PLACE

Analogous to the developmental pattern in animals in which the sensitive period has been established experimentally, it might be possible to estimate the period in which developmental sequelae could take place in the human brain. This point has already been mentioned in relation to the lesions found in schizophrenia and autism, and may also be illustrated by our work on the human hypothalamus.

In rats, sexual differentiation of the sexually dimorphic nucleus (SDN) of the preoptic area in the hypothalamus can be affected around the first postnatal week. This is the period in which the rat SDN becomes sexually dimorphic (Gorski, 1984). In the human brain, sexual differentiation of the SDN was found to take place only after 2–4 years postnatally (Figure 3.1). By analogy with the raw data this may be the period in which the SDN is sensitive to factors, either chemical or psychological in nature such as stress, (Swaab & Hofman, 1988) which influence sexual differentiation. Whether such alterations really take place in the human brain has to be studied in subjects who were exposed to factors which possibly could influence sexual differentiation of the human brain in different prenatal and postnatal periods, e.g. Klinefelter, Turner, Prader–Willi and adrenogenital syndromes.

Another example of possible timing of the period of an alteration in development can be given for the suprachiasmatic nucleus, the clock of the hypothalamus. In adulthood, the SCN has some 7000 vasopressin neurons whereas the total cell number of this nucleus is about 46,000. Cell numbers are stable until about 80 years of age (Swaab et al., 1985). Much to our surprise, in two male-to-female transsexuals of 44 and 50 years of age, vasopressin cells and total cell numbers were doubled. This was probably not due to steroid hormone treatment in adulthood, since a patient displaying similar high SCN cell numbers was a 30-year-old woman with Prader–Willi syndrome. This syndrome is characterized by a congenital lack of LHRH and thus a lack of sex hormones (Swaab et al., 1987). Recently, similar high cell numbers have been observed in the SCN of 14 male homosexuals who died of acquired immune deficiency
Figure 3.1. Development 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 (▲) and girls (○) and contains only about 22% of the cell number found at 2–4 years of age. The SDN cell number of a female neonate with a pituitary aplasia (A) is fully within the range of other neonates. Cell numbers reach a peak value around 2–4 years postnatally, 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 cell number decreases for the second time after the age of about 60, after a period of relative stability, dropping to values which are only 10–15% of the cell number found at 2 years postnatally. Note that in men the reduction in cell number in senescence is less dramatic. The largest discrepancy 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 at least around the age of 60. The SDN cell number in homosexual men (■) does not differ from that in the male reference group. The cell number of the SDN of two male-to-female transsexuals (T) is within the female range, whereas the SDN of a woman with a Prader–Willi syndrome (P) is small. The curves for quintic polynomial functions fitted to the original data for males (drawn line) and females (dashed line), with $F_{1}[5, 49] = 10.05$, $P < 0.001$ and $F_{1}[5, 39] = 7.32$, $P < 0.001$, respectively (from Swaab & Hofman, 1988, with permission).
syndrome (AIDS) (Swaab & Hofman, 1990). The similar large SCN in homosexuals, transsexuals and Prader–Willi syndrome might be related to an alteration in the interaction of sex hormones and brain development. Since it is hard to explain an increased neuron number arising in adulthood, we have studied the developmental pattern of vasopressin cell formation in the SCN (Figure 3.2). It seems that vasopressin expression in the human SCN neurons starts mainly in the neonatal period. Around the first year postnatally, cell numbers are high, i.e. more than twice the number of vasopressin and total cells in the adult (Swaab et al., 1990). These values are comparable to those found in the subject with Prader–Willi syndrome, transsexuals and male homosexuals. After the first postnatal years, cell numbers decrease gradually towards levels seen in adults (Figure 3.2). This developmental study indicates that in these three conditions a factor is preventing the programmed cell death of SCN neurons around the first year of age. Whether or not this factor may be a change in sex hormones should be investigated experimentally. The question whether a large SCN and homosexuality are not directly related but due to a common factor

Figure 3.2. Development of the human suprachiasmatic nucleus (SCN) of the hypothalamus. Log-log scale. The period at term (38–42 weeks of gestation) is indicated by the vertical bar. Note that total cell number is low at the moment of birth (21% 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 1–1½ years postnatally are more than twice the adult cell numbers. After these high levels a decrease to adult total cell number is found. (From Swaab et al., 1990, with permission.)
during brain development or alternatively the SCN is indeed directly involved in partner preference, should also be studied in animal experiments.

Consequently, life-span studies might be useful to pinpoint a particular period in development in which a certain factor might have been active. The ultimate proof that such a relationship is a causal one has to come, however, from animal experiments. This means that studies on human material obtained by a well-organized brain bank on subjects with psychosocial disorders of a possible developmental origin, and on animal experiments should go hand in hand in order to reveal the putative relationship mentioned in the title.

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