Fetal and Neonatal Neurology and Neurosurgery

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21. Functional teratogenic effects on the developing brain

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Probably because of the thalidomide tragedy, our awareness of the dangers of drug ingestion has cautioned us against the indiscriminate use of medicines mainly during the initial stage of pregnancy. However, even medicines that do not cause any gross physical malformations may cause microscopic defects or alter the intricate structure or chemical composition of fetal brain tissue, also during the second part of pregnancy, to such an extent that permanent behavioural deviations later develop. The latter field, which is known as 'functional or behavioural teratology' is the subject of this chapter. In the Netherlands some 80% of pregnant women take medicines, whereas in a prospective study in the UK 35% of pregnant women took drugs (Rubin et al 1986). Most of these drugs are of the type that easily cross the placenta. They readily reach the fetal brain, since the blood-brain barrier at this stage of development is not capable of preventing their passage. The same holds for addictive compounds taken by the mother, e.g heroin, morphine, methadone, alcohol, marihuana and cigarette smoking.

There are several reasons why such effects of medicines were not recognized until recently, the most important one being that chemical compounds do not generally give rise to a syndrome in the child that can easily be recognized by the clinician as being specific to a particular compound taken by the mother during pregnancy. 'Functional teratology' is rather expressed much later in life by cognitive disturbances, mental retardation, reproductive or motor defects, disturbed language development or sleep disturbances. This, and the long time-interval between the use of medicines and the functional disturbances often makes it difficult to establish the relationship with intra-uterine sequela of chemicals.

CHEMICALS AFFECTING BRAIN DEVELOPMENT

Many different chemicals might affect the developing brain. In fact, all those chemical compounds which are of importance for adult brain function appear to be involved in brain development as well (Swaab 1980). At present this is established for sex hormones, corticosteroids, thyroid hormones, and neurotransmitters. Substances which alter the balance of any of these neuro-active compounds during the vulnerable periods in ontogeny are therefore capable of altering brain development in a permanent way.

Sex hormones

In the rat sex hormones act during the perinatal period by affecting maturation of the brain, both structurally and functionally, inducing in this way a sexual differentiation of the brain. For example, a light microscopically evident sexual dimorphism occurs in the size of a part of the nucleus preopticus medialis (the sexual dimorphic nucleus of the preoptic area, SDN-POA), which is determined in the rat by the levels of testosterone present around the time of birth (Gorski et al 1978, Jacobson et al 1980). The SDN-POA has recently been described by us in the human brain. The volume of this nucleus is 2.5 times as large in men as it is in women and contains 2.2 times as many cells (Swaab & Fliers 1985). Sex hormones coming from the fetus probably constitute in normal development, the biological basis for sex-related brain and behavioural differences in animals as well as in humans. It is therefore a matter of considerable concern that progestagens, oestrogens and/or combinations thereof have frequently been prescribed to pregnant women (Reinish & Karow 1977), in the mistaken belief that they would prevent impending miscarriages. In the USA 1—4.5 million pregnant women used diethylstilboestrol (DES) from 1945 until 1971. It was taken off the market due only to a probable carcinogenic effect on the cervix and vagina in female offspring (Herbst et al 1981). However, not only are these drugs ineffective in sustaining pregnancy but their use entails a real possibility of inducing personality disorders in the offspring. Oestrogen-exposed children have been found between 4 and 21 years of age to be generally less self-confident, less sensitive, and more dependent and group oriented than normal children (Reinischi & Karow 1977). In addition, a high percentage (25%) of infertility and possible interference with sexual function
Function of the developing brain and its development

1. Functional teratogenic effects on the developing brain

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The developing brain is exposed to numerous teratogenic factors, which can lead to functional abnormalities. These factors can act at different stages of development, leading to various consequences on brain function. The understanding of these effects is crucial for the development of strategies to prevent or mitigate their impact.

In the past, most studies focused on the identification and quantification of these factors, but recent research has shifted towards understanding the mechanisms underlying their effects. This has led to a better understanding of the developmental processes involved and the potential for intervention.

The article discusses the current knowledge on the functional teratogenic effects on the developing brain and highlights the need for further research to improve our understanding of these processes.
was found following intra-uterine exposure to oestrogens (Beral & Colwell 1981, Stenchever et al 1981). Prenatal administration of oestrogen and progesterone in boys has been reported to influence noticeably certain aspects of postnatal psychosexual development (i.e. ‘masculinity’, ‘aggressiveness’, and athletic abilities) (Yalom et al 1973). Recent studies show that DES daughters have an increased incidence of bisexuality and homosexuality (Ehrhardt et al 1985). It is worth mentioning that sexual differentiation of the brain is not only affected by sex hormones. Similar developmental effects affecting sexual differentiation have been described for serotonin, noradrenaline (see later) and dopamine-related drugs (Hull et al 1984), nicotine (Lichtensteiger & Schlumpf 1985), alcohol (McGivern et al 1984), cimetidine (Anand & Van Thiel 1982), morphine (Vathy et al 1983), barbiturates (Reinisch & Sanders 1982), and maternal stress (Dörner 1979). When pregnant rats are exposed to stress, the SDN-POA of the male offspring becomes permanently smaller, i.e. of female size (Anderson et al 1985). Consequently, all types of neuro-active compounds might affect sexual differentiation of the brain. This is another characteristic of the functional teratology of chemical compounds that makes this field hard to study; different compounds, when given during development, may lead to similar aspects of functional sequelae in later life.

**Corticosteroids**

Corticosteroids are used during pregnancy, for example in cases of allergic reactions, and to promote lung development in the child in cases of imminent parturition. However, in quite a number of patients this treatment did not seem to have the expected effect (Gariete et al 1981). In addition, animal experiments indicate that exposure to corticosteroids can retard brain development and affect behaviour in later life (Balazs et al 1975, Taesch 1975, Sobel 1978, Dahlol et al 1980, Johnson et al 1981, Marton & Szondy 1982) found a retardation of psychomotor development, which persisted at least up to 2 years of life in prematurely born children who had been exposed to corticosteroids. A good prospective study is badly needed in this area.

**Thyroid hormones**

Thyroid hormones have been injected directly into the amniotic fluid to enhance fetal lung maturation (Mashiah et al 1978). No follow-up investigation of these children has been carried out to our knowledge, although it is known from animal experiments that such treatment may hamper brain development (Balazs 1979).

**Neurotransmitters**

Recent research indicates also that neurotransmitters which can be subdivided into the following groups: acetylcholine, biogenic amines, amino acids and peptides are essential for normal brain development.

**Acetylcholine**

Pyridostigmine (an acetylcholinesterase inhibitor), when administered to neonatal rats, induces premature puberty and increased male sexuality in both sexes of the offspring (Hinz et al 1978). Nicotine (an acetylcholine receptor agonist) enhances cell death in the rat fetal brainstem (Kraus et al 1981) while neonatal administration of chlorisodamine (a nicotine-receptor blocker) prevented the normal postnatal increase in volume and cell number of the mouse superior cervical ganglion (Black & Geen 1974). This might be one of the mechanisms by which smoking of the pregnant mother may have a permanent effect on brain development and school performance of the child (Butler & Goldstein 1973, Abel 1980).

**Biogenic amines**

Apart from the serotonin-reuptake blocker (chlorimipramine) and the alpha-adrenergic agonist (clonidine), see later, there are many examples of medicines which, if used during pregnancy, impair normal brain development by upsetting the balance of the monoamines and/or influencing the sensitivity of the receptors. In animal experiments, reserpine (used as an antihypertensive drug as well as a tranquilizer) decreases the monoamine levels in the brain and has been shown to induce permanent brain and behavioural changes in the offspring. These include reduced formation of neurones, hyperactivity, and increased susceptibility to audiogenic seizures. Amphetamine, which increases the release of catecholamines in the brain, is used as a dieting aid but is also given to children in cases of enuresis nocturna or minimal brain dysfunction. Offspring of pregnant rats treated with such drugs show behavioural changes, most notably an inability in adulthood to adapt to new surroundings. Alpha-methyldopa (a false transmitter precursor for noradrenaline) and propranolol (a beta-adrenergic blocker), when taken by the pregnant mother, result in a reduced head circumference in the human neonate. The use of neurotoxics such as chlorpromazine (a dopamine antagonist) during pregnancy has been reported to lead to extrapyramidal disturbances in the newborn child, while in animal experiments it impaired learning ability (for references on this section see Swaab & Mirmiran 1984). 3-Chlorophenylalanine, which blocks serotonin synthesis, affects cell division in regions of the posterior diencephalon known to become innervated by serotonergic fibres (Lauder et al 1983, Lauder & Krebs 1984a,b). Barbiturates, which also stimulate dopamine receptors (Yanai & Feigenbaum 1981), are commonly used as hypnotics, sedatives, anticonvulsants and for preventing neonatal jaundice. They may induce a withdrawal syndrome.
Structural and functional changes in the developing brain, and interactions among the brain and the environment, provide a foundation for acquisition of knowledge and development of cognitive skills. These processes are influenced by a variety of factors, including genetic predispositions, environmental exposures, and social interactions. In the prenatal period, the brain undergoes rapid development, with significant changes in cell proliferation, migration, and synaptogenesis. During the postnatal period, the brain continues to develop, with myelination and synaptic pruning occurring in response to environmental stimuli. These processes are facilitated by the release of neurotransmitters and growth factors, which regulate neuronal growth and connectivity. The developing brain is particularly vulnerable to environmental toxins, stress, and infections, which can lead to neurodevelopmental disorders if exposure occurs during critical periods of development.
lasting as long as 3 months (Thornburg & Moore 1976). In animal studies, barbiturates have been shown to impair reproductive function and maze-learning ability of the offspring (Middaugh et al. 1975, Clemens et al. 1979, Gupta et al. 1980). Beta-mimetics, such as ritodrine, that are used frequently to prevent premature delivery, may lead to less good school performance of the children later on in life (Hadders-Algra et al. 1986).

**Amino acids**

Many compounds influencing amino acid transmitters are used during pregnancy and postnatal development. Prenatal or early postnatal treatment of rats with the often used tranquilizer diazepam (which acts upon gamma-aminobutyric acid (GABA)-benzodiazepine receptor complex) produces long-lasting effects on brain enzymes and behavioural disturbances such as hyperactivity, lack of acoustic startle reflexes, and sleep disturbances (Fonseca et al. 1976, Jakoubek 1978, Kellog et al. 1980, Livezey et al. 1985). It also reduces choline uptake in the male rat frontal cortex (Grimm 1984), induces a permanent decrease in noradrenaline level, turnover and release in the hypothalamus (Simmons et al. 1984, Kellogg & Retell 1986), and a permanent decline in diazepam binding sites (Livezey 1985). Diphénylhydantoin, administered to the pregnant rat, caused increased levels of GABA in adulthood (Vorhees 1985). Diazepam administration during pregnancy in humans results in low Apgar scores, depressed respiration and impaired suckling (Patrick et al. 1972, Cree et al. 1973). Long-term follow-ups of such children are lacking. Dairy cow milk formulae might be too low in taurine to secure optimal brain development (Gaul 1985).

**Peptides**

Little is known about the possible long-term effects on brain development of this recently discovered group of neurotransmitters. They were originally thought to be simply hormones produced by the hypothalamus but later appeared to have important central effects as well (for review, see Swaab 1982, Boer & Swaab 1985).

Oxytocin is routinely used in obstetrics and may cause fetal distress, including a rise in core temperature and possibly retarded motor and speech development. Observations in the rat revealed a permanent decrease in water metabolism following administration of oxytocin to the developing rat (for references see Boer & Swaab 1983). Vasotocin administered to kittens induced delayed eye-opening and brain lipid content while locomotion was diminished and periods of active sleep were enhanced (Goldstein 1984). Vasopressin, which may permanently alter osmoregulation following perinatal administration (Boer & Swaab 1983, Boer et al. 1984), and its analogues have been given to mentally retarded children (Waggoner et al. 1978, Anderson et al. 1979, Eisenberg et al. 1984a,b), and for the treatment of enuresis nocturna (Stegner et al. 1986). DGAVP has been given to children following brain trauma (Wit et al. 1986). Such children should consequently be followed up for possible long-term sequelae due to the peptide treatment in development. One of the problems of such a long-term follow-up is, however, that not enough experimental studies have been done yet to tell the clinician which functions might be disturbed due to the administration of neuropeptides.

Postnatal treatment with thyrotropin-releasing hormone (TRH) increased rat hypothalamic weight and impaired T-maze learning (Stratton et al. 1976). Corticotropin-releasing factor (CRF) accelerated eye-opening, enhanced rearing in an open-field and impaired body temperature regulation. Substance P increased pain perception and induced upregulation of its receptors (Handelmann et al. 1984), while neonatal exposure to a high level of ACTH 4-10 impairs adult learning behaviour (McGivern et al. 1986).

Opioids and compounds influencing this system have strong effects on brain development. Methadone exposure of developing rats caused, for example, a delay in reflex development, eye-opening, somatic and brain growth, a regional alteration of catecholamines, hyperactivity, increased emotionality, learning disabilities and dysfunction of thermoregulation and nociception. In children whose mothers had been exposed to opioids, abstinence symptoms were found, a high rate of mortality, sleep disturbances, delays in the sensorimotor development, retardation in somatic growth, smaller head circumference, delays in walking, problems in visual and auditory systems, aberrations in neuro-ontogeny, less alert, poor attention spans, hyperactivity, learning disabilities and social problems (Zagon & McLaughlin 1984).

Naloxone, an opiate antagonist, is administered clinically to normalize fetal heart rate (Goodlin 1981). Animal experiments have implicated naloxone as the cause of a permanent impairment of sensitivity to thermal stimuli (Sandman et al. 1979) and of maze-learning ability (Vorhees 1981). Beta-endorphin, used during delivery as an analgesic (Oyama et al. 1980), induces similar disturbances in the rat (Sandman et al. 1979). This treatment causes a reduced beta-endorphin immunocytochemical staining in various brain regions (Moldow et al. 1981).

**MECHANISMS OF ACTION OF CHEMICALS ON THE DEVELOPING BRAIN**

Drugs taken by the pregnant mother may impair the developing child's brain in different ways:

1. This action may be *indirect*, as in the case of aspirin which, when taken by the pregnant mother, may result in a higher incidence of intracranial bleeding and perinatal mortality (Collins 1981, Rumack et al. 1981). Another action of this kind is the alcohol-induced impairment of
umbilical circulation producing hypoxia and acidosis in the fetus (Mukherjee & Hodgen 1982). Prenatal exposure to barbiturates might also influence brain development indirectly by altering liver metabolism of sex hormones (Reinsch & Sanders 1982).

2. Drugs may affect brain development by interacting directly with the formation of the neuronal and glial network, e.g. by affecting cell division, cell death, cell migration, or the formation of neurites, synapses and receptors. Most, if not all, medicines appear to affect Cell division is reported to be slowed down by a number of medicines, both in vivo and in vitro. Barbiturates were found to cause a 30% reduction in the number of cerebellar Purkinje cells and a 15% reduction in hippocampal pyramidal cells. Other compounds which have similar deleterious effects include corticosteroids, chlorpromazine, alcohol, reserpine, thyroid hormone and sex hormones (for references see Swaab & Mirmiran 1984). Indirect evidence for decreased brain cell division is provided by the smaller head circumferences which have been found at birth following treatment with sex hormones (Huisjes H J, personal communication); with alpha-methylldopa or propranolol, and diphenylhidantoin, or by the use of alcohol during human pregnancy (for references see Swaab & Mirmiran 1984).

Cell death is augmented by nicotine (Kraus et al 1981), accelerated by alcohol exposure prior to birth in the rat (Yanai 1981), and delayed by morphine in the chick embryo (Merney et al 1985).

Cell migration may be disturbed by alcohol (Jones et al 1976), anticonvulsants (Trice & Ambler 1983) and monosodium glutamate (Marani et al 1982).

The formation of neurites and synapses is known to be affected by sex hormones, corticosteroids, morphine, methadone, anticonvulsive agents and by alcohol (for references see Swaab & Mirmiran 1984). Receptors may also be permanently altered by neuroactive compounds given during development. Haloperidol, which blocks dopamine receptors, induced in this way a permanent decrease in the number of dopamine receptors in the striatum (Rosengarten & Friedhoff 1979). L-Dopa, which increases dopamine synthesis, permanently increased receptor density (Friedhoff et al 1977). Prenatal morphine exposure in the rat increases the adult number and affinity of spinal cord opiate receptors (Kirby 1984), while prenatal exposure to diazepam results in enduring reductions in diazepam binding sites in the rat thalamus (Livezey et al 1985).

3. The third mechanism involves effects of medicines on spontaneous behavioural states, namely wakefulness, quiet sleep and rapid eye movement (REM) sleep. In a study at our institute in which the long-term effect of REM sleep (active sleep, AS) deprivation on brain and behaviour development was studied, experimental suppression of AS during early postnatal life by means of clomipramine or clonidine in rats revealed a clear-cut reduction of cortical size, a higher level of open field activity, deficient masculine sexual behaviour, and disturbed sleep patterns in adulthood (Mirmiran et al 1981, 1983a, Swaab & Mirmiran 1984). These results, and those of others using different pharmacological as well as non-pharmacological approaches, argue in favour of AS as a mediating factor for normal brain maturation (Mitler 1971, Juvenes & Nowaczky 1975, Sauzier & Astick 1975), in which, of course, several mechanisms as discussed before may be involved.

The specific reduction of cortical weight, together with decreased protein content, in the absence of any significant change in cell number, was highly reminiscent of the picture seen in rats reared under sensorily impoverished conditions (Rozenzweig & Bennett 1978). Furthermore, concomitant AS deprivation by means of clomipramine neutralizes the effect that environmental enrichment normally exerts upon cortical growth (Mirmiran & Uylings 1983). Another intriguing finding is that prolonged AS deprivation by means of clomipramine even prior to the period of enrichment rearing interferes with the expected extra brain growth (Mirmiran et al 1983b). Apparently, cortical mechanisms underlying ‘plasticity’ in later life can be adversely affected by the absence of AS and/or noradrenaline disturbances in early development. Such a phenomenon may implicate abnormal sleep patterns as a potential contributory factor to learning deficiencies in humans as well.

The drugs used in the AS-deprivation studies, namely clomipramine (Anafranil) and clonidine (Catapresan) are also used in clinical practice (for treating depression, hypertension, migraine, nocturnal enuresis, sleep apnoea, opiate withdrawal, minimal brain dysfunction, etc.). A recent follow-up study examined the effects of prenatal clonidine treatment of hypertensive mothers on the development of children who are now 6–8 years of age. In the exposed group compared with non-treated hypertensives an excess of sleep disturbances was found (Huisjes et al 1986), indicating that animal experiments in the field of functional teratology might give useful clues on the functions that have to be examined in children by means of long-term follow-up studies.

CLINICAL AWARENESS AND ANIMAL EXPERIMENTS REQUIRED

It is both surprising and a source of concern that practically no follow-up studies appear to have been carried out on the possible long-lasting functional consequences of treatments during human pregnancy. A wide variety of chemical compounds having comparable effects upon monoamine systems and/or AS, as described above, are currently in clinical use (cf. Swaab & Mirmiran 1984) and many consequently cause functional deficits. Effects of chemicals administered during development might even be carried over to following generations (Friedler 1974),
possibly by affecting autodulation of genes (Campbell & Zimmerman 1982).

It is important to point out that almost all drugs used during gestation easily cross the placenta, and their level in the fetus (especially in the brain) may even be higher than in the maternal circulation (Mirkin & Singh 1976). In addition, humans are often more sensitive than animals to teratogenicity of drugs (Council on Environmental Quality 1981). One report does demonstrate a prolonged disturbance of sleep in babies born from heroin-addicted mothers (Davis & Glass 1980). Similar sleep disturbances might be responsible for the smaller head circumferences in boys, up to 4 years of age, born to mothers treated with alpha-methylldopa during late gestation (Moar et al 1978, Ounsted et al 1980). A problem is that long-term follow-up without a strong indication of what behaviour or function has to be studied in later life will most probably fail to find disturbances. The sleep disturbances found in children where the mother used clonidine during pregnancy (Huisjes et al 1986) indicate that animal experimental studies might allow a selection of the right functions to study in human follow-up studies. This means that systematic search for functional teratological effects of chemicals should be encouraged.

The direct and indirect effects of a variety of clinically used drugs upon the development of the brain have been discussed here. Taken together, the literature on this subject points to a potential health hazard not only during the first trimester of pregnancy but also throughout the entire period of gestation, and during lactation. The possibility that similar mechanisms are still present in later development cannot be excluded at present. Obstetricians, neonatologists, and paediatricians should, therefore, be aware that the immediate beneficial effects of many drugs may be offset by the induction of permanent behavioural and psychological defects within the children's developing brains. This is a relevant consideration, e.g. in cases involving children suffering from minimal brain dysfunction who are often subjected to extremely high doses of imipramine or amphetamine-like drugs (for review, see Gross & Wilson 1974) even though improvement often occurs eventually even in the absence of any medication whatsoever. The same point can be made, of course, for the treatment of nocturnal enuresis by means of antidepressants. It is an unfortunate commentary at the present time that the mothers themselves are often more aware of the potential dangers inherent in the use of medicines during pregnancy than are the physicians who prescribe them. Since recent work indicates the functional developmental effects of anaesthetics (Chalon et al 1981, Blair et al 1984, Koëter & Rodier 1986, Rodier & Koëter 1986, Rodier et al 1986), it should be a point of concern not only for operations on pregnant mothers but also for pregnant staff in operating and recovery rooms. We suggest that the investigation of the link between experimental and clinical medicine in this area, namely the question of functional teratological sequelae of medications administered during early development, ought to be encouraged (Swaab 1985, Swaab & Mirmiran 1985).

For those diseases that have to be treated during pregnancy it is of utmost importance to select, in the future, only those compounds which combine high therapeutic potencies with low functional teratological side effects.

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REFERENCES


Boer G J, Swaab D F 1985 Neuropeptide effects on brain development to be expected from behavioral teratology. Peptides 6 (suppl 2): 21–28


The development of in vivo protein expression systems has provided a powerful tool for understanding the function of proteins. These systems allow for the expression of recombinant proteins in living organisms, which can then be studied in a physiological context. The use of these systems has been particularly useful in the study of proteins that are difficult to express in vitro or that require specific post-translational modifications. In vivo expression systems have been used to study the localization, stability, and function of proteins, as well as their interactions with other molecules. These studies have provided important insights into the biology of proteins and have had a significant impact on the field of molecular biology.
Handelmann G E, Selsky J H, Helke C J 1984 Substance P administration to neonatal rats increases adult sensitivity to substance P. Physiology and Behavior 33: 297–300
Koeter H B W M, Rodier P M 1986 Behavioral effects in mice exposed to nitrous oxide or halothane: prenatal vs postnatal exposure. Neurobehavioral Toxicology and Teratology 8: 189–194
Middaugh L D, Santos C A, Zemp J W 1975 Effects of phenobarbital given to pregnant mice on behavior of mature offspring. Developmental Psychology 8: 305–313
early and late effects of neonatal cortisone on physical growth and skeletal maturation. Pediatric Research 12: 945-947.


Trice J E, Ambler M 1985 Multiple cerebral defects in an infant exposed in utero to anticonvulsants. Archives of Pathology and Laboratory Medicine 109: 521-523.


Vorhees C V 1985 Fetal anticonvulsant syndrome in rats: effects on postnatal behavior and brain amino acid content. Neurobehavioral Toxicology and Teratology 7: 471-482.


Yanai J 1981 Comparison of early barbiturate and ethanol effects on CNS. Substance and Alcohol Actions Misuse 2: 79-91.
