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PSYCHOTROPIC DRUGS AND BRAIN DEVELOPMENT: EFFECTS ON CELL REPLICATION *IN VIVO* AND *IN VITRO*

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Summary—The effects of certain psychotropic drugs, which are suspected behavioural teratogens, on cell replication in the developing brain were investigated using both biochemical and morphological techniques. Reserpine (a monoamine depletor), chlorpromazine, thioridazine and haloperidol (which mainly acts on post-synaptic receptors as a dopamine antagonist), and lysergic acid diethylamide (believed to affect serotonin systems) resulted in a severe reduction of cell replication, measured in terms of the *in vivo* rate of [^{3}H]thymidine incorporation into brain DNA of 11-day old rats. In the forebrain the rate of DNA labelling was about one third to one half of controls during the period 4–30, 14–30, 10–24 and 1–2 hr after subcutaneous administration of reserpine (2.5 mg/kg), chlorpromazine (50 mg/kg), haloperidol (20 mg/kg) and lysergic acid diethylamide (1 mg/kg), respectively. The depression was dose-dependent and the half maximal effect was produced with approx. 0.15 mg/kg reserpine, 10 mg/kg chlorpromazine, 15 mg/kg thioridazine, 7 mg/kg haloperidol and 0.5 mg/kg lysergic acid diethylamide. The effect on the cerebellum was similar but less marked. Parallel histological studies showed that in both reserpine- and chlorpromazine-treated rats, the turnover time of cell replication was markedly increased in the forebrain subependymal layer, while in the cerebellar external granular layer there was a decrease in the mitotic index and an increase in the degeneration of post-mitotic cells. The role of the drugs affecting neurotransmitter balance in the regulation of cell replication was also studied *in vitro*, using morphologically preserved brain slices. The drugs affecting monoamine neurotransmitter systems reduced the rate of DNA labelling in a dose-dependent manner. Agonist and antagonists both showed similar inhibition and the half-maximal effect was obtained with approx. 10^{-4} M concentrations. The drugs affecting cholinergic systems were apparently ineffective. The results of both *in vivo* and *in vitro* experiments suggest that the claimed behavioural teratogenic action of the psychotropic drugs tested may be related, in part, to a chronologically-determined interference with the formation of certain cell types.

There is a growing mass of information suggesting that psychotropic and other drugs, acting on the central nervous system (CNS), produce persistent behavioural changes when given to experimental animals during the period of rapid brain development (Werboff and Gottlieb, 1963; Lundborg and Engel, 1978; Vorhees, Brunner and Butcher, 1979; and other papers in this Symposium). In current medical practice, treatment of pregnant and lactating mothers with CNS-active drugs is routine, and in some circumstances the period of therapy may be prolonged. Under these conditions, the foetus or neonate may be exposed not just to an acute drug dose but also to a sustained level of drug throughout a significant proportion of the time during which the brain is developing. The basis for any behavioural changes produced by a pharmacological agent or other adverse developmental influence is likely to be complex, involving both physical factors and environmental interactions. Physical aspects to be considered include disturbances of cell acquisition and of cell differentiation in the brain. Changes in these processes, occurring either singly or together, are capable of disrupting the balance and timing of neural network formation. Investigation of possible structural correlates of behavioural abnormality thus offers a number of different approaches. A body of data has accumulated to show changes in cell replication induced in the developing brain by a variety of drugs and associated in some cases with behavioural alterations (Langman, Webster and Rodier, 1975; Patel and Lewis, 1981). This paper follows this approach and describes the effect of certain psychotropic drugs, which are suspected behavioural teratogens, on *in vivo* and *in vitro* cell replication in the rat brain, using both biochemical and histological techniques.

Key words: psychotropic drugs, cell proliferation, developing brain, chlorpromazine, reserpine, haloperidol, cell kinetics, *in vitro* cell replication, DNA labelling.

CELL PROLIFERATION IN THE BRAIN

Cell proliferation in the central nervous system has been extensively reviewed in recent years (Balázs, Patel and Lewis, 1977; Jacobson, 1978; Patel and Balázs, 1980; Lewis, 1981; Patel and Lewis, 1981) and here we will list briefly only certain facts. The "birth days" of many different nerve cell populations, as determined in the mammalian brain by [^{3}H]thymidine autoradiography, are fixed, indicating that the chronology of neurogenesis follows a rigid time-table. In many mammalian species, including rat and man, a large proportion of the nerve cells in the forebrain are

formed before birth. However, in the rat, significant neurogenesis has been shown to occur after birth in certain places in the forebrain, such as the hippocampus and the olfactory bulbs, and in the cerebellum (where total cell number is 40% higher than in the forebrain) nearly all the interneurones, which accounts for about half of the cell population, are formed postnatally. There is convincing evidence that in man neurogenesis also takes place on a significant scale in the cerebellum and elsewhere after birth (Gadsdon and Emery, 1976; Patel and Lewis, 1981). Postnatal cell formation occurs over a relatively restricted period, about 2 years in humans and probably the first 3 weeks in the rat. It accounts in man for about 70% of the final cell number and in the rat for about 40, 97, 60 and 80% of the total neural complement in the forebrain, cerebellum, hippocampus and olfactory bulbs respectively. Although dispersed replicating cells, presumably glial precursors, are detected throughout the brain, postnatal cell proliferation occurs largely in circumscribed secondary germinal sites, such as the forebrain subependymal layer, the cerebellar external granular layer and the hippocampal fascia dentata.

The characterization of the major germinal sites in the various parts of the brain, in terms of the classes of cells they generate, permits the differential analysis of proliferation kinetics of predominantly neuronal precursors (as in external granular layer) on the one hand and of glial precursors (as in lateral ventricular subependymal layer) on the other. Findings in vehicle-treated controls can be compared with those following exposure to psychotropic drugs during early life. Morphologically-derived indices of cell proliferation can thus be obtained and compared with biochemical changes. In the experiments reviewed here, 11-day old rats were used because at this age cell replication is occurring at a high level throughout the brain (Patel, Balázs and Johnson, 1973).

In vivo *studies*

Reserpine. This widely used central nervous system depressant and antihypertensive agent, which reversibly depletes brain monoamine stores produced, when given to developing rats in a single subcutaneous injection of 2.5 mg/kg, a severe reduction of DNA labelling (Lewis, Patel, Béndek and Balázs, 1977; Patel, Béndek, Balázs and Lewis, 1977). The depression of the rate of [^{3}H]thymidine incorporation into DNA was already detectable 2 hr after reserpine administration, and during the period 4–30 hr after administration the rate was about 30 and 60% of control in the forebrain and cerebellum, respectively (Fig. 1). The reduced incorporation of labelled thymidine into DNA did not seem to result from side effects of reserpine treatment, including effects on body temperature, circulating levels of corticosteroids, food intake, entry of [^{3}H]thymidine into the brain, thymidine kinase activity, the *in vivo* conversion of [^{3}H]thymidine into [^{3}H]thymine nucleotides and the availability of ATP, which may influence biosynthetic reactions such as DNA synthesis (Patel *et al.*, 1977; Patel, Bailey and Balázs, 1979). Furthermore, the effect of reserpine was dose-dependent and the half-maximal effect was obtained with a dose of approx. 0.15 mg kg in the forebrain and approx. 0.5 mg/kg in the cerebellum (Patel *et al.*, 1979).

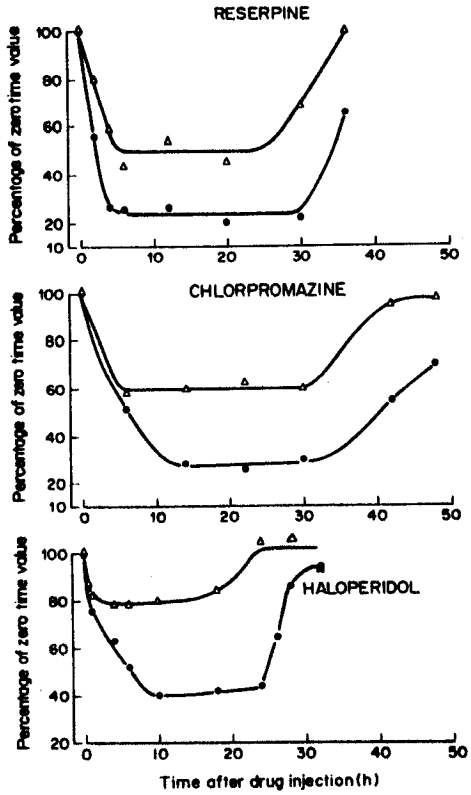

Fig. 1. Effect of reserpine, chlropromazine and haloperidol on DNA labelling in the forebrain and cerebellum. Eleven day old rats received a subcutaneous injection of either reserpine (2.5 mg/kg body weight), or chlorpromazine (50 mg/kg body weight), or haloperidol (20 mg/kg body weight); the control received same volume of respective vehicle only. At the times indicated after the administration of the drug, the animals received a subcutaneous injection of [^{3}H]thymidine and were killed 30 min later. The specific radioactivity of DNA (d.p.m./μg atom-P) was corrected on the basis of [^{3}H]water-freed acid soluble radioactivity concentration, and the results of drug treated rats were expressed as a percentage of the control value at time "0". The time curves for drug treated rats differed significantly from controls in both brain areas ($P < 0.01$). ●——● Forebrain; △——△, cerebellum. The results are from Lewis *et al.* (1977), Patel *et al.* (1980) and Backhouse *et al.* (1982).

Parallel histological studies (Lewis *et al.*, 1977) confirmed a depression of cell acquisition in both forebrain and cerebellar germinal sites (Table 1). In the subependymal layer, the rate of cell acquisition was markedly reduced (turnover time was increased), and cell cycle time and duration of DNA synthesis phase were clearly prolonged. In the cerebellar external granular layer, the major effect on the dividing cell

Table 1. Comparison of effects of psychotropic drugs on cell death, cell cycle parameters and cell acquisition in rat cerebellar external granular layer and forebrain subependymal layer

	External granular layer			Subependymal layer		
	Reserpine	Chlorpromazine	Haloperidol	Reserpine	Chlorpromazine	Haloperidol
Pyknotic index	268*	316*	135	142	182	118
Mitotic index	79*	77*	95	105	91	124
Labelling index	93	108	103	71*	77*	100
Cell cycle time	113	101	98	149	117	106
Duration of G_2-phase	107	176	181	119	129	115
Duration of S-phase	104	116	94	114	109	118
Turnover time	104	124	91	160	143	118

The results are expressed as a percentage of control values. Comparisons were made on median values for cell cycle parameters and means for indices. Significant differences ($P < 0.05$) in indices between control and drug treated groups are indicated by an asterisk. The results are from Lewis *et al.* (1977), Patel *et al.* (1980) and Backhouse *et al.* (1982).

population was a dramatic increase in cell death, paralleling a fall in the number of mitoses.

Chlorpromazine. The neuropharmacological effects of chlorpromazine differ from those of reserpine; this drug is believed to act primarily on dopamine receptors. However, like reserpine, chlorpromazine produced a marked reduction of the rate of [^{3}H]thymidine incorporation into brain DNA (Patel, Vértes, Lewis and Lai, 1980b). The depression in DNA labelling was detectable by 6 hr after drug treatment (50 mg/kg, s.c.) and the rate of DNA synthesis was less than 30 and 60% of controls during the period 14–30 hr in the forebrain and 6–30 hr in the cerebellum respectively (Fig. 1). The depression was dose-dependent and a half-maximal effect was produced with approx. 10 mg/kg of chlorpromazine. This effect was seen with a related phenothiazine drug, thioridazine, which produced a marked reduction in DNA labelling, the half-maximal effect in the forebrain occurring with a dose of approx. 15 mg/kg (Table 2). These drugs caused some retardation in the rate of conversion of [^{3}H]thymidine to [^{3}H]thymine nucleotides in the brain, but severe depression in DNA labelling was also evident after correcting the values on the basis of [^{3}H]thymine nucleotide concentrations. Similarly, the reduced incorporation of labelled thymidine into DNA in the developing brain did not seem to result from other side effects of these drugs, such as sedation, which can lead to hypothermia, and food deprivation due to lack of maternal care. Chlorpromazine, when given to 16-day pregnant female rats also produced dose-dependent depression in the rate of [^{3}H]thymidine incorporation into foetal brain DNA (Vértes, Patel and Balázs, 1980).

Parallel morphological analyses (Patel *et al.*, 1980b) revealed changes similar to those seen with reserpine (Table 1). The labelling index in subependymal cells was significantly depressed and turnover time increased, suggesting a decreased cell acquisition rate. In the external granular layer there was a reduction in

Table 2. Effect of various pharmacological agents on brain cell replication *in vivo*

Drug	Clinical daily dose range (mg/kg)	Dose (mg/kg)	Period of treatment (hr)	DNA labelling (as a % of controls)	Half maximal effect (mg/kg)
Reserpine	0.004–0.06	2.5	4–30	30*	0.15
Tetrabenazine	1–3	10	1	66*	—
RO4-1284	0.3–2	10	1	57*	—
Propranolol	0.3–10	10	5	75*	—
Phenoxybenzamine	0.2–3	20	5	71*	—
Chlorpromazine	2–30	50	14–30	40*	10
Haloperidol	0.1–1.5	20	6–24	50*	7
Thioridazine	0.5–12	15	3	70*	15
LSD	0.001–0.05	1	1–2	54*	0.5
Atropine	0.004–0.06	50	0.5	91	—

Eleven day old rats received either a single subcutaneous injection of drug or vehicle. Body temperature and nutritional status of the animals were maintained normal. At time indicated after the administration of the drug, the animals received a subcutaneous injection of [^{3}H]thymidine. At 30 min later they were killed and forebrains were removed and processed as described previously by Patel *et al.* (1977). The specific radioactivity of forebrain DNA (d.p.m. μg atom-P) were corrected on the basis of [^{3}H]water-freed acid soluble radioactivity (d.p.m. g) and the results in the drug treated animals were expressed as a percentage of the control values. Significant differences ($P < 0.05$) between vehicle and drug treated groups are marked with an asterisk. The range of clinical daily doses are from Goodman and Gilman (1970) and Wade (1977). The other results are from Barochovsky *et al.* (1979), Patel *et al.* (1979, 1980), Backhouse *et al.* (1982) Patel and Lewis (1981).

RO4-1284, (−)-threo-α-phenyl-α-piperid-2-yl methyl acetate hydrochloride; LSD, (+)-*N,N*-diethyl-lysergamide.

numbers of mitoses and a marked increase in cell degeneration, reflecting the pattern of inhibition of DNA synthesis (Table 1). Furthermore, autoradiographs showed labelling of pyknotic nuclei at 6 hr or more after injection of [^{3}H]thymidine, suggesting that nuclear degeneration was a postmitotic phenomenon. Feulgen cytophotometry confirmed that cell death occurred after division, though the relationship of this to the reduction in mitotic index was not clear.

Haloperidol. Haloperidol, one of the butyrophenones, and a drug with a very similar pharmacological action to that of the phenothiazines, was also found to produce a marked depression of DNA labelling (Backhouse, Barochovsky, Malik, Patel and Lewis, 1982). The reduction of *in vivo* DNA synthesis in the rat was detectable by 4 hours after haloperidol administration (20 mg/kg, s.c.) and through the period 10–24 hr after drug treatment the rate was less than 50% of that of controls in the forebrain (Fig. 1). Incorporation returned to control values by 32 hr. The effect on the cerebellum was similar but less pronounced. The depression was also dose-dependent and a half-maximal effect was produced with approx. 7 mg/kg haloperidol. Parallel histological studies indicated that, even though the changes are relatively less severe, they are in the same direction as those seen with reserpine and chlorpromazine (Table 1).

Other drugs acting on central nervous system. Several neurohumoral substances, including monoamines, are known to affect cell proliferation and differentiation in a variety of tissues (Buznikov, Sakharova, Manukhin and Markova, 1972; Vernadakis and Gibson, 1974; Lanier, Dunn and Van Hartesveldt, 1976; Balázs *et al.*, 1977; Lauder and Krebs, 1978; Patel and Lewis, 1981). Similar control mechanisms have been shown to operate for cell lines derived from transformed nervous tissue, such as neuroblastomas and glioma tumors (Prasad, 1975; Hamprecht, 1977). Therefore, it seems possible that neurotransmitters at certain stages of brain development could act as neurohumors controlling cell replication, and drugs which affect neurotransmitter balance could thereby disturb the production of cells. The preliminary results reported in Table 2 showed that the monoamine depletors, tetrabenazine and RO4-1284 (see Table 2), like reserpine, severely inhibited [^{3}H]thymidine incorporation into brain DNA. The results also indicate that both α- and β-adrenergic systems may be involved in control of brain cell replication: the administration of both phenoxybenzamine (α-blocker) and propranolol (β-blocker) caused marked reduction in DNA labelling. This effect was not restricted to drugs influencing only catecholaminergic systems, since lysergic acid diethylamide, a drug known to interfere mainly with serotoninergic transmitter systems in the brain, also produced a severe reduction in the rate of DNA synthesis (Barochovsky, Patel and Balázs, 1979). On the other hand, the anticholinergic drug, atropine, had relatively little effect on brain cell replication *in vivo*. It should however be noted that some of the studies were carried out at one time point and with one dose concentration; therefore, definitive observations to elucidate mechanisms whereby these drugs acting on the central nervous system may influence cell replication in the developing brain may yet have to be made. While, in these *in vivo* studies various side effects of the drugs could be excluded as being responsible for the depressed rate of cell proliferation, the findings nevertheless provide only circumstantial evidence for a role of transmitters in the control of cell replication in the developing brain.

In vitro *studies*

In order to investigate further the role of neurotransmitters and drugs affecting neurotransmitter balance in the regulation of cell replication in CNS tissue procedures have recently been developed for the study of cell proliferation *in vitro* using brain slices (Barochovsky and Patel, 1981). Brain slices from 11-day old rats when incubated under conventional conditions (Garthwaite, Woodhams, Collins and Balázs, 1980) were found to incorporate [^{3}H]thymidine into DNA. Using this estimate as an index of cell replication the optimal conditions were established; these included the incubation of slices in Eagle basal medium supplemented with 10% foetal calf serum, at pH 7.4 in a 95% O_2–5% CO_2 atmosphere. Under these conditions, the rate of *in vitro* [^{3}H]thymidine incorporation into DNA was linear for periods much in excess of the S-phase length: more than 20 hr in the forebrain and 14 hr in the cerebellum. Furthermore, during this period the *in vitro* rate of DNA labelling was about 3.36 times higher in the cerebellum than in the forebrain (Table 3), this estimate being similar to the previously calculated ratio of *in vivo* cell acquisition rates (3.64) in these two brain regions (Patel *et al.*, 1973). Parallel histological studies showed good morphological preservation of the slices, while [^{3}H]thymidine autoradiography experiments demonstrated incorporation predominantly in cells of the germinal layers (Barochovsky and Patel, 1981).

The effects of certain psychotropic drugs on brain cell replication were studied using this *in vitro* system (Table 4). All three dopamine antagonists, chlorpromazine, thioridazine and haloperidol, severely inhibited brain cell replication. The reduction was dose-dependent and the half-maximal effects were obtained at about $3–5 \times 10^{-4}$ M concentrations. The effect was not restricted to dopamine antagonists, since piribedil, a dopamine agonist, also resulted in a marked reduction in DNA labelling in a dose-dependent manner (Barochovsky and Patel, 1981). Similar inhibition was observed with drugs affecting serotoninergic transmitter systems but not with compounds affecting cholinergic transmitter systems. Both the serotonin agonist TFMTP and the "antagonist" lysergic acid diethylamide resulted in a marked reduction in [^{3}H]thymidine incorporation into DNA. Once again

Table 3. Comparison of *in vivo* and *in vitro* rate of cell acquisition in 11-day old rat

	Cerebellum	Forebrain	Cerebellum/forebrain ratio
Amount of DNA-P (μg atom/brain part)	1.5	2.2	—
In vivo			
DNA-P deposition (ng atom/24 hr)	273	75	3.64
Rate of thymine deposition (nmol/24 hr)	77.5	21.3	3.64
In vitro			
[^{3}H]thymidine incorporation (pmol/hr per μg atom DNA-P)	644	130	—
Rate of [^{3}H]thymine deposition (nmol/24 hr)	23.2	6.9	3.36

Values for the amount and the deposition of DNA are from Patel *et al.* (1973). The rate of thymine deposition was calculated by taking 28.4% of DNA as thymine. The *in vitro* rate of [^{3}H]thymidine incorporation into DNA was linear for 20 hr in the forebrain and for 14 hr in the cerebellum. Using the values of the amount of DNA and slope of *in vitro* DNA labelling curves, the rate of [^{3}H]thymine deposition were calculated in the forebrain and the cerebellum. The *in vitro* results are from Barochovsky and Patel (1981).

the inhibition was dose-dependent with half-maximal effects at about $1\text{–}4 \times 10^{-4}$ M concentrations. The pharmacological agents affecting the monoaminergic systems showed at 1 mM concentration 69–98% inhibition in DNA labelling; in contrast, at this concentration, atropine and carbamylcholine, the drugs affecting cholinergic systems, were apparently ineffective (Table 4). However, at high concentrations (about 10^{-3} M) some of the effective drugs were shown to be either toxic or to affect the processes of cell replication directly (for references see, Patel *et al.*, 1980b; Patel and Lewis, 1981). Therefore, even though these *in vitro* results indicated that monoamines and drugs affecting monoamine neurotransmitter systems may be involved in the regulation of cell replication in the developing brain, the exact mechanisms of action remain to be determined.

Clinical implications

The belief that prenatal influences can have profound and long-lasting effects on the life of the newborn is very old (see Ferreira, 1965). However, the development of the science of teratology, including its behavioural aspects, has occurred only over the last two decades, spurred by such continuing human tragedies as the thalidomide disaster and Minimata disease.

The present findings show that a number of widely used psychotropic drugs are capable of producing a severe reduction in cell replication in the developing brain. The effects are greatest at high dose levels, but for certain drugs they are also found at a dose of the same order of magnitude as the largest doses given in clinical practice (Table 2). These effects on brain cell proliferation are of interest in relation to possible long-term functional effects of these drugs on the nervous system. As stated previously, a given nerve cell population is produced only at a certain period of development. If an adverse influence on neuronal acquisition were to be present on its "birth day", its number might be permanently depleted. This could result in significant and persistent alterations of brain circuitry and integrated brain function (Balázs, *et al.*,

Table 4. Effect of various pharmacological agents on forebrain cell replication *in vitro*

Drug	Known action	Half-maximal effect (mM)	% Inhibition at 1 mM
Chlorpromazine	DA-antagonist	0.31	98
Haloperidol	DA-antagonist	0.55	71
Thioridazine	DA-antagonist	0.55	97
Piribedil	DA-agonist	0.53	87
LSD	"5-HT-antagonist"	0.12	69
TFMTP	5-HT-agonist	0.41	95
Atropine	Muscarinic antagonist	—	5
Carbamylcholine	Cholinergic agonist	—	0

The forebrain slices were incubated in nutrient medium containing various concentrations of drugs. At 4 hr later the rate of [^{3}H]thymidine incorporation into DNA was measured and this is used as a measure of cell replication. The results are from Barochovsky and Patel (1981).

TFMTP, *N*-(α,α,α-Trifluoro-*m*-tolyl)piperazine.

1977; Patel, Balázs, Smith, Kingsbury and Hunt, 1980a).

In current clinical practice, drugs acting on the central nervous system are prescribed to pregnant and lactating mothers on a large scale. Medication with such drugs may be indicated not only for psychiatric disorders but for such physical conditions as *hyperemesis gravidarum*, hypertension and premature uterine contraction. In the puerperium, the lactating mother may receive treatment with psychotropic drugs. Thus, initially via the placenta and later via breast milk, the brain of the infant may be exposed to a sustained or repeated pharmacological challenge. Effects on cell proliferation of the type described in this paper could be brought into play, and the possibility of behavioural teratogenicity is clearly significant and worrying. A large body of experimental and clinical data has accumulated to suggest adverse effects of maternal psychotropic drug intake on the neurological and behavioural development of the offspring (see, Introduction; and Forfar and Nelson, 1973; Hill and Stern, 1979), though some of the human epidemiological surveys have produced conflicting results (Nahas and Goujard, 1979). However, there can be no doubt that, for a variety of reasons—such as pressure from patients and pharmaceutical companies, and desire to relieve symptoms which are not necessarily health threatening—physicians are tempted to overprescribe during pregnancy and lactation. They do so without a full knowledge of possible adverse effects of the preparations they prescribe, and often in the mistaken belief that significant developmental events are restricted to the first trimester of pregnancy. The data presented in this paper, seen against the relevant scientific background, argue strongly for greatly increased caution in the administration of drugs acting on the nervous system to pregnant and breast feeding mothers.

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