Neuropeptide Effects on Brain Development to be Expected From Behavioral Teratology¹

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BOER, G. J. AND D. F. SWAAB *Neuropeptide effects on brain development to be expected from behavioral teratology. PEPTIDES 6: Suppl. 2, 21-28, 1985.*—A wealth of literature has become available about lasting functional consequences of perinatal psychotropic drug exposure, having affected brain development in a subtle rather than gross structural way (behavioral teratology or functional neuroteratology). The underlying mechanism is thought to result from changing levels of neurotransmitters during neurogenesis induced by these neuroactive drugs, which as a consequence appears to lead to impaired cell acquisition and receptor setting i.e., to irreversible changes in particular neuronal circuitries. Neuropeptides are true candidates for a neurotransmission function as well, and are also present early in brain development. As for the classical neurotransmitters, a role for neuropeptides in the growth and functional organization of the nervous system, might therefore be expected. Anomalies in neuropeptide levels also would lead to functional neuroteratology. Although not overwhelming, several studies support this view, and the current state is summarized in this paper: a trophic role for some neuropeptides as well as neuroteratological effects upon perinatal manipulation for others were revealed. However, more detailed studies are necessary, certainly also because of the crying need for exposing possible adverse effects at a time when clinical applications of neuropeptides and their analogues are becoming a mode.

Neuropeptides Brain development Behavioral teratology

FETAL brain development involves a specific sequence of events including (a) cell generation in the germinal layers, (b) subsequent migration, and (c) differentiation into mature neurons which become incorporated into functional nervous circuitries by outgrowth of neurites and formation of synapses. Different types of neurons are generated in different regions and stages of ontogeny [2,82], so that the functional integration of a given type of neuron with others in the network has a well-defined timing. It is easy to understand therefore that the development of complex neuronal circuitries will be sensitive to delay or advancement, and to increase or decrease in any of the events mentioned above, i.e., neurogenesis, migration and maturation. Such changes might give rise not only to altered organization at the cellular level, but also to changes visible at the macroscopical level.

The overall process of neurogenesis is controlled by intrinsic genetic programs in interaction with epigenetic factors such as trophic or growth factors (e.g., somatomedins [89] and hormones (e.g., thyroid hormones [66,77]; corticosteroids [20, 32, 77]; gonadal hormones [69]), and functional factors involved directly or indirectly in neurotransmission (see below). Neuropeptides are a set of substances which may have representatives in all these classes of compounds.

BEHAVIORAL TERATOLOGY

Over the last decade a wealth of literature has become

available about lasting functional consequences of perinatal psychotropic drug exposure (behavioral teratology or functional neuroteratology; Table 1). Since gross structural changes of the brain are usually not patently visible, such drugs are supposed to affect brain development in a subtle way. Nonetheless, they may drastically change the functional capacity of the brain. Many of these neuroactive drugs have been shown to interfere specifically with particular neurotransmission systems in the adult brain. For this very reason the underlying mechanisms of perinatal drug-induced behavioral alterations are thought to result from changing levels of neurotransmitters during brain development [64, 78, 79]. Or to put it differently, neurotransmitters are indispensable factors during development for the normal organization of the brain.

Biochemical and morphological evidence to support this last statement is accumulating. First of all, classical neurotransmitters such as acetylcholine and the catecholamines (adrenaline, noradrenaline, dopamine and serotonin) are present in the developing brain from the outset, long before they have any apparent transmitter function. They can be detected in fertilized egg cells [28,53] and in the initial phases of neural tube formation [53]. Various embryonic structures can, moreover, accumulate amines during early morphogenesis, while as the process of neurulation nears completion, the capacity of the neural tube to concentrate noradrenaline is gradually lost [64]. Drug-induced alterations

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TABLE I

SELECTIVE LIST OF NEUROPSYCHOTROPIC DRUGS SHOWN TO IMPAIR BEHAVIOR AND NEUROBIOLOGY OF RAT OFFSPRING PERMANENTLY WHEN GIVEN PERINATALLY

antihypertensives	antidepressant
propanolol	imipramine
methyl dopa	
reserpine	amphetamine
clonidine	
anticonvulsants	methylxanthines
valproate	(caffeine, theophylline)
barbiturates	
hydantoin	nicotine
carbamazepine	
diazepam	marihuana
neuroleptics	opiates
clozapine	
chlorpromazine	LSD
haloperidol	
pimozide	6-OH-dopa
	aspirin

Taken from reviews [5, 24, 35, 42, 65, 78, 79, 81, 104].

in catecholamine metabolism in the very early stages of neurulation are able to disturb processes like neural tube closure, brain organogenesis and somite differentiation [59]. These phenomena indicate that some, if not all, transmitters may play a neurohumoral role during embryonic brain development.

The aminergic systems described for the adult brain appear later, but at any rate long before neurogenesis has been completed fully. In the rat, morphological evidence is available that adrenergic and serotonergic pathways are present from respectively gestational day 15 [90] and 19 onwards [68]. Blockade of such systems during prenatal and postnatal development has been shown to impair neurogenesis. For instance, Lauder and Krebs [63, 64, 65] have shown that maternal treatment with p-chlorophenylalanine (which blocks serotonin synthesis) exhibits clearcut changes in [³H]thymidine incorporation, i.e., DNA or cell acquisition between days 12 and 16 prenatally. This treatment revealed either a suppression of the onset of cell generation or of its peak value, as well as a prolongation of cell synthesis in regions of the posterior diencephalon known to become innervated by serotonergic fibers. In addition, the application of a variety of psychotropic drugs has been reported to reduce cell acquisition in cerebral cortex and cerebellum both in vivo and in vitro in 11-day-old rat pups [78,79]. Apart from the above-mentioned effects on cell generation, persistent changes in neurotransmission result from early exposure to neuroactive drugs. For instance, haloperidol (which blocks dopamine receptors) induced a permanent decrease in number of dopamine receptors in the striatum when administered to pregnant rat dams [83], whereas L-dopa (which increases dopamine synthesis) permanently increases receptor density [43]. The early development of dopamine receptor systems therefore appeared to be sensitive to the availability of ligand and may show a persistent up-down regulation depending on dopamine access. Such effects are the opposite of those produced in adult brain, where the system adapts to changes in the overall magnitude of the incoming signal [43,91]. For haloperidol, this reversal of effects already takes

place before the end of the second postnatal week [42]. Apparently, the eventual effect observed is dependent on the developmental stage in which a change in transmitter level is brought about. This presumably explains why, e.g., 6-hydroxydopamine given neonatally in rats in order to block noradrenergic transmission can have opposite, lasting effects on the regional total number of beta-adrenergic receptors [57]. It revealed higher levels in the cerebral cortex and lower levels in the cerebellum, the latter being less mature at the time of treatment.

From the type of observations described above, and reviewed recently by Patel *et al.* [78,79], Brown and Fishman [24], Swaab and Mirmiran [104], and Lauder and Krebs [64,65], it can be concluded that neurotransmitters act as neurohumors controlling cell replication and neurite and receptor formation at certain stages of brain development. Anomalies in the nerve cell acquisition could consequently result in significant and persistent alterations in brain circuitry and integrated brain function. The same holds true for changes in the developmental setting of the final number of receptors and maybe their responsiveness. The latter may turn out to be a key-factor in behavioral teratology, because this is the place where information is passed on.

NEUROPEPTIDES

The observations of De Wied *et al.* [38], and others (cf. [102]) that pituitary hormones like vasopressin (VP), adrenocorticotrophic hormone (ACTH) and α -melanocyte stimulating hormone (α -MSH) interact with behavior in the rat, kindled interest in brain-born peptides with central functions (neuropeptides). Immunocytochemistry has contributed a great deal to support the concept that neuropeptides act directly upon the nervous system by providing morphological evidence for the existence of neurons that synthesize neuropeptides and innervate other parts of the brain by means of peptidergic fibers. Immunocytochemistry is also continuously enlarging the list of neuropeptides which already far outnumbers the 'classical' neurotransmitters, viz., acetylcholine, monoamines and aminoacids [99,102]. Some of these neuropeptides were known earlier as neurohormones (e.g., the neurohypophyseal peptides VP and oxytocin (OT) along with their phylogenetic homologues, as well as the hypophyseotropic hormones such as luteinizing hormone-releasing hormone (LHRH), somatostatin, etc.) or as pituitary hormones (mainly the family of pro-opiomelanocortin peptides). Later, peripheral peptides (e.g., vasoactive intestinal peptide (VIP), angiotensin II, cholecystokinin) and peptides originally characterized in invertebrates (e.g., bombesin, hydra-head-activator) were also shown to be present in certain mammalian neurons, and, at last, also previously unknown neuropeptides were discovered in mammalian brain tissue (neuropeptide Y (NP-Y), dynorphin, etc.) (for reviews see [99,102]).

Neuropeptides seem to influence information processing within the brain. Their candidacy as putative neurotransmitters has been clearly established by ultrastructural immunocytochemical evidence for their presence in nerve terminals, as well as the identification of extensive peptidergic pathways in the nervous system (e.g., hypothalamic VP cells innervate neurons from bulbus olfactorius all the way down to the spinal cord [37]) [12,25]. In addition, neuropeptides may act as neuromodulators thus influencing inter-neuronal communication through a variety of mechanisms. Going through the list of criteria which a 'true' neurotrans-

TABLE 2
TROPIC EFFECTS OF NEUROPEPTIDES

Neuropeptide	Observed Effects	References
VP	mitogenic in various in vitro cell systems of rat thymocytes rat chondrocytes mouse 3T3 fibroblasts rat renal epithelial cells rat adrenal glomerulosa cells mitogenic in bone marrow cells at haemorrhage stimulates liver regeneration promotes neurite outgrowth of spinal cord neurons in vitro (<i>Xenopus</i>)	[115] [71] [84] [113] [80] [52] [86] [47]
α -MSH	stimulates brain cell maturation promotes peripheral nerve regeneration induces faster recovery from central lesions prevents bone resorption in hypophysectomized rat stimulates dendrite formation in melanocytes in newborn mice stimulates liver regeneration counteracts corticosteroid-reduced somatic growth of chick embryos	[98] [11] [110] [95] [51] [106] [100]
ACTH	promotes peripheral nerve regeneration	[96]
subst. P	counteracts 6-OH-dopamine-induced denervation	[58]
bombesin	mitogenic for in vitro cell systems of mouse 3T3 fibroblasts human bronchial mucosa cells	[85] [114,116]

mitter should meet, as reformulated by Barchas *et al.* [4]. Buijs [25] concluded in 1982 that for VP and OT neurons many of the criteria already had been met. Since then, evidence both for the stimulus-secretion phenomena of VP [26] and for the presence of VP [7, 39, 107] and OT binding sites [21,108] in the brain has been provided, which puts both VP and OT squarely in the group of neuropeptides such as the enkephalins and substance P, for which evidence for a neurotransmitter action is very strong.

Neuropeptides can be demonstrated immunocytochemically early in development [101]. Data analogous to that available for the classical neurotransmitters demonstrating their presence even prior to the first peak of neurogenesis (around day 13 prenatally in rat), are (yet?) lacking, but they are certainly present shortly thereafter. NP-Y is assayable on gestational day 13 [41] and somatostatin and substance P on day 14 [70]. VP and OT are assayable from fetal day 14 and 17 onwards [92], whereas vasopressinergic fibers appear immunocytochemically on day 16 [13,27]. Peptides of the pro-opiomelanocortin family are present on gestational day 15 [8,100], thyroid stimulating hormone releasing hormone (TRH) on day 18 [31] and the earliest observations for LHRH were made on day 20 of gestation [36]. VIP and bombesin appeared on the day of birth [70], whereas neurotensin seems not to be present prior to day 10 postnatally [3].

Considering that (a) neuropeptides are sometimes not only neurohormones but form also an important class of putative neurotransmitters, (b) they are present in the fetal brain soon after the first peak of neurogenesis and long before neurogenesis ceases (day 22 postnatally in rat [2]), and (c) both hormones and classical neurotransmitters have been found to be of importance in the growth and functional

organization of the nervous system, a role for neuropeptides in brain development is to be expected [12,99]. In addition, therefore administration of such compounds during brain development or blocking the action of the endogenous substances might thus cause behavioral teratology [103]. The possible importance of neuropeptides in early development is further stressed by studies on VP and α -MSH revealing that these peptides are present early prenatally (day 17) in even higher concentrations than just before birth (day 19–20) [27, 100, 101]. In another study, somatostatin immunostaining even shows its presence in the developing cerebellum but not later on [55].

GROWTH EFFECTS OF NEUROPEPTIDES

In addition to the trophic effects of circulating neuropeptide hormones (whether or not synthesized in the brain) on their target system, the literature provides some support for growth effects in other systems as well. Table 2 summarizes these scanty data, and shows that VP is frequently found to be mitogenic while α -MSH is a potent trophic factor for nerve cells.

With respect to VP, the only observation related to nerve cell growth comes from Gruener *et al.* [47], who reported increased outgrowth and thickening of neurites from dispersed neurons of embryonic *Xenopus* spinal cord grown in culture together with VP. In addition, a number of peripheral metabolic actions of VP are known such as inhibition of the generation of free fatty acids upon ketonurea [29], stimulation of glucagon release [40], of ornithine decarboxylase [54] and of brain uptake of the RNA-precursor orotic acid [62]. These findings, along with observation that fetal growth dis-

TABLE 3
SURVEY OF BRAIN FUNCTIONAL TERATOLOGY OF NEUROPEPTIDES IN RAT

Neuropeptide Level		Period of Change	Anomalies Observed	References
VP	↓	life time (Brattleboro rat)	among other observations: disturbed brain growth changed catecholamine innervation "memory" deficits impaired temperament-related tests hyperresponsiveness	[13, 17, 19, 46] e.v. [111] e.v. [109] [22,23] [44]
VP	↑	postnatal	central diabetes insipidus?	[18]
VT (kitten)	↑	postnatal	reduced total lipid brain content delayed eye opening reduced locomotion increased active sleep	[45]
α -MSH	↓	late prenatal	disturbs brain cell maturation	[98]
α -MSH	↑	postnatal	improves learning, memory and attention tasks accelerated development of motor behavior advances eye opening reduced feedback of α -MSH on DA neurons	[9, 10, 30, 73, 74, 75] [87] [105] [67]
TRH	↑	postnatal	increased weight hypothalamus impaired T-maze learning	[97]
CRF	↑	postnatal	eye opening accelerated rearing enhanced body temperature regulation impaired	[119]
subst. P	↑	postnatal	increase brain receptors Substance P increased pain perception	[50] [49]

Effects of endogenous opioids have been omitted, see text.

turbances enhance VP release [76], led to the conclusion that VP may be regarded as a neuropeptide possessing potency for the stimulation of body and brain growth.

BRAIN FUNCTIONAL TERATOLOGY OF NEUROPEPTIDES

Rat studies on the teratological effects of prenatally applied *opioids* were greatly stimulated by clinical observations concerning the neonatal narcotic abstinence syndrome and behavioral disturbances in children of narcotic mothers (see review [121]). Rats exposed to methadon during development showed delays in (a) reflex ontogeny, (b) eye-opening, as well as (c) somatic and brain growth (the brain cell content was regionally reduced and catecholamine systems were altered). In addition, (d) hyperactivity, (e) increased emotionality, (f) learning disabilities and (g) dysfunctions of thermoregulation and (h) increased nociceptive thresholds were found. Several of these effects were also seen following morphine and heroin treatment in rats. Detrimental effects on development have been shown to be blocked by concomitant administration of the opioid antagonist naloxone [33], whereas this drug alone accelerated postnatal behavioral development [112]. Naltrexone, another potent narcotic antagonist, appeared capable of en-

hancing general brain growth and cerebellar cell generation [120]. All together these data have been interpreted as indicating that opioid antagonist actions on brain development may reside at the opiate receptor [120,121]. In fact, opioid receptors are present in spinal cord at day 16 prenatally and prenatal morphine exposure increases adult number of spinal cord opiate receptors as well as their affinity [61].

With the large body of evidence for neurodysgenesis and permanently changes in behavior after perinatal exposure with opioids of plant and of synthetic origin, a possible role of the endogenous opioids in normal brain development is clear, but is not often discussed in relation with the pro-opiomelanocortin family of peptides. Plant and synthetic opioids certainly interfere with the endogenous opioid systems of enkephalins and endorphins in adult rat [94], and therefore may well be capable of affecting brain developmental processes in which these neuropeptides are involved. In fact, some studies with the endogenous opioids themselves have proven to have organizational effects on the nervous system. Neonatal injections with β -endorphin induce an elevated threshold for the tail flick response in adult rats [88], and correlate with a reduced β -endorphin immunostaining in several brain regions [72]. Similar treatment with Met-enkephalin facilitates maze learning [60].

Considerably less data are available about possible effects of other neuropeptides on brain development and their functional teratology (Table 3). The absence of centrally synthesized *vasopressin* in the developing Brattleboro rat coupled with peculiar regional brain growth deficits, has been interpreted as an argument in favor of a role for VP in brain development [14]. Since this impaired nervous growth could not be cured by long-term postnatal VP treatment [18], and since at birth a stunted neurogenesis is already present [17], a prenatal requirement for VP must be assumed. Moreover, a variety of behavioral and functional deficits are well-known for the adult Brattleboro rat, a few of which are mentioned in Table 3. Some of these abnormalities cannot be reversed by VP supplementation in adulthood [44], whereas blood pressure regulation [3], in which VP is centrally involved is quite normal in the Brattleboro rat despite its VP deficiency. Such observations may reflect permanent changes in central systems regulating some types of behavior and on the other hand complete functional compensation in the case of a life-sustaining action, that of blood pressure regulation.

A 3-week neonatal treatment with two daily injections of 2.5 μ g VP has no effect on brain size (Snijdwint and Boer, unpublished observations), but, as reported in other studies [48,118], induces a permanent increase in urine output. Kidney responsiveness decreased because of down-regulation of number of VP receptors [48,50]. Since a similar effect was seen in the polyurinating VP-deficient Brattleboro rat after a short exposure to VP in the first postnatal week, and kidney responsiveness in this case seemed normal [18], the possibility of permanent changes in other, especially central, systems involved in body water regulation mechanisms can by no means be excluded (Table 3). Opposite effects were recently seen for maternal *oxytocin* exposure, i.e., decreased urine production of rat offspring [15,16]. Since such opposite reactions of VP and OT are also seen in the development of the aorta VP receptors [34], these heterologues may have opposite effects on the CNS during ontogeny just as they have opposite effects on avoidance behavior in adult rats [38,109].

In a recent study of Goldstein [45], who administered chronically either VP, OT, [Lys⁸]VP (LVP) or *vasotocin* (VT) to kittens, only VT turned out to produce detrimental effects on the brain. Eye-opening was delayed and brain lipid content reduced, while behaviorally reduced locomotion and increased periods of rapid eye-movement sleep were induced.

Blockade of fetal α -melanocyte stimulating hormone late in pregnancy by means of *in utero* subcutaneously injected MSH antiserum in rat, reduced body and brain growth [98]. Brain cell number proved not to be affected, but there was a decrease in average cell size, pinpointing α -MSH as a necessary maturation factor in normal brain ontogeny. Such a role correlates with the preferential processing of the pro-opiomelanocortin into α -MSH as compared to ACTH, during early developmental stages [1, 6, 100]. In the adult, the stimulatory capacity of α -MSH (but also of ACTH and MSH-fragments) on regeneration after either peripheral nerve crush or a central lesion (Table 2) also points to a neurotrophic effect of this

molecule. Increased α -MSH levels during early postnatal life in the rat advances eye-opening [105] and accelerates the ontogeny of motor behavior [87], while several learning, memory and attention tasks are better performed even long after the treatment has been terminated (Table 3). Therefore α -MSH treatment could be a promising approach for counteracting subnormal brain growth [100]. On the other hand, side-effects of the use of MSH-like compounds may be expected. For example, Lichtensteiger *et al.* [67] showed that neonatal treatment with α -MSH causes lasting effects on the feedback of this peptide on the dopamine neurons that inhibit α -MSH release from the intermediate lobe of the pituitary.

Finally there are a few published observations on lasting neuropeptidergic effects upon rat brain development by TRH, corticotroph hormone releasing factor (CRF) and substance P. Postnatal treatment with TRH increased hypothalamic weight while impairing T-maze learning, CRF accelerated eye-opening, enhanced rearing in an open field but impaired body temperature regulation, and substance P increased pain perception and substance P brain receptors were upregulated regionally (see Table 3).

FINAL REMARKS

Although the amount of data reviewed above is not overwhelming, indications already exist that neuropeptidergic changes induced in developing brain can affect both nerve cell acquisition and receptor setting in a way similar to that reported for the behavioral teratological effects of neuroactive drugs interacting with the classical neurotransmitter systems. However, more detailed studies are necessary to further disclose possible subtle disturbances in brain functions as well as in the underlying mechanisms. One obvious way in which to perform such studies is to look, following perinatally changed neuropeptide function, for the control of neurogenesis in areas innervated by the neuropeptide under investigation, to assay its receptors and to test for possible disorientation of behavior known to be related. On the other hand, since next to its transmitter action the route to the target areas of a neuropeptide can be the cerebrospinal fluid [99] or even the systemic blood circulation (blood-brain barrier in rat appears to be mature only by the end of the third week of postnatal life [56]), other parts of the brain should also be subjected to biochemical and psycho-teratogenic tests.

The importance of such animal studies resides not only in the scientific interest in the early neurobiological role of neuropeptides in the complex timetable of nervous system development, but certainly also in the crying need for revealing possible adverse effects at a time where clinical applications of neuropeptides are starting to become the mode [15,103].

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REFERENCES

1. Alessi, N. E. and P. Quinhan. Postnatal development of ACTH and α -MSH in the medulla oblongata of rat: α -MSH is the predominant peptide. *Peptides* 6: Suppl 2, 137-141, 1985.
2. Altman, J. DNA metabolism and cell proliferation. In: *Handbook of Neurochemistry*, vol II. New York: Plenum Press, 1969, pp. 137-182.

3. Aperia, A., P. Herin, A.-C. Eklöv and V. Johnsson. Importance of AVP for blood pressure control during development: a study in the Brattleboro rat. *Ann NY Acad Sci* 394: 350-360, 1982.
4. Barchas, J. D., H. Akil, G. R. Elliott, R. B. Holman and S. J. Watson. Behavioral neurochemistry: neuro-regulators and behavioral states. *Science* 200: 964-973, 1978.
5. Barlow, S. M. and F. M. Sullivan. Behavioural teratology. In: *Teratology*, edited by C. L. Berry and D. E. Poswilllo. Berlin: Springer-Verlag, 1975, pp. 103-120.
6. Barnea, A., G. Cho and J. C. Porter. Disproportionate accumulation of immunoreactive corticotropin, melanotropin and lipotropin in the brain of the maturing brain. *Endocrinology* 109: 1990-1998, 1981.
7. Baskin, D. G., F. Petracca and D. M. Dorsa. Autoradiographic localization of specific binding sites for (³H)-arginine 8-vasopressin in the septum of the rat brain with tritium-sensitive film. *Eur J Pharmacol* 90: 155-158, 1983.
8. Brayon, A., W. J. Shoemaker, F. F. Bloom, A. Manns and R. Guillemain. Perinatal development of the endorphin- and enkephalin-containing systems in the rat brain. *Brain Res* 179: 93-101, 1979.
9. Beckwith, B. E., R. K. O'Quin, M. S. Petro, A. J. Kastin and C. A. Sandman. The effects of neonatal injections of α -MSH on the open-field behavior of juvenile and adult rats. *Physiol Psychol* 5: 295-299, 1977.
10. Beckwith, B. E., C. A. Sandman, D. Hothersall and A. J. Kastin. Influence of neonatal injections of α -MSH on learning, memory and attention in rats. *Physiol Behav* 18: 63-71, 1977.
11. Bijlsma, W. A., P. Schotman, F. G. I. Jennekens, W. H. Gispen and D. De Wied. The enhanced recovery of sensorimotor function in rats is related to the melanotropic moiety of ACTH/MSH neuropeptides. *Eur J Pharmacol* 92: 231-236, 1983.
12. Boer, G. J., D. F. Swaab, H. B. M. Uylings, K. Boer, R. M. Buijs and D. N. Velis. Neuropeptides in rat brain development. *Prog Brain Res* 53: 207-227, 1980.
13. Boer, G. J., R. M. Buijs, D. F. Swaab and G. J. De Vries. Vasopressin and the developing rat brain. *Peptides* 1: Suppl 1, 203-209, 1980.
14. Boer, G. J., C. M. F. van Rheenen-verberg and H. B. M. Uylings. Impaired brain development of the diabetes insipidus Brattleboro rat. *Dev Brain Res* 3: 557-575, 1982.
15. Boer, G. J. and D. F. Swaab. Long-term effects on brain and behavior of early treatments with neuropeptides. In: *Application of Behavioral Pharmacology in Toxicology*, edited by G. Zbinden, V. Cuomo, G. Racagni and B. Weiss. New York: Raven Press, 1983, pp. 251-263.
16. Boer, G. J. and J. Kruisbrink. 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, 1984.
17. Boer, G. J., M. H. Dozy and H. B. M. Uylings. Cerebellar DNA and tissue water changes in the brain of diabetes insipidus Brattleboro rats are already present at birth. *Int J Dev Neurosci* 2: 301-304, 1984.
18. Boer, G. J., R. Kragten, J. Kruisbrink and D. F. Swaab. Vasopressin fails to restore postnatally the stunted brain development in the Brattleboro rat, but affects water metabolism permanently. *Neurobehav Toxicol Teratol* 6: 103-109, 1984.
19. Boer, G. J. Vasopressin and brain development; studies using the Brattleboro rat. *Peptides* 6: Suppl 1, 49-62, 1985.
20. Bohn, M. C. Glucocorticoid induced teratologies of the nervous system. In: *Neurobehavioral Teratology*, edited by J. Yanai. Amsterdam: Elsevier, 1984, pp. 365-387.
21. Brinton, R. E., J. K. Wamsley, K. W. Gee, Y.-P. Wan and H. I. Yamamura. [³H]oxytocin binding sites in the rat brain demonstrated by quantitative light microscopic autoradiography. *Eur J Pharmacol* 102: 365-367, 1984.
22. Brito, G. N. O., G. J. Thomas, S. I. Gingold and D. M. Gash. Behavioral characteristics of vasopressin-deficient rats (Brattleboro strain). *Brain Res Bull* 6: 71-75, 1980.
23. Brito, G. N. O., G. J. Thomas, D. M. Gash and J. H. Kitchen. The behavior of Brattleboro rats. *Ann NY Acad Sci* 394: 740-748, 1982.
24. Brown, R. M. and R. H. B. Fishman. An overview and summary of the behavioral and neural consequences of perinatal exposure to psychotropic drugs. In: *Neurobehavioral Teratology*, edited by J. Yanai. Amsterdam: Elsevier, 1984, pp. 3-54.
25. Buijs, R. M. The ultrastructural localization of amines, amino acids and peptides in the brain. *Prog Brain Res* 55: 167-183, 1982.
26. Buijs, R. M. and J. J. Van Heerikhuizen. Vasopressin and oxytocin release in the brain—a synaptic event. *Brain Res* 252: 71-76, 1982.
27. Buijs, R. M., D. N. Velis and D. F. Swaab. Ontogeny of vasopressin and oxytocin in the fetal rat: Early vasopressinergic innervation of the fetal brain. *Peptides* 1: 315-324, 1980.
28. Buzkinov, G. A., A. V. Sakkarova, B. N. Manukhin and L. V. Markova. The role of neurohumors in early embryogenesis. IV. Fluorometric and histochemical study in cleaving eggs and larvae of sea urchins. *J Embryol Exp Morphol* 27: 339-351, 1972.
29. Caisová, D., J. Sáva and A. Stajner. Modification of fat and carbohydrate metabolism by neurohypophyseal hormones. II. Effects of lysine-vasopressin on non-esterified fatty acid, glucose, triglyceride and cholesterol levels in the serum of male rats. *Endocrinologie* 76: 326-332, 1980.
30. Champney, T. F., T. L. Sahley and C. A. Sandman. Effects of neonatal cerebral ventricular injection of ACTH 4-9 and subsequent adult injection on learning in male rat and female albino rats. *Pharmacol Biochem Behav* 5: 3-9, 1976.
31. Conklin, P. M., W. J. Schindler and S. F. Hull. Hypothalamic thyrotropin releasing factor. Activity and pituitary responsiveness during development of the rat. *Neuroendocrinology* 11: 197-211, 1973.
32. Cotterrell, M., R. Balász and A. L. Johnson. Effects of corticosteroids on the biochemical maturation of rat brain: postnatal cell formation. *J Neurochem* 19: 2151-2167, 1972.
33. Crofford, M. and A. A. Smith. Growth retardation in young mice treated with dl-methadone. *Science* 181: 947-949, 1973.
34. Csaba, G., A. Rónai, V. László, Z. Darvas and I. Berzsetai. Amplification of hormone receptors by neonatal oxytocin and vasopressin treatment. *Horm Metab Res* 12: 28-31, 1980.
35. Cuomo, V., R. Cagiano, I. Mochetti, E. Coen, F. Cattabeni and G. Racagni. Biochemical and behavioral effects after early postnatal administration of neuropeptides in rats. In: *Application of Behavioral Pharmacology in Toxicology*, edited by G. Zbinden, V. Cuomo, G. Racagni and B. Weiss. New York: Raven Press, 1983, pp. 173-185.
36. Daikoku, S., H. Kanano, H. Matsumura and S. Saito. In vivo and in vitro studies on the appearance of LHRH neurons in the hypothalamus of perinatal rats. *Cell Tissue Res* 194: 433-445, 1978.
37. De Vries, G. J., R. M. Buijs, F. W. Van Leeuwen, A. R. Caffé and D. F. Swaab. The vasopressinergic innervation of the brain in normal and castrated rats. *J Comp Neurol* 233: 236-254, 1985.
38. De Wied, D., B. Bohus and Tj. B. Van Wimersma-Greidanus. The hypothalamo-neurohypophyseal system and the preservation of conditioned avoidance behavior in rats. *Prog Brain Res* 41: 417-427, 1974.
39. Dorsa, D. M., L. A. Majumdar, F. M. Petracca, D. G. Baskin and L. E. Cornett. Characterization and localization of [³H] arginine vasopressin binding to rat kidney and brain tissue. *Peptides* 4: 699-706, 1983.
40. Dunning, B. E., J. H. Moltz and C. P. Fawcett. The effects of oxytocin and vasopressin on release on insulin and glucagon from pancreatic islets in vitro. *Neuroendocrinol Lett* 4: 89-93, 1982.
41. Foster, G. A. and M. Schultzberg. Immunohistochemical analysis of the ontogeny of neuropeptide Y immunoreactive neurons in foetal rat brain. *Int J Dev Neuroscience* 2: 387-407, 1984.

42. Friedhoff, A. J. and J. C. Miller. Prenatal psychotropic drug exposure and the development of central dopaminergic and cholinergic neurotransmitter systems. *Monogr Neural Sci* 9: 91-98, 1983.
43. Friedhoff, A. J., K. A. Bonnet and H. Rosengarten. Reversal of two manifestations of dopamine receptor supersensitivity by administration of L-dopa. *Res Commun Chem Pathol Pharmacol* 16: 411-423, 1977.
44. Gash, D. M., P. H. Warren, L. B. Dick, J. R. Sladek, Jr. and J. R. Ison. Behavioral modification in Brattleboro rats due to vasopressin administration and neural transplantation. *Ann NY Acad Sci* 394: 672-681, 1982.
45. Goldstein, R. The involvement of arginine vasotocin in the maturation of the kitten brain. *Peptides* 5: 25-28, 1984.
46. Greer, E. R., M. C. Diamond and J. M. W. Tang. Effect of age and enrichment on certain brain dimensions in Brattleboro rats deficient in vasopressin. *Exp Neurol* 75: 11-22, 1982.
47. Gruener, R., R. E. Brinton and H. I. Yamamura. Vasopressin promotes neurite outgrowth in culture. 13th Annual Meeting of the Soc. for Neuroscience, Boston, 206, 1983.
48. Handelsmann, G. E., J. T. Russell, H. Gainer, R. Zerbe and M. Bayorh. Vasopressin administration to neonatal rats reduces antidiuretic response in adult kidney. *Peptides* 4: 827-832, 1984.
49. Handelsmann, G. E., J. H. Selsky and C. J. Helke. Substance P administration to neonatal rats increases adult sensitivity to substance P. *Physiol Behav* 33: 297-300, 1984.
50. Handelsmann, G. A role for neuropeptides in the development of their receptors. *Peptides*, 1985, in press.
51. Hirobe, T. Stimulation of dendritogenesis in the epidermal melanocytes of newborn mice by melanocyte stimulating hormone. *J Cell Sci* 33: 371-383, 1978.
52. Hunt, N. H., A. D. Perris and P. A. Sandford. Role of vasopressin in the mitotic response of rat bone marrow cells to haemorrhage. *J Endocrinol* 72: 5-16, 1977.
53. Ignarro, L. J. and F. E. Shideman. Appearance and concentrations of catecholamines and their biosynthesis in embryonic and developing chick. *J Pharmacol Exp Ther* 159: 38-48, 1968.
54. Ikeno, T. and G. Gureff. The effect of vasopressin on the activity of ornithine decarboxylase in rat brain and liver. *J Neurochem* 33: 973-975, 1979.
55. Inagaki, S., S. Shiosaka, K. Takatsuki, H. Iida, M. Sakanaka, E. Senba, Y. Hara, T. Matsuzaki, Y. Kawai and M. Tokuyama. Ontogeny of somatostatin-containing neuron system of the rat cerebellum including its fiber connections; an experimental and immunohistochemical analysis. *Dev Brain Res* 3: 509-527, 1982.
56. Johanson, C. E. Permeability and vascularity of the developing brain: Cerebellum vs. cerebral cortex. *Brain Res* 190: 3-16, 1980.
57. Jonsson, G. and H. Hallman. Changes in β -receptor binding sites in rat brain after neonatal 5-hydroxydopamine treatment. *Neurosci Lett* 9: 27-32, 1978.
58. Jonsson, G. and H. Hallman. Substance P modifies the 6-hydroxydopamine induced alteration of postnatal development of central noradrenaline neurons. *Neuroscience* 7: 2909-2918, 1982.
59. Jurand, A. Malformations of the central nervous system induced by neurotropic drugs in mouse embryos. *Dev Growth Diff* 22: 61-70, 1980.
60. Kastin, A. J., R. M. Kostrzewa, A. V. Schally and D. H. Coy. Neonatal administration of met-enkephalin facilitates maze performance of adult rats. *Pharmacol Biochem Behav* 13: 883-886, 1980.
61. Kirby, M. L. Alterations in fetal and adult responsiveness to opiates following various schedules of prenatal morphine exposure. In: *Neurobehavioral Teratology*, edited by J. Yanai. Amsterdam: Elsevier, 1984, pp. 233-248.
62. Landgraf, R., J. Hess and A. Ermisch. The influence of vasopressin on the regional uptake of [3 H]orotic acid by rat brain. *Acta Biol Med Germ* 37: 655-658, 1978.
63. Lauder, J. M., J. A. Wallace, M. B. Wilkie, A. DiNome and H. Krebs. Roles for serotonin in neurogenesis. *Monogr Neural Sci* 9: 3-10, 1983.
64. Lauder, J. M. and H. Krebs. Neurotransmitters in development as possible substrates for drugs of use and abuse. In: *Neurobehavioral Teratology*, edited by J. Yanai. Amsterdam: Elsevier, 1984, pp. 289-314.
65. Lauder, J. M. and H. Krebs. Humoral influences on brain development. *Adv Cell Neurobiol* 5: 3-50, 1984.
66. Legrand, J. Effects of thyroid hormones on central nervous system development. In: *Neurobehavioral Teratology*, edited by J. Yanai. Amsterdam: Elsevier, 1984, pp. 331-363.
67. Lichtensteiger, W., M. Schlumpf and M. D. Davis. Pituitary peptide actions on early neuroendocrine organization. 4th Int. Congress of the Int. Soc. for Developm. Neurosci., Salt Lake City, 6, 1983.
68. Lidov, H. G. W. and M. E. Molliver. Immunohistochemical study of the development of serotonergic neurons in the rat CNS. *Brain Res Bull* 9: 559-604, 1982.
69. McEwen, B. S. Gonadal steroid influences on brain development and sexual differentiation. *Int Rev Physiol* 27: 99-145, 1983.
70. McGregor, G. P., P. L. Woodmans, D. J. O'Shaughnessy, M. A. Ghatei, J. M. Polak and S. R. Bloom. Developmental changes in bombesin, substance P, somatostatin and vasoactive intestinal polypeptide in the rat brain. *Neurosci Lett* 28: 21-27, 1982.
71. Miller, R. P., F. Husain, M. Svensson and S. Lohin. Enhancement of [3 H-methyl]thymidine incorporation and replication of rat chondrocytes grown in tissue culture by plasma, tissue extracts and vasopressin. *Endocrinology* 100: 1365-1447, 1977.
72. Moldow, R. L., A. J. Kastin, C. S. Hollander, D. H. Coy and C. A. Sandman. Brain beta-endorphin-like immunoreactivity in adult rats given beta-endorphin neonatally. *Brain Res Bull* 7: 638-686, 1981.
73. Nyakas, C. Behavioral effects of infantile administration of glucocorticoid hormones. In: *Application of Behavioral Pharmacology in Toxicology*, edited by G. Zbinden, V. Cuomo, C. Racagni and B. Weiss. New York: Raven Press, 1983, pp. 265-276.
74. Nyakas, C. Influence of corticosterone and ACTH on the postnatal development of learning and memory functions. In: *Hormones and Brain Function*, edited by K. Lissak. New York: Plenum Press, 1975, pp. 83-89.
75. Nyakas, C., G. Levay, J. Viltsek and E. Endroczi. Effects of neonatal ACTH 4-10 administration on adult adaptive behavior and brain tyrosine hydroxylase activity. *Dev Neurosci* 4: 225-232, 1981.
76. Oosterbaan, H. P., D. F. Swaab and G. J. Boer. Increased amniotic vasopressin levels in experimentally growth-retarded rat fetuses. *J Dev Physiol* 7: 89-97, 1985.
77. Patel, A. J. and R. Balász. Hormones and cell proliferation in the rat brain. In: *Progress in Psychoneuroendocrinology*, edited by F. Brambilla, G. Racagni and D. De Wied. Amsterdam: Elsevier, 1980, pp. 621-632.
78. Patel, A. J., O. Barochovsky and P. D. Lewis. Psychotropic drugs and brain development: effects on cell replication in vivo and in vitro. *Neuropharmacology* 20: 1243-1249, 1981.
79. Patel, A. J., O. Barochovsky, S. Borges and P. D. Lewis. Effects of neurotropic drugs and brain cell replication in vivo and in vitro. *Monogr Neural Sci* 9: 99-110, 1983.
80. Payet, N., Y. Déziel and J.-G. Lehoux. Vasopressin: a potent growth factor in adrenal glomerulosa cells in culture. *J Steroid Biochem* 20: 449-454, 1984.
81. Racagni, G., I. Mucchetti, N. Brunello, G. Renna and V. Cuomo. Early biochemical and behavioral changes after prolonged postnatal exposure to antidepressant drugs. In: *Application of Behavioral Pharmacology in Toxicology*, edited by G. Zbinden, V. Cuomo, G. Racagni and B. Weiss. New York: Raven Press, 1983, pp. 161-172.

82. Rodier, P. M. Correlations between prenatally-induced alteration in CNS cell populations and postnatal function. *Teratology* 16: 235-246, 1977.
83. Rosengarten, H. and A. J. Friedhoff. Enduring changes in dopamine receptor cells of pups from drug administration to pregnant and nursing rats. *Science* 203: 1133-1135, 1979.
84. Rozengurt, E., A. Legg and P. Pettican. Vasopressin stimulation of mouse 3T3 cell growth. *Proc Natl Acad Sci USA* 76: 1284-1287, 1979.
85. Rosengurt, E. and J. Sinnott-Smith. Bombesin stimulation of DNA synthesis and cell division in cultures of Swiss 3T3 cells. *Proc Natl Acad Sci USA* 80: 2936-2940, 1983.
86. Russell, W. E. and N. L. R. Bucker. Vasopressin modulates liver regeneration in the Brattleboro rat. *Am J Physiol* 245: G321-324, 1983.
87. Saint-Côme, C., G. R. Acker and F. L. Strand. Peptide influences on the development and regeneration of motor performance. *Peptides* 3: 439-449, 1982.
88. Sandman, C. A., R. F. McGivern, C. Berka, J. M. Walker, D. H. Coy and A. J. Kastin. Neonatal administration of β -endorphin produces "chronic" insensitivity to thermal stimuli. *Life Sci* 25: 1755-1760, 1979.
89. Sara, V. R., K. Hail and L. Witterberg. Growth hormone dependent polypeptides and the brain. In: *Hormones and the Brain*, edited by D. De Wied and P. A. Van Keep. London: MTP Press, 1980, pp. 63-72.
90. Schlumpf, M., W. J. Schoemaker and F. E. Bloom. Innervation of embryonic rat cerebral cortex by catecholamine-containing fibers. *J Comp Neurol* 192: 361-376, 1980.
91. Seeman, P. Brain dopamine receptors. *Pharmacol Rev* 32: 229-313, 1980.
92. Sinding, C., A. G. Robinson and S. M. Seif. Neurohypophyseal peptides in the developing rat fetus. *Brain Res* 195: 177-186, 1980.
93. Smith, C. M. and F. L. Strand. Neuromuscular response of the immature rat to ACTH/MSH 4-10. *Peptides* 2: 197-205, 1981.
94. Snyder, S. H. and R. Simantov. The opiate receptor and opioid peptides. *J Neurochem* 28: 13-20, 1977.
95. Stenström, A., L. I. Hansson and K. G. Thorngren. Influence of α -MSH and ACTH on cortical bone remodelling in hypophysectomized rats. *Experientia* 35: 132-133, 1979.
96. Strand, F. L. and T. T. Kung. ACTH accelerates recovery of neuromuscular function following crushing of peripheral nerve. *Peptides* 1: 135-138, 1980.
97. Stratton, L. O., C. A. Gibson, K. G. Kolar and A. J. Kastin. Neonatal treatment with TRH affects development, learning and emotionality in the rat. *Pharmacol Biochem Behav* 5: Suppl 1, 65-67, 1976.
98. Swaab, D. F., G. J. Boer and M. Visser. The fetal brain and intrauterine growth. In: *Paediatrics and Growth*, edited by D. Bartrop. London: Fellowship Postgraduate Medicine, 1978, pp. 63-69.
99. Swaab, D. F. Neuropeptides and brain development—a working hypothesis. In: *A Multidisciplinary Approach to Brain Development*, edited by C. Ci Benedetta, R. Balász and G. Gombos. Amsterdam: Elsevier, 1980, pp. 181-196.
100. Swaab, D. F. and J. T. Martin. Functions of α -melanotropin and other opiomelanocortin peptides in labour, intrauterine growth and brain development. *Ciba Found Symp* 81: 196-217, 1981.
101. Swaab, D. F. and J. P. Ter Borg. Development of peptidergic systems in the rat brain. *Ciba Found Symp* 86: 271-294, 1981.
102. Swaab, D. F. Neuropeptides. Their distribution and function in the brain. *Prog Brain Res* 55: 97-122, 1982.
103. Swaab, D. F. and G. J. Boer. Neuropeptides and brain development. Current perils and future potential. *J Dev Physiol* 5: 67-75, 1983.
104. Swaab, D. F. and M. Mirmiran. Possible mechanisms underlying the teratogenic effects of medicines on the developing brain. In: *Neurobehavioral Teratology*, edited by J. Yanai. Amsterdam: Elsevier, 1984, pp. 55-71.
105. Van der Helm-Hylkema, H. and D. DeWied. Effects of neonatally injected ACTH and ACTH analogues on eye opening of the rat. *Life Sci* 18: 1099-1104, 1976.
106. Vándor, E., G. Simon, E. Anda and A. Budavári. The effect of α -melanophore-stimulating hormone on liver regeneration and incorporation of amino acid in rat's liver protein. *Endokrinologie* 66: 81-87, 1975.
107. Van Leeuwen, F. W. and P. Wolter. Light microscopic autoradiographic localization of [3 H]arginine-vasopressin binding sites in the rat brain and kidney. *Neurosci Lett* 41: 61-66, 1983.
108. Van Leeuwen, F. W. and G. J. De Vries. Vasopressin and oxytocin binding sites in the rat brain, kidney and mammary gland as demonstrated by light microscopic autoradiography. In: *Neurosecretion and the Biology of Neuropeptides*, edited by H. Kobayashi, H. A. Bern and A. Urano. Berlin: Springer Verlag, 1985, pp. 375-382.
109. Van Wimersma Greidanus, Tj. B. Disturbed behavior and memory of the Brattleboro rat. *Ann NY Acad Sci* 394: 655-662, 1982.
110. Veldhuis, H. D., C. S. Nyakas and D. De Wied. ACTH-related peptides facilitate functional recovery after brain damage. Proceedings 26th Federation Meeting, Amsterdam, 1985, in press.
111. Versteeg, D. M., M. Tanaka and E. R. De Kloet. Catecholamine concentration and turnover in discrete regions of the brain of the homozygous Brattleboro rat deficient in vasopressin. *Endocrinology* 103: 1654-1661, 1978.
112. Vorhees, C. V. Effects of prenatal naloxone exposure on postnatal behavioral development of rats. *Neurobehav Toxicol Teratol* 3: 295-301, 1981.
113. Walsh-Reitz, M. M. and F. G. Toback. Vasopressin stimulates growth of renal epithelial cells in culture. *Am J Physiol* 245: C365-370, 1983.
114. Weber, S., J. E. Zuckerman, D. G. Bostwick, K. G. Bensch, B. I. Sikic and T. A. Raffin. Gastrin releasing peptide is a selective mitogen for small cell lung carcinoma in vitro. *J Clin Invest* 75: 306-309, 1985.
115. Whitfield, J. F., J. P. MacManus and D. J. Gillan. The possible mediation by cyclic AMP of the stimulation of thymocyte proliferation by vasopressin and the inhibition of this mitogenic action by thyrocalcitonin. *J Cell Physiol* 76: 65-76, 1970.
116. Willey, J. C., J. F. Lochner and C. C. Harris. Bombesin and the C-terminal tetradecapeptide of gastrin-releasing peptide are growth factors for normal human bronchial epithelial cells. *Exp Cell Res* 1985, in press.
117. Wolfson, B., R. W. Manning, L. G. Davis and F. Baldino, Jr. A quantitative, immunocytochemical demonstration of the postnatal development of neurotensin in the medial preoptic area of the rat. *Dev Brain Res* 18: 241-250, 1985.
118. Wright, W. A. and C. L. Kutscher. Vasopressin administration in the first month of life: effects on growth and water metabolism in hypothalamic diabetes insipidus rats. *Pharmacol Biochem Behav* 6: 505-509, 1977.
119. Zadina, J. Perinatal peptide administration: Effects on development, behavior and receptors in the rat. *Peptides*, 1985, in press.
120. Zagon, I. S. and P. J. McLaughlin. Increased brain size and cellular content in infant rats treated with an opiate antagonist. *Science* 221: 1179-1180, 1983.
121. Zagon, I. S. and P. J. McLaughlin. An overview of the neurobehavioral sequelae of perinatal opioid exposure. In: *Neurobehavioral Teratology*, edited by J. Yanai. Amsterdam: Elsevier, 1984, pp. 197-234.