Influence of Drugs on Brain Neurotransmitters and Behavioral States during Development¹

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Abstract. Neurotransmitters are present at very early stages of brain development. They may have trophic effects on maturation of target neurons and mediate the behavioral repertoire of the immature brain. Many centrally acting drugs which are used during pregnancy and early childhood for the treatment of e.g. hypertension, depression, epilepsy, sleep disorders, or hyperkinesia influence brain neurotransmitters and behavioral states. Disturbances observed later in life in animal and man, due to *perinatal* interference of such drugs with brain neurotransmitters and behavioral states, are not gross physical malformations but rather subtle behavioral and neurological symptoms such as hyperactivity, emotional lability, perceptual motor disturbances, attentional distractibility and sleep disturbances.

Introduction

The unborn child is exposed to a variety of chemicals used by the mother during gestation. This includes drugs administered for treatment of conditions threatening the fetus, e.g. hypertension, epilepsy, depression and premature labour. In addition, self-medication is widely present. Disturbances caused by such chemicals are mostly not of a gross physical nature, but rather based upon permanent microscopic and biochemical alterations of transmitter systems. The effect of functional deficits induced

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that way in the child is called behavioral teratogenicity. This topic recently gained much interest and the potentiality of it has been reviewed in several publications [Hutchings, 1978; Schwartz and Yaffe, 1980; Yanai, 1984]. These teratogenic factors may act on brain neurotransmitters, neuroendocrine systems and behavioral states and induce subtle behavioral changes when used during the period of rapid brain growth, which in humans takes place during the last trimester of gestation and lactation. The intention of the present paper is to review the development of brain neurotransmitters, with emphasis on monoamines, and the behavioral states in both rat and man. We want to point especially to the sensitivity of man to the hazards of medicines and to put forward the hypothesis that neurochemical or behavioral changes due to drug therapy may mediate the appearance of long-lasting behavioral changes.

Development of Brain Neurotransmitters: Influence of Drugs

Neurotransmitters can be subdivided into the following groups: acetylcholine, biogenic amines, amino acids and peptides. Monoamines, i.e. noradrenaline (NA), serotonin (5HT) and dopamine (DA), are among the first neurotransmitters present at early stages of brain development. Rats, the experimental animal model commonly used for behavioral teratology experiments, are born very immature. The brain of a newborn rat is in various aspects comparable to a 7-month-old human fetal brain [Dobbing and Sands, 1973, 1979]. In the rat that has a gestational period of about 21 days, NA neurons of the nucleus locus ceruleus differentiate on days 10–13 of gestation, whereas dopaminergic cells of the substantia nigra appear on days 11–15. In the serotonergic neurons of the dorsal raphe the peak of heavy labelling occurs on day 14 [Lauder and Bloom, 1974]. Early appearance of monoamines has been shown in 3- to 4-month-old human fetus [Nobin and Björklund, 1973]. Although these studies demonstrate that the neurons are able to synthesize the neurotransmitter, they do, in principle, not give information on their level of activity. In order to demonstrate the functional capacity and sensitivity of the monoamines to drugs in the immature brain several biochemical, electrophysiological and behavioral studies (see below) have been carried out. Drugs such as imipramine, which are able to inhibit NA uptake, are as effective in newborn rats as in adults [Nomura et al., 1978]. Reserpine which release the amines from

nerve terminals, thereby inducing a functional denervation, is also effective even in fetal life [Tennyson et al., 1983]. Nomura et al. [1979] have shown that depolarizing membranes by increasing the potassium concentration in the medium induced the release of NA, DA and 5HT from brain slices of 2- to 3-day-old rats to the same extent as in brain slices of adult rats. He concluded that NA, DA and 5HT are already stored in a functionally releasable pool at the nerve terminals of central monoamine neurons in the newborn rat and that these compounds act as neurotransmitters at birth as they do in adulthood.

Although the time course of the development of other neurotransmitters will not be discussed in detail, it is important to note that a similar pattern of development with rapid changes during the last week of prenatal and the first 3 weeks of postnatal life in rat is found for amino acids, acetylcholine and peptides [McDonald et al., 1982; Kvale et al., 1983; Nobu et al., 1985; Swaab, 1980]. This is also the period of vulnerability of the neurotransmitters to the unintentional hazards such as drug therapy, as a result of which (1) the development of brain neurotransmitter target area will be affected and (2) the activity of the neurotransmitter system itself and its receptor sensitivity will be permanently hampered leading to behavioral abnormalities.

Animal studies have indeed improved our knowledge about neurotransmitter disturbances as one of the mechanisms of the neuroteratological disturbances induced by chemicals. Such developmental studies can of course not be easily carried out in humans. However, in the near future some parameters of neurotransmitter activity such as contents of neurotransmitters and their breakdown products in cerebrospinal fluid, receptor binding studies using blood platelet or placental tissue, PET scan and NMR techniques, and – in case brain material is available – immunocytochemical and biochemical studies may be carried out in humans. To our knowledge only one study has been carried out in humans which in this way addressed the hypothesis of whether prenatal drug exposure may result in neurotransmitter system changes which, in turn, cause behavioral teratology. On the assumption that placental tissue neurotransmitter regulation may mirror fetal brain receptor regulation, placental neurotransmitter receptors from control and substance-abusing pregnant women were examined. Increased amounts of opiate receptors and adrenergic receptors were found in placenta of mothers who used opiates or amphetamine during pregnancy [Perry et al., 1984].

Development of Behavioral States: Influence of Drugs

Developmental aspects of sleep and wakefulness have been studied extensively in different mammals including humans by various pioneers in the field [Prechtl, 1974; Jouvet-Monier et al., 1969; Dreyfus-Brisac, 1979]. Behavioral states are generated exclusively in the brain. These are: wakefulness (W), quiet sleep (QS) and rapid eye movement sleep (REM). The advantage of an altricial animal such as the rat, which is born very immature, is that it enables us to study early development of the behavioral states *ex utero*. Postnatal studies of the development of the behavioral states of the rat have demonstrated a very high level of REM (90%) at birth, which declines dramatically around 2 weeks of age in expense of increased W and QS. This high proportion of REM characterized by high frequency of REM periods, high intensity and frequency of both eye movements as well as other phasic activation during this state is believed to be an indicator of the immaturity of the central nervous system. In addition, REM might be of importance for brain development [Roffwarg et al., 1969; Mirmiran et al., 1983]. In humans prenatal studies have been carried out using ultra sound techniques. Different criteria for definition of the behavioral states develop at different stages. Motility appears at 7.5 weeks after menstrual age, and by 15 weeks all the different types of movements closely resembling those observed in preterm and fullterm babies can be distinguished [De Vries et al., 1982]. Eye movements appear at 16 weeks gestation and by 24 weeks different types of eye movements (rapid-slow, single and complex) can be observed [Birnholtz, 1981]. In humans, it seems that although separate criteria of the behavioral states are present at a very early age, the mechanisms which are responsible for coherence of these characteristic criteria develop much later. In prenatal human studies a high level of REM can be observed at 32 weeks, which declines gradually to 50–60% at birth and later to a level comparable to adult values (25%) by 2 years of age [Nijhuis et al., 1982; Casaer and Devlieger, 1984; Roffwarg et al., 1969; Dreyfus-Brisac, 1979].

Since behavioral states are generated in the brain and show a very close relationship to the stage of brain development, they may be used as a good indicator of hazards of chemicals to the developing brain. Unfortunately, only the effects of few drugs on behavioral states have been tested during development [Hutchings et al., 1979]. Our own studies have shown clear disturbances of sleep-wake patterns during chronic administration of antidepressant drugs (such as clomipramine) or antihypertensives (such as

clonidine or α -methyldopa) in developing rats [Mirmiran et al., 1981, 1983, 1985]. Either of these drugs chronically administered to developing rats dramatically reduced the amount of time spent in REM as well as the density of eye movements during sleep. In adulthood, neonatally clomipramine- or clonidine-exposed rats showed a high percentage of REM together with an increased amount of motoric activation during this state. Although clonidine treatment did not change the level of NA in the brain, a reduced NA turnover was observed in hypothalamus and amygdala in experimental animals [Mirmiran et al., 1985]. Another example of behavioral states and neurotransmitter disturbances as a result of exposure to centrally acting drugs has recently been observed in rat prenatally exposed to diazepam. Following 6 days of valium exposure during the last week of gestation, mature prenatally exposed rats at 4 months of age displayed significantly less deep QS compared to controls [Livezey et al., 1985]. Although it was not stated by the authors, from the results presented it could also be concluded that the amplitude of the circadian rhythmicity of the REM was reduced in prenatally diazepam-exposed animals. These rats also had a smaller number of benzodiazepine receptors in thalamus than the vehicle-exposed controls.

Although ultrasound techniques are frequently used in clinics, no one has so far systematically investigated the influence of drugs that are used by expecting mothers on the behavioral states of the fetus during chronic exposure. It is known from adult human as well as both developmental and adult animal studies that the majority of centrally acting drugs suppress REM and disturb the sleep-wake rhythm [Swaab and Mirmiran, 1984]. These drugs influence sleep by affecting different brain neurotransmitters such as NA (e.g. α -methyldopa, clonidine and propranolol), 5HT (e.g. imipramine, clomipramine and zimelidine), γ -aminobutyric acid (e.g. diazepam), acetylcholine (e.g. atropine or scopolamine), and drugs which suppress reticular formation activity and indirectly influence brain neurotransmitters (e.g. phenobarbital). Several investigators have examined behavioral states of passively dependent human infants during opiate withdrawal. A significant decrease in QS and increased REM was found in neonates prenatally exposed to opiates, a profile shared by many newborn at high risk for central nervous system impairment [Schulman, 1969; Dinges et al., 1980]. A decrease in both QS and REM was found in newborn babies born to addicted mothers [Sisson et al., 1974]. Sisson concluded that since protein synthesis is stimulated during REM, treatment of withdrawal may be essential not only to relieve symptoms but also to pro-

mote normal and necessary sleep patterns required for brain development [Mirmiran et al., 1983]. Disturbances of the sleep-wake pattern with increased motor activity during sleep and increased sleep complaints have also been found in children born to hypertensive mothers treated with clonidine or α -methyldopa [Huisjes et al., 1984; Shimohira, 1984].

Despite growing literature in the field of human behavioral teratogenicity it is very difficult to be certain about a causal relationship between pre- and perinatal drug exposure and behavioral outcomes. These subtle behavioral disturbances have symptoms such as hyperactivity, emotional lability, perceptual motor disturbances, attentional distractability, poor self-adjustment, increased activity in situations requiring motor inhibition and sleep disturbances. One of the major difficulties in studying subtle changes in human behavior is the presence of the enormous range of 'normal' behavior. Other factors such as the lack of adequate controls, e.g., for the social and economic environment of the child, and his genetic and educational background further complicate the study of drug teratogenicity in humans. Since in experimental animal studies many of these factors can be controlled and because the implication of animal results to man might be better than has been presumed earlier (see above), such experiments should become routine in screening for putative side effects of new (and old!) drugs.

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