CHAPTER 1

Concept of functional neuroteratology and the importance of neurochemistry

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Interest in congenital malformations may be traced back to the Assyrians, Babylonians and Egyptians. In ancient Greek mythology, too, cyclops and other types of malformations are mentioned. However, a causal link with chemicals during development was not made in those days, as far as we know. Until a century ago, factors other than chemicals were generally thought to be the causes of congenital defects, e.g., excess or paucity of semen, undersized uterus, abdominal trauma during pregnancy or the influence of devils and demons (Paré, 1537, see Health Council report, 1985). Only alcohol was considered as a potential danger. Consumption of alcohol on wedding days was prohibited even in ancient Carthage, out of fear that an abnormal child might be begotten (cf. Jones and Smith, 1975).

It was only in the last century that Etienne Geoffroy de Saint-Hilaire was able to induce experimentally anencephaly and spina bifida and coined the term teratology (cf. Schmid, 1987). The concept that more subtle behavioural or functional defects might be due to chemicals during pregnancy was put forward for the first time by Werboff and Gottlieb (1963), who introduced the term behavioural teratology in scientific literature. Neurochemical alterations in relation to functional teratology were reported soon after these observations (e.g., Eiduson, 1966; Tonge, 1972; Huttunen, 1971).

Functional neuroteratology is, consequently,

quite a new concept in *neurosciences*. Yet, the *idea* that emotions of the mother might affect the fetus is certainly much older as is evident from old wives' tales. The birth of the Siamese twins Judith and Helena, who were exhibited in The Netherlands during fairs from 1701 to 1723 was thought to be due to the copulating dogs their mother had watched during pregnancy ('verzien', Naaktgebooren, 1985).

For the acceptance of the importance of the concept of behavioural teratology catastrophes, e.g., in Minamata Bay and Iraq (Weiss, 1988) and the description of the fetal alcohol syndrome have been important. Lemoine et al. (1968) were the first to report disturbed brain development in children following alcohol use by the pregnant mother. Such an effect had, in fact, been suggested as early as in the beginning of the eighteenth century (cf. Warner et Rosett, 1975) and in a more recent past by Huxley, when he described in '*Brave New World*' how 'Gammas' were created by dosing the eggs almost to death with alcohol during the 'Bokanovsky Process' ("They say somebody made a mistake when he was still in the bottle – thought he was a Gamma and put alcohol into his blood surrogate. That is why he is so stunted." (Huxley, 1932)). Lemoine's findings were published in French and had, therefore, to be rediscovered and published in English by Jones et al. (1973) before it became known as 'the fetal alcohol syndrome', which is in serious

cases characterized by microcephaly and mental retardation.

Alcohol now appears to be only one example of the many compounds that may cause functional teratology. Such effects may also be due to, e.g., opiates, marihuana, caffeine and nicotine (for references see Butler and Goldstein, 1973; Vorhees, 1986a; Tanaka et al., 1987; Swaab and Mirmiran, 1988), to medicines (e.g., the kinds of medicine that 80% of pregnant Dutch women seem to use; Eskes et al., 1983), to radiation from atomic bomb explosions (Miller and Blott, 1972) or X-ray examinations (Granroth, 1979), to food additives (Olney, 1988; Weiss, 1988), to heavy metals (Annau, 1988) or other environmental chemicals.

Functional neuroteratology, the iceberg under the classical teratological tip?

Some chemicals may cause only classic teratology (e.g., limb malformations), while others cause only functional teratological defects but no gross CNS malformations (Hutchings, 1983; Leonard, 1981). However, increasing numbers of chemicals appear to have the capacity of causing both functional neuroteratological and classical teratological birth defects. On the one hand, various well-established functional teratogens, such as alcohol, are also found to increase the frequency of gross morphological birth defects (Vorhees, 1986a). In general, the causal relationship between chemicals and classical teratological defects is, however, often hard to prove because of the extremely high number of exposed individuals that is needed in order to result in statistically significant numbers of affected children. On the other hand, compounds known as classical teratogens also seem capable of inducing functional defects.

It is still not well established whether or not preconceptional or early gestational exposure to sex hormones may induce teratogenic defects, but dysmorphic features have been reported in the offspring of mothers using such compounds

(Lorber et al., 1979). The enormous attention the diethylstilboestrol (DES) catastrophe has received was mainly based upon the small chance (1:10000) that DES daughters would develop cervical or vaginal carcinomas (Herbst et al., 1981). However, functional defects seem to be more common. About 25% of the DES daughters and sons might develop functional, reproductive defects (Bercel and Colwell, 1981; Stenchever et al., 1981). The reported 5- to 7-times increased chance that DES daughters become bi- or homosexual (Ehrhardt et al., 1985) are of a similar order to the reproductive defects, and in any case much more frequent than the chance of gross morphological birth defects.

There is a growing concern that valproate may cause spina bifida in about 1% of exposed pregnancies (Robert and Guibaud, 1982; Bjerkedal et al., 1982). This compound is also teratogenic in rat and mouse (Trotz et al., 1987). However, valproic acid may be a functional teratogen even at lower doses that do not induce such gross malformations (Chapman and Cutler, 1984; Vorhees, 1987).

Corticosteroids, which may be teratogenic in high doses (Baxter and Fraser, 1950; Warrel and Taylor, 1968; Schardein, 1985) may inhibit psychomotor development (Marton and Szondy, 1982) or affect behaviour when given during pregnancy at much lower dose levels (cf. De Kloet et al., 1988).

In conclusion, some compounds are capable of causing both gross morphological CNS birth defects and functional teratological defects. Such compounds might seem to induce functional defects at a higher frequency and lower dose level than the classical malformations. Consequently, for such compounds functional teratology seems a more sensitive way to find their effects than the study of classical teratological defects.

What compounds might cause functional neuroteratology?

An increasing number of chemicals is reported to affect the developing brain. In fact, all those

chemical compounds which are capable of influencing adult brain function may also affect brain development (Swaab, 1980). At the present time this principle has been established, e.g., for sex hormones, corticosteroids and thyroid hormones, for neurotransmitters, i.e., acetylcholine, biogenic amines, amino acids and peptides, for stimulating compounds (alcohol, nicotine, caffeine, marijuana), anaesthetics and metals. Exogenous substances which alter the balance of any of the endogenous neuroactive compounds seem to act on the formation of the materials of the brain, e.g., cell number, migration, connections and receptors. They are therefore capable of altering brain development in a permanent way (for review see Swaab and Mirmiran, 1988). When peripherally administered compounds are 'neuro'-active in adulthood, they are evidently capable of easily crossing biological barriers such as the placenta and will readily reach the fetal brain. Their level in the fetal brain might even be higher than in the maternal circulation (Mirkin and Singh, 1976) and might persist in the fetus for a much longer time than in the maternal tissues (Madsen et al., 1981; Simmons et al., 1983). Compounds which are not neuroactive in adulthood because of poor blood-brain barrier (BBB) penetration may be neuroactive in development as the fetal and neonatal BBB are known to be open for many more chemicals than the adult one (Werboff and Gottlieb, 1963).

By what mechanisms do these compounds act?

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

Firstly, this action may be *indirect*, i.e., not by a direct action on brain cells. This is e.g., the case with aspirin which, when taken by the pregnant mother, may result in a higher incidence of intracranial bleeding (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 and Hodgen, 1982). Prenatal expo-

sure of barbiturates might also influence brain development indirectly by altering liver metabolism of sex hormones (Reinisch and Sanders, 1982).

Secondly, 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 or the synthesis of transmission-related molecules such as receptors. Most, if not all, medicines in fact appear to affect several of these processes.

Cell acquisition 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, e.g., corticosteroids, chlorpromazine, alcohol, reserpine, thyroid hormone and sex hormones (for references see Swaab and Mirmiran, 1984; Patel and Lewis, 1988; Vaccari, 1988). Indirect evidence for decreased brain cell division is provided by the smaller head circumferences which have been found at birth following maternal treatment with sex hormones (Huisjes, personal communication), alpha-methyldopa, propranolol or diphenylhydantoin, or by the use of alcohol during human pregnancy (for references see Swaab and 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 (Meriney et al., 1985).

Cell migration may be disturbed by alcohol (Jones et al., 1976), anticonvulsants (Trice and Ambler, 1985) and monosodium glutamate (Marani et al., 1982).

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 and Mirmiran, 1984).

Receptors may also be permanently altered by neuroactive compounds given during development. Just to mention a few examples, haloperidol, which blocks dopamine receptors, induced in this way a persisting decrease in the number of dopamine receptors in the striatum (Rosengarten and Friedhoff, 1979), whereas L-Dopa, which increases dopamine synthesis, enhanced receptor density (see Miller and Friedhoff, 1988). Prenatal morphine exposure in the rat increases the adult number and affinity of spinal cord opiate receptors (Kirby, 1984), and prenatal exposure to diazepam results in enduring reductions of its binding sites in the rat thalamus (Livezey et al., 1985).

It might be good to point out that only recently has it become clear that paternal factors may be of importance as well (Soyka and Joffe, 1980). The mechanism of action of such factors might be similar to maternal factors, as is true, e.g., in case of passive smoking. On the other hand, not-yet-revealed mechanisms of action on the gametes (Campbell and Perkins, 1988) might also exist, e.g., in the case of developmental defects in the offspring in case of paternal alcohol abuse, or in the adverse effects on the offspring of male rats pretreated with opiates, lead, ethanol, caffeine, thalidomide, anaesthetics and cigarettes before mating (Soyka and Joffe, 1980).

The biochemical basis of functional neuroteratology

All effects of neuroactive compounds on the developing brain will ultimately be based upon biochemical mechanisms, whether the effect involves cell death, cell migration, cell division, cellular outgrowths or subcellular mechanisms. With the emergence of functional neuroteratology as a scientific discipline, its biochemical basis has attained attention. It is striking in this respect that the early reviewers on this field (Barlow and Sullivan, 1975; Coyle et al., 1976) already treated the subtle morphological, biochemical and behavioural disturbances as parts of one complex, in

which morphological and biochemical changes underlie the behavioural changes. In addition, it was suggested that biochemical measurements might be more sensitive than behavioural screening procedures. However, the discovery of underlying chemical mechanisms has only recently obtained momentum. In this respect one may wonder whether the term 'behavioural teratology' (Werboff and Gottlieb, 1963) is still useful. The term 'functional teratology' may be more appropriate.

Knowledge of the anatomy and chemistry of neurotransmission has increased enormously in the last decades and has made clear that many neuroactive compounds interact selectively with particular neurotransmitter systems in the brain. It is tempting to suggest that these compounds produce long-term or lasting effects on the developing brain by disturbing the neurochemical processes that they are known to affect in adulthood. Studies following this approach in development have reported both similarities and differences in the mechanisms of action as compared with the adult brain. This is of interest not only because of the relation to the mechanism by which neuroteratological effects are produced, but also since it gives information relevant to the developmental biology of the nervous system and to the study of chronic treatments with neuroactive drugs in adulthood (e.g., Miller and Friedhoff, 1988; Kellogg, 1988; Del Rio et al., 1988).

As stated by Hutchings (1980, 1983), the trade-off between human and animal neurobehavioural studies cross-validates both. Meaningful neurochemical data are difficult to obtain in the human situation, making a neurochemical cross-validation at present impossible. In the future, neurochemical measurements may certainly contribute to our knowledge of neonatal neuropathology, in particular with the emergence of non-invasive techniques. However, in our opinion the importance of neurochemistry to functional neuroteratology lies not in the detection of abnormalities in the human condition, but rather in the elucidation of the mechanism by

which neuroactive compounds have produced these abnormalities. It is generally accepted that Wilson's principles of teratology (Wilson, 1977) apply to functional neuroteratology (Vorhees, 1986b) and that it is not only the developmental phase which defines the outcome of the teratological action of a chemical, but also the access, dose, chemical nature and, above all, the mechanism of action of that compound (Coyle et al., 1976; Leonard, 1981; Hutchings, 1983). Only knowledge of the mechanism of action will enable us to predict from animal studies the teratological potential of chemicals and this will be of great importance not only for the development of safer medicines, but also for unravelling the chemical steps that are essential for optimal brain development.

In which developmental period do chemicals affect the developing brain?

A central principle in teratology is that the various stages of development provide critical periods in which the developing organism is more vulnerable than in other stages. 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, although later in gestation medicines do not cause any gross physical malformations, they may cause permanent behavioural or other functional deviations. Literature suggests that at present there is not only a potential health hazard of chemicals during the second half of gestation, but during lactation as well (e.g., PCPs; Schardein, 1985). This is also an important item in relation to the increasing amounts of chemicals that are prescribed during lactation.

It seems logical that permanent alterations may be induced as long as brain areas are still developing. Recent observations of ours on the sexual differentiation of the human hypothalamus suggest that the sensitive period for functional teratology might even extend into the first postnatal

years. Observations in rat have indicated that the sexually dimorphic nucleus (SDN) of the hypothalamus is sensitive to sex hormones during the period that the sexual dimorphism of this area is just arising, i.e., during the first postnatal week (Gorski, 1984). It has been presumed that in humans this vulnerable phase of sexual differentiation would take place during mid-gestation. The SDN has been localized in the human hypothalamus as well, and in adulthood contains twice as many cells in man as in woman (Swaab and Fliers, 1985). However, in a recent developmental study (Swaab and Hofman, in prep.), the SDN appeared not to become sexually dimorphic during mid-gestation, but only after several years postnatally. This suggests that, at least for a few postnatal years, this process might be affected by chemicals.

A postnatal vulnerable period is also a relevant consideration for compounds other than sex hormones, e.g., in children suffering from minimal brain dysfunction, who are often subjected to extremely high doses of imipramine- or amphetamine-like drugs (for review see Gross and Wilson, 1974), despite the fact that improvement often occurs even in the absence of any medication whatsoever. The same concerns may be expressed with respect to the treatment of nocturnal enuresis by means of antidepressants (Huygen, 1979), about steroids used in order to inhibit growth in extremely tall boys and girls (Van der Werff ten Bosch and Bot, 1981; Van der Werff ten Bosch et al., 1986), about clonidine treatment of children for short stature (Pintor et al., 1987) and about oxytocin used to induce lactation (Boer and Kruisbrink, 1984).

When might symptoms of functional teratology occur?

One of the main problems of recognizing symptoms of functional teratology is the long time interval between the moment that chemicals are acting upon the developing brain and the occurrence of symptoms. Often, the first occasion that altered

functions may become apparent is at school-going age. This has been reported, e.g., for smoking by the pregnant mother, which affects the school performance of the child (Butler and Goldstein, 1973; Abel, 1980). It goes without saying that, for this reason, it is often hard to prove a relationship between early exposure to chemicals and functional defects. Effects might even occur very late in life. In a study on the effect of clonidine on the development of rat brain and behaviour, this compound appeared to affect masculine sexual behaviour in adulthood (Mirmiran et al., 1983). Many other chemicals, when given during development might affect reproduction (for references see Swaab and Mirmiran, 1988). If such reproductive effects would also hold for human subjects, the time interval between the use of chemicals and the appearance of symptoms might be as long as several decades in humans. The observation that the effects of prenatal stress or chemicals administered during development might even be carried over to following generations (Friedler, 1974; Pollard, 1986; Campbell and Perkins, 1988) has added a new dimension to this long time interval.

What symptoms belong to functional neuroteratology?

Another set of clinical problems of functional neuroteratology is 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. In fact the symptoms, e.g., cognitive disturbances, mental retardation, reproductive or motoric defects, disturbed language development or sleep disturbances, may have so many different causes that one usually does not even make the connection between symptoms and chemicals at all. For instance, sexual differentiation of the brain may be affected not only by sex hormones, but also by serotonin, noradrenaline, dopamine-related drugs, nicotine, alcohol, cimetidine, morphine barbiturates, and maternal stress (for references

see Swaab and Mirmiran, 1988). In addition, one compound, such as alcohol, may cause many different symptoms, e.g., prematurity (Berkowitz et al., 1982; Kaminski et al., 1981), cognitive disturbances and mental retardation (Spaans and Verspreet, 1981), language disturbances (Gusella and Fried, 1984) and disturbances of sexual differentiation (McGivern et al., 1984), possibly depending on the stage of development in which it was acting on the fetal brain (cf. Rodier, 1988).

A third problem in functional teratology is that clinicians do not find defects caused by chemicals during development, because they do not know what symptoms exactly they should look for. The sleep disturbances in 6- to 8-year-old children whose mothers had been treated with clonidine (Huisjes et al., 1986) were only found because Mirmiran et al. (1983) had described sleep defects before in rat, after early postnatal treatment with the same compound. In this respect animal experiments might be very valuable in focussing the clinician's attention to a particular set of symptoms to look for in relation to functional teratology.

A fourth problem is that functional teratology might contribute to more-or-less circumscribed neurologic or psychiatric disease entities that at present are generally considered to be multi-causal. One of such diseases is *schizophrenia*. Jakob and Beckmann (1986) described histological alterations in limbic regions, e.g., heterotopic displacement of nerve cells in entorhinal cortex, suggesting a disturbance of neuronal migration during mid-gestation in which environmental factors might play a role. The *autistic* brain might also have been affected during development (Bauman and Kemper, 1984; Sanua, 1986). Pregnancy and birth complications have repeatedly been mentioned as possible causes of infantile autism. However, unexpected differences in preconceptional histories of the families of autistic children have been reported as well. An increased incidence of exposure to chemicals of the parents of such children was noted in a retrospective study (Coleman, 1979). In a follow-up study of 40

babies who had died of *sudden infant death syndrome* (SIDS) Einspieler and Kenner (1985) found that 78% of the mothers concerned had labour induced by oxytocin. Others have related SIDS to the use of cigarettes or barbiturates by the mother (cf. Naeye, 1980). It has been suggested that the *hyperactive child syndrome* is an effect of maternal alcohol use during pregnancy (Warner and Rosett, 1975). *Depression* and *anxiety* have been found to be more frequent in the sons and daughters of women who had been treated with DES during pregnancy than in controls (Vessey et al., 1983; Meyer-Bahlburg and Ehrhardt, 1987).

Of course, most possible relationships between functional teratological effects of chemicals and certain disease entities are at present highly speculative. However, the idea certainly seems worthy of a follow-up. It should be noted that there might also occur unexpected seemingly positive functional teratological effects. Maternal alcohol ingestion was associated with a decreased risk of respiratory distress syndrome (Ioffe and Chernick, 1987), and psychological development in children born to mothers treated with bromocriptine appeared to be more precocious (Raymond et al., 1985). However, the presence of yet undetected disadvantageous effects in such children should certainly be considered.

May animal experiments predict functional teratology in man?

Functional neuroteratological effects are, for various reasons, often hard to prove in humans (see before). Confirmation of effects in experimental animals may, therefore, be a crucial step in the establishment of such effects. On the other hand, the question whether animal experiments might provide good models for predicting functional teratological effects, e.g., of new chemicals, is of course of great importance too.

The few experimental animal and human investigations that have studied related phenomena owing to chemicals in development, indicate that

animal experiments may indeed give useful predictions in this field. Clonidine administered during the first postnatal weeks in rat caused, among other defects, sleep disturbances in adulthood (Mirmiran et al., 1983). A recent follow-up study examined the effects of prenatal clonidine treatment of hypertensive mothers on the development of their children, who were then 6–8 years of age. Compared to non-treated hypertensives a seven-times-as-high excess of sleep disturbances was observed in the exposed group (Huisjes et al., 1986).

In the mistaken belief that it would prevent miscarriages, 1–4.5 million pregnant American women used DES. The observation that DES daughters have an increased incidence of bisexuality and homosexuality (Ehrhardt et al., 1985) could have been suspected from the masculinizing effect of DES on brain differentiation (Döhler et al., 1986). The dysmorphic facial changes of the fetal alcohol syndrome can be mimicked in animal experiments (Leonard, 1988). The same is true for the smaller head circumference due to alpha-methyl dopa (Swaab and Mirmiran, 1984).

These few examples illustrate that animal experiments in the field of functional teratology might give useful clues concerning the defects that clinicians have to look for in children exposed to chemicals in development by means of long-term follow-up studies. Of course, species differences in sensitivity to the chemicals should be taken into consideration. However, in contrast to what is generally believed, humans are often more sensitive than animals to the teratogenicity of drugs (Council on Environmental Quality, 1981).

Summary and conclusions

Functional neuroteratology – i.e., the existence of behavioural or other functional defects owing to the effect of chemicals during brain development – is a quite new development in the neurosciences with important fundamental and clinical consequences. The classical gross morphological

birth defects owing to the effects of chemicals during the first months of pregnancy appear to form only the tip of the teratological iceberg. Functional teratological defects may generally be induced at lower dose levels and higher frequencies than the classical birth malformations. All neuroactive compounds may affect brain development, e.g., sex hormones, corticosteroids, thyroid hormones, neurotransmitters, addictive and stimulating compounds, anaesthetics and organometals.

Chemicals might affect the developing nervous system indirectly (e.g., by affecting the umbilical cord circulation) or directly by affecting cell division, cell death, cell migration, the formation of neurites, synapses or the synthesis of transmission-related molecules. A paternal factor may be of importance too. Functional teratological effects are generally due to chemicals taken after the first trimester of gestation. The possibility, though, that such mechanisms are still present during lactation, and even later in development, seems to exist.

The use of medicines during pregnancy and lactation and prior to conception should therefore be strictly limited to therapeutic necessity. For such cases a list of relatively safe medicines should be made, and the children exposed to such chemicals should be followed up carefully for a long-term period, using indications on the type of possible defects derived from animal experiments.

Clinical recognition of functional teratological defects may be hampered by the long time interval (up to decades) between the administration of chemicals and the occurrence of the symptoms. In addition, symptoms are not specific to certain chemicals and may include cognitive disturbances, mental retardation, reproductive or motoric defects, disturbed language development or sleep disturbances, while one compound may cause different symptoms. Moreover, one process, such as sexual differentiation, might be affected by many compounds.

In addition, functional teratology may contribute to multicausal disease entities, e.g. schizo-

phrenia, autism, SIDS, hyperactive child syndrome, depression or anxiety. An additional problem is that clinicians generally do not know what symptoms of functional teratology they have to look for. In this respect, animal experiments may provide both a selection of the right functions to be studied in human follow-ups as well as a suggestion of the underlying biochemical mechanisms. This means that systematic searching for functional teratological effects and mechanisms of chemicals should be encouraged. On the other hand, as we have learned from the thalidomide tragedy, species differences in sensitivity to chemicals might exist. This means that safety of a compound can never be proved by animal experiments. Consequently, information must be obtained from children exposed to chemicals in prospective trials as well. This concerns not only clinical follow-up studies on possible developmental defects. Also, systematic neuropathological investigation of the exposed newborn might contribute considerably to our knowledge. Last but not least, functional teratology may give very valuable clues concerning the chemical processes that are crucial in normal brain development. This aspect is one of the ultimate purposes of the present volume.

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References

- Abel, E. (1980) Smoking during pregnancy: a review of effects on growth and development of offspring. *Hum. Biol.*, 52: 593-625.
- Annau, Z. (1988) Organometals and brain development. Ch. 19 this volume.
- Barlow, S.M. and Sullivan, F.M. (1975) Behavioural teratology. In C.L. Berry and D.E. Poswillo (Eds.), *Teratology. Trends and Applications*. Springer, Berlin, pp. 103-120.
- Bauman, M.L. and Kemper, T.L. (1984) The brain in infantile autism: a histoanatomic case report. *Neurology*, 34: 275.

- Baxter, H. and Fraser, F. C. (1950) Production of congenital defects in offspring of female mice treated with cortisone. *McGill Med. J.*, 19: 245–249.
- Bercl, V. and Colwell, L. (1981) Randomized trial of high doses of stilboestrol and ethisterone therapy in pregnancy: long-term follow-up of the children. *J. Epidem. Community Hlth*, 35: 155–160.
- Berkowitz, G. S., Holford, T. R. and Berkowitz, R. L. (1982) Effects of cigarette smoking, alcohol, coffee and tea consumption on preterm delivery. *Early Hum. Dev.*, 7: 239–250.
- Bjerkedal, T., Czeizel, A., Goujard, J., Kallen, B., Mastroiacova, P., Nevin, N., Oakley, G. Jr. and Robert, E. (1982) Valproic acid and spina bifida. *Lancet*, November 13: 1096.
- Boer G. J. and Kruisbrink, J. (1984) Effects of continuous administration of oxytocin by an Accurel device on parturition in the rat and on development and diuresis in the offspring. *J. Endocrinol.*, 101: 121–129.
- Butler, N. R. and Goldstein, H. (1973) Smoking in pregnancy and subsequent child development. *Br. Med. J.*, 4: 573–575.
- Campbell, J. H. and Perkins, P. (1988) Transgenerational effects of drug and hormonal treatments in mammals: A review of observations and ideas. Ch. 33, this volume.
- Chapman, J. B. and Cutler, M. G. (1984) Sodium valproate: effects on social behaviour and physical development in the mouse. *Psychopharmacology* (Berlin), 83: 390–396.
- Coleman, M. (1979) Studies of the autistic syndromes. In R. Katzman (Ed.) *Congenital and Acquired Cognitive Disorders*. Raven Press, New York, pp. 265–275.
- Collins, E. (1981) Maternal and fetal effects of acetaminophen and salicylates in pregnancy. *Obstet. Gynecol.*, N.Y., 58 (Suppl 578): 57S–62S.
- Council on environmental quality (1981) *Chemical hazards to human reproduction*. Prepared by Clement Association Inc. for the Council on Environmental Quality, Government Printing Office, Washington.
- Coyle, I., Wayner, M. J. and Singer, G. (1976) Behavioral teratogenesis: A critical evaluation. *Pharmacol. Biochem. Behav.*, 4: 191–200.
- De Kloet E. R., Rosenfeld, P., Van Eekelen, J. A. M., Sutanto, W. and Levine, S. (1988) Stress, glucocorticoids and development. Ch. 8, this volume.
- Del Rio, J., Montero, D. and De Ceballos, M. L. (1988) Long-lasting changes after perinatal exposure to antidepressants. Ch. 12, this volume.
- Döhler, K. D., Coquelin, A., Davis, F., Hines, M., Shryne, J. E. and Gorski, R. A. (1986) Aromatization of testicular androgens in physiological concentrations does not defeminize sexual brain functions. *Monogr. Neural Sci.*, 12: 28–35.
- Ehrhardt, A. A., Meyer-Bahlburg, H. F. L., Rosen, L. R., Feldman, J. F., Veridiano, N. P., Zimmerman, I. and McEwen, B. S. (1985) Sexual orientation after prenatal exposure to exogenous estrogen. *Arch. Sexual Behav.*, 14: 57–75.
- Eiduson, S. (1966) 5-Hydroxytryptamine in the developing chick brain: its normal and altered development and possible control by end product repression. *J. Neurochem.*, 13: 993–932.
- Einspieler, C., Kenner, T. (1985) A possible relation between oxytocin for induction of labor and sudden infant death syndrome. *New Engl. J. Med.*, 313: 1660.
- Eskes, T. K. A. B., Nijdam, W., Buys, M. J. R. M. and Van Rossum, J. M. (1983) Drug therapy during pregnancy. Effects on mother, fetus and newborn. In: T. K. A. B. Eskes and M. Finster (Eds.) *Drug Therapy During Pregnancy*. Butterworths, London, Boston, Wellington etc., pp. 1–8.
- Friedler, G. (1974) Long-term effects of opiates. In: J. Dancis and J. C. Hwang (Eds.), *Perinatal Pharmacology: Problems and Priorities*, Raven Press, New York, pp. 207–219.
- Granroth, G. (1979) Defects of the central nervous system in Finland. IV. Association with diagnostic x-ray examinations. *Am. J. Obstet. Gynecol.*, 133: 191–194.
- Gorski, R. A. (1984) Critical role for the medial preoptic area in the sexual differentiation of the brain. In: G. J. De Vries, J. P. C. De Bruin, H. B. M. Uylings and M. A. Corner (Eds.), *Sex Differences in the Brain. Prog. Brain Res.*, Vol. 61, Elsevier, Amsterdam, pp. 129–146.
- Gross, M. B. and Wilson, W. C. (1974) *Minimal Brain Dysfunction*. Brunner/Mazel, New York.
- Gusella, J. L. and Fried, P. A. (1984) Effects of maternal social drinking and smoking on offspring at 13 months. *Neurobehav. Toxicol. Teratol.*, 6: 13–17.
- Health Council of the Netherlands (1985) *Report on the Evaluation of the Teratogenicity of Chemical Substances*. No. 1985/6E, the Hague, The Netherlands.
- Herbst, A. L., Hubby, M. M., Azizi, F. and Makii, M. M. (1981) Reproductive and gynecologic surgical experience in diethylstilbestrol-exposed daughters. *Am. J. Obstet. Gynecol.*, 141: 1019–1028.
- Huisjes, H. J., Hadders-Algra, M. and Touwen, B. C. L. (1986) Is clonidine a behavioural teratogen in the human? *Early Hum. Dev.*, 13: 1–6.
- Hutchings, D. E. (1980) Neurobehavioral effects of prenatal origin: Drugs of use and abuse. In R. H. Schwartz and S. J. Yaffe (Eds.) *Drugs and Chemical Risks to the Fetus and Newborn. Prog. Clin. Biol. Res.*, Vol. 36, Alan R. Liss Inc., New York, pp. 109–114.
- Hutchings, D. E. (1983) Behavioral teratology: a new frontier in neurobehavioral research. In: E. M. Johnston and D. M. Kochlar (Eds.), *Handbook of Experimental Pharmacology*, Vol. 65, Springer Verlag, Berlin, pp. 207–235.
- Huttunen, M. O. (1971) Persistent alteration of turnover of brain noradrenaline in the offspring of rats subjected to stress during pregnancy. *Nature*, 230: 53–55.
- Huygen, F. J. A. (1979) De behandeling van enuresis nocturna. *Ned. Tijdschr. Geneesk.*, 123: 748–752.

- Huxley, A. (1932) *Brave New World*. Grafton Books, London, Great Britain.
- Ioffe, S. and Chernick, V. (1987) Maternal alcohol ingestion and the incidence of respiratory distress syndrome. *Am. J. Obstet. Gynecol.*, 156: 1231–1235.
- Jakob, H. and Beckmann, H. (1986) Prenatal development disturbances in the limbic allocortex in schizophrenics. *J. Neural Transm.*, 65: 303–326.
- Jones, K.L., Smith, D.W., Ulleland, C.N. and Streissgut, A.P. (1973) Pattern of malformation in offspring of chronic alcoholic women. *Lancet*, i: 1267–1271.
- Jones, K.L. and Smith, D.W. (1975) The fetal alcohol syndrome. *Teratology*, 12: 1–10.
- Jones, K.L. and Smith, D.W. and Hanson, J.W. (1976) The fetal alcohol syndrome: clinical delineation. *Ann. N.Y. Acad. Sci.*, 273: 130–137.
- Kaminski, M., Franc, M., Lebouvier, M., Du Mazau-Brun, C. and Rumeau-Rouquette, C. (1981) Moderate alcohol use and pregnancy outcome. *Neurobehav. Toxicol. Teratol.*, 3: 173–181.
- Kellogg, C.K. (1988) Benzodiazepines: Influence on the developing brain. Ch. 14, this volume.
- Kirby, M.L. (1984) Alterations in fetal and adult responsiveness to opiates following various schedules of prenatal morphine exposure. In: J. Yanai (Ed.) *Neurobehavioral Teratology*. Elsevier, Amsterdam, pp. 235–248.
- Kraus, H.F., Campbell, G.A., Fowler, A.C. and Farber, J.P. (1981) Maternal nicotine administration and fetal brain stem damage: a rat model with implications for sudden infant death syndrome. *Am. J. Obstet. Gynecol.*, 140: 743–746.
- Lemoine, P., Haronsseau, H., Borteyru, J.-P. and Menuet, J.-C. (1968) Les enfants des parents alcooliques; anomalies observées à propos de 127 cas. *Ouest Méd.* 25: 476–482.
- Leonard, B.E. (1988) Alcohol as a social teratogen for the developing brain. Ch. 20, this volume.
- Leonard, B.E. (1981) Effect of psychotropic drugs administered to pregnant rats on the behaviour of the offspring. *Neuropharmacology*, 20: 1237–1242.
- Livezey, G.T., Radulovacki, M., Isaac, L. and Marczyński, T.J. (1985) Prenatal exposure to diazepam results in enduring reductions in brain receptors and deep slow wave sleep. *Brain Res.* 334: 361–365.
- Lorber, C.A., Cassidy, S.B. and Engel, E. (1979) Is there an embryo-fatal exogenous sex steroid exposure syndrome (efesses)? *Fert. Steril.*, 31: 21–24.
- Madsen, J.R., Campbell, A. and Baldessarini, R.J. (1981) Effects of prenatal treatment of rats with haloperidol due to altered drug distribution in neonatal brain. *Neuropharmacology*, 20: 931–939.
- Marani, E., Rietveld, W.J. and Boon, M.E. (1982) Monosodium glutamate accelerates migration of hypothalamic perikarya at puberty. *Histochemistry*, 75: 145–150.
- Marton, I.S. and Szondy, M. (1982) Possible neuroendocrine hazards of prenatal steroid exposure. In: Endröczy et al. (Eds.), *Neuropeptides, Neurotransmitters and Regulation of Endocrine Processes*. Akademia Press, Budapest, pp. 535–543.
- McGivern, R.F., Clancy, A.N., Hill, M.A. and Noble, E.P. (1984) Prenatal alcohol exposure alters adult expression of sexually dimorphic behavior in the rat. *Science*, 224: 896–898.
- Meriney, S.D., Gray, D.B. and Pilar, G. (1985) Morphine-induced delay of normal cell death in the avian ciliary ganglion. *Science*, 228: 1451–1452.
- Meyer-Bahlburg, H.F.L. and Ehrhardt, A.A. (1987) A prenatal-hormone hypothesis for depression in adults with a history of fetal DES exposure. In: U. Halbreich (Ed.), *Hormones and Depression*. Raven Press, New York, pp. 325–338.
- Miller, J.C. and Friedhoff, A.J. (1988) Prenatal neurotransmitter programming of postnatal receptor function. Ch. 31, this volume.
- Miller, R.W. and Blott, W.J. (1972) Small head size after in utero exposure to atomic radiation. *Lancet*, ii: 784–787.
- Mirkin, B.L. and Singh, S. (1976) Placental transfer of pharmacologically active molecules. In: B.L. Mirkin (Ed.) *Perinatal Pharmacology and Therapeutics*. Academic Press, New York, pp. 1–69.
- Mirmiran, M., Scholtens, J., Van de Poll, N.E., Uylings, H.B.M., Van der Gugten, J. and Boer, G.J. (1983) Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior. *Dev. Brain Res.*, 7: 277–286.
- Mukherjee, A.B. and Hodgen, G.D. (1982) Maternal ethanol exposure induces transient impairment of umbilical circulation and feral hypoxia in monkeys. *Science*, 218: 700–702.
- Naaktgeboren, C. (1985) Over waarneming en verklaring in de verloskunde. Deel II: 'Het verzien'. *Tijdschr. Verloskundigen*, 10: 329–341.
- Naeye, R.L. (1980) Sudden infant death. *Sci. Am.*, 242: 52–58.
- Olney, J.W. (1988) Excitotoxic food additives: functional teratological aspects. Ch. 18, this volume.
- Patel, A.J. and Lewis, P.D. (1988) Brain cell acquisition and neurotropic drugs with special reference to functional teratogenesis. Ch. 25, this volume.
- Pintor, C., Cella, S.G., Loche, S., Puggioni, R., Corda, R., Locatelli, VI and Müller, E.E. (1987) Clonidine treatment for short stature. *Lancet*, May 30: 1226–1230.
- Pollard, I. (1986) Prenatal stress effects over two generations in rats. *J. Endocrinol.*, 109: 239–244.
- Raymond, J.P., Goldstein, E., Konopka, P., Leleu, M.F., Merceron, R.E. and Loria, Y. (1985) Follow-up of children born of bromocriptine-treated mothers. *Hormone Res.*, 22: 239–246.

- Reinisch, J.M. and Sanders, S.A. (1982) Early barbiturate exposure: the brain, sexually dimorphic behavior and learning. *Neurosci. Biobehav. Rev.*, 6: 311-319.
- Robert, E. and Guibaud, P. (1982) Maternal valproic acid and congenital neural tube defects. *Lancet*, 23: 937.
- Rodier, P.M. (1988) Structural-functional relationships in experimentally-induced brain damage. Ch. 22, this volume.
- Rosengarten, H. and Friedhoff, A.J. (1979) Enduring changes in dopamine receptor cells of pups from drug administration to pregnant and nursing rats. *Science*, 203: 1133-1135.
- Rumack, C.M., Guggenheim, W.R., Rumack, B.H., Peterson, R.G., Johnson, M.L. and Braithwaite, W.R. (1981) Neonatal intracranial hemorrhage and maternal use of aspirin. *Obstet. Gynecol., N.Y.*, 58: 52S-56S.
- Sanua, V.D. (1986) The organic etiology of infantile autism: a critical review of the literature. *Int. J. Neurosci.*, 30: 195-225.
- Schardein, J.L. (1985) *Chemically Induced Birth Defects*. Marcel Dekker, Inc., New York and Basel.
- Schmid, B. (1987) Old and new concepts in teratogenicity testing. *TIPS*, 8: 133-138.
- Simmons, R.D., Miller, R.K. and Kellogg, C.K. (1983) Prenatal diazepam: distribution and metabolism in perinatal rats. *Teratology*, 28: 181-188.
- Soyka, L.F. and Joffe, J.M. (1980) Male mediated drug effects on offspring. In: R.H. Schwarz and S.J. Yaffe (Eds.), *Drug and Chemical Risks to the Fetus and Newborn*. *Prog. Clin. Biol. Res.*, Vol. 36. Alan R. Liss, New York, pp. 49-66.
- Spaans, C. and Verspreet, F.A.M. (1981) Het foetale alcohol-syndroom: vier patiënten in één gezin. *Ned. Tijdschr. Geneesk.*, 125: 452-454.
- Stenchever, M.A., Williamson R.A., Leonard, J., Karp, L.E., Ley, B., Shy, K. and Smith, D. (1981) Possible relationship between in utero diethylstilbestrol exposure and male fertility. *Am. J. Obstet. Gynecol.*, 140: 186-193.
- Swaab, D.F. (1980) Neuropeptides and brain development: a working hypothesis. In: C. Di Benedetta, R. Balazs, G. Gombos and P. Procellati (Eds.), *A Multidisciplinary Approach to Brain Development*. Proceedings of the International Meeting, Selva di Fasano. Elsevier/North Holland Biomedical Press, Amsterdam, pp. 181-196.
- Swaab, D.F. and Fliers, E. (1985) A sexually dimorphic nucleus in the human brain. *Science*, 228: 1112-1115.
- Swaab, D.F. and Mirmiran, M. (1984) Possible mechanisms underlying the teratogenic effects of medicines on the developing brain. In: J. Yanai (Ed.), *Neurobehavioral Teratology*. Elsevier, Amsterdam, pp. 55-71.
- Swaab, D.F. and Mirmiran, M. (1988) Permanent effects of chemicals on the developing brain; functional or behavioral teratology. In: M.I. Levere, M.J. Bennett and J. Punt (Eds.) *Fetal and Neonatal Neurology and Neurosurgery*. Churchill, Livingstone, United Kingdom, in press.
- Tanaka, H., Nakazawa, K. and Arima, M. (1987) Effects of maternal caffeine ingestion on the perinatal cerebrum. *Biol. Neonate*, 51: 332-339.
- Tonge, S.R. (1972) Permanent changes in brain monoamine metabolism induced by the administration of psychotropic drugs during the pre- and neonatal periods in rats. *J. Pharm. Pharmacol.*, 24, Suppl.: 149P-150P.
- Trice, J.E. and Ambler, M. (1985) Multiple cerebral defects in an infant exposed in utero to anticonvulsants. *Arch. Pathol. Lab. Med.* 109: 521-523.
- Trotz, M., Wegner, Chr. and Nau, H. (1987) Valproic acid-induced neural tube defects: reduction by folinic acid in the mouse. *Life Sci.*, 41: 103-110.
- Vaccari, A. (1988) Teratogenic mechanisms of dysthyroidism in the central nervous system. Ch. 6, this volume.
- Van der Werff ten Bosch, J.J. and Bot, A. (1981) Growth of tall girls without and during oestrogen treatment. *Neth. J. Med.*, 24: 52-57.
- Van der Werff ten Bosch, J.J., Bot, A. and Goslings, B.M. (1986) Growth of tall boys without and during androgen treatment. *Neth. J. Med.*, 29: 73-78.
- Vessey, M.P., Fairweather, D.V.I., Norman-Smith, B. and Buckley, J. (1983) A randomized double-blind controlled trial of the value of stilboestrol therapy in pregnancy: long-term follow-up of mothers and their offspring. *Br. J. Obstet. Gynaecol.* 90: 1007-1017.
- Vorhees, C.V. (1986a) Origins of behavioral teratology. In: E.P. Riley and C.V. Vorhees (Eds.), *Handbook of Behavioral Teratology*. Plenum Press, New York and London, pp. 3-22.
- Vorhees, C.V. (1986b) Principles of behavioral teratology. In: E.P. Riley and C.V. Vorhees (Eds.), *Handbook of Behavioral Teratology*. Plenum Press, New York, pp. 23-48.
- Vorhees, C.V. (1987) Behavioral teratogenicity of valproic acid: selective effects on behavior after prenatal exposure to rats. *Psychopharmacology* (Berlin), 92: 173-179.
- Warner, R.H. and Rosett, H.L. (1975) The effects of drinking on offspring. *J. Stud. Alcohol*, 36: 1395-1420.
- Warrel, D.W. and Taylor, R. (1968) Outcome for the fetus of mothers receiving prednisolone during pregnancy. *Lancet*, i: 117-118.
- Weiss, B. (1988) Implications of behavioral teratology for assessing the risks posed by environmental and therapeutic chemicals. Ch. 3, this volume.
- Werboff, J. and Gottlieb, J.S. (1963) Drugs in pregnancy: behavioral teratology. *Obstet. Gynecol. Surv.*, 18: 420-423.
- Wilson, J.G. (1977) Current status of teratology: General principles and mechanisms derived from animal studies. In: J.G. Wilson and F.C. Fraser (Eds.), *Handbook of Teratology, Vol. 1, General Principles and Etiology*, Plenum Press, New York, pp. 47-74.
- Yanai, J. (1981) Comparison of early barbiturate and ethanol effects on the CNS. *Substance Alcohol Actions/Misuse*, 2: 79-91.

Discussion

A.J. Patel: Your results indicate that an increase in cell number in the sexually dimorphic nucleus (SDN) is similar in both males and females up to about 2 to 5 years. Thereafter, in comparison with males, the cell number decreases much faster in females. Do you think that the reduction of cell content of the SDN is due to cell death and more so in females? Furthermore, I would appreciate your comments on testosterone, which is believed to be associated with reduction in cell formation or cell death, as a missing trophic factor in females.

D.F. Swaab: The most probable explanation for the equally high SDN cell number in boys and girls and the major diminution in cell numbers later in female is indeed that testosterone is preventing pre-programmed cell death in the SDN in a similar way as has been reported for the spinal nucleus of the bulbocavernosus (SNB) (Breedlove, 1984). The sexual dimorphism of the SNB also concerns neurons. However, an influence via steroid receptors of the muscle in which these SNB neurons terminate can certainly not be excluded. It is also not clear whether the SDN neurons contain steroid receptors themselves, or are innervated by steroid sensitive neurons.

B.S. McEwen: Could the late appearance of the sex difference in the human sexually dimorphic nucleus (SDN) be due to the second, postnatal surge of testosterone secretion in the human male, rather than the first, prenatal testosterone peak?

D.F. Swaab: Indeed, in the first postnatal months high sex hormone levels have been reported in human neonates. This is also the period in which we find the SDN increasing rapidly in cell number, whereas its cells show signs of high activity. The nucleus of SDN cells is larger in this period than in any other period in life. Although it is tempting to assume a direct relationship between the hormone levels and SDN alterations, it will of course be hard to prove its possible causal nature.

Z. Annau: The ultimate effect of chemicals on the offspring may be related very significantly to the stress (hormonal) effects induced in the mother by these chemicals, at doses which do not cause overt toxicity.

D.F. Swaab: In principle such an interaction seems quite possible.

G.J. Boer: You distinguished between direct and indirect effects of chemicals on brain development by assuming either direct or indirect effects on brain cells. But are there not many teratogens that have general effects on maturing or proliferating cells as such? This would mean that a distinction between direct and indirect effects specifically on brain cells might sometimes not exist.

D.F. Swaab: It is true that one chemical might have various effects. For instance, alcohol might affect the developing brain indirectly by causing constriction of the umbilical cord and directly by affecting cell division, migration, formation of neurites, etc. In fact, most chemicals seem to act by various mechanisms, being direct or indirect.

B. Weiss: Carcinogenesis is almost the only endpoint in toxicology for which we conduct lifetime studies. Yet, longitudinal studies with neurotoxicants are rare. Apart from methylmercury, I cannot think of any cogent studies. Are you aware of any such studies?

D.F. Swaab: Dr. Kellogg has made an important start into this direction by showing alterations in the rat offspring at 24 months of age following benzodiazepine treatment in development (Kellogg, 1988). It seems logical to expect effects on the ageing process when the organism has been exposed to chemicals during development. For such a study one would need rats around the age of 32 months. This would not only be an extremely time-consuming type of experiment, but also an extremely expensive one, since such rats cost a few thousand dollars each.

References

- Breedlove, S.M. (1984) Steroid influences on the development and function of a neuromuscular system. In G.J. De Vries, J.P.C. De Bruin, H.B.M. Uylings and M.A. Corner (Eds.) *Sex Differences in the Brain. The Relation between Structure and Function, Progress in Brain Research, Vol. 61*, Elsevier, Amsterdam, pp. 147–170.
- Kellogg, C.K. (1988) Benzodiazepines: influence on the developing brain. Ch. 14, this volume.