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LOYOLA UNIVERSITY CHICAGO

THE EFFECTS OF ETHANOL ON THE DEVELOPING SEROTONERGIC NEURONS

A DISSERTATION SUBMITTED TO

THE FACULTY OF THE GRADUATE SCHOOL

IN CANDIDACY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

NEUROSCIENCE PROGRAM

BY

JUNG-AE KIM

CHICAGO, ILLINOIS

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Dedicated to

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for their unmeasurable love, support and encouragement

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for their endless support throughout my life

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotrophin
ADH	alcohol dehydrogenase
AMG	amygdala
ANOVA	analysis of variance
BAL	blood alcohol level
bFGF	basic fibroblast growth factor
BLA	basolateral nucleus of amygdala
CA	Cornu Ammonis
C-AD	control ad libitum
CAM	cell adhesion molecule
CCM	control conditioned media
CM	conditioned media
CMF-HBSS	calcium- and magnesium-free Hank's balanced salt solution
CNS	central nervous system
C-PF	control pair-fed
DG	dentate gyrus
DR	dorsal raphe
5,7-DHT	5,7-dihydroxytryptamine

ECM	ethanol conditioned media
ED	embryonic day
EGF	epidermal growth factor
E-PF	ethanol pair-fed
FAE	fetal alcohol effect
FAS	fetal alcohol syndrome
FBS	fetal bovine serum
FCx	frontal cortex
FGF	fibroblast growth factor
FN	fibronectin
GFAP	glial fibrillary acidic protein
HBSS	Hank's balanced salt solution
5-HIAA	5-hydroxyindole acetic acid
5-HT	5-hydroxytryptamine
IGF	insulin-like growth factor
LH	lateral hypothalamus
LS	lateral septum
LTP	long term potentiation
MAO	monoamine oxidase
MEM	Eagle's minimum essential medium
MFB	medial forebrain bundle
MnR	median raphe

MS	medial septum
5-MT	5-methoxytryptamine
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide
8-OH-DPAT	8-hydroxy-2-(N,N-dipropylamino)tetralin
*p*CPA	para-chlorophenylalanine
PCx	parietal cortex
PN	postnatal day
PORN	poly-ornithine
QAR	quantitative autoradiography
SDN-POA	sexually dimorphic nucleus of the preoptic area
SN	substantia nigra
TPH	tryptophan hydroxylase

LIST OF DRUGS

Drug	Action
5-Methoxytryptamine	5-HT_1 receptor agonist
p-Chlorophenylalanine	Tryptophane hydroxylase inhibitor
8-OH-DPAT	5-HT_{1A} receptor agonist
Spiperone	5-HT_2 receptor antagonist
Citalopram	5-HT reuptake site blocker
Pargyline	Monoamine oxidase inhibitor
Paroxetine	5-HT reuptake site blocker

CHAPTER I

INTRODUCTION

Fetal alcohol syndrome (FAS) refers to the characteristic anomalies found in the children born to alcoholic women. It is composed of facial dysmorphology, pre- and post-natal growth retardation and central nervous system (CNS) abnormalities. FAS is one of the leading causes of mental retardation in the western world. In the United States alone, more than 1200 children are born with FAS each year (Abel and Sokol, 1991). As children grow, facial abnormalities and retarded growth become less marked. However, CNS abnormalities, especially abnormal behavior, learning and memory deficits, and mental retardation, become persistent devastating problems.

During the last 20 years, neuroanatomical and neurochemical studies in animal models of FAS have been performed in order to understand the underlying causes of abnormal behavior and mental retardation. These studies have shown that many brain structures and major neurotransmitter systems were abnormally developed. The serotonergic system, one of the most expansive neurotransmitter systems in the brain, has been shown to be affected by *in utero* ethanol exposure at the level of the concentrations of the neurotransmitter and its metabolites, uptake sites and certain receptors (Rathbun and Druse, 1985; Druse et al., 1991; Druse and Paul, 1989; Tajuddin and Druse, 1989).

However, the mechanism by which ethanol acts on the developing serotonergic system is unknown. The purpose of this dissertation is to assess two potential underlying

mechanisms by which *in utero* ethanol exposure contributes to the abnormal development of the serotonergic system. (1) The decreased serotonin (5-hydroxytryptamine, 5-HT) content in ethanol-exposed fetuses (Druse et al., 1991) could lead to decreased stimulation of raphe 5-HT_{1A} receptors and astroglial 5-HT_{1A} receptors. Decreased stimulation of raphe 5-HT_{1A} receptors may result in altered development of serotonergic neurons. Decreased stimulation of astroglial 5-HT_{1A} receptors leads to decreased production of neurotrophic factors for fetal 5-HT neurons (Azmitia et al., 1990; Whitaker-Azmitia et al., 1990). (2) In addition, a generalized decrease in astroglial protein synthesis and content (Guerri et al., 1990; Snyder et al., 1992) could lead to decreased production of important astroglial growth factors, which are essential for neuronal growth and differentiation. Thus, the hypothesis of this dissertation is that part of abnormal development of serotonergic system caused by *in utero* ethanol exposure may be due to in part a decreased level of essential growth factors such as serotonin and/or astroglial-derived growth factors.

In order to examine this hypothesis, an *in vivo* animal model and *in vitro* cell culture studies were used. In the *in vivo* rat model, quantitative autoradiographic studies were performed. Specifically, I examined the effects of *in utero* ethanol exposure on the extent of abnormalities in 5-HT reuptake sites and 5-HT_{1A} receptor sites in offspring. In addition, I assessed the possibility that maternal treatment with a 5-HT_{1A} receptor agonist, buspirone, could prevent abnormalities involving 5-HT reuptake and 5-HT_{1A} receptors in ethanol-exposed rats. In *in vitro* cell culture studies, the effects of ethanol treatment on the production of astroglial-derived growth factors were studied. Conditioned media from

control and ethanol-treated astroglia were used to culture fetal serotonergic neurons. The effects of the conditioned media on the growth of serotonergic neurons was determined.

CHAPTER II

REVIEW OF RELATED LITERATURE

Fetal Alcohol Syndrome (FAS)

Human Studies

A characteristic pattern of anomalies was found in children whose mothers chronically abused alcohol during pregnancy. These symptoms were described as FAS (Jones and Smith, 1973). FAS is characterized by facial dysmorphology, growth deficiency and signs of CNS dysfunction (Jones et al., 1973; Clarren and Smith, 1978; Clarren et al., 1978; Kyllerman et al., 1985; Aronson et al., 1985; Streissguth et al., 1988). It has been recognized that all infants exposed to prenatal alcohol may not develop the full spectrum of symptoms. Affected infants may show symptoms ranging from mild to severe. The lesser affected infants may be described as having fetal alcohol effects (FAE). The facial abnormalities in FAS include malformations in the major components of the face: short palpebral fissures, low nasal bridge, upturned nose, indistinct philtrum, thin upper lip, and flat midface (Jones and Smith, 1973). Malformations of other organs also occur in the children with FAS. These include cardiac, genital and renal malformations (Sandor et al., 1981; Clarren and Smith, 1978). The growth retardation of FAS children exhibited by decreased weight, height and head circumference, starts

prenatally and persists throughout adolescence (Clarren and Smith, 1978; Golden et al., 1982; Streissguth et al., 1985). Intrauterine growth retardation is directly proportional to the degree of maternal alcohol intake (Streissguth et al., 1980).

There are a number of CNS abnormalities associated with FAS. Microcephaly, reflecting deficient brain growth, has been observed in greater than 80% of children with FAS. The microcephaly has a prenatal onset and becomes quite apparent as the child matures (Clarren and Smith, 1978). Neural tube defects such as anencephaly and meningomyelocele also occur at a higher rate in FAS children (Freidman, 1982). Other neurological abnormalities such as altered cerebellar function, hypotonicity and increased incidence of cerebral palsy and seizure are also found in FAS children (Burd and Martsolf, 1989; Hanson et al., 1978; Olegard et al., 1979). Behaviorally, newborns born with FAS usually show irritability, tremulousness, and poor suckling and eye-hand coordination (Golden et al., 1982; Hanson et al., 1976). Young children with FAS show hyperactivity, emotional instability, and poor motor skills and eye-hand coordination (Aronson and Olegard, 1987; Harris et al., 1993; Kyllerman et al., 1985; Steinhausen et al., 1993). In addition, FAS children have higher rates of speech and language problems and visual-perceptual problems (Aronson et al., 1985; Greene et al., 1990). Mental retardation is one of the most common and serious problems associated with *in utero* ethanol exposure. IQ studies of FAS children showed an average score of around 65 with a range from 16 to 105 (Streissguth et al., 1988). There is a close association between the severity of physical abnormalities and the severity of intellectual deficits. However, brain malformation and intellectual deficits also occur in the absence of

detectable physical abnormalities in the offspring of moderate drinking mothers (Clarren et al., 1978; Peiffer et al., 1979; Conry, 1990; Streissguth et al., 1990).

Animal Studies

In human studies it is extremely difficult to separate the effects of alcohol from other complicating factors because chronic alcoholic women are frequently malnourished, are heavy smokers, and may abuse other drugs (Abel, 1984). These factors could also independently affect the fetal development. Animal models make it possible to control the compounding variables of human research. Among the animal species, mice and rats have been popularly used to study fetal alcohol effects because of their inexpensiveness and short gestation periods.

Ethanol is administered in animal models using a number of different methods. Alcohol has been administered in drinking water (Borges and Lewis, 1982), in a liquid diet (Barnes and Walker, 1981), by vapor inhalation (Phillips and Cragg, 1982) or by stomach intubation (Abel et al., 1983; West et al., 1981). Since most animals do not voluntarily consume sufficient quantities of ethanol to obtain and sustain blood alcohol levels comparable to those of human alcoholics, administration of alcohol in drinking water is considered the least preferable procedure. By the intubation method high blood alcohol levels can be achieved. However, the procedure is stressful and may cause an irritation of the gastrointestinal tract, which leads to inhibited absorption of essential nutrients. The intubation procedure has been reported to significantly reduce maternal body weights compared to rats given the same quantity of alcohol by liquid diet (Vorhees

and Butcher, 1982). This may have an adverse effect on fetal development that is unrelated to the direct effect of ethanol. Therefore, the most appropriate method seems to be a liquid diet procedure which is not stressful to the animal and provides stable intoxicating blood alcohol levels as well as adequate nutrition. In addition, this method allows one to control alterations of food intake by using a pair-feeding paradigm.

In the rat model of FAS neonatal alcohol exposure has also been studied because the first postnatal week of the rat pups is equivalent to the third trimester in humans. This time period corresponds to the brain growth spurt (Dobbing and Sands,1979). Postnatal alcohol exposure in rats has been accomplished using a variety of techniques: through the milk of lactating alcohol-consuming mother (Abel, 1974), through gastric intubation (Diaz and Samson, 1980) and artificial rearing (Bonthius and West, 1990; Grant et al., 1983). The artificial rearing technique seems to be the best because it allows stable blood alcohol levels and adequate nutrition. This technique involves maintaining the rat pup in a cup which floats in the 37°C water bath. Alcohol-containing milk formula is infused into the pup through an implanted intragastric feeding tube.

Many of the symptoms seen in human infants prenatally exposed to alcohol, have also been found in animal models of FAS (Chernoff, 1977; Randall et al., 1977). These include microcephaly (Tze and Lee, 1975; Randall et al., 1977; Lancaster et al., 1982; Miller, 1987), hyperactivity as measured in the open-field (Bond and DiGiusto, 1976, 1977; Branchey and Friedhoff, 1976; Shaywitz et. al., 1979) and impairment in learning and memory (Abel, 1979; Lochry and Riley, 1980; Shaywitz et. al., 1979). Prenatal alcohol exposure also impairs maternal behavior in rats. Adult female rats that had been

exposed to alcohol prenatally, displayed poor nest building and pup-retrieving behaviors (Hard et al., 1985a).

In addition, ethanol-exposed offspring exhibit abnormalities of neuroendocrine responses which are associated with hypothalamic neuronal activity. Adrenocortical responses to stressor or drug challenges were disturbed in rats exposed to ethanol *in utero*. These disturbances induced by *in utero* ethanol exposure were found in two opposite directions: the stress responses were decreased in both neonatal and prepubertal ages (Taylor et al., 1986; Weinberg, 1989), but the responses were increased in adulthood (Weinberg, 1988; Taylor et al., 1982). Male rats exposed to ethanol *in utero* had a feminized pattern of sexually dimorphic reproductive (Parker et al., 1984) and nonreproductive behaviors (McGivern et al., 1984). Fetuses and neonates exposed to ethanol *in utero* had a decreased testosterone surge (McGivern et al., 1988, 1993; Redei and McGivern, 1988). Since testosterone plays a role in the masculinization and defeminization of sexually dimorphic behavior and brain morphology (Dohler et al., 1984, 1986), the reduced size of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in male rats prenatally exposed to ethanol (Barron et al., 1988) may be the result of a decreased testosterone surge.

In Utero Ethanol Effects on Brain Structures

The evidence that *in utero* ethanol exposure results in abnormalities of learning and motor function suggests that ethanol-induced defects may be caused by abnormalities in the neocortex, hippocampus and cerebellum. Anatomical investigations have confirmed

this hypothesis.

In prenatal ethanol-exposed rats, the total number of neurons and glia is decreased in the somatosensory cortex. These changes are accompanied by decreased neuronal and increased glial cell body size. The noted changes result in a decrease of overall volume of the somatosensory cortex (Miller and Potempa, 1990). The decreased number of neurons could be due to either a decrease in neuronal generation, or an increase in neuronal death, or both. In the cerebral cortex, neurons are generated between embryonic day (ED; day of insemination = ED0) 12 and ED 21. These neurons are generated by the proliferation of neuronal precursors particularly within the ventricular and subventricular zones (Angevine and Sidman, 1961). In ethanol-exposed rats generation of cortical neurons was decreased between ED12 and ED19, but after ED19 more neurons were generated (Miller, 1988). Decreased neurogenesis between ED12 to ED19 corresponds to the decreased cell proliferation in the ventricular zone, whereas increased neurogenesis after ED19 corresponds to the stimulated cell proliferation in the subventricular zone (Miller, 1989). Overall neuronal generation in the cortex of developing ethanol-exposed rats appears to be delayed. In contrast, specific neurons such as corticospinal neurons are increased in the ethanol-exposed rat offspring. This suggests that *in utero* ethanol exposure may also affect the process of axonal pruning (Miller, 1987).

Disturbed neuronal migration has also been observed. Normally, neuronal migration occurs in an inside-to-outside pattern, which means that early-generated neurons reside in deep layers and late-generated neurons migrate along the earlier

neurons, and ultimately reside in the outer layer of cortex (Angevine and Sidman, 1961; Rakic, 1972). However, in ethanol-exposed rat offspring, late-generated neurons are found in the deep cortical layer (Miller, 1988). In addition, dendritic arborization of cortical pyramidal cells was stunted and less matured by *in utero* ethanol exposure (Hammer and Scheibel, 1981; Schapiro et al., 1984; Stoltenburg-Didinger and Spohr, 1983).

A number of abnormalities have been found regarding hippocampal and dentate gyrus neurons. Anatomical and electrophysiological vulnerability of the hippocampal formation to the neurotoxic effects of alcohol may contribute to the learning and cognitive deficits seen in FAS (Hoff, 1988). Although no change was observed in the number of hippocampal CA3 and dentate gyrus (DG) granule cells, the neuronal connections between CA3 and DG were affected. Mossy fibers are the axons of granule cells of the dentate gyrus innervating pyramidal cells of CA3. The mossy fibers were hypertrophied in rat offspring exposed to ethanol *in utero* (West et al., 1981). Prenatal alcohol exposure also delayed the appearance of complex synapses and multiple synaptic contacts on single neurons in dentate gyrus (Hoff, 1988). In addition to these morphological disturbances, electrophysiological responses of the hippocampal neurons, such as long-term potentiation and potassium-induced excitability, were impaired (Swartzwelder, et. al., 1988). In contrast to the CA3 and DG regions, the number of pyramidal neurons in the hippocampal CA1 region is decreased, and the dendritic morphology of these neurons is altered in rats exposed to ethanol prenatally (Barnes and Walker, 1981; Abel et al., 1983). Excitability of the neurons in the CA1 region was

increased in rats exposed to ethanol *in utero* (Hablitz, 1986; Tan et. al., 1990). Behaviorally, these rats exhibit impaired passive avoidance learning (Tan et. al., 1990).

In the cerebellum, Purkinje cells are generated prenatally and granule cells are generated postnatally. Ethanol exposure during the gestation period decreased the number of Purkinje cells, and delayed both the maturation of those cells and synaptogenesis in the molecular layer (Volk et al., 1981; Volk, 1984). Purkinje cell loss has been also shown in rats exposed to ethanol during the neonatal period, a time when these neurons are in the process of maturation. The influence of neonatal ethanol exposure on the degree of Purkinje cell loss in different regions correlates with the maturational state of the cells. The lobules I, IX, and X, in which Purkinje cells tend to mature early, were the most severely affected. In contrast, lobules VI and VII, in which Purkinje cells tend to mature late, were the least affected (Bonthius and West, 1990). In contrast to the hippocampus where no granule cell loss occurred, there is a loss of cerebellar granule cells in rats exposed to ethanol neonatally (Borges and Lewis, 1983; Bonthius and West, 1990).

Ethanol has been shown to affect neurogenesis, neuronal migration and/or neuronal cell death (Borges and Lewis, 1983; Bonthius and West, 1990). However, the effects of ethanol on the development of neurons appear to depend on the brain regions where the cells are located and the timing of ethanol exposure relative to the birth or development of the neurons.

<u>*In Utero* Ethanol Effects on the Development of Serotonergic System</u>

Neurotransmitters and their receptors play an important role in neuronal activity. The effects of ethanol on the adult alcoholic brain have been studied in terms of the metabolism, turnover, storage and uptake of neurotransmitters, and receptor concentration (Rawat, 1974; Walsh et al., 1970; Kuriyama, et al., 1971). Similarly, the effects of *in utero* ethanol exposure on the levels of neurotransmitters, their metabolites and receptors have been studied in developing brains of animal models of FAS in order to elucidate the underlying causes of the CNS disorders associated with FAS. These studies have reported that development of many neurotransmitter systems are disturbed by *in utero* ethanol exposure (see Druse, 1992 for review). The serotonergic system, the most expansively distributed system throughout the brain (Tork, 1990), is one of the affected neurotransmitter systems.

A number of studies suggested that the behavioral changes in FAS may be associated with altered serotonin activity. *In utero* ethanol-exposed mice and rats have shown hyperactivity, aggression, decreased sensory-motor responses and altered maternal behavior, all of which are signs of decreased 5-HT activity. In fact, the same animals have decreased brain serotonin content (Krsiak et al., 1977; Hard et al., 1985a, 1985b). Rat offspring exposed to ethanol prenatally have also been shown to have a significant decrease in the content of serotonin and its metabolites in its cell body region (the brain stem) and in its terminal regions, motor and somatosensory cortex at PN19 and PN35 (Rathbun and Druse, 1985; Druse et al., 1991). One of the serotonergic terminal regions, motor cortex, also has decreased 5-HT uptake sites in prenatally ethanol-exposed rats (Druse and Paul, 1989). In addition, 5-HT_1 receptors are decreased in the motor and

somatosensory cortex (Tajuddin and Druse, 1989). Cortical 5-HT_2 receptors, however, are not affected by *in utero* ethanol exposure (Tajuddin and Druse, 1989a).

Interestingly, the deficit of serotonin in the brain stem, cell body region, has been found as early as ED15. Since fetal serotonin plays an important role for the development and maturation of the serotonergic neuron and its target areas (Whitaker-Azmitia and Azmitia, 1986; Whitaker-Azmitia et al., 1987; Lauder and Krebs, 1978, 1984; Chubakov et al., 1986), the early deficiency of 5-HT may disturb the outgrowth of 5-HT fibers, and lead to abnormal CNS development seen in FAS.

Effects of Ethanol on Astrocytes

Astrocytes exert important roles in neuroembryogenesis by promoting neuritic growth over the astroglial surfaces and by providing trophic factors for the survival and functional maintenance of neurons (reviewed by Manthorpe et al., 1986). Abnormalities in the development of radial glial cells and astroglial cell dysfunction caused by ethanol treatment may contribute to the abnormal CNS development as in FAS. This may happen by disrupting neurite outgrowth and/or neuronal survival. Radial glial cells transform into astroglial cells after they serve as guides for migrating neurons (Schmechel and Rakic, 1979). *In utero* ethanol exposure has been shown to induce premature degradation of radial glia, and accelerate the transformation of radial glia into astrocytes (Miller and Robertson, 1993). This may cause abnormal migration of cortical neurons seen in ethanol-exposed rats (Miller, 1988). Ethanol-exposed astrocytes exhibit abnormal morphology. They have a smoothened cell surface, less developed processes, decreased

content of glial fibrillary acidic protein (GFAP) and ultrastructural alterations (Babu et al., 1994; Davies and Cox, 1991; Mayordomo et al., 1992; Renau-Piqueras et al., 1989). In addition to the morphological changes, the activity of astroglial glutamine synthetase has been decreased by ethanol treatment (Babu et al., 1994; Davies and Vernadakis, 1984). 5-HT uptake by astroglial cells was also decreased by ethanol exposure. The decrement of 5-HT uptake was due in part to decreased protein content in astroglial cells because 5-HT uptake was reduced to the same extent as astrocytic proteins (Lokhorst and Druse, 1993b). Astrocytes, cultured in the presence of ethanol, or astrocytes from ethanol-treated rats, cultured in the absence of ethanol have decreased protein content and decreased synthesis of DNA, RNA and protein (Babu et al., 1994; Davies and Vernadakis, 1984; Guerri et al., 1990; Snyder et al., 1992). Since astrocytes are known to synthesize and secrete neurotrophic factors which are essential for neuronal survival and growth, any alteration in the synthetic machinery may result in abnormal production of neurotrophic factors, which, in turn, can cause abnormal development of neurons.

Possible Mechanisms

Many clinical reports have demonstrated that ethanol can induce a wide range of detrimental effects on the developing fetus. Recently, many possible mechanisms by which *in utero* ethanol exposure adversely affects the development of CNS have been proposed.

These effects of ethanol on the developing fetus could arise indirectly through the actions on the mother. For example, ethanol could affect maternal hormonal or

nutritional status, or altered placental function. The placental abnormalities can affect the developing fetus because it is totally dependent on the maternal oxygen and nutrient supply through placenta. Decreased placental transport of ^{14}C-valine and placental-facilitated diffusion of glucose have been observed. The latter has been correlated with intrauterine growth retardation (Henderson et al., 1981; Snyder et al., 1986). Since ethanol has been shown to decrease fetoplacental blood flow and cause umbilical artery spasm (Jones et al., 1981; Altura et al., 1983; Savoy-Moore et al., 1989), fetal hypoxia has been suggested as a mechanism by which *in utero* ethanol exposure causes the abnormal development of the brain (Michaelis, 1990). The regions of cell loss induced by *in utero* ethanol exposure include the CA1 region of the hippocampus and cerebellar Purkinje cells (Barnes and Walker, 1981; Volk, 1984). Interestingly, cell loss in these regions is similar to those induced by hypoxia or ischemia (Jorgensen and Diemer, 1982; Auer et al., 1989).

Ethanol is a lipophilic molecule which freely crosses the placental barrier (Waltman and Iniquez, 1972; Idanpaan-Heikkila, et al., 1972; Cook et al., 1975). In the human, the transfer of ethanol occurs within a minute and the alcohol concentration in the fetus can be as high as that in the mother (Idanpaan-Heikkila, et al., 1971; Waltman and Iniquez, 1972). In comparison with adults, fetuses have a relatively low activity of alcohol dehydrogenase, an ethanol-metabolizing enzyme (Pikkarainen, 1971). Thus, elimination of fetal alcohol depends on passive diffusion along the concentration gradient generated by effective maternal elimination of alcohol. Furthermore, it has been also reported that alcohol elimination from the amniotic fluid takes a much longer time than

from the maternal circulation (Brien et al., 1983). Thus, ethanol can be present for a longer period of time in the fetus than in the mother. Indeed, the direct effect of ethanol on fetal development has been shown in the chick embryo (Heaton et al., 1992). In addition, an investigation which cultured rat embryos in an ethanol-containing medium showed impaired development of the fetus in an ethanol concentration-dependent manner (Brown et al., 1979).

Another possible mechanism by which ethanol can directly affect the fetal CNS may be through the alteration of either second messenger systems or the level of neurotransmitters. Neurotransmitters, such as serotonin, dopamine and norepinephrine, and second messengers, such as cyclic AMP, diacylglycerol, inositol phosphates and Ca^{+2} are important mediators of cell proliferation and differentiation (reviewed by Lauder, 1993). In fact, ethanol-exposed fetuses and neonates had a decreased level of cyclic AMP and decreased cyclic AMP binding to the regulatory subunit of protein kinase A (Pennington, 1988). ^{3}H-Forskolin binding sites (associated with cAMP) and [^{3}H]-phorbol ester binding sites (associated with protein kinase C) were increased in the hippocampus and cortex of rat offspring exposed to ethanol *in utero* (Nio et al., 1991).

Direct inhibition of protein synthesis in the fetal tissue by ethanol may also contribute to the damage associated with FAS. *In utero* ethanol administration has been shown to inhibit the synthesis of protein, RNA and DNA in fetal and neonatal brain tissue (Rawat, 1975; Sharma and Rawat, 1989). The neuronal cell loss in ethanol-exposed rats may be due in part to decreased DNA synthesis (Michaelis, 1990). In addition, diminished neuronal growth and differentiation may be related to decreased

synthesis of proteins such as the astroglial growth factors (Dow and Riopelle, 1985; Heaton et al., 1992; Walker et al., 1990).

In utero ethanol exposure may alter gene expression. Fetuses of the ethanol-treated mice showed hypomethylation of DNA, and decreased nuclear methylase activity (Garro et al., 1991). Generally, hypermethylated regions of DNA are not transcribed, but hypomethylated regions are transcribed (Cedar, 1988). Thus, alterations of DNA methylation during embryogenesis can affect normal fetal gene expression, and thus fetal development. *In vitro* ethanol treatment has been shown to increase gene expression in neuronal cell lines. In N1E-115 neuroblastoma cells, ethanol increased tyrosine hydroxylase mRNA expression in a dose-dependent manner (Gayer et al., 1991). Ethanol also induced the gene transcription of the stress protein, Hsc70, and two related molecules, GRP94 and GRP78, in the NG108-15 neuroblastoma x glioma cell line (Miles et al., 1991; Wilke et al., 1994). In the pheochromocytoma cell line (PC12) chronic ethanol exposure increased expression and function of dihydropyridine-sensitive calcium channels and enhanced neurite outgrowth induced by nerve growth factor (Roivainen et al., 1994). These effects of ethanol appear to be mediated through the activation of protein kinase C (Roivainen et al., 1994). Altered gene expression by prenatal ethanol exposure was also demonstrated in *in vivo*. Myelin basic protein mRNA levels (Kojima et al., 1994) and the mRNA for insulin-like growth factors I and II (Singh et al., 1994) were decreased in ethanol-exposed rat offspring.

The Serotonergic Neurotransmitter System

Serotonin Receptors

Fourteen serotonin receptor subtypes have been identified so far. It has been recently suggested that serotonin receptors could be classified according to the amino acid sequence homology, pharmacological properties, and transductional components (Humphrey et al., 1993). Thirteen serotonin receptor subtypes belong to the G protein-linked superfamily and one belongs to the ligand-gated ion channel superfamily. Those which belong to the G protein superfamily can be divided into two subfamilies, the 5-HT_1 and 5-HT_2 receptor families, based on the second messenger system with which they are coupled. The 5-HT_1 receptor family is linked to the inhibition of adenylyl cyclase and includes 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}. The 5-HT_2 receptor family is linked to the stimulation of phospholipase C and includes 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. The serotonin receptors linked to a ligand-gated ion channel are the 5-HT_3 receptors. The other subtypes which are linked to G proteins are 5-HT_4, 5-HT_{5A}, 5-HT_{5B}, 5-HT_6, and 5-HT_7. Among these, three (5-HT_4, 5-HT_6 and 5-HT_7) subtypes are linked to the stimulation of adenylyl cyclase.

Binding affinity for spiperone was initially used to discriminate high affinity 5-HT_{1A} receptors from the low affinity 5-HT_{1B} sites (Pedigo et al., 1981). Now, a more selective agonist, 8-hydroxy-2-(N,N-dipropylamino)tetralin (8-OH-DPAT), can be used to label the 5-HT_{1A} receptor sites. The regional distribution of 5-HT_{1A} receptors is similar in many species including rat, mouse, guinea pig, calf, cat, pig, monkey, and humans (Pazos and Palacios, 1985; Waeber et al., 1989; Kohler et al., 1986; Pazos et al., 1987). The highest density of 5-HT_{1A} receptors are localized in limbic structures such as

hippocampus, septum, amygdala and entorhinal cortex. Astroglia cells in the septum and hippocampus of adult rats have been shown to express dense 5-HT_{1A} receptors (Whitaker-Azmitia et al., 1993). 5-HT_{1A} receptors are present on the serotonergic cell bodies in dorsal and median raphe nuclei (Hall et al., 1985; Verge et al., 1985) where they serve as somatodendritic autoreceptors regulating 5-HT neuronal cell firing activity and thus serotonin release (Sprouse and Aghajanian, 1987). In the terminal region, for example, hippocampus and cortex, 5-HT_{1A} receptors are located postsynaptically (Hall et al., 1985; Verge et al., 1986). These postsynaptic receptors are involved in the modulation of the release of other neurotransmitters. Stimulation of 5-HT_{1A} receptors enhances acetylcholine release in the rat cortex (Sinniscalchi et al., 1990). In addition, postsynaptic 5-HT_{1A} receptors are involved in the hypothalamo-hypophyseal axis. Stimulation of postsynaptic 5-HT_{1A} receptors results in the release of adrenocorticotrophin (ACTH), corticosterone, corticotrophin-releasing factor, prolactin and β-endorphin (reviewed by Van de Kar, 1991).

5-HT_{1B} sites have previously been identified by their low affinity for spiperone. Few drugs can bind to the 5-HT_{1B} receptors with a nanomolar affinity. 5-HT_{1B} sites are found only in some species such as rat and mouse brain in which they are densely localized in the substantia nigra and globus pallidus (Pazos and Pallacios, 1985; Hoyer et al., 1985). 5-HT_{1B} sites have been demonstrated to be presynaptic terminal autoreceptors in the frontal cortex, where they regulate the release of 5-HT (Middlemiss et al., 1988; Engel et al., 1986). These receptors are also located postsynaptically in the terminals of cholinergic fibers in the hippocampus, where they modulate the release of

acetylcholine (Maura and Raiteri, 1986).

$5\text{-}HT_{2A}$ receptors are the classical $5\text{-}HT_2$ receptors. Initially [^{3}H]-spiperone was used to label the $5\text{-}HT_2$ sites. A high density of $5\text{-}HT_2$ receptor sites is found in the cortex. Low levels are present in the hippocampus, caudate, putamen and nucleus accumbens (Pazos et al., 1985; Biegon et al., 1986). $5\text{-}HT_2$ receptors are involved in the release of other neurotransmitters in a region-specific way. In the cortex and hippocampus activation of $5\text{-}HT_2$ receptors inhibits the K^+-evoked acetylcholine release (Muramatsu et al., 1988), whereas in the striatum $5\text{-}HT_2$ receptor agonists increase the basal release of acetylcholine and inhibit dopamine release (Bianchi et al., 1989; Muramatsu et al., 1988a).

The receptors previously named as $5\text{-}HT_{1C}$ have characteristics similar to the $5\text{-}HT_2$ receptor family in terms of the nucleotide sequence homology, pharmacological profiles and second messenger system (Hoyer, 1988; Hartig et al., 1990). The $5\text{-}HT_{1C}$ receptor has been renamed the $5\text{-}HT_{2C}$ receptor. These receptors are highly localized in the choroid plexus and control the exchange between CNS and cerebrospinal fluid (Hartig et al., 1990). Low levels of $5\text{-}HT_{2C}$ receptors are also present in the substantia nigra, globus pallidus, cortex and olfactory tubercles (Pazos et al., 1985a).

$5\text{-}HT_3$ receptors are ligand-gated ion channels, composed of four hydrophobic transmembrane regions (Maricq et al., 1991). $5\text{-}HT_3$ receptors are localized highly in the area postrema and some other areas such as cortex, amygdala, hippocampus, nucleus accumbens, thalamus and hypothalamus (Kilpatrick et al., 1987; Waeber et al, 1989a). Activation of $5\text{-}HT_3$ receptors facilitates the nonselective movement of monovalent

cations, resulting in the depolarization of cells (Yakel and Jackson, 1988; Neijt et al., 1988). Activation of 5-HT_3 receptors seems to stimulate the release of dopamine in the nucleus accumbens and amygdala (Hagan et al., 1990; Imperato and Angelucci, 1989).

Roles of Serotonin in Various Regions

5-HT and the Basal Ganglia

The striatum and substantia nigra (SN) receive serotonergic projections mainly from the dorsal raphe (Dray et al., 1976; Fibiger and Miller, 1977; Palkovits, et al., 1974; Steinbusch, 1981a). A majority of 5-HT terminals in the SN appear to be collaterals of the dorsal raphe-striatal projection (Van der Kooy and Hattori, 1980; Imai et al., 1986). In the striatum, dorsal raphe stimulation or local application of 5-HT suppresses spontaneous firing activity of most striatal neurons (Olpe and Koella, 1977). Selective destruction of 5-HT fibers entering the striatum decreased dopamine turnover and tyrosine hydroxylase activity within the striatum, suggesting that 5-HT terminals in the striatum facilitate dopamine transmission (Giambalvo and Snodgrass, 1978). These observations were extended by behavioral studies. Generally, animals turn towards the side where nigrostriatal neurons are less stimulated (Pycock, 1980). Striatal infusion of the serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), facilitated turning behavior towards the ipsilateral side which implies that destruction of 5-HT terminals with 5,7-DHT results in less stimulation of nigrostriatal dopamine neurons (Jacobs et al., 1977).

In the SN, dorsal raphe stimulation inhibits the activity of most compacta cells and some reticulata cells (Fibiger and Miller,1977). Serotonergic terminals in the SN synapse onto dopaminergic dendrites (Nedergaard et al., 1988). Microiontophoretic application of 5-HT increased dendritic calcium conductance of dopaminergic neurons in SN, which could result in dendritic dopamine release (Nedergaard et al., 1988; Trent and Tepper, 1991; Williams and Davies, 1983). Dopamine, released from dendrites following stimulation by 5-HT, may interact with somatodendritic D_2 autoreceptors, and then cause decreased somatodendritic excitability of dopaminergic neurons (Groves et al., 1975; Bunney and Aghajanian, 1978). Behavioral effects caused by alterations of 5-HT transmission in the SN have been studied. Local infusion of 5,7-DHT into the SN facilitated spontaneous locomotor activity (Carter and Pycock, 1979). Unilateral infusion of 5,7-DHT or *p*-chlorophenylalanine (*p*CPA) elicited turning behavior towards the contralateral side (Giambalvo and Snodgrass, 1978). Raphe nuclei lesions or *p*CPA treatment also facilitated amphetamine-induced locomotor hyperactivity (Costall et al., 1979). It has been demonstrated that microinjection of the 5-HT uptake blocker, fluoxetine, into SN exerts an anticonvulsant action in the forebrain (Pasini et al., 1992). Behavioral studies, thus, suggest that 5-HT terminals in the SN play an important inhibitory role in controlling motor behavior.

In the basal ganglia the actions of 5-HT appear to be modified by other neurotransmitters and 5-HT itself. Several neurotransmitters such as glutamate, GABA, substance P, acetylcholine, and dopamine have been shown to modify 5-HT release in the SN (Reisine et al., 1982; Soubrie et al., 1981; Hery et al., 1980). For example, SN

contains high levels of GABA, which is localized in nerve terminals of the striato- and pallido-nigral neurons (Di Chiara et al., 1980; Ribak et al., 1980). GABA tonically inhibits 5-HT transmission in the SN (Soubrie et al., 1981) through GABA receptors located on nigral 5-HT terminals (Gale, 1979). 5-HT itself released from dendrites in the raphe nuclei controls the terminal 5-HT release in the SN through the activation of the somatodendritic autoreceptors (Adell et al., 1993; Ferre and Artigas, 1993).

5-HT and the Hippocampus

Although the hippocampus receives fibers from both the median raphe and dorsal raphe, the median raphe is its major source of serotonergic innervation (Azmitia and Segal, 1978). 5-HT fibers from the median raphe project to the Cornu Ammons (CA) areas and polymorphic layers of dentate gyrus (DG) where they innervate calbindin- and calretinin-containing GABAergic neurons (Freund et al., 1990; Acsady et al., 1993). Dorsal raphe neurons project to the molecular layer of DG where 5-HT fibers innervate dendrites of the granule cells (Azmitia and Segal, 1978).

Either stimulation or application of 5-HT to the median raphe elicits long lasting inhibition of spontaneous activity of hippocampal pyramidal cells through an increase in potassium conductance (Segal, 1975, 1976; Beck and Choi, 1991). Lesioning of hippocampal serotonergic innervation results in hyperexcitability of granule cells in DG (Richter-Levin et al., 1994).

The hippocampus appears to play an important role in the learning and memory process. The long-term potentiation (LTP) of synaptically evoked responses, believed to

be the cellular mechanism of learning and memory, has been described in a number of excitatory pathways in the hippocampus (Bliss, 1979; Milner, 1972). LTP can be recorded in the granule cells of DG and in the pyramidal cells of the CA3 by the electrical stimulation of the perforant path and mossy fibers, respectively. Studies with serotonergic fiber lesioning and raphe tissue grafts confirmed that serotonergic modulation of hippocampal activity is important in cognitive function. LTP in the DG was decreased in the rats treated with 5,7-DHT (Bliss et al., 1983). The activation of 5-HT_3 receptors seems to inhibit the induction of LTP in the CA3 (Maeda, et al., 1994). Lesioning of hippocampal serotonergic innervation results in impairment of spatial learning (Richter-Levin et al., 1994). There is a recovery of learning when raphe tissue is grafted into the hippocampus of animals which had combined serotonergic and cholinergic lesions (Richter-Levin et al., 1993). However, these results were not consistent. There is a report that serotonergic deafferentation of the hippocampus enhances spatial discrimination learning in rats (Altman et al., 1990).

5-HT and the Amygdala

The amygdala receives serotonergic inputs from both rostral and caudal raphe groups. Especially, the basolateral nucleus of the amygdala uniformly receives dense 5-HT input from the dorsal raphe (Imai et al., 1986). Electrical stimulation or iontophoretic application of 5-HT on dorsal raphe inhibited the spontaneous firing of amygdaloid cells (Wang and Aghajanian, 1977). This inhibition was prevented in 5,7-DHT or *p*CPA treated rats. A marked decrease of 5-HT and 5-HIAA in *p*CPA-treated rats was

associated with increased sexual activity and aggressive behavior (Ferguson et al., 1970). Also, lesions of the amygdala showed alterations in sexual and aggressive behavior (Goddard, 1964). The inhibitory influence of dorsal raphe nucleus on amygdaloid cells, thus, has been connected with its important influence over sexual and aggressive behavior.

5-HT and the Lateral Hypothalamus

The lateral hypothalamic area contains medial forebrain bundle and the lateral hypothalamic nucleus (Sipe and Moore, 1977). The lateral hypothalamic nucleus (LHN) receives input from the brainstem reticular formation, medial hypothalamus, other lateral hypothalamus and basal forebrain (Millhouse, 1969; Nauta and Haymaker, 1969; Raisman, 1970). The neurons of LHN project into the adjacent medial hypothalamic zone. The LH is involved in the regulation of behaviors such as feeding (Baillie and Morrison, 1963; Teitelbaum and Epstein, 1962), locomotor activity (Balagura et al., 1969; Gladfelter and Brobeck, 1962), sensorimotor integration (Marshall et al., 1971; Turner, 1973) and reward (Ols, 1973: Rolls, 1975). LH also contains serotonergic terminals (Moore, et al., 1978; Steinbusch, 1981a; Heym and Gladfelter, 1982; Kai et al., 1988). 5-HT in LH plays an inhibitory role in food intake (Blundell, 1979; Coscina et al., 1972).

5-HT and the Septum

The septum is a part of the limbic system (Köhler et. al., 1982; Swanson and

Cowan, 1979) and is involved in the process of learning and memory, and neuroendocrine and autonomic regulation (De France, 1976). The septum can be divided into two parts, the lateral septal and the medial septal nuclei (Raisman, 1966; Chafetz et al., 1981). The lateral septum contains dense 5-HT terminals whereas the medial septum receives 5-HT fibers en route to other parts of the septum (Dinopoulos, et al., 1993; Kohler et al., 1982). The lateral septum sends efferents to dorsal and medial raphe nuclei (Staiger and Nurnberger, 1991). Functionally, 5-HT in the lateral septum seems to play a role in memory consolidation. Direct infusion of the 5-HT uptake blocker, fluoxetine, into the lateral septum enhanced memory in rats (Lee et al., 1992). In the medial septum 5-HT appears to be involved in the regulation of neuronal discharge of both the medial septum and hippocampus. Some neurons in the medial septum discharge in an irregular manner which is synchronous with hippocampal theta spikes (Apostol and Creutzfeldt, 1974). Repetitive stimulation of the median raphe nucleus disrupts the bursting discharge of septal neurons and hippocampal theta spikes (Assaf and Miller, 1978).

5-HT and the Frontal Cortex

Serotonergic fibers innervate whole cortex, but the motor regions in the frontal lobe receive a lower extent than other cortical areas (Azmitia and Gannon, 1986). Laminar distribution of 5-HT fibers in monkeys shows that the highest densities are in layer I and IV (Azmitia and Gannon, 1986; Morrison et al., 1982). In adult rat brain, serotonergic fibers are localized in layer V whereas layer IV is densely innervated in the

developing brain (Blue and Molliver, 1985). 5-HT fibers projecting to cortex have been distinguished by the morphology and origin (Kosofsky and Molliver, 1987; Tork, 1990). One type is the thick fibers with large, spherical varicosities. These fibers arise from the median raphe nuclei and innervate equally to parietal, occipital and frontal cortex (O'Hearn and Molliver, 1984). Another type of 5-HT fibers is the thin fibers with small varicosities. These axons arise from the dorsal raphe nucleus and project heavily to frontal cortex (O'Hearn and Molliver, 1984). The exact functional role of dual serotonergic projections to the frontal cortex is unknown. However, serotonergic projections from the dorsal raphe nucleus to the frontal cortex could be involved with basal ganglia-motor system since frontal cortex is associated with motor function and DR nucleus also project to the caudate putamen (Steinbusch et al., 1980, 1981b). Additional studies suggest that the serotonergic system in the frontal cortex is associated with affective disorders and cognitive function (Stanley and Mann, 1983; Stanley et al., 1982; Bennett et al., 1979; Morris et al., 1993).

Development of The Brain Serotonergic System

Ontogeny

5-HT containing neurons in the rat brain first appear at the ventricular zone along the border between the metencephalon and the rostral myelencephalon, and develop as bilateral superior and inferior cell groups (Lidov and Molliver, 1982; Wallace and Lauder, 1983; Aitken and Tork, 1988). Autoradiographic studies showed that

neurogenesis of 5-HT neurons in the superior cell groups occurs between ED11 and ED15 in the rat. 5-HT immunopositive cells in the superior cell group can be identified as early as ED12 (Lauder and Bloom, 1974; Levitt and Moore, 1978; Lidov and Molliver, 1982; Lauder et al., 1982; Aitken and Tork, 1988). The results of immunohistochemistry and [^{3}H]-thymidine autoradiography studies indicate that serotonergic neurons synthesize their transmitter soon after they have completed their terminal mitosis (Lauder et al., 1982). 5-HT neurons in the superior cell group then differentially migrate during ED14 - ED19 to form subgroups of cells. These subgroups correspond to rostral raphe nuclei; dorsal raphe, median raphe, caudal linear raphe nuclei, and the B9 group in the adult rat brain. Midline fusion of bilateral superior cell groups starts at ED18.

In contrast to the superior cell group, 5-HT immunoreactive cells in the inferior group are first detected at sites away from the ventricular zone around ED14 in rat embryo (Lidov and Molliver, 1982). Thus, inferior 5-HT cells seem to complete much of their initial migration prior to the onset of phenotypic expression. These inferior cells give rise to the raphe magnus, raphe obscurus, and raphe pallidus and B3 group, found in the medulla of adult rat brain. Around the end of the embryonic period changes in the surrounding brainstem structures re-shape both the superior and inferior 5-HT cell groups to resemble that of adult 5-HT nuclei (Aiken and Tork, 1988; Lidov and Molliver, 1982). In addition, most of these 5-HT neurons acquire more complex dendritic trees and undergo decreased cellular packing density.

Nearly all ascending projections of the raphe nuclei are derived from the rostral

group of 5-HT neurons, whereas neurons in the caudal group give rise to the majority of descending fibers. Ascending projections seem to begin at ED13 and enter the basal forebrain as axons in the medial forebrain bundle (MFB) (Lidov and Molliver, 1982a; Lauder, 1990). According to the whole mount study of Aiken and Tork (1988), the 5-HT fibers contained within the MFB appear to be segregated into medial and lateral components which project to different regions of the forebrain. The medial part of the MFB contains fibers destined for the frontal pole of the telencephalon, whereas the lateral part contains fibers which project medially into the hypothalamus and cross in the supraoptic commissure. 5-HT axons reach the vicinity of all structures in the brain that are to receive a serotonergic innervation by ED19 and arrive at the cerebral cortex by the end of gestation. However, parieto-occipital areas of the cerebral cortex remain devoid of 5-HT fibers until the first postnatal week. The branching or arborization of axon terminals develop during the postnatal period. Initial development of ascending 5-HT axons seems to follow a very circumscribed and directional growth. In all primary sensory areas of the cortex, dense patches of serotonergic innervation are seen in the rat brain from the neonatal period to postnatal day 21. This innervation pattern becomes more uniform in the adult rat neocortex (D'Amato, et al., 1987). By adulthood, 5-HT axons ramify extensively and innervate most of the brain (Steinbusch, 1981).

Descending projections from the caudal raphe nuclei to the spinal cord course in the marginal zone along the ventricular and lateral funiculi and adult pattern of cord innervation seems to be achieved by postnatal day 21 (Rajaofetra et al., 1989).

5-HT Synthesis

Although 5-HT-containing neuroblasts first appear as early as the twelfth day of gestation in the rat central nervous system, as shown using histofluorescence and immunohistochemistry (Olson and Seiger, 1972; Lauder et al., 1982; Lidov and Molliver, 1982), measurable amounts of 5-HT are first detected at ED15 (Liu et al., 1987). This low concentration of 5-HT remains almost constant until the end of gestation. At birth, the concentration of whole brain 5-HT increases dramatically (Liu, et al., 1987; Herregodts, et al., 1990). However, its levels are still 25-50% of those in adult rats (Zeisel et al., 1981; Baker and Quay, 1969; Lauder and Bloom, 1974; Tissari, 1973). 5-HT levels in the cell body and terminal regions show differences in the developmental profile. In the brain stem, the cell body region, 5-HT levels at birth are 32-75% of those found in the same region of adult rats (Bourgoin et al., 1977; Nomura et al., 1976). The levels increase progressively till the end of the third postnatal week to a value slightly higher than those of adult rats. After that they level off to the adult level. In the forebrain, one of the terminal regions of 5-HT neurons, 5-HT levels at birth are about 22% of those in adult rat brain. 5-HT levels increase much more slowly in the forebrain region than in the brain stem, and are only 75% of the adult levels at the end of the fifth postnatal week (Bourgoin et al., 1977).

The developmental changes in 5-HT content seem to parallel the changes in the activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme in the synthesis of 5-HT (Schmidt and Sansers-Bush, 1971). Low levels of whole brain TPH activity have been detected at ED16 with *in vitro* assay (Renson, 1973) and ED15 with *in vivo* assay

(Liu et al., 1987). At birth, brain stem TPH activity is about 30-50% of that in adult rats (Deguchi and Barchas, 1972; Hamon and Bourgoin, 1982). The activity rapidly increases and reaches peak activity at the end of third postnatal week. It then decreases to adult levels (Park et al.,1986; Hamon and Bourgoin, 1982; Deguchi and Barchas, 1972). In the terminal regions, TPH activity is much lower than that in the cell body region, and reaches adult levels at around postnatal day 30 (Park et al., 1986; Hamon and Bourgoin, 1982; Deguchi and Barchas, 1972). The apparent K_m of the TPH in the newborn is about twice as high as in adult rats. The concentration of tryptophan, the substrate for TPH, is very high in the brain of newborn rat. For the first 2 days following birth, the brain tryptophan concentration is four to eight times higher than in adult rats (Zeisel et al., 1981; Bourgoin et al., 1974). In the adult brain, the tryptophan concentration is between 30 and 40 μM which is close to the K_m value (50 μM) of TPH (Kaufman, 1974). The ratio of the tissue concentration of tryptophan to the apparent K_m of TPH is considered as an index of the saturation state of the enzyme (Bourgoin et al., 1974). Although the affinity of TPH is low in neonate brain, the enzyme is in a highly saturated state compared to that of the adult because the concentration of the substrate is very high in the neonate brain.

Such a high concentration of tryptophan is characteristic of the developing brain. The high tryptophan is due in part to the high activity of the tryptophan carrier in brains of newborn rats. In addition, the binding of circulating tryptophan to serum albumin is extremely low during the early postnatal period (Bourgoin et al., 1974). Therefore, free serum tryptophan is almost totally available for the tryptophan carrier. The increased

activity of the tryptophan carrier seems to be associated with an increased V_{max} since the apparent affinity of the tryptophan uptake carrier is similar to that of adults (Hamon and Bourgoin, 1979).

5-HT Metabolism

5-HT is taken up by the serotonin transporter after it is released from the neuron. It is then degraded to 5-hydroxyindolacetic acid (5-HIAA) by monoamine oxidase (MAO). *In vitro* synaptosomal uptake of 5-HT increases rapidly in various brain regions for the first 2 weeks following birth (Kirksey and Slotkin, 1979). The K_m values for 5-HT reuptake are indistinguishable from adult values (Nomura et al., 1976). The increment in the 5-HT reuptake in the developing brain has been associated with an increase in the number of nerve endings or reuptake sites per ending (Kirksey and Slotkin, 1979) during the critical period of a developmental stage of the rat brain (Davison and Dobbing, 1968).

The level of 5-HIAA is higher than that of 5-HT in the developing brain of rats. 5-HIAA levels can be detected at ED17 and increase significantly around birth (Ribary et al., 1986). At birth, 5-HIAA levels are already as high as that of adults in the brain stem and about 50% of the adult value in the forebrain (Bourgoin et al., 1977). 5-HIAA levels increase progressively during the first three postnatal weeks in both the brain stem and the forebrain. Especially in the brain stem, the levels are significantly higher (85%) than adult values at the end of the third postnatal week (Bourgoin et al., 1977; Zeisel et al., 1981). Thereafter, the levels decrease to adult values by the sixth postnatal week.

The ratio of 5-HIAA/5-HT is very high during the fetal period and the first three postnatal weeks. During this period the ratio of 5-HIAA/5-HT remains two to four times higher than in adult rats (Herregodts et al., 1990; Bourgoin et al., 1977). In adult rats, the ratio of 5-HIAA/5-HT is dependent on the electrical activity of serotonergic neurons (Aghajanian et al., 1967). Similarly, the high value of the 5-HIAA/5-HT ratio in young brain may be the consequence of an enhanced rate of neuronal firing. However, the neurons of the dorsal raphe in neonate brain showed a regular discharge pattern, and the firing rate is not significantly different from that of the adult neurons (Lanfumey and Jacobs, 1982; Gallager, 1982). Alternatively the high turnover of 5-HT in the young brain may be due to either poor storage capacity of vesicles or enhanced enzymatic degradation. However, poor storage capacity may not play a role in the high turnover of 5-HT in newborn rat brain, since the conversion of $[^3H]$5-HT into $[^3H]$5-HIAA is faster in the newborn than in adult rats even after reserpine treatment, which depletes 5-HT storage capacity in tissues (Bourgoin et al., 1977). Rather, the high rate of enzymatic degradation of 5-HT seems to be the main cause of the high 5-HT turnover in developing rat brain. In fact, monoamine oxidase (MAO) activity peaks around birth. At ED15, total activity of MAO is composed of an equal proportion of MAO type A and type B (Liu et al., 1987). The activity of MAO A increases faster than that of type B. The high MAO A activity found in young rats seems to be associated with a high V_{max} (Nelson et al., 1979). The apparent affinity of MAO A for 5-HT does not change significantly throughout the development (Bourgoin et al., 1977).

Receptors

In the human brain, 5-HT_{1A} binding shows a prenatal peak (Bar-Peled et al., 1991) and decreases with age in the cortex, hippocampus and raphe nuclei (Dillon et al., 1991). However, in the rat brain, the levels of 5-HT_{1A} receptors in the cortex, hippocampus and septum, are increased during the first three weeks after birth (Daval et al., 1987; Gozlan et al., 1990). In the cerebellum, high levels of 5-HT_{1A} receptors are present at birth but disappear with age, suggesting that 5-HT_{1A} receptors are involved in the regulation of the brain development (Daval et al., 1987; Hamon et al., 1990). Transcripts of 5-HT_{1A} receptors are detected as early as ED 12 in the rat. The level of transcripts reaches its maximum concentration at ED15 and decreases progressively to very low concentrations at ED20 (Hillion et al., 1993). Even though the level of 5-HT_{1A} receptor mRNA at postnatal day (PN) 18 is higher than at ED18, its level is still markedly lower than at ED15.

In the developing rat brain, 5-HT_{1B} receptors appear in a vibrissa-related pattern in the primary somatosensory cortex. This pattern is no longer present in the adult brain (Leslie et al., 1992). The developmental profile of the 5-HT_{1B} receptor is closely matched to that of 5-HT fiber immunoreactivity, which implies 5-HT_{1B} receptors are expressed in the terminals of the developing 5-HT fibers (Leslie et al., 1992). The expression of 5-HT_{1B} receptor mRNA is detected at ED17 in whole brain (Voigt et al., 1991). The mRNA level is unchanged during development, whereas high levels of 5-HT_{1B} receptor mRNA are expressed in the striatum, thalamus, and cerebellum at birth up to PN 12 and then remarkably decreases at adulthood (Voigt et al., 1991).

The $5\text{-}HT_2$ receptors are present prenatally in the rat cortex, hippocampus, caudate, pontine tegmentum (Morilak and Ciaranello, 1993). The expression of $5\text{-}HT_2$ receptors in the developing brain parallels mRNA levels. $5\text{-}HT_2$ transcripts are detected as early as ED14 (Hellendall et al., 1993), and the expression of $5\text{-}HT_2$ receptor mRNA in whole brain increases 13-fold between ED 17 and PN 5 (Roth et al., 1991). The increase of the number of $5\text{-}HT_2$ receptors is about 8-fold between ED 17 and PN13 and then the number is reduced by 50% at PN 27 (Roth et al., 1991). These $5\text{-}HT_2$ receptors in immature brain are functionally active. $5\text{-}HT_2$ receptor-induced phosphatidyl inositide breakdown was greater in immature rat cortex than in adult (Claustre et al., 1988). $5\text{-}HT_{1C}$ receptor mRNA is also detected at ED14 (Hellendall et al., 1993). $5\text{-}HT_{1C}$ receptor levels increased 2-fold between ED17 and PN13 and its mRNA levels increased 5-fold between PN2 and PN13 (Roth et al., 1991).

Serotonin as a Developmental Signal

In the developing brain, monoamine neurotransmitters appear early, especially one to several days before the generation of their target cells (Lauder and Bloom, 1974). These monoamines are believed to act as developmental signals in fetal brain before they act as neurotransmitters. Neurotransmitters seem to be released from the growing axon terminals into the surrounding environment (Hume et al, 1983; Young and Poo, 1983). They regulate neurite outgrowth of neighboring axons and the morphology of target cells during development (Handa et al., 1986; Lankford, 1987).

The specific actions of serotonin as an extrinsic factor for the growth cone have

been demonstrated in cultures of identified neurons from the CNS of the snail helisoma. Application of serotonin to the culture medium inhibited growth cone motility, neurite elongation and electrical synaptic connections of identified snail buccal neurons (Haydon et al., 1987; McCobb et al., 1988). Altered 5-HT fiber growth was also observed in the snail nervous system by treating snail embryo with 5,7-DHT, which transiently lowers 5-HT levels (Goldberg and Kater, 1989).

Serotonin also regulates the growth of its own neurons (Whitaker-Azmitia and Azmitia, 1986). In co-cultures of fetal serotonergic neurons and target cells, low concentrations of 5-methoxytryptamine (5-MT) inhibited the growth of 5-HT neurons, whereas high concentration of 5-MT stimulated the growth of 5-HT neurons. These dual effects of serotonin on the growth of its own neurons were also demonstrated in whole animal studies, in which 5-HT terminal density was decreased with a low concentration of 5-MT treatment and increased with a high concentration of 5-MT (Shemer et al., 1991). Serotonin has been shown to initiate and autoamplify its own synthesis in embryonic hypothalamic neurons (De Vitry et al., 1986). The autoregulatory function of 5-HT is further demonstrated by the observation that *Drosophila* mutants, incapable of 5-HT synthesis, exhibit altered branching of serotonergic axons (Budnik et al., 1989).

The involvement of 5-HT in the target cell morpho-functional development was demonstrated in organotypic cultures. When 5-HT was added to cultured neurons from 5-HT target tissues (e.g. cerebral cortex or hippocampus), it stimulated growth and differentiation of neurons, and enhanced synaptogenesis (Chumasov et al., 1980; Chubakov et al., 1986). The role of 5-HT in the target cell differentiation *in vivo* has

also been studied by manipulating the content of 5-HT in the embryonic brain. Maternal administration of *p*CPA, a 5-HT synthesis inhibitor, delayed neurogenesis of 5-HT target cells (Lauder and Krebs, 1978).

These actions of 5-HT as a developmental signal seem to be mediated through 5-HT receptors in the developing brain. 5-HT receptors are expressed in the fetal brain (Hillion et al., 1993; Hellendall et al., 1993; Morilak and Ciaranello, 1993; Roth et al., 1991), and the number of 5-HT_1 receptors was altered in the offspring treated with maternal *p*CPA or 5-MT (Whitaker-Azmitia et al., 1987). Also, stimulation of 5-HT_{1A} receptors has been shown to modify neuritic branching of developing rat cortical neurons *in vitro* (Sikich et al., 1990).

The effect of serotonin on neuronal development can also be mediated through astroglia. Cultured astroglia possess a high affinity 5-HT uptake system (Katz and Kimelberg, 1985), serotonin binding proteins (Hertz and Tamir, 1981), the serotonin degrading enzyme, MAO (Fitzgerald et al., 1990), and 5-HT receptors (Fillion et al, 1980, 1983; Whitaker-Azmitia and Azmitia, 1986a). Serotonin decreases the levels of GFAP and its mRNA in cultured astroglia derived from rat brainstem (Le Prince et al., 1990). Application of a 5-HT_{1A} receptor agonist shifted astroglial morphology to a more mature state (Whitaker-Azmitia et al., 1990). Stimulation of 5-HT_{1A} receptors on cultured astroglial cells caused increased synthesis and release of a serotonergic growth factor, S100β (Whitaker-Azmitia et al., 1990; Azmitia et al., 1990). The effect of 5-HT on the production of glial-derived trophic factors for dopaminergic neurons has also been demonstrated. The growth of dopaminergic neurons in the presence of mesencephalic glia

cells was stimulated by addition of 5-HT to the culture medium (Liu and Lauder, 1992), but no such effect was found in neurons cultured on the polylysine substrate (Liu and Lauder, 1991).

The Effects of Buspirone on the CNS

Buspirone is a non-benzodiazepine anxiolytic drug which has been used clinically for the treatment of anxiety (Feighner et al., 1982). Buspirone has little or no affinity for any of the major CNS receptors except dopamine D_2 and 5-HT_{1A} receptors. Binding of ^{3}H-buspirone occurs in the regions rich in 5-HT_{1A} receptors (Matheson and Tunnicliff, 1991). Electrophysiological studies have shown that either systemic administration or iontophoretic application of buspirone produces a dose-dependent inhibition of the activities of serotonergic raphe neurons (VanderMaelen et al., 1986). Acute intraperitoneal administration of buspirone in rats reduces the synthesis of 5-HT in the brain (Hjorth and Carlsson, 1982). These effects of buspirone are due to the activation of 5-HT_{1A} autoreceptors in the dorsal raphe. In addition, buspirone treatment results in increased plasma levels of corticosterone and reduced hippocampal rhythmical slow activity, both of which are mediated through the activation of the postsynaptic 5-HT_{1A} receptors (Coop and McNaughton, 1991; Cowan et al., 1990).

Buspirone also binds to dopamine D_2 autoreceptors as an antagonist, and thus enhances dopaminergic neurotransmission (reviewed by Riblet et al., 1984). In the striatum where dopamine autoreceptors have been identified, buspirone increases tyrosine hydroxylase activity and levels of dopamine metabolites (McMillen et al., 1983).

Although buspirone does not alter the activities of striatal choline acetyltransferase or acetylcholinesterase, buspirone reduces acetylcholine levels in the striatum (Kolasa et al., 1982). Since dopamine inhibits the firing of acetylcholine neurons, the decreased level of acetylcholine induced by buspirone can be attributed to its antagonistic action on D_2 receptors. Buspirone has been shown to increase plasma prolactin levels (Metzer et al., 1982; Urban et al., 1986), but this increase was only significant from postnatal day 12 onward (Hockl et al., 1993).

CHAPTER III

METHODS

In Vivo Studies

Animals and Diet

Virgin female Sprague-Dawley rats (Harlan) weighing 180 to 200 grams, were housed in individual cages and maintained at 23°C - 25°C and on a light/dark cycle (12/12 hour). The rats were allowed to adjust to the environment for 2 days before initiating liquid diets.

On the first day of diet administration, all rats were given the control liquid diet. The caloric composition of the diet was 21% protein, 29% fat, and 50% carbohydrate. The caloric concentration was 1 kcal/ml (Noronha and Druse, 1982). Water was given ad libitum. After 3 days of adjustment to the liquid diet, rats were divided into two weight-matched groups: i) ethanol-consuming dams; ii) pair-fed dams. The ethanol-fed rats were given liquid diet containing 6.6% (v/v) ethanol, while pair-fed rats received a volume of control liquid diet equal to the volume consumed by the ethanol-fed rats during the previous day. Ethanol accounts for approximately 35% of the total calories in the ethanol diet, and replaces isocaloric amounts of carbohydrates (e.g. maltose-dextrin) in the control diet. Diets were replenished daily. An ad libitum control group

which was fed with control diet ad libitum was included in experiments of specific aim 1. The ethanol and control diets were pair-fed to rats for 5-6 weeks prior to breeding and during gestation.

Female rats were mated. Breeding was confirmed by the detection of sperm in vaginal smears; that day was defined as embryonic day 0 (ED0). At parturition, rat mothers were given free access to chow plus either the control liquid diet (pair-fed controls) or half-strength (3.3% v/v) ethanol-liquid diet. On the third day after parturition, all rats were fed standard lab chow exclusively and litters were adjusted to 9 pups.

Drug Treatment

Buspirone (RBI, Natick, MA) was administered from ED13 to ED20, when 5-HT neuronal differentiation and growth take place. Buspirone was dissolved in 0.9% sterile saline solution at a concentration of 3 mg/ml. After weighing, control- and ethanol-fed rats in drug treatment groups were given either a subcutaneous injection of saline or buspirone, at a dose of 4.5 mg/kg body weight. Injections were made once a day between 2 pm and 3 pm. Rats were monitored for a minimum of 30 minutes for any visible side effects.

Blood Ethanol Level

Blood ethanol levels were determined by using an enzymatic kit (Sigma St. Louis, MO). This kit measures ethanol by assessing the formation of reduced nicotinamide

adenine dinucleotide (NADH) in the oxidation-reduction reaction, in which ethanol plus nicotinamide adenine dinucleotide (NAD) are converted, respectively, to acetaldehyde and NADH. Alcohol dehydrogenase (ADH) catalyzes this reaction. The increase in absorbance at 340 nm occurs when NAD is converted to NADH, is directly proportional to the ethanol concentration in the sample.

Blood samples were obtained from the tail vein two hours after the introduction of a fresh ration of diet to rats that had been fasted for 12 hours. Blood samples were collected in heparinized tubes (Becton Dickinson Inc., Lincoln Park, NJ) and deproteinized by the addition of trichloroacetic acid (6.25% w/v). Each tube was tightly capped and allowed to stand at room temperature for 5 minutes. The tube was then centrifuged at 2000 rpm for 5 minutes (Sorvall RT6000). One hundred microliters of supernatant were added to 2.9 ml of the NAD-ADH solution. The latter solution is prepared by adding 16 ml of glycine buffer to a multi-assay vial containing NAD-ADH. Mixed solutions were incubated for 10 minutes at room temperature. The solutions were transferred to cuvets, and the absorbance was measured at 340 nm (Gilford Response spectrophotometer).

Dissection and Tissue Sectioning

Rat offspring were sacrificed by decapitation on either postnatal day 5 (PN5), PN19 or PN35. The brains were quickly removed and frozen on dry ice. To obtain brains from ED19 rats, mothers were decapitated, and the uterine horn was removed and placed on an ice-cold glass plate. The brains from the fetuses were quickly removed and

frozen. Frozen brains were stored at -80 °C. Rats were sacrificed between 7 am and 9 am.

Twenty micron thick coronal sections of brains were cut at -18 °C using a cryostat (Leitz, Germany). Sections were thaw-mounted onto gelatin-coated microscope slides (Fisher, Pittsburg, PA). Four consecutive sections were put on one slide and every fifth section was set aside for staining with cresyl violet . These sections were put in an air-tight slide box and stored at -80°C until use.

Staining of Tissue Sections

Every fifth section was stained with a 0.5% (w/v) cresyl violet (Sigma, St. Louis, MO) solution. These sections were compared with those in an atlas (Paxinos and Watson, 1986) for identification of anatomical structures. In radioligand binding assays, brain sections from the same anatomical levels were selected.

Tissue sections were warmed to room temperature and dehydrated by sequentially immersing the sections for 5 minute intervals in each of the following: xylene, xylene, 100% (v/v) ethanol, 95% (v/v) ethanol, and 70% (v/v) ethanol. Slides were then dipped in distilled water and stained in the 0.5% (w/v) cresyl violet in acetate solution (0.06 M sodium acetate and 0.34 M glacial acetic acid, pH 3.9) for 25 minutes. Following staining, excess cresyl violet was removed by dipping the sections twice in distilled water for 3 minutes. Slides were then dehydrated by immersing them in 70% (v/v), 95% (v/v), and 100% (v/v) ethanol respectively. After dehydration, slides were put in xylene. A coverslip coated with Depex mounting medium (BDH Laboratory Suppliers, England)

was affixed to each slide.

Labeling of 5-HT Recognition Sites

5-HT Reuptake Sites

Serotonin reuptake sites were measured according to the method of D'Amato et al. (1987a). Slide-mounted tissue sections were brought to room temperature and preincubated for 15 minutes in 50 mM Tris buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl and 0.001% bovine serum albumin (BSA), in order to remove endogenous ligands. Tissue sections were then incubated for 60 minutes at room temperature in the incubation buffer containing 0.8 nM or 2.4 nM [^{3}H] citalopram (NEN, Boston, MA). The K_D for [^{3}H]citalopram is approximately 0.8 nM (D'Amato et al., 1987a). A concentration approximately three times the K_D concentration was used to estimate the binding at the Bmax. Non-specific binding was determined in the presence of 1 μM paroxetine (gift from Smith Kline Beecham Pharmaceuticals). Sections were then dipped (1-2 seconds) and washed twice for 10 minutes in ice-cold preincubation buffer, dipped in ice-cold distilled water for a few seconds, and dried in a cold air stream. Radiolabeled sections and a tritium-radiolabeled reference microscale were exposed to tritium sensitive Hyperfilm (Amersham, Arlington Heights, IL) at 4 °C for 4 weeks (for PN19 and PN35 brain sections) or 60 days (for ED19 and PN5 brain sections).

5-HT_{1A} Receptor Sites

5-HT_{1A} receptor sites were labeled with [^{3}H]8-hydroxy-2-(N,N-di-n-propylamino) tetralin ([^{3}H]8-OH-DPAT; NEN) according to a modified protocol of Marlier et al. (1991). Slides were warmed to room temperature and preincubated in 170 mM Tris buffer (pH 7.6) containing 4 mM $CaCl_2$, 0.01% ascorbic acid, and 0.001% BSA. Tissue sections were then incubated for 60 minutes at room temperature in the incubation buffer containing 1 μM paroxetine, 10 μM pargyline, and 1.1 nM or 2.75 nM [^{3}H]8-OH-DPAT. Non-specific binding was determined in the presence of 1 μM 5-HT. Paroxetine was included to prevent [^{3}H]8-OH-DPAT binding to 5-HT reuptake sites. Pargyline was used to prevent degradation of 5-HT. Following incubation, sections were washed twice for 5 minutes in ice-cold preincubation buffer, dipped in ice-cold distilled water for 5 seconds and dried in a cold air stream. Radiolabeled sections and [^{3}H]-reference microscales were exposed at room temperature to tritium sensitive Hyperfilm for 4 weeks (for PN19 and PN35 brain sections) or 60 days (for ED19 and PN5 brain sections).

Development of [^{3}H]-Exposed Film

Autoradiograms were generated by developing the [^{3}H] exposed film in the developing solution for X-ray film (Doehren Co., IL) for 40 seconds, washed in water for 20 seconds and fixed for 5 minutes at room temperature. Films were then rinsed in running water for 15 minutes and air-dried.

Analyses of Autoradiograms

The illuminated image of each autoradiogram was collected by a solid state

camera (Sony CCD Video Camera, Japan) and quantitated using a Macintosh-based image analysis system, NIH Image. The optical density of the reference microscale was interpolated to the radioactivity (DPM/mg protein) using tritium labeled brain mash standards. The optical density of tissue sections was converted to radioactivity based upon the calibration curve of the reference microscale. The radioactivity of tissue sections was then converted to pmol/mg protein depending on the specific activity of the radioligand. Brain structures on autoradiograms were identified in reference to the rat brain atlas (Paxinos and Watson, 1986). 5-HT reuptake sites were analyzed in the regions of raphe nuclei, lateral hypothalamus, substantia nigra, medial septal area, CA3 and frontal cortex. 5-HT_{1A} receptor sites were examined in the raphe nuclei, amygdala, dentate gyrus, CA1, lateral septal area and frontal cortex.

In Vitro Cell Culture Studies

Astroglial Cell Cultures

Astroglial-conditioned media was obtained by the modified protocol of Rudge et al. (1985). Primary cultures of astroglial cells were prepared from the cerebral cortex of embryos from timed-pregnant Sprague-Dawley rats (Harlan) at 20 days of gestation (ED20; day of insemination = ED0).

The uterine horn was aseptically removed from the body of a decapitated rat and placed into a sterile petri dish containing cold calcium- and magnesium-free Hank's balanced salt solution (CMF-HBSS; 5.4 mM KCl, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$,

140 mM NaCl, 0.34 mM Na_2HPO_4, 5.6 mM glucose). The embryos were removed from the uterus and transferred twice to the other petri dishes containing fresh cold CMF-HBSS. The brain was separated from the fetus; meninges were carefully removed under the dissecting microscope (Zeiss, Germany). Cerebral hemispheres were dissected and cleaned by removing the olfactory bulbs, striatum and hippocampal formation. Cortical tissues were cut into small pieces and collected in sterile CMF-HBSS. Minced cortical tissues were transferred to the CMF-HBSS containing 0.25% (w/v) trypsin/0.02% (w/v) EDTA (Sigma, St. Louis, MO) and 0.1% (w/v) DNAse (Boehringer Mannheim, Indianapolis, IN), and incubated for 30 minutes at 37 °C. An equal volume of medium containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY) was added to inactivate trypsin. After centrifugation at 1000 g for 2 minutes, cells were resuspended and tritulated in a D-valine substituted Eagle's minimum essential medium (MEM), containing 10% FBS (heat-inactivated at 56°C for 1 hour) (MEM-FBS). D-Valine substituted MEM was used to inhibit the growth of fibroblasts. The media was modified to contain 26.4 mM $NaHCO_3$ (Sigma), 33.3 mM glucose (Sigma), 25 mM HEPES (Gibco), 25 μg/ml gentamicin (Gibco) and 0.25 μg/ml amphotericin (Gibco). Tritulated cells were filtered through a sterile 70 μm nylon mesh (Falcon, Lincoln Park, NJ). The number of viable cells was determined by trypan blue exclusion using a hemacytometer. Trypan blue excluding cells were placed in 162 cm^2 flasks (Costar, Cambridge, MA) at the density of 7 x 10^4 cells/cm^2 (11.34 x 10^6 cells/flask). The cultures were maintained in a humidified atmosphere containing 5% CO_2. The culture medium was changed with fresh MEM-FBS on the day following seeding, and on every third day thereafter. After

2 weeks, astrocytes were subcultured.

The day before subculture, 6-well plates (Costar, Cambridge, MA) were coated with poly-ornithine (0.1 mg/ml in 1.15 M borate buffer) at room temperature. Two hours later, poly-ornithine (PORN; Sigma) solution was aspirated and plates were washed three times with sterile water. Plates were then covered with 0.001% fibronectin (FN; Sigma, St. Louis, MO.) and placed in a 37°C overnight. The plates were carefully washed three times with MEM and then covered with MEM until use (Plates should not be air-dried).

On the day of subculture, astrocyte cultures were washed two times with warm CMF-HBSS and treated with 0.05% trypsin/ 0.02%EDTA/ 0.1% DNAse solution for 15 minutes. Trypsin action was stopped with MEM-FBS and the lifted cells were gently tritulated, pooled and centrifuged at 1000 g for 5 minutes. Cells were resuspended in 1 ml of ovalbumin (1% v/v)-containing MEM. After the number of viable cells were determined, the cell suspension was diluted with serum- and ovalbumin-free MEM to a density of 3 x 10^5 cells/ml. The density of 4.5 x 10^5 cells (1.5 ml) was added to each well, coated with PORN-FN. After 2 hours of incubation, media was changed to remove any residual ovalbumin and FBS.

On the next day, the culture medium was changed and a portion of the cells were treated with ethanol (100 mM). Conditioned media from either control astroglia (CCM), or ethanol treated astroglia (ECM) were collected at the second and fourth day of ethanol treatment. CMs were centrifuged at 3500 rpm for 10 minutes and the supernatants were preserved at -80 °C until use.

Neuronal Cell Cultures

Primary neuronal cell cultures were generated from rhombencephalon of ED14 fetuses from timed pregnant Sprague-Dawley rats (Harlan). The same dissecting protocol used for the astroglial cultures was used for the rhombencephalic neuronal cultures. Rhombencephalon from between the rhombencephalic isthmus and the cervical flexure was dissected into small pieces in CMF-HBSS. The tissues were incubated with a 0.05% trypsin/EDTA/DNAse solution at 37 °C. After 30 minutes, an equal volume of 0.05% (w/v) soybean trypsin inhibitor was added to stop the trypsin action. The cells were centrifuged at 1000 g for 5 minutes. Precipitated cells were resuspended and tritulated in serum-free medium. Serum-free medium was made of Dulbecco's modified Eagle's medium and Ham's nutrient F-12 (DMEM/F12) containing 1% penicillin-streptomycin (Sigma) and Bottenstein's N_2 supplements (N_2), 5 μg/ml insulin (Sigma), 100 μg/ml transferrin (Sigma), 20 nM progesterone (Sigma), 100 μM putrescine (Sigma) and 30 nM sodium selenite (Sigma). After the number of viable cells was determined, cells were seeded in the poly-D-lysine (33 μg/ml, Sigma) coated wells at a density of 8 x 10^4 cells/cm^2 (7.6 x 10^5 cells/well). Five hours later, 6 μM fluorodeoxyuridine (ICN, Cleveland, OH) and 12 μM uridine (Sigma) solution were added to inhibit proliferation of mitotic cells.

On the day following seeding and on every other day thereafter, neuronal media was changed to either absolute N_2 media or N_2 media containing 30% CCM (30-CCM), 60% CCM (60-CCM), 30% ECM (30-ECM), 60% ECM (60-ECM). On the fifth and sixth day of culture, neuronal cells were assayed.

Immunohistochemistry

Pure astroglial cultures were identified by staining glial fibrillary acidic protein (GFAP), an astrocyte marker protein, using a peroxidase immunohistochemistry kit (Sigma). Astrocyte cultures were washed three times with warm phosphate-buffered saline (PBS: 2.7 mM KCl, 1.5 mM KH_2PO_4, 137 mM NaCl, 8 mM Na_2HPO_4, 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$) and fixed with cold 4% paraformaldehyde for 1 hour. To block non-specific binding, cultures were incubated for 10 minutes with blocking reagent containing 1% normal goat serum in PBS. After the blocking reagent was removed, cultures were incubated with primary anti-GFAP for 1 hour. Cultures were then washed three times with PBS and incubated with a biotinylated secondary antibody for 20 minutes. After washing with PBS, cultures were incubated with peroxidase reagent for 20 minutes, washed, and exposed to peroxidase substrate solution, containing chromogen, 3-amino-9-ethylcarbazole (AEC) in N,N-dimethylformamide.

Serotonin containing neurons were stained with anti-serotonin (Incstar, Stillwater, MN) using the peroxidase vectastain ABC kit (Vector Laboratory Inc., Burlingame, CA). Neuronal cultures were incubated with 100 μM L-tryptophan (Sigma) and 10 μM pargyline (Sigma) for 24 hours before staining. The staining procedures were the same as that for GFAP. For the 5-HT staining peroxidase substrate was composed of 0.03% H_2O_2 and 0.01% diaminobenzidine (Sigma) in 0.1M Tris buffer. 5-HT immunostained neuronal cultures were photographed using a Nikon inverted microscope at a magnification of 200x. The percentage of 5-HT immunopositive neurons was determined by counting the number of positively stained and unstained neurons.

Measurement of Neurite Length

The neurite length was measured on the film negatives using the Macintosh based image analysis system, NIH Image. The longest neurite of each neuron was measured starting from the cell body to the end of neurite.

5-HT Uptake

5-HT uptake was determined by measuring the accumulation of [^{3}H]5-HT by the neuronal cultures. Neuronal cultures were washed three times with warmed Hank's balanced salt solution (HBSS; 5.4 mM KCl, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 140 mM NaCl, 0.34 mM Na_2HPO_4, 0.5 mM $MgCl_2$, 1.26 mM $CaCl_2$, 0.41 mM $MgSO_4$, 5.6 mM glucose) and then incubated with 60 nM [^{3}H]5-HT (Amersham) in HBSS containing 0.1 mM L-cysteine (Sigma) for 20 minutes at 37°C. Nonspecific uptake was determined using 10 μM fluoxetine (Lily, Indianapolis, IN). After incubation, the [^{3}H]5-HT containing solution was removed and the cultures were washed three times with ice-cold HBSS. Cultures were then air-dried, and extracted with 1 ml of 95% (v/v) ethanol for 1 hour. At the end of extraction procedure, cells were scraped from the bottom and the wells were rinsed with 0.5 ml of 95% ethanol. Two extracts were combined prior to tritium counting. An aliquot was saved for protein determination.

Neuronal Number

The number of surviving neurons was determined by counting with a hemacytometer. Neuronal cultures were rinsed with CMF-HBSS and incubated with 500

μl of 0.05% trypsin/EDTA/DNAse for 30 minutes at 37 °C. An equal volume of FBS-MEM was added to stop the action of trypsin. Lifted cells were tritulated with 10 ml pipet and 9 inch Pasteur pipet. Dissociated cells were counted with hemacytometer.

DNA Content

DNA content was measured using a colorimetric assay developed by Burton (1956). Cultures were rinsed three times with HBSS. To each well of neuronal cultures was added 800 μl of 1 N NaOH. The bottom of each well was scraped after 30 minutes. Duplicate aliquots of 250 μl were transferred to test tubes. The remaining 300 μl was saved for protein determination. Standard DNA (Sigma), dissolved in 1 N NaOH was used at 10 different concentrations. Samples and standards were hydrolyzed for 30 minutes at room temperature using 250 μl of 20% perchloric acid. During incubation, the acetaldehyde stock solution (16 mg/ml in distilled water) was diluted 1:10. After the 30 minute incubation, 500 μl of diphenylamine (40 mg/ml in glacial acetic acid) and 25 μl of acetaldehyde (1.6 mg/ml) were added and the tubes were mixed. Test tubes were capped and shaken overnight in a 30 °C waterbath. The tubes were then centrifuged at 800 g (Sorvall RT6000) for 10 minutes. The optical density of the samples and standards was read at 595 nm on a spectrophotometer (Gilford).

Protein Determination

Protein content was measured by a micromodification of the Lowry method (1951). Samples for protein measurement were previously collected from the same

cultures used for DNA measurements. Standards containing 0 and 25 μg protein were generated. Samples and standards were hydrolyzed in 100 μl of 1 N NaOH for 30 minutes. During the incubation 1% (w/v) cupric sulfate, 2% (w/v) potassium-sodium tartrate and 2% (w/v) sodium carbonate were mixed at a ratio of 0.1:0.1:10 (v/v/v) (solution A). To each sample and standard, 1 ml of solution A was added. The tubes were then vortexed. After 10 minutes 100 μl of Folin-phenol reagent was added and allowed to react for 30 minutes. The optical density of the standards and samples was read at 700 nm on a Gilford spectrophotometer.

Statistical Analysis

In the study of *in utero* ethanol effects on the development of 5-HT reuptake sites and 5-HT_{1A} sites the results were analyzed using a two way analysis of variance (ANOVA) and a post-hoc Tukey's protected t test. The ANOVA with randomized block design was used. Each experiment was a block. In each experiment, samples from a given brain region from age-matched dietary groups were included. The results of cell culture studies were analyzed using a one way ANOVA followed by Tukey's protected t test. P values less than 0.05 were considered statistically significant.

CHAPTER V

RESULTS

In Vivo Studies

Maternal Weight Gain and Blood Alcohol Levels

After 6 weeks of diet consumption, the average blood alcohol level (BAL) of ethanol-consuming rats was ~ 100 mg/dl; the BALs ranged from 75 to 120 mg/dl. Although the female rats consumed an increasing quantity of diet as pregnancy progressed, the amount of ethanol consumed (g/kg body weight) was constant because the maternal weight gains paralleled the increased diet consumption (Figure 1). During pregnancy (ED0 to ED21) the maternal weight gain by pair-fed and ad lib controls (C-PF, C-AD) and ethanol-fed rats (E-PF) was comparable (Table 1). The weights of offspring from these rats were also comparable.

Effects of *In Utero* Ethanol Exposure on the Development of 5-HT Reuptake Sites

Since 5-HT reuptake sites are localized in nerve terminals, their concentration has been used as an index of serotonergic nerve fiber innervation of discrete brain areas. 5-HT reuptake sites were radiolabeled with [^{3}H]citalopram, a specific blocker of 5-HT

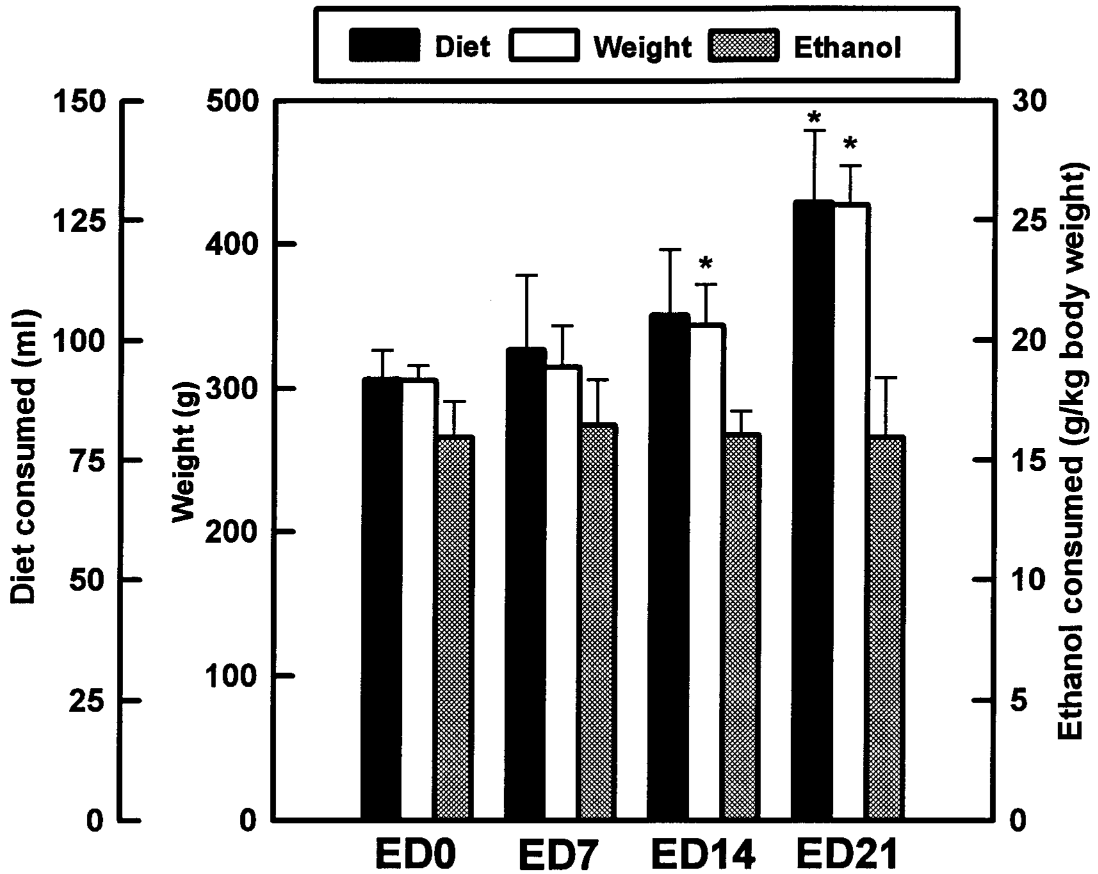


Fig. 1. Maternal weight gain and diet and ethanol consumption during pregnancy (ED0-ED21) in the ethanol-fed rats. The results are presented as the mean values obtained from five dams. The error bars represent SD. The asterisk denotes values which are significantly different from those of ED0 ($p<0.05$). ED represents embryonic day.

TABLE 1

EFFECTS OF *IN UTERO* ETHANOL EXPOSURE ON MATERNAL WEIGHT GAIN DURING PREGNANCY (ED1 - ED21) AND ON OFFSPRING WEIGHTS

	Weight (g)					
	C-PF	(n)	E-PF	(n)	C-AD	(n)
Maternal	115.7±18.1	(7)	108.6±20.5	(7)	153.0±47.3	(4)
			PN5			
Offspring	9.6±2.1	(6)	11.0±0.8	(8)	8.9±1.4	(4)
			PN19			
	51.0±6.1	(8)	48.2±5.1	(8)	50.6±6.0	(3)

All the data are presented as the mean ± SD.
Abbreviations are as follows: C-PF, pair-fed control; E-PF, pair-fed ethanol; C-AD, ad libitum control; PN, postnatal day.

reuptake sites. Binding was done at 2 ligand concentrations. Binding at 0.8 nM [^{3}H]citalopram approximated the K_D, and that at 2.4 nM was used as an estimate of binding at the Bmax. Figure 2 depicts autoradiograms of brain regions from control rats, which were radiolabled with [^{3}H]citalopram. Table 2 summarizes the specific binding of 0.8 nM and 2.4 nM [^{3}H]citalopram to brain regions from control rats, aged PN5 to PN35. In this table, the mean and standard deviation of all control values are provided. Data from the three groups of offspring are presented in Figures 3-10. These figures depict the binding in ethanol-exposed and ad lib offspring in comparison with that of age-matched control offspring that were analyzed in the same experiment. Multiple (6 - 8) individual experiments were performed. Rather than pooling the values obtained from all experiments, each experiment was analyzed as a block. The results of a 2-way analysis of variance (ANOVA) using a randomized block design is summarized in the legends to the figures.

On PN5, 5-HT reuptake sites could be quantitated in both the PCx and DR. However, binding was below the sensitivity of our method in other serotonergic brain areas at this age. By PN19, binding had increased in both the PCx and DR, as well as in the other brain regions listed in Table 2. At this age, the highest binding was found in the DR and MnR. The rank order of binding was DR ~ MnR > LH > SN > AMG > CA3 > PCx ~ FCx. With few exceptions the rank order of binding was similar at PN19 and PN35. Other than one data point (of a total of 18), the values for the specific binding of 0.8 nM and 2.4 nM [^{3}H]citalopram to serotonergic brain regions was comparable in ad lib (C-AD) and pair-fed control rats (C-PF).

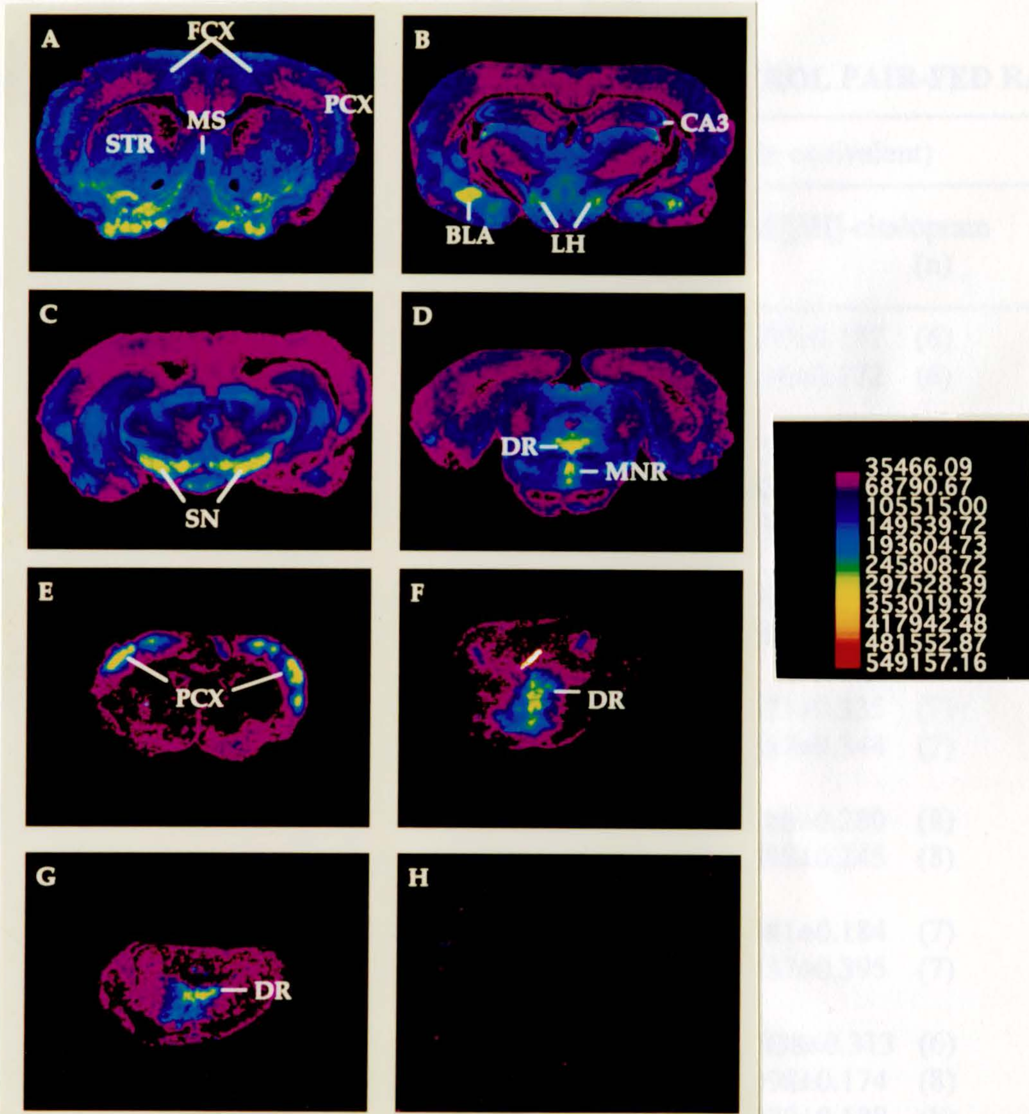


Fig. 2. Autoradiographic images of serotonin reuptake sites in rat brain labeled with [^{3}H]citalopram. Images of 20 μm thick coronal sections, labeled with 0.8 nM [^{3}H]citalopram, are presented. A - D represent the level of forebrain (A-B), midbrain (C) and brainstem (D) of PN35 rats. E - F represent the level of forebrain (E) and brainstem (F) of PN5 rats. G represents a coronal section of brain stem from embryonic day 19 fetuses. H represents image of PN35 brain section showing nonspecific binding of [^{3}H]citalopram in the presence of 1 μM paroxetine. The color scale and corresponding radioactivity (DPM/mg protein) is shown on the right side. The warmer colors (red and yellow) correspond to higher densities and the cooler colors (blue and violet) correspond to lower densities. Abbreviations are as follows: FCX, frontal cortex; PCX, parietal cortex; MS, medial septum; STR, striatum; LH, lateral hypothalamus; BAL, basolateral nucleus of amygdala; CA3, hippocampus Cornu Ammon 3; SN, substantia nigra; DR, dorsal raphe; MNR, median raphe.

TABLE 2

SPECIFIC BINDING OF [^{3}H]-CITALOPRAM IN CONTROL PAIR-FED RATS

		Specific binding (pmol/mg protein equivalent)			
Region	Age	0.8nM [3H]-citalopram	(n)	2.4nM [3H]-citalopram	(n)
FCx	PN19	0.286±0.094	(6)	0.507±0.197	(6)
	PN35	0.301±0.090	(6)	0.616±0.172	(6)
PCx	PN5	0.119±0.080	(6)	0.196±0.104	(6)
	PN19	0.343±0.120	(6)	0.530±0.152	(6)
	PN35	0.271±0.073	(6)	0.509±0.159	(6)
CA3	PN19	0.541±0.301	(8)	0.910±0.268	(8)
	PN35	0.714±0.284	(8)	1.103±0.277	(8)
LH	PN19	1.249±0.313	(7)	1.671±0.335	(7)
	PN35	0.959±0.234	(7)	1.557±0.344	(7)
AMG	PN19	0.903±0.378	(8)	1.288±0.280	(8)
	PN35	1.128±0.291	(8)	1.698±0.245	(8)
SN	PN19	0.798±0.312	(6)	1.441±0.184	(7)
	PN35	1.105±0.312	(6)	1.537±0.395	(7)
DR	PN5	0.515±0.224	(6)	0.838±0.313	(6)
	PN19	1.713±0.160	(8)	2.098±0.174	(8)
	PN35	1.571±0.179	(8)	2.079±0.188	(8)
MnR	PN19	1.459±0.420	(7)	2.071±0.162	(7)
	PN35	1.129±0.194	(7)	1.803±0.193	(7)

All the data are presented as the mean ± SD.
Abbreviation: FCx, frontal cortex; PCx, parietal cortex; CA3, hippocampus cornu ammonis 3; LH, lateral hypothalamus; AMG, amygdala; SN, substantia nigra; DR, dorsal raphe; MnR, median raphe; PN, postnatal day.

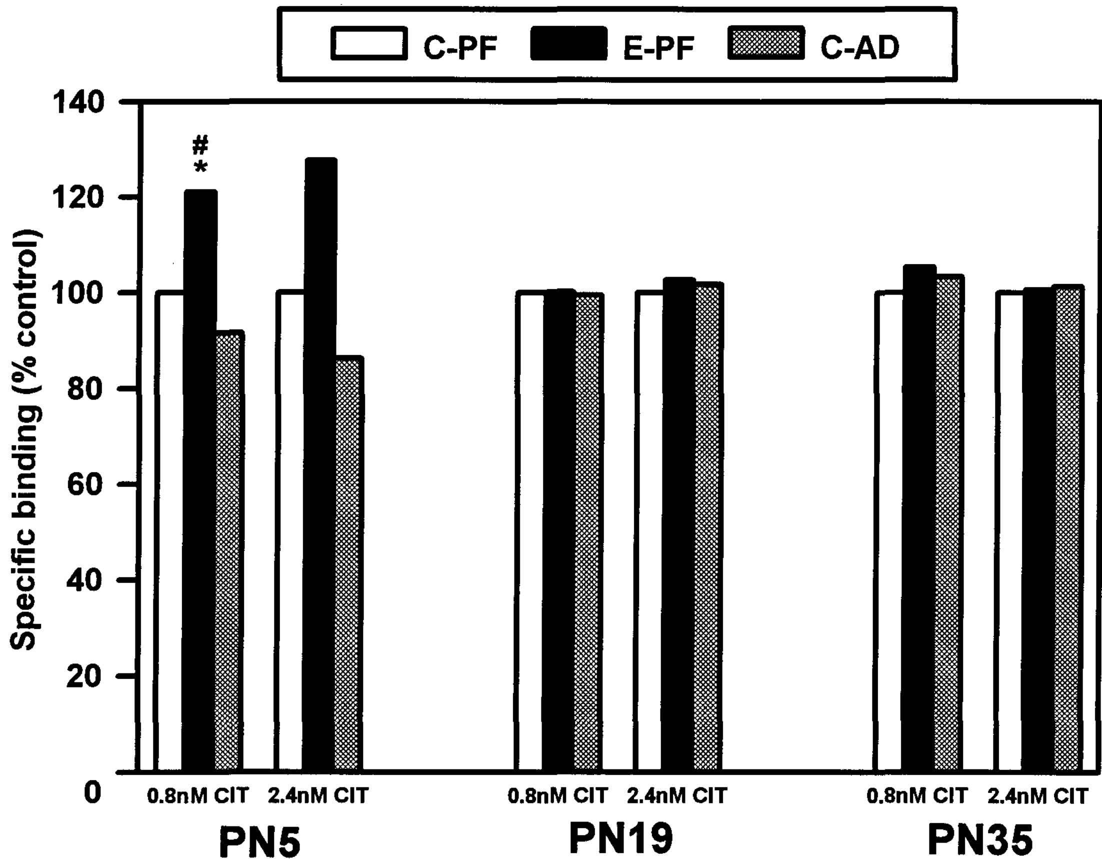


Fig. 3. Effects of *in utero* ethanol exposure on the binding of [^{3}H]citalopram in the dorsal raphe. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 2. The asterisk (*) denotes values which are significantly different from those in the age-matched pair-fed control group ($F = 19.4$, $p = 0.0004$), and the pound (#) denotes values which are significantly different from those in the age-matched ad libitum control group ($F = 19.4$, $p = 0.0004$). Abbreviations are as follows: C-PF, pair-fed control; E-PF, pair-fed ethanol; C-AD, ad libitum control; PN, postnatal day.

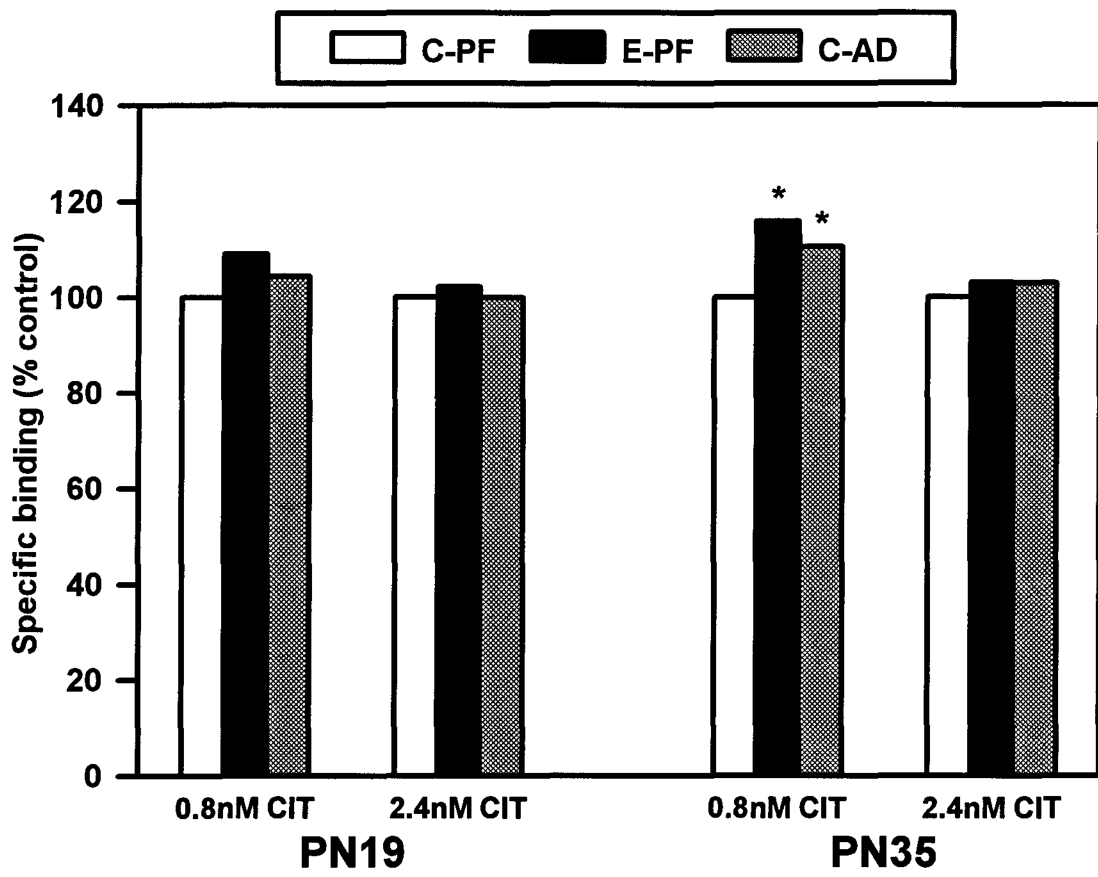


Fig. 4. Effects of *in utero* ethanol exposure on the binding of [^{3}H]citalopram in the median raphe. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 2. The asterisk (*) denotes values which are significantly different from those in the age-matched pair-fed control group ($F = 7.7$, $p = 0.0071$). Abbreviations are the same as those in Fig. 3.

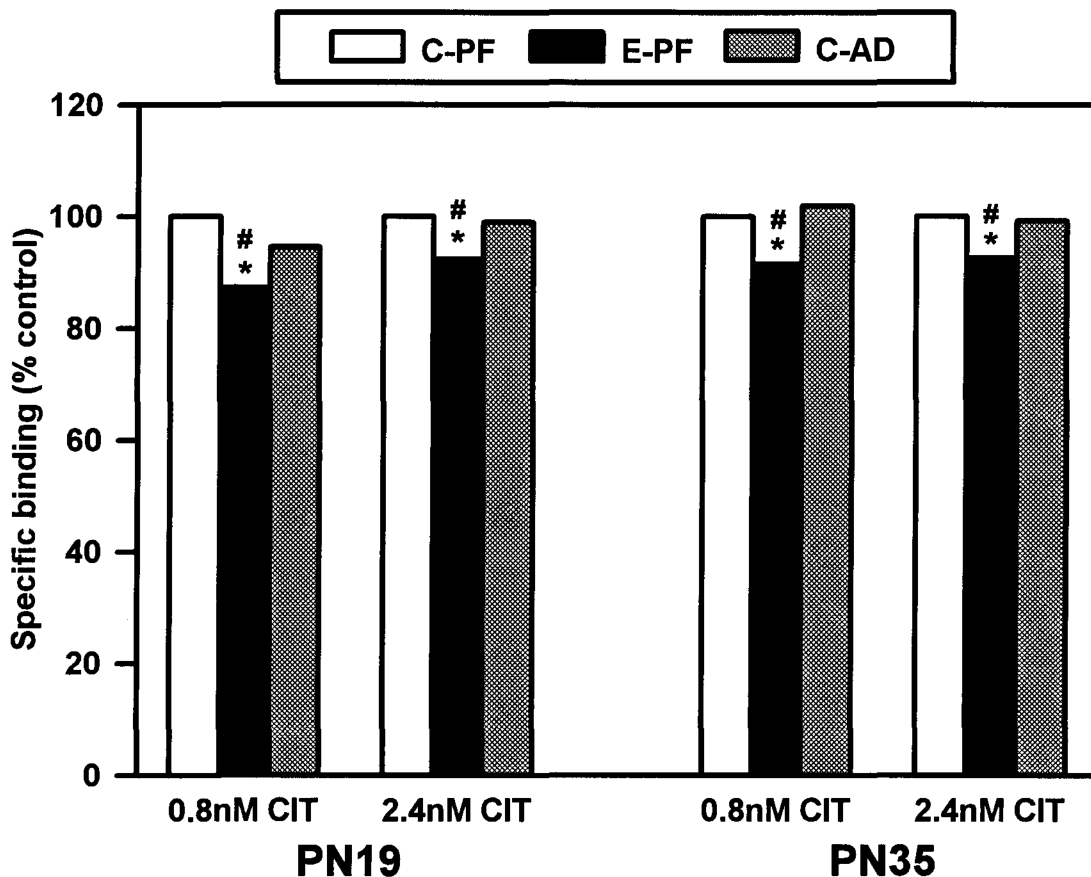


Fig. 5. Effects of *in utero* ethanol exposure on the binding of [^{3}H]citalopram in the lateral hypothalamus. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 2. The asterisk (*) denotes values which are significantly different from those in the age-matched pair-fed control group, and the pound (#) denotes values which are significantly different from those in the age-matched ad libitum control group (at PN19, 0.8 nM [^{3}H]citalopram: $F = 12.2$, $p = 0.0013$; at PN19, 2.4 nM [^{3}H]citalopram: $F = 8.2$, $p = 0.006$; at PN35, 0.8 nM [^{3}H]citalopram: $F = 5.3$, $p = 0.02$; at PN35, 2.4 nM [^{3}H]citalopram: $F = 12.1$, $p = 0.001$). Abbreviations are the same as those in Fig. 3.

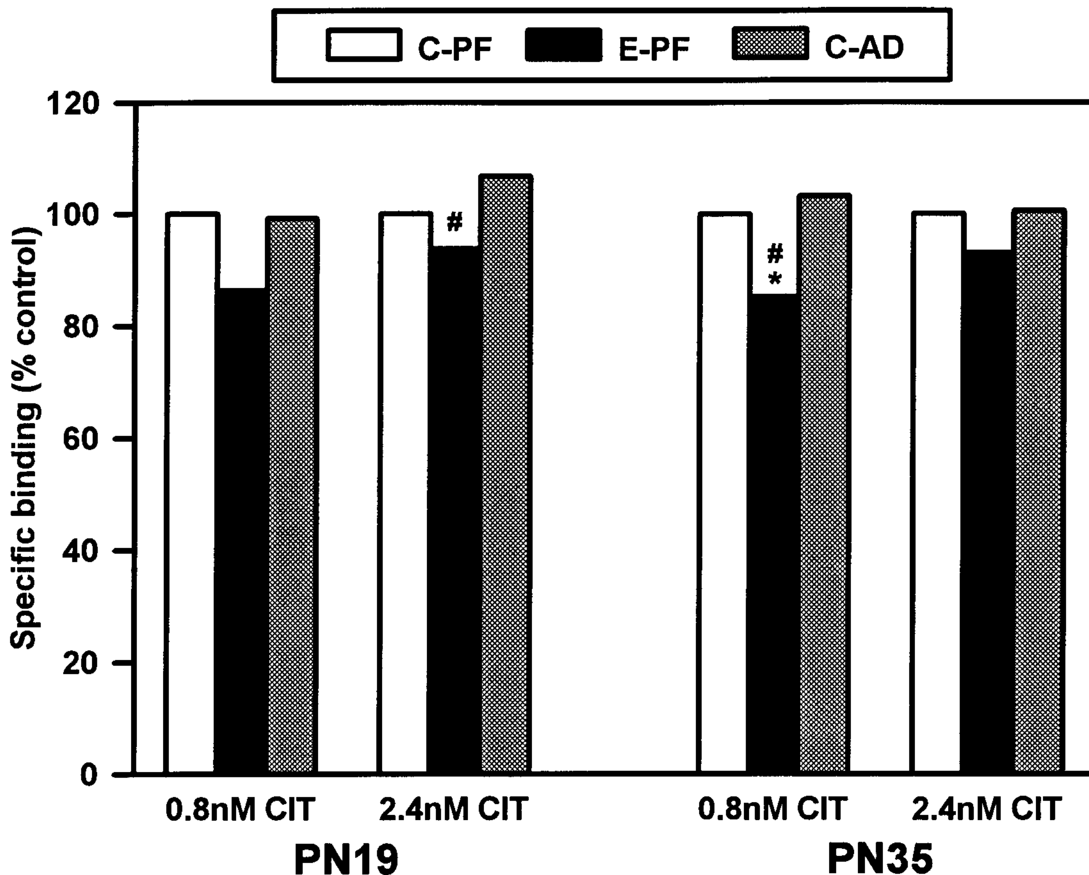


Fig. 6. Effects of *in utero* ethanol exposure on the binding of [^{3}H]citalopram in the substantia nigra. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 2. The asterisk (*) denotes values which are significantly different from those in the age-matched pair-fed control group, and the pound (#) denotes values which are significantly different from those in the age-matched ad libitum control group (at PN19, 2.4 nM [^{3}H]citalopram: $F = 17.8$, $p = 0.0005$; at PN35, 0.8 nM [^{3}H]citalopram: $F = 6.6$, $p = 0.01$). Abbreviations are the same as those in Fig. 3.

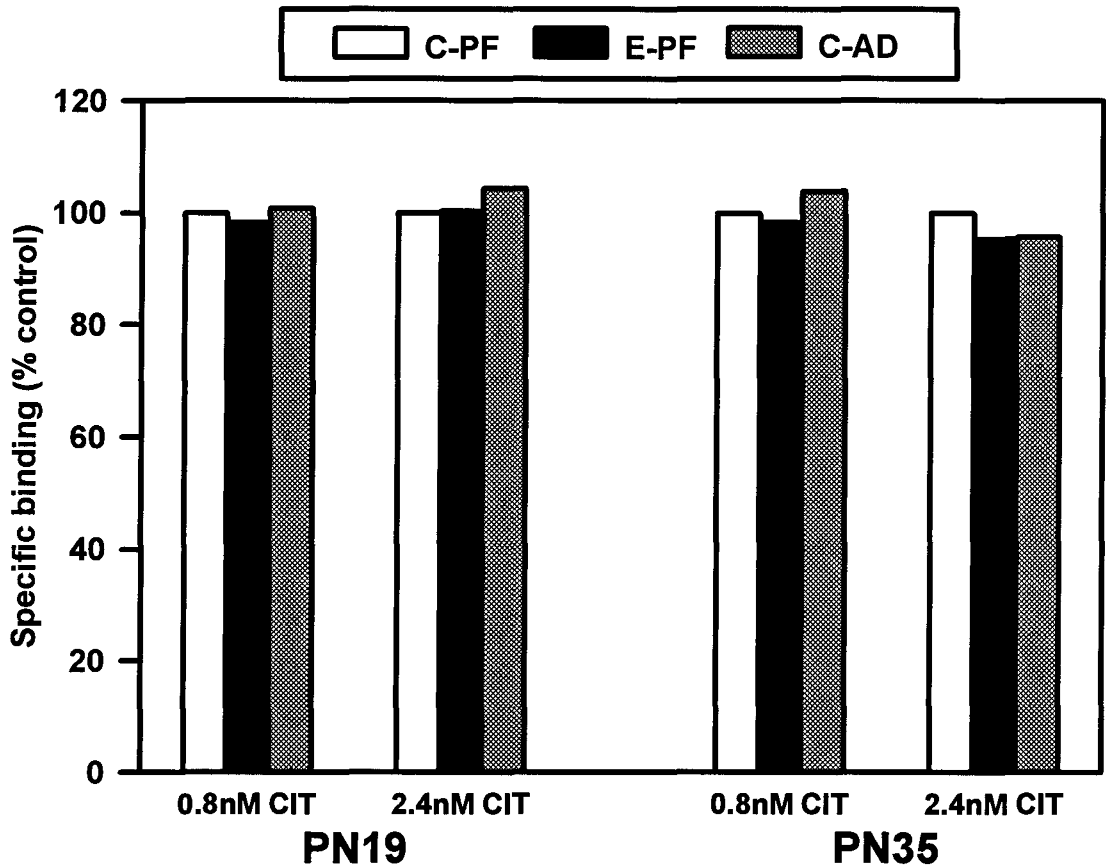


Fig. 7. Effects of *in utero* ethanol exposure on the binding of [^{3}H]citalopram in the basolateral nucleus of amygdala. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 2. *In utero* ethanol exposure did not affect the binding of [^{3}H]citalopram in this region at either age. Abbreviations are the same as those in Fig. 3.

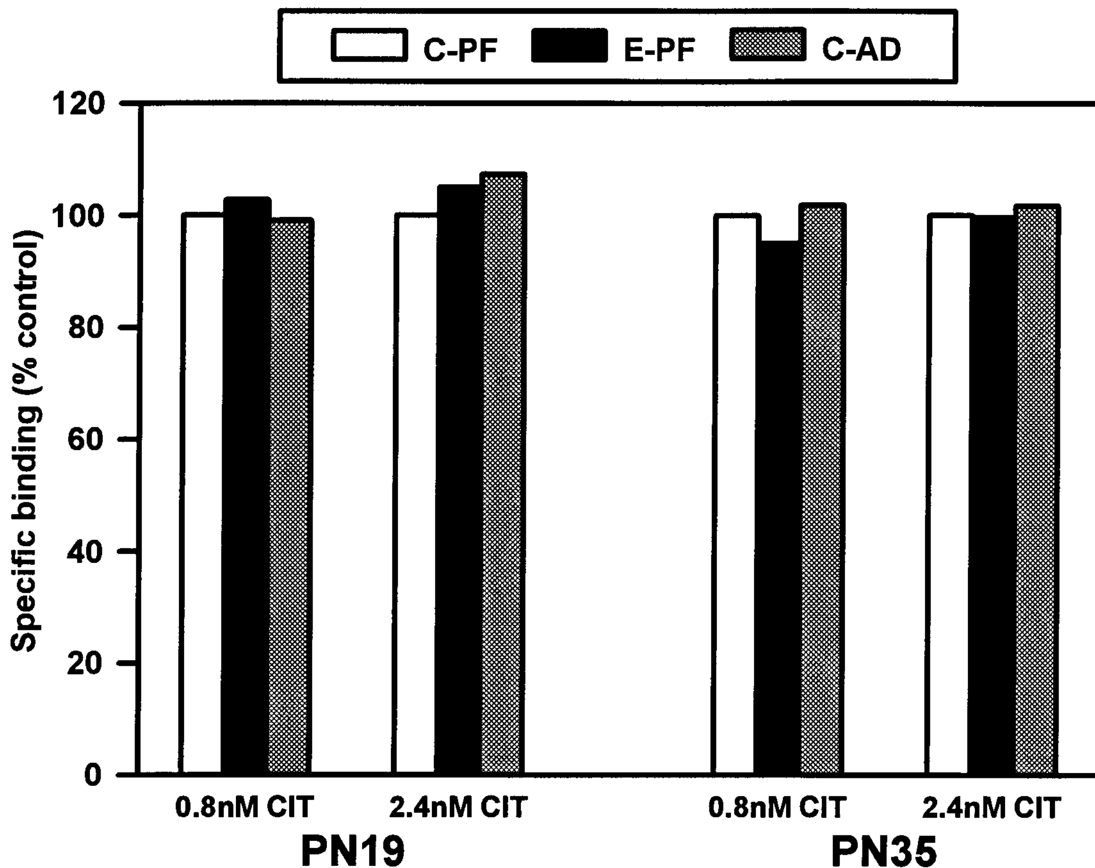


Fig. 8. Effects of *in utero* ethanol exposure on the binding of [^{3}H]citalopram in the hippocampal CA3. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 2. *In utero* ethanol exposure did not affect the binding of [^{3}H]citalopram in this region at either age. Abbreviations are the same as those in Fig. 3.

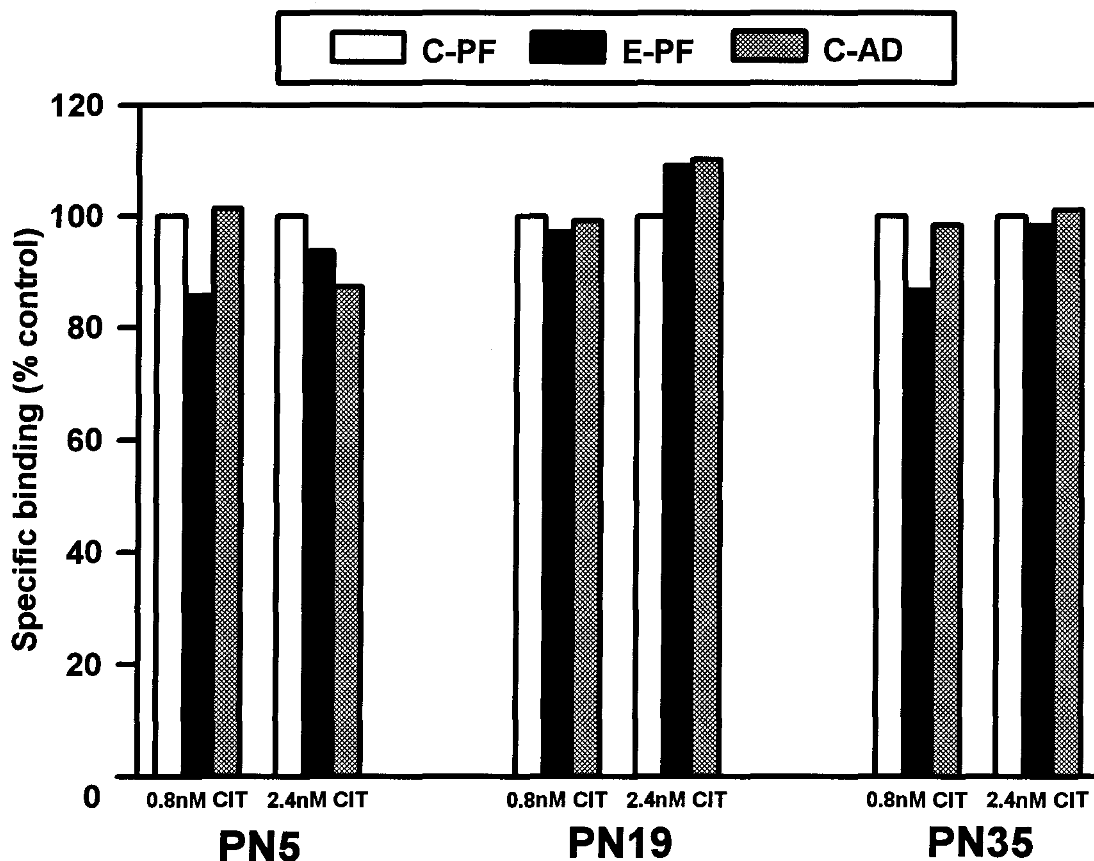


Fig. 9. Effects of *in utero* ethanol exposure on the binding of [^{3}H]citalopram in the parietal cortex. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 2. *In utero* ethanol exposure did not affect the binding of [^{3}H]citalopram in this region at all ages. Abbreviations are the same as those in Fig. 3.

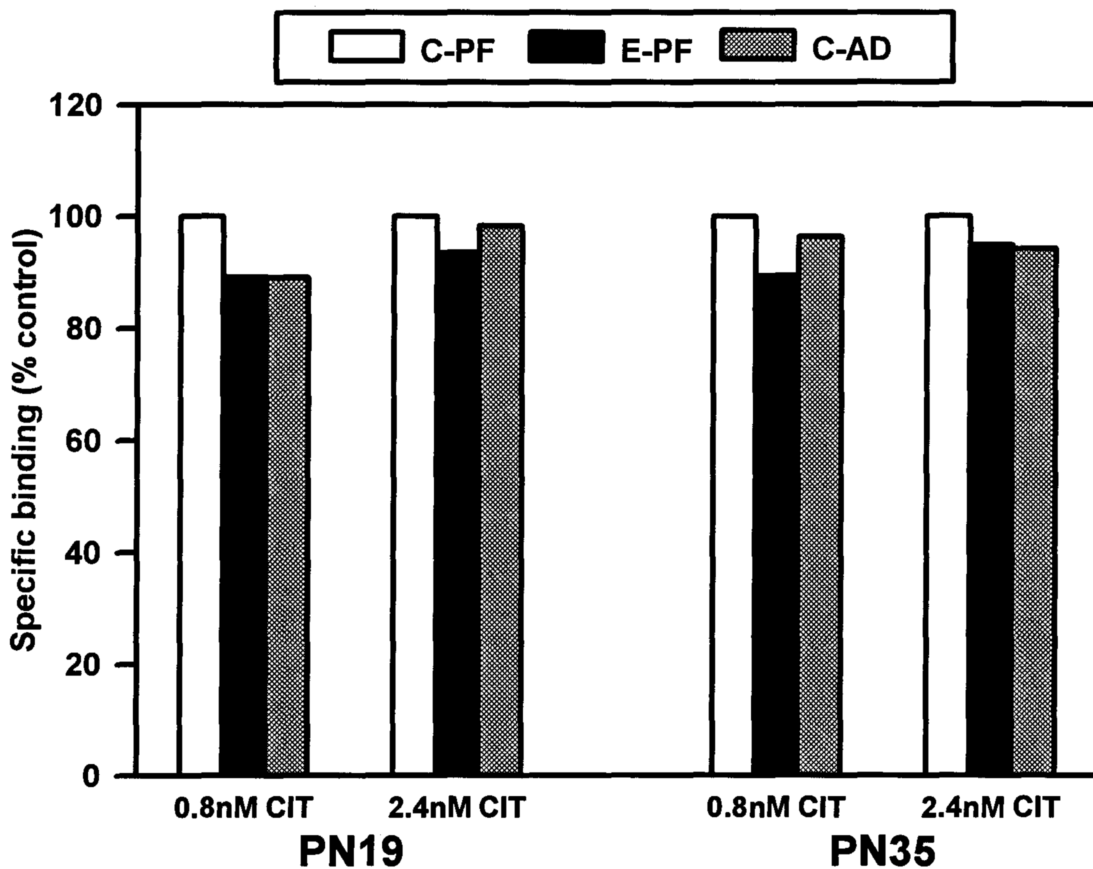


Fig. 10. Effects of *in utero* ethanol exposure on the binding of [^{3}H]citalopram in the frontal cortex. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 2. *In utero* ethanol exposure did not affect the binding of [^{3}H]citalopram in this region at either age. Abbreviations are the same as those in Fig. 3.

Several significant abnormalities were found in the developing ethanol-exposed offspring. There was a transient increase in [^{3}H]citalopram binding in the DR at PN5 (Fig. 3), and in the MnR on PN35 (Fig. 4). There was also a decrease in binding in the LH at PN19 and PN35 (Fig. 5). In the SN (Fig. 6), binding was decreased at both PN19 and PN35. In some cases, the significantly altered binding was detected at one but not both ligand concentrations. At the other ligand concentrations, a similar, though nonsignificant trend was noted.

Effects of *In Utero* Ethanol Exposure on the Development of 5-HT_{1A} Receptors

5-HT_{1A} receptors were radiolabeled with either 1.1 nM or 2.75 nM [^{3}H]-8-OH-DPAT, a specific 5-HT_{1A} receptor agonist. Figure 11 depicts autoradiograms of control brain regions from rats aged PN5 and PN35. Table 3 contains the values for the mean and standard deviations for the specific binding of [^{3}H]-8-OH-DPAT to brain regions from control rats. In each binding assay, samples from C-PF, C-AD, and E-PF rats were included. The radiolabeled sections from a given experiment were exposed to a single film. The results of the multiple experiments were compared using the randomized block design, in order to adjust for differences between individual experiments. Figures 12 - 17 depict the values for C-AD and E-PF rats as a percentage of the values for C-PF rats, included in the same experiment. Statistical differences were calculated using a 2-way ANOVA with random block design.

At PN5, binding of [^{3}H]-8-OH-DPAT was detectable only in the DG. However,

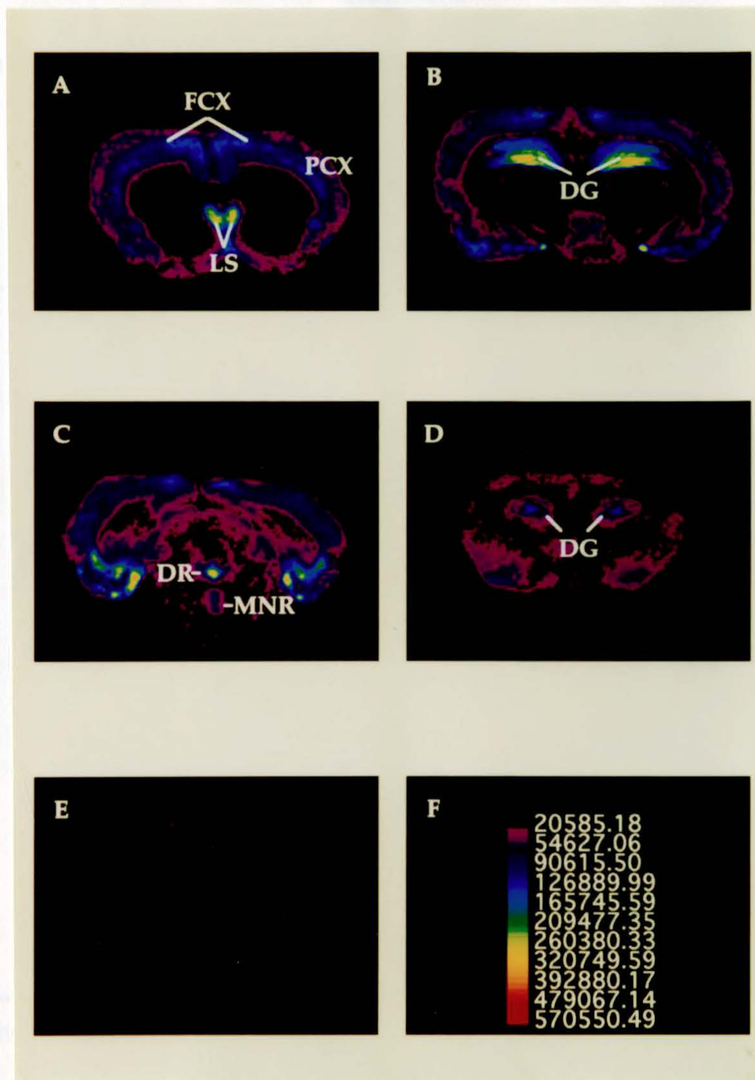


Fig. 11. Autoradiographic images of 5-HT$_{1A}$ receptor sites labeled with [^{3}H]-8-OH-DPAT. Images of 20 μm thick coronal sections, labeled with 1.1 nM [^{3}H]-8-OH-DPAT, are presented. A - C represents sections of forebrain (A and B) and brainstem (C) of PN35 rats. D represents the section of forebrain of PN5. Nonspecific binding of [^{3}H]-8-OH-DPAT in the presence of 1 μM 5-HT is shown in E. The color scale and corresponding radioactivity are shown in F. The warmer colors (red and yellow) correspond to higher densities and the cooler colors (blue and violet) correspond to lower densities. Abbreviations are as follows: FCX, frontal cortex; PCX, parietal cortex; LS, lateral septum; DG, dentate gyrus; DR, dorsal raphe; MNR, median raphe.

TABLE 3

SPECIFIC BINDING OF [^{3}H]-8-OH-DPAT IN CONTROL PAIR-FED RATS

Region	Age	Specific binding (pmol/mg protein equivalent) 1.1nM [^{3}H]-8-OH-DPAT	(n)	2.75nM [^{3}H]-8-OH-DPAT	(n)
FCx	PN19	0.099±0.033	(7)	0.164±0.076	(6)
	PN35	0.166±0.042	(7)	0.242±0.069	(6)
PCx	PN19	0.154±0.070	(7)	0.226±0.071	(7)
	PN35	0.212±0.057	(7)	0.279±0.084	(7)
LS	PN19	0.580±0.168	(6)	0.709±0.180	(6)
	PN35	0.535±0.066	(6)	0.658±0.119	(6)
DG	PN5	0.232±0.071	(6)	0.265±0.089	(6)
	PN19	0.584±0.132	(6)	0.737±0.185	(6)
	PN35	0.731±0.073	(6)	0.825±0.191	(6)
DR	PN19	0.575±0.142	(6)	0.685±0.191	(6)
	PN35	0.581±0.140	(6)	0.646±0.100	(6)
MnR	PN19	0.270±0.080	(6)	0.354±0.115	(6)
	PN35	0.150±0.040	(6)	0.245±0.082	(6)

All the data are presented as the mean ± SD.
Abbreviation: FCx, frontal cortex; PCx, parietal cortex; LS, lateral septum; DG, dentate gyrus; DR, dorsal raphe; MnR, median raphe; PN, postnatal day.

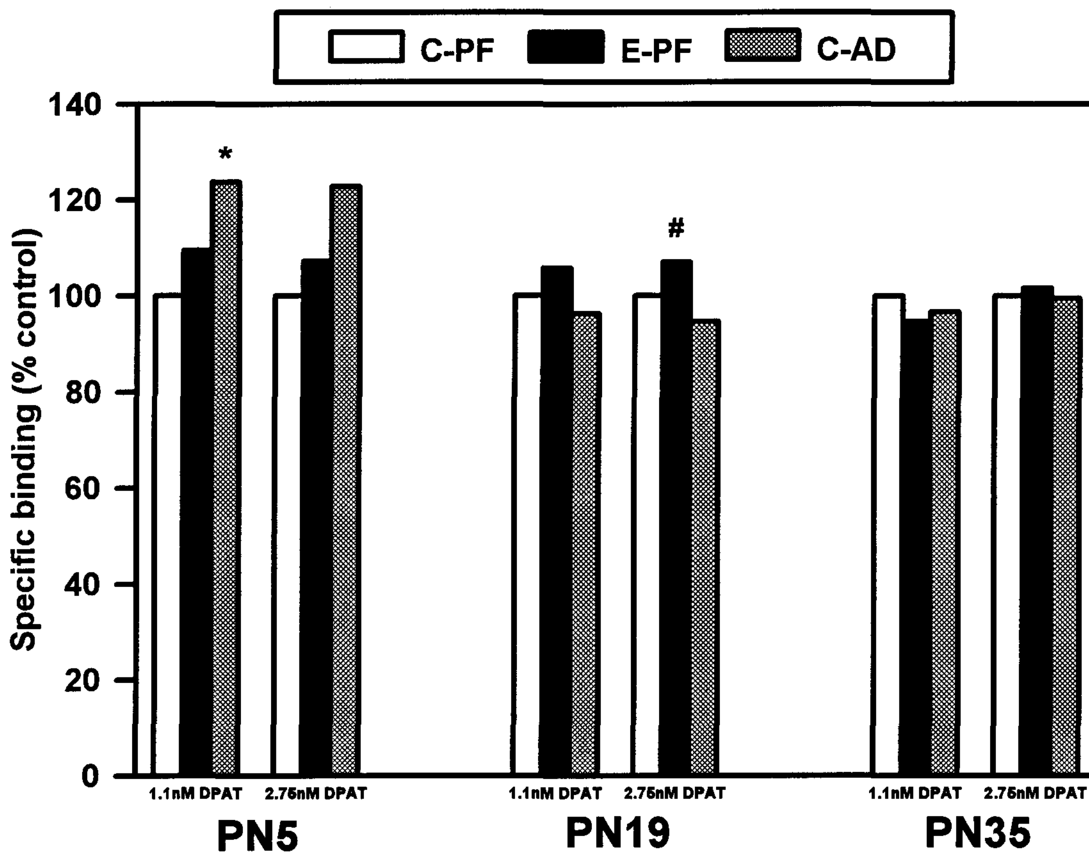


Fig. 12. Effects of *in utero* ethanol exposure on the binding of [^{3}H]-8-OH-DPAT in the dentate gyrus. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 3. The asterisk (*) denotes values which are significantly different from those in the age-matched pair-fed control group ($F = 3.8$, $p = 0.058$), and the pound (#) denotes values which are significantly different from those in the age-matched ad lib control group ($F = 6.5$, $p = 0.01$). Abbreviations are the same as those in Fig. 3.

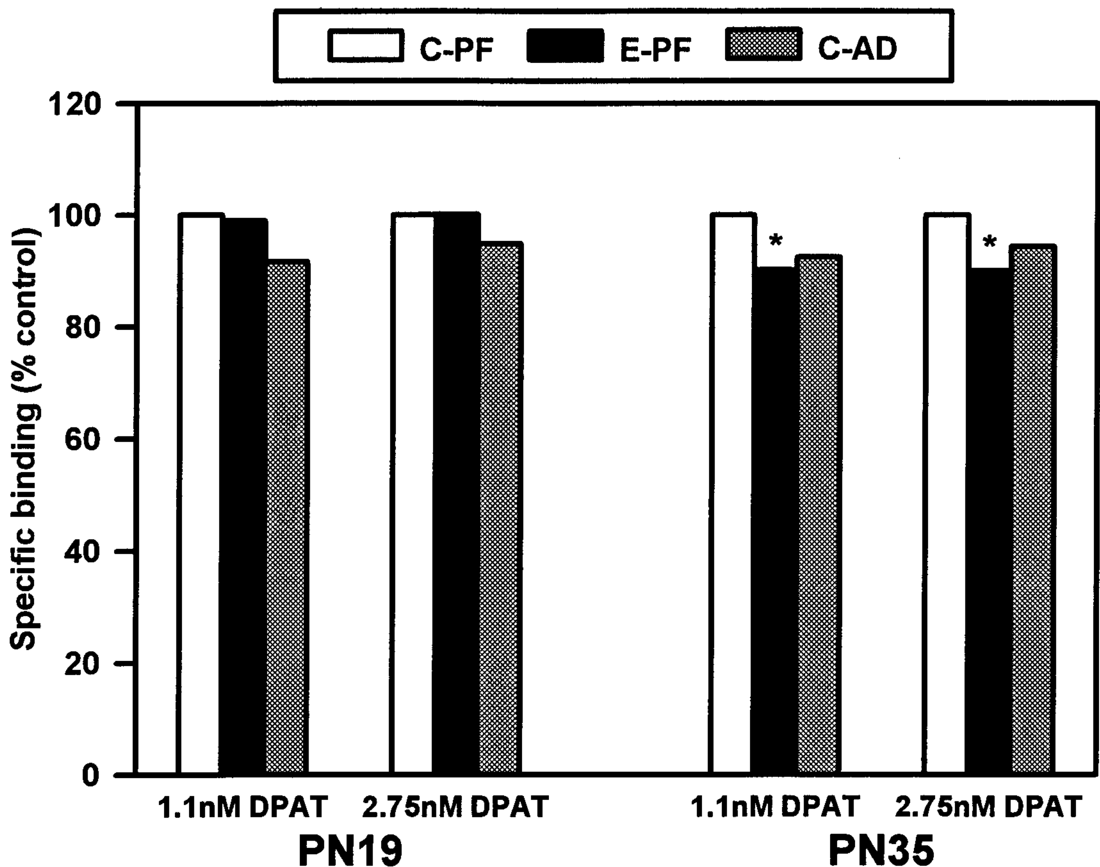


Fig. 13. Effects of *in utero* ethanol exposure on the binding of [^{3}H]-8-OH-DPAT in the parietal cortex. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 3. The asterisk (*) denotes values which are significantly different from those in the age-matched pair-fed control group (at PN35, 1.0 nM [^{3}H]-8-OH-DPAT: $F = 4.2$, $p = 0.04$; at PN35, 2.7 nM [^{3}H]-8-OH-DPAT: $F = 4.3$, $p = 0.04$). Abbreviations are the same as those in Fig. 3.

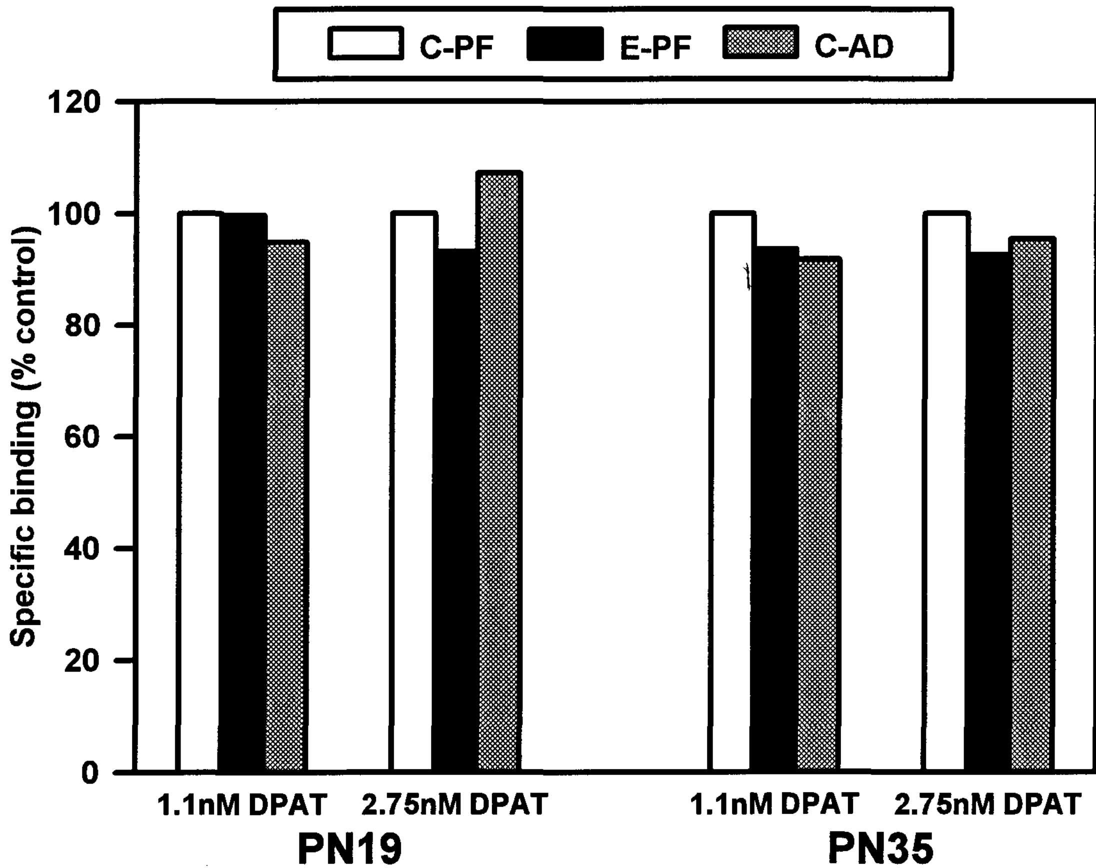


Fig. 14. Effects of *in utero* ethanol exposure on the binding of [^{3}H]-8-OH-DPAT in the frontal cortex. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 3. In utero ethanol exposure did not affect the binding of [^{3}H]-8-OH-DPAT in this region at either age. Abbreviations are the same as those in Fig. 3.

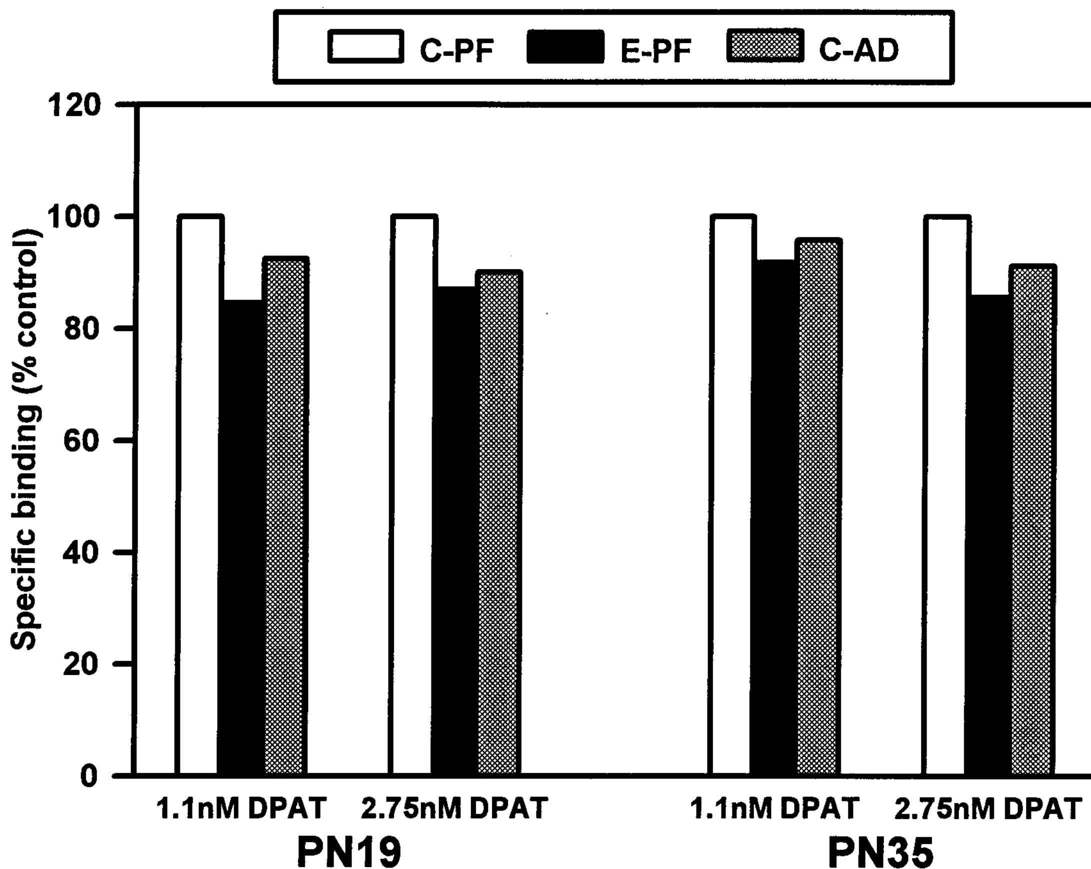


Fig. 15. Effects of *in utero* ethanol exposure on the binding of [^{3}H]-8-OH-DPAT in the lateral septum. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 3. *In utero* ethanol exposure did not affect the binding of [^{3}H]-8-OH-DPAT in this region at either age. Abbreviations are the same as those in Fig. 3.

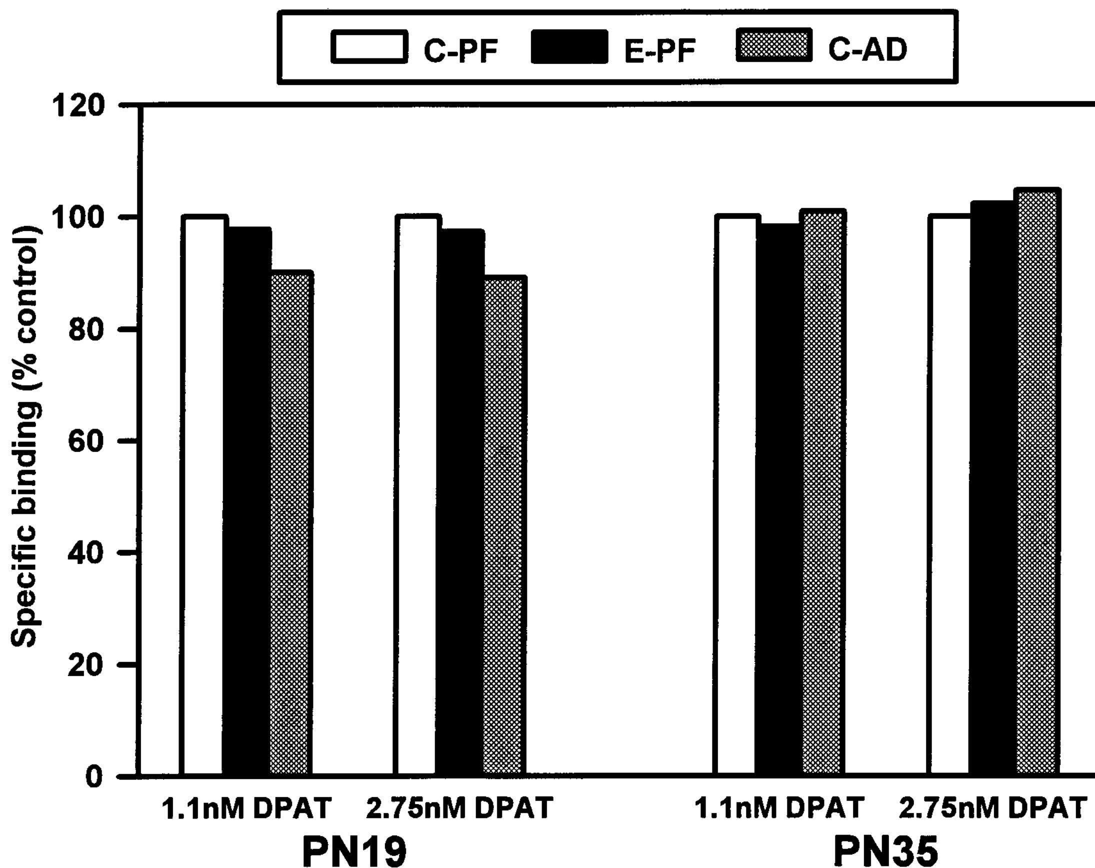


Fig. 16. Effects of *in utero* ethanol exposure on the binding of [^{3}H]-8-OH-DPAT in the dorsal raphe. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 3. *In utero* ethanol exposure did not affect the binding of [^{3}H]-8-OH-DPAT in this region at either age. Abbreviations are the same as those in Fig. 3.

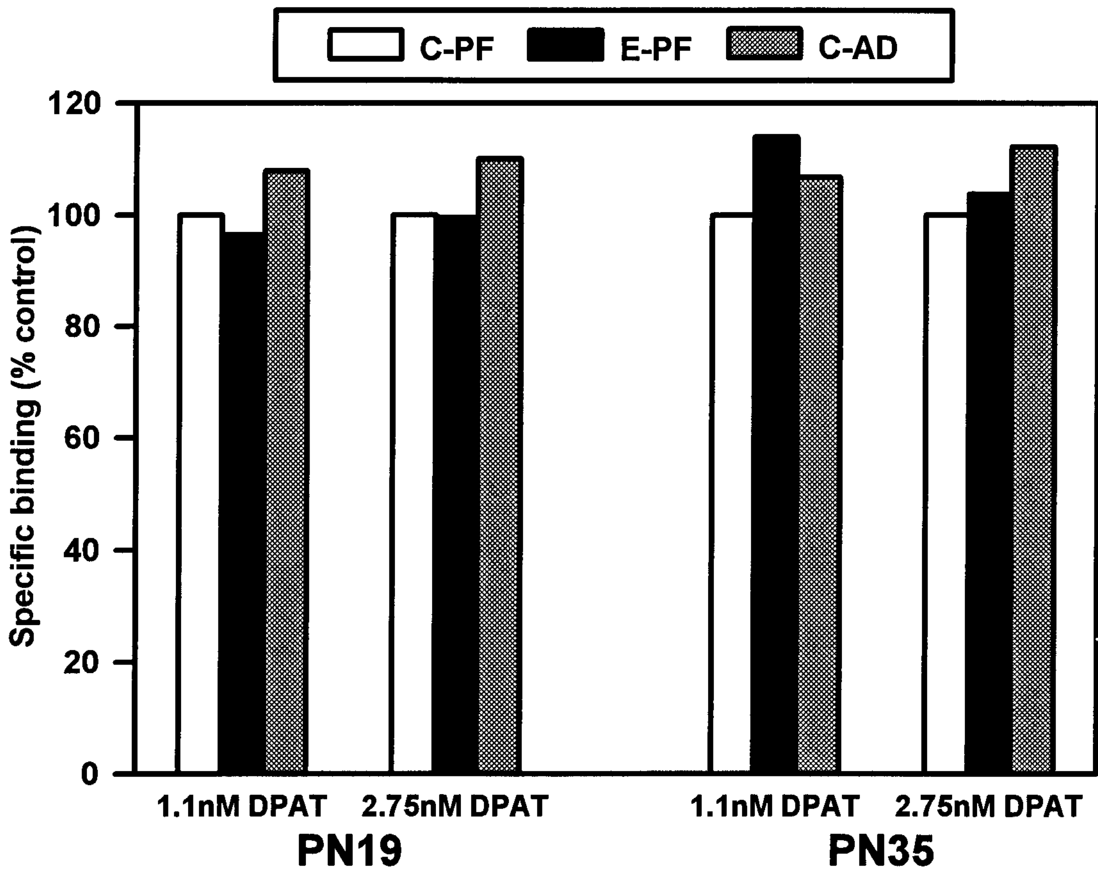


Fig. 17. Effects of *in utero* ethanol exposure on the binding of [^{3}H]-8-OH-DPAT in the median raphe. Results are expressed as percent of the specific binding obtained from the pair-fed control group at each age. The pair-fed control values are given in Table 3. *In utero* ethanol exposure did not affect the binding of [^{3}H]-8-OH-DPAT in this region at either age. Abbreviations are the same as those in Fig. 3.

by PN19 5-HT_{1A} receptors could be quantitated in several brain areas. At both PN19 and PN35 binding was highest in the DR, DG and LS. Somewhat lower binding was noted in the MnR, PCx and FCx. With one exception, there were no significant differences in the binding of [^{3}H]-8-OH-DPAT to tissue from pair-fed and ad lib control offspring. However, in ethanol-exposed offspring there was a transient increase in [^{3}H]-8-OH-DPAT binding in the DG (PN19, Fig. 12) and a transient decrease in binding in the PCx (PN35, Fig. 13).

Effects of Buspirone Treatment on the Ethanol-Associated Developmental Abnormalities in 5-HT Reuptake Sites and 5-HT_{1A} Receptors

Previous investigations from this laboratory (Druse et al., 1991) indicated that 5-HT content was decreased by ~50% in the brain stem of ED15 rats. Since fetal 5-HT appears to function as an essential trophic factor for the development of serotonergic as well as other types of neurons, it was hypothesized that the ethanol-induced fetal deficit of 5-HT could contribute to the subsequent abnormal development of serotonergic neurons by decreasing stimulation of 5-HT_{1A} receptors. In order to test this hypothesis, pregnant control and ethanol-fed dams were treated with buspirone, a 5-HT_{1A} agonist, from ED13 to ED20 to determine whether treatment with a 5-HT_{1A} agonist could prevent or reverse ethanol-associated developmental abnormalities. Additional control and ethanol-fed dams were treated with saline instead of buspirone. The offspring of these dams were used for analysis of 5-HT reuptake and 5-HT_{1A} receptors. All rats used in

these studies were distinct from those described in sections 2 and 3. Nonetheless, a portion of these studies replicated the work reported in those sections. In addition, it expanded the earlier studies to include additional brain regions and/or ages, and strengthened the observations by including a large sample number.

Following subcutaneous injection of buspirone, the pregnant rats sometimes exhibited brief and mild symptoms of the serotonin syndrome (Smith and Peroutka, 1986). These rats exhibited a flattened body posture and outstretched forepaws. In addition, the buspirone-treated control (CB) dams exhibited a smaller weight gain than that noted in either of the pair-fed groups (Table 4). In contrast, the weight gain in both of the ethanol-treated groups (ethanol-saline, ES; ethanol-buspirone, EB) was comparable to that of the control-saline (CS) group. Despite the noted effects on the dams, the weight gain in the offspring of buspirone-treated control- and ethanol-fed rats was not significantly different at PN5, PN19 and PN35 (Fig. 18).

Table 5 contains the values for the specific binding of 0.8 nM and 2.4 nM [^{3}H]citalopram to brain sections obtained from the offspring of saline-treated, pair-fed control rats (CS). Specific binding was readily detected in the DR by ED19 and in the PCx by PN5. On PN19 and PN35 the highest binding was found in the DR and MnR. The rank order of binding was DR >MnR> LH ~ SN ~ AMG > MS ~ CA3 > Striatum ~ PCx ~ FCx. This pattern was generally similar to that noted in the earlier experiment (Table 2).

When [^{3}H]citalopram binding was compared in the offspring of pair-fed control and ethanol dams, we noted that the ethanol-exposed offspring had a significant decrease

in binding in several regions. Binding was decreased at one or both ligand concentrations in the FCx (PN19, Fig. 19), PCx (PN19, Fig. 20), LH (PN19 & PN35 Fig. 21), SN (PN19 & PN35, Fig. 22), MS (PN19, Fig. 23) and striatum (PN19 & PN35, Fig.24). In contrast, binding was increased in the DR (Fig. 25) on PN5 and MnR (Fig. 26) on PN19 and PN35. The noted observations in the DR, LH and SN confirm earlier findings; those in the MS and ST expand the first study which did not examine the latter brain areas. It should also be noted that in both investigations, we did not detect any ethanol-associated changes in the hippocampus (CA3, Fig. 27) or amygdala (Fig.28). A few significant differences were noted in one, but not both studies. For example, the latter study detected significantly decreased [^{3}H]citalopram binding in the frontal and parietal cortex. A similar, though nonsignificant trend was found in the frontal cortex in the first investigation.

When the [^{3}H]citalopram binding was compared in the offspring of saline- and buspirone treated dams, maternal treatment with buspirone prevented or reversed some of the ethanol-associated developmental abnormalities in 5-HT reuptake sites. Buspirone prevented the decline in binding of [^{3}H]citalopram at either the low or high concentration noted in the FCx (PN19, Fig. 19), LH (PN19 & PN35, Fig. 21), SN (PN35, Fig. 22), and MS (PN19, Fig. 23). Similarly, buspirone treatment reversed the ethanol-associated increase in binding in the DR (PN5, Fig. 25) and MnR (PN19, Fig 26). In contrast, maternal buspirone treatment had no effect on the decreased number of reuptake sites in the PCx (Fig. 20) and Striatum (Fig. 24). Buspirone treatment alone had little effect on [^{3}H]citalopram binding to reuptake sites.

TABLE 4

WEIGHT INCREASE DURING PREGNANCY (From ED1 to ED20)

	n	Weight increase (g)
CS	5	136.0±13.4[a]
CB	5	110.4±14.3*
ES	5	124.8±14.3
EB	5	118.4±11.7

[a]Data are presented as the mean ± SD.
*$p<0.05$ compared to CS.

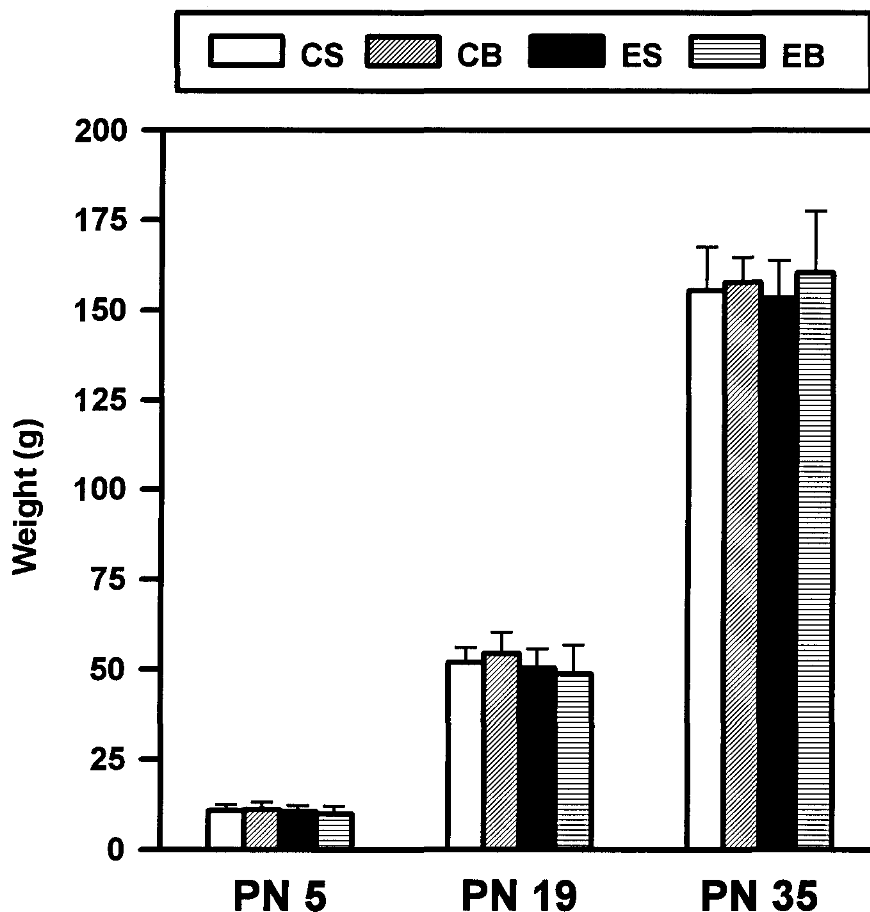


Fig. 18. Effect of buspirone treatment on the weight of offspring at PN5, PN19 and PN35. The results are presented as the mean values obtained from pups of eight different dams. The error bars represent SD. Abbreviations are as follows: CS, saline treated pair-fed control; CB, buspirone-treated pair-fed control; ES, saline-treated ethanol-fed; EB, buspirone-treated ethanol-fed; PN, postnatal day.

TABLE 5

SPECIFIC BINDING OF [^{3}H]-CITALOPRAM IN THE CONTROL SALINE GROUP

		Specific binding (pmol/mg protein equivalent)			
Region	Age	0.8nM [^{3}H]-citalopram	(n)	2.4nM [^{3}H]-citalopram	(n)
FCx	PN19	0.194±0.116	(7)	0.509±0.180	(6)
	PN35	0.243±0.141	(7)	0.719±0.208	(6)
PCx	PN 5	0.278±0.147	(5)	0.334±0.223	(8)
	PN19	0.325±0.172	(6)	0.607±0.260	(6)
	PN35	0.290±0.151	(6)	0.687±0.159	(6)
MS	PN19	0.619±0.245	(7)	1.045±0.306	(7)
	PN35	0.570±0.304	(7)	1.103±0.286	(7)
Striatum	PN19	0.322±0.155	(7)	0.701±0.173	(7)
	PN35	0.343±0.170	(7)	0.713±0.208	(7)
CA3	PN19	0.441±0.219	(8)	0.922±0.265	(8)
	PN35	0.534±0.277	(8)	0.981±0.242	(8)
LH	PN19	1.091±0.397	(7)	1.919±0.397	(7)
	PN35	0.975±0.264	(7)	1.658±0.327	(7)
AMG	PN19	0.781±0.303	(7)	1.298±0.489	(7)
	PN35	1.013±0.322	(7)	1.626±0.425	(7)
SN	PN19	1.129±0.309	(7)	1.629±0.474	(7)
	PN35	1.071±0.227	(7)	1.710±0.278	(7)
DR	ED19	0.386±0.207	(6)	0.471±0.282	(6)
	PN5	0.330±0.109	(5)	0.320±0.188	(7)
	PN19	1.769±0.209	(7)	2.547±0.582	(7)
	PN35	1.757±0.214	(7)	2.485±0.308	(7)
MnR	PN19	1.592±0.234	(6)	2.400±0.405	(7)
	PN35	1.343±0.263	(6)	1.910±0.315	(7)

All the data are presented as the mean ± SD.
Abbreviations are same as in Table 2 except MS, medial septum.

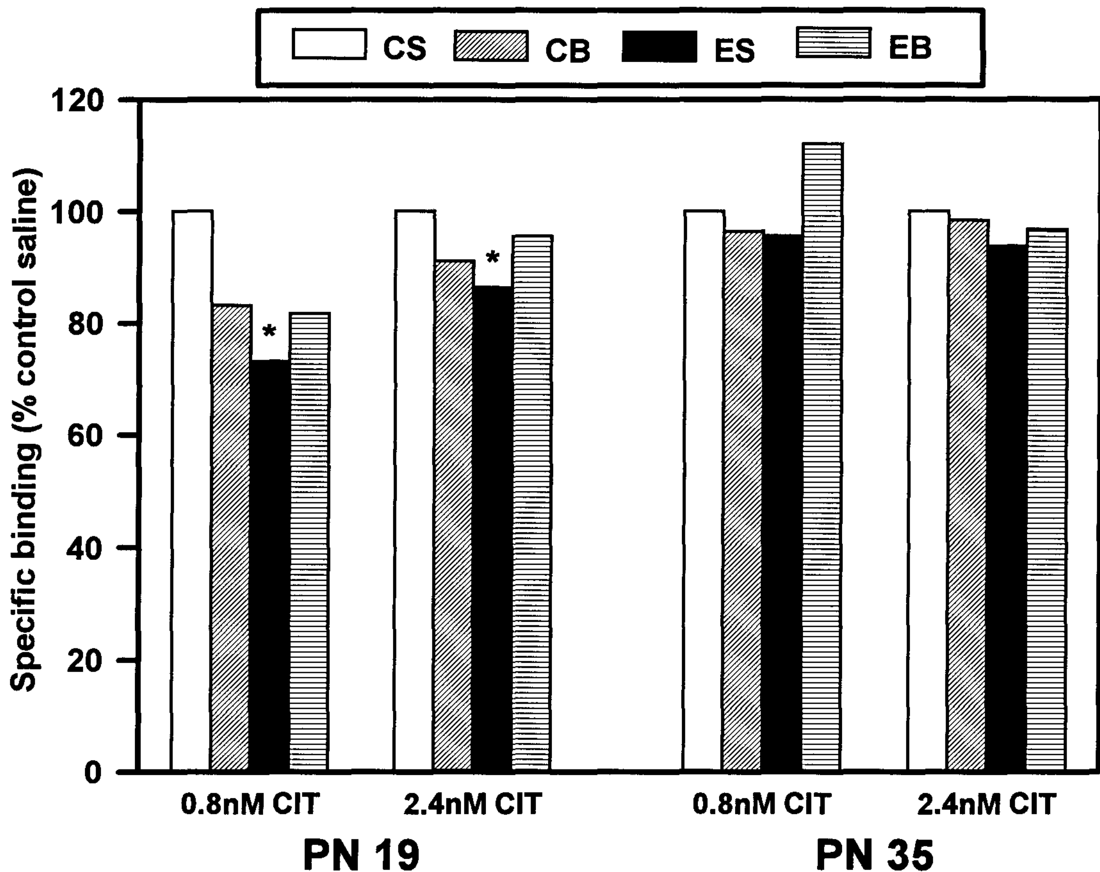


Fig. 19. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the frontal cortex. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group. Abbreviations are as follows: CS, control saline; CB, control buspirone; ES, ethanol saline; EB, ethanol buspirone; ED, embryonic day; PN, postnatal day.

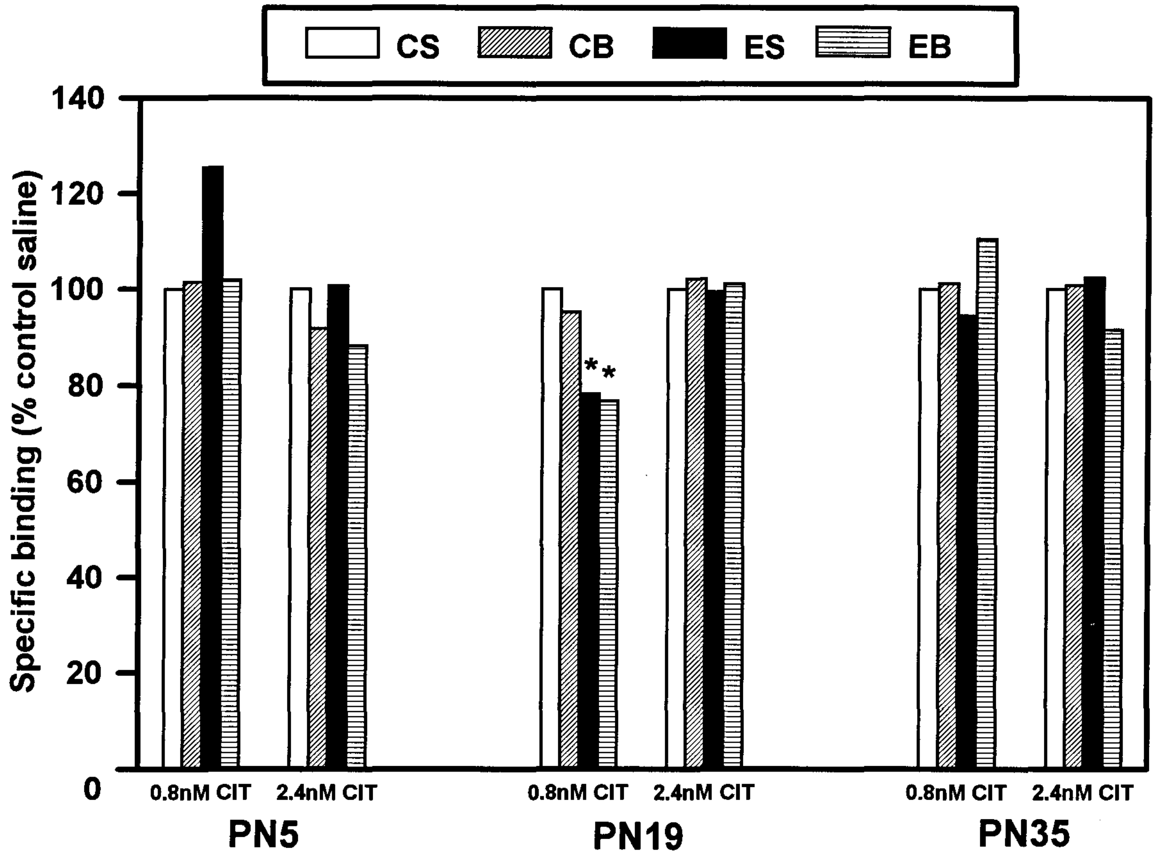


Fig. 20. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the parietal cortex. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (PN19, 0.8 nM [^{3}H]citalopram: $F = 8.7$, $p = 0.03$). Abbreviations are the same as those in the Fig. 19.

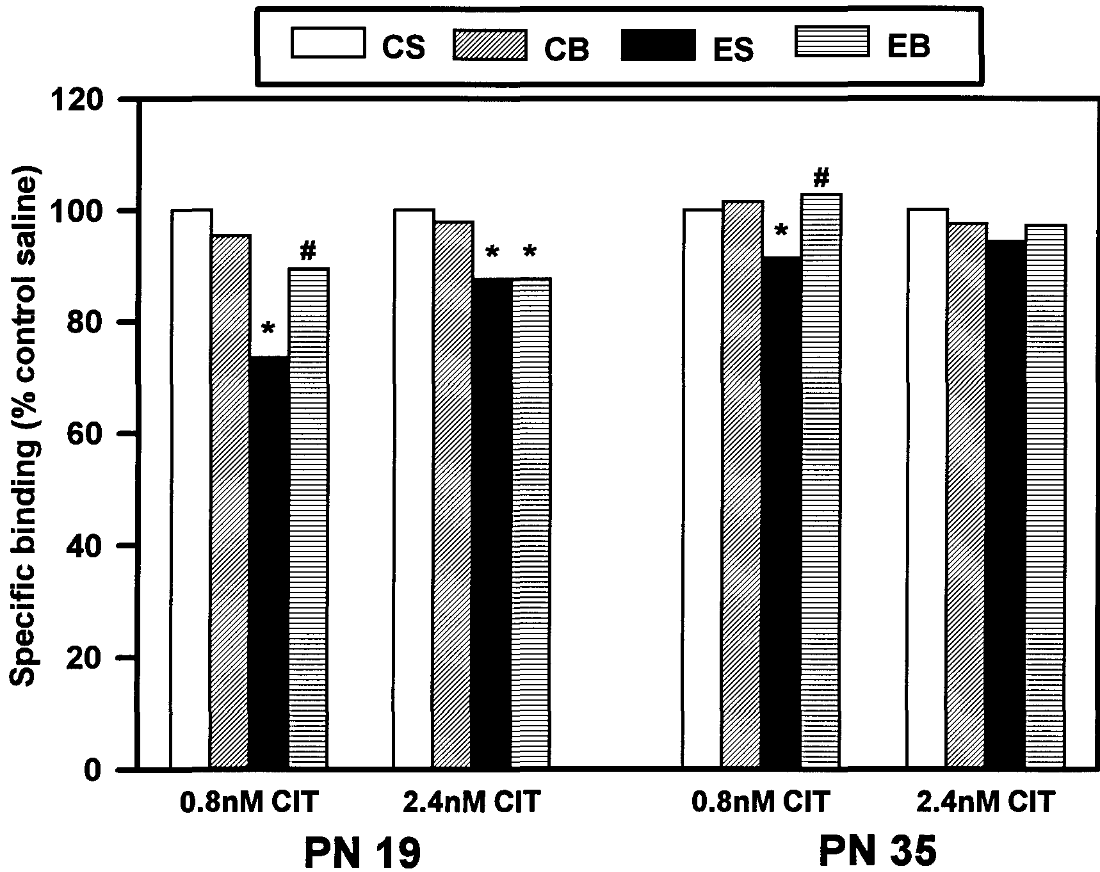


Fig. 21. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the lateral hypothalamus. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (PN19, 0.8 nM [^{3}H]citalopram: $F = 13.7$, $p = 0.01$; PN19, 2.4 nM [^{3}H]citalopram: $F = 7.4$, $p = 0.03$). The pound (#) denotes values which are significantly different from those in the age-matched ethanol saline group (PN35, 0.8 nM [^{3}H]citalopram: $F = 12.5$, $p = 0.01$). There was also a significant ethanol x buspirone interaction at PN19, 0.8 nM [^{3}H]citalopram ($F = 10.0$, $p = 0.02$) and at PN35, 0.8 nM [^{3}H]citalopram ($F = 11.6$, $p = 0.01$). Abbreviations are the same as those in the Fig. 19.

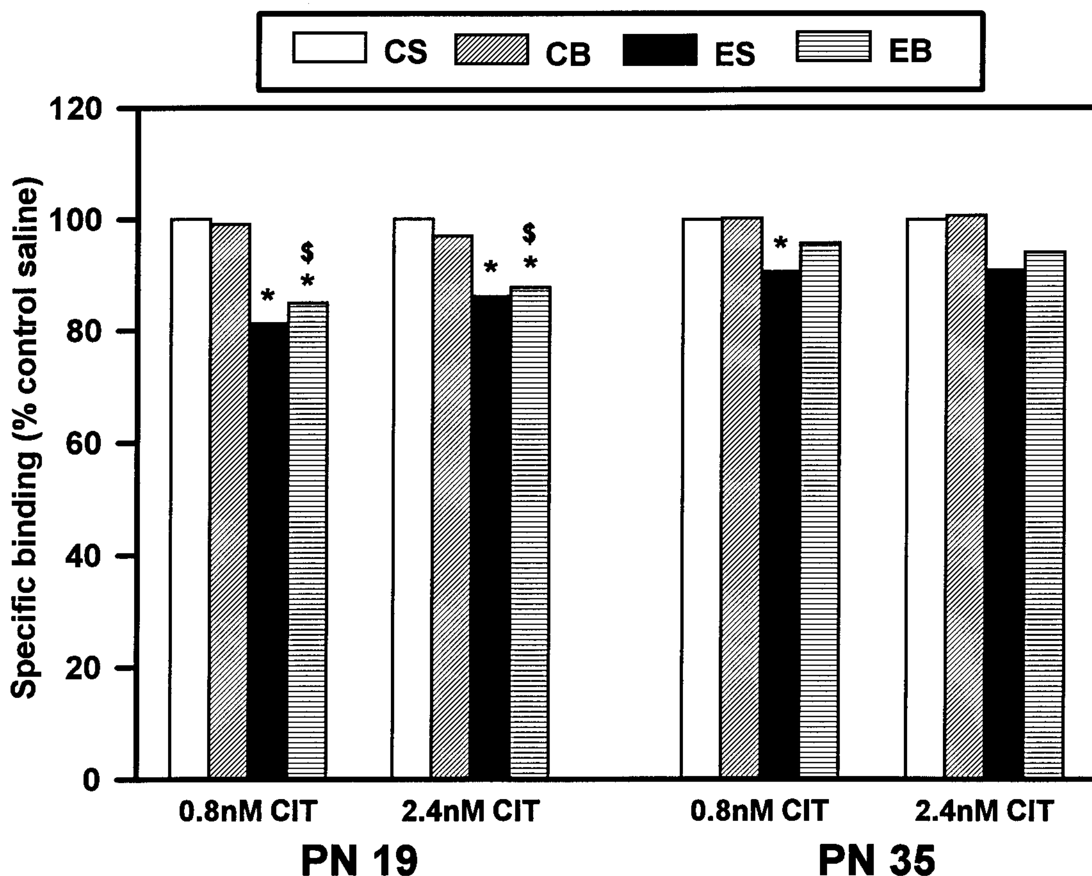


Fig. 22. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the substantia nigra. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (PN19, 0.8 nM [^{3}H]citalopram: $F = 27.0$, $p = 0.002$; PN19, 2.4 nM [^{3}H]citalopram: $F = 12.1$, $p = 0.01$). The dollar sign ($) denotes values which are significantly different from those in the age-matched control buspirone group (PN19, 0.8 nM [^{3}H]citalopram: $F = 27.0$, $p = 0.002$; PN19, 2.4 nM [^{3}H]citalopram: $F = 12.1$, $p = 0.01$). Abbreviations are the same as those in the Fig. 19.

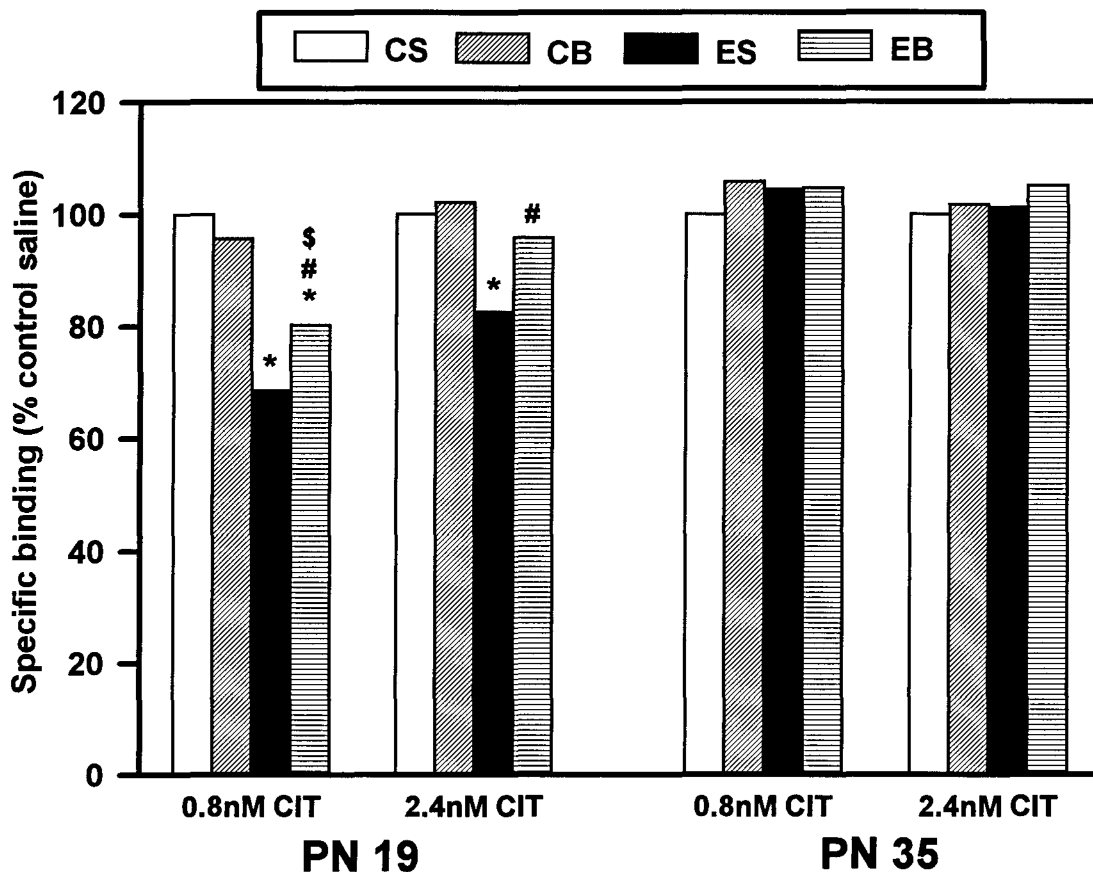


Fig. 23. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the medial septum. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (PN19, 0.8 nM [^{3}H]citalopram: $F = 8.0$, $p = 0.03$). The pound (#) and the dollar signs ($) denotes values which are significantly different from those in the age-matched ethanol saline and control buspirone, respectively (PN19, 2.4 nM [^{3}H]citalopram: $F = 7.1$, $p = 0.04$). There was a significant ethanol x buspirone interaction (PN19, 0.8 nM [^{3}H]citalopram: $F = 6.4$, $p = 0.04$). Abbreviations are the same as those in the Fig. 19.

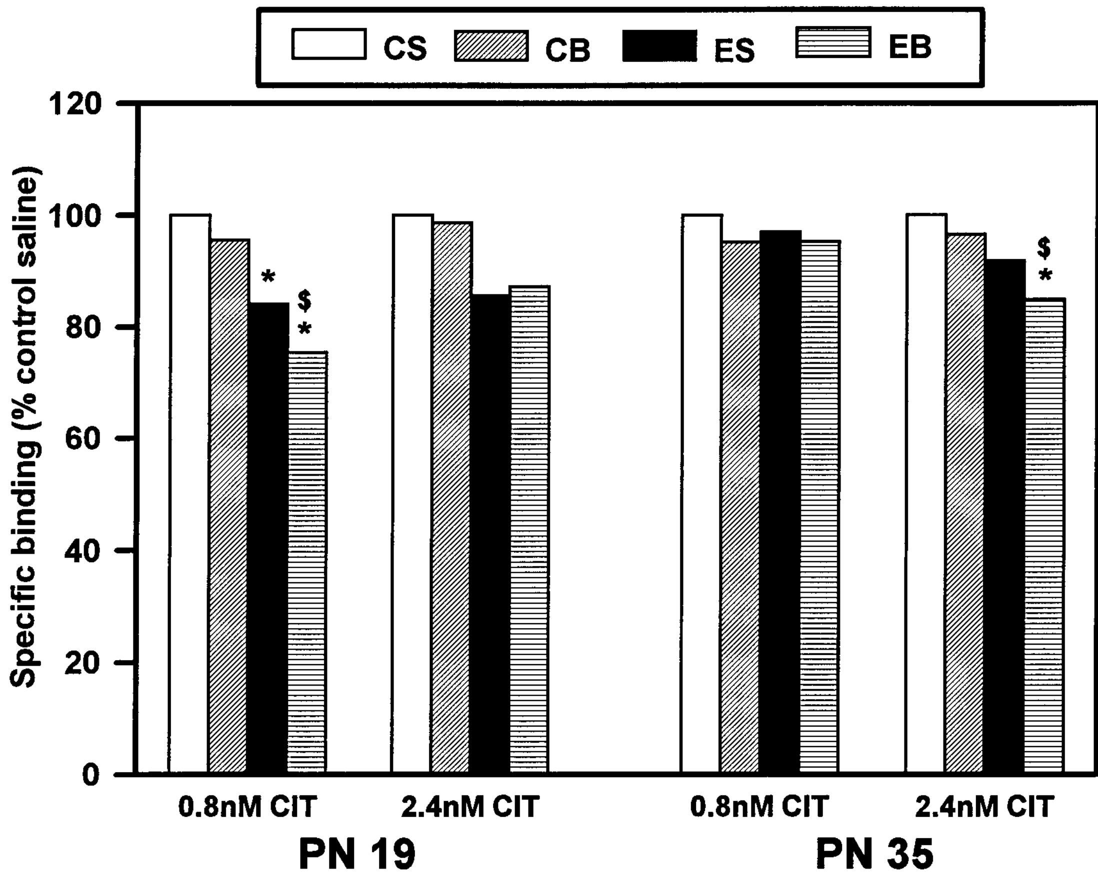


Fig. 24. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the striatum. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (at PN19, 0.8 nM [^{3}H]citalopram: $F = 8.7$, $p = 0.02$; PN35, 2.4 nM [^{3}H]citalopram: $F = 7.3$, $p = 0.04$). The dollar sign ($) denotes values which are significantly different from those in the age-matched control buspirone group (PN19, 0.8 nM [^{3}H]citalopram: $F = 8.7$, $p = 0.02$; PN35, 2.4 nM [^{3}H]citalopram: $F = 7.3$, $p = 0.04$). Abbreviations are the same as those in the Fig. 19.

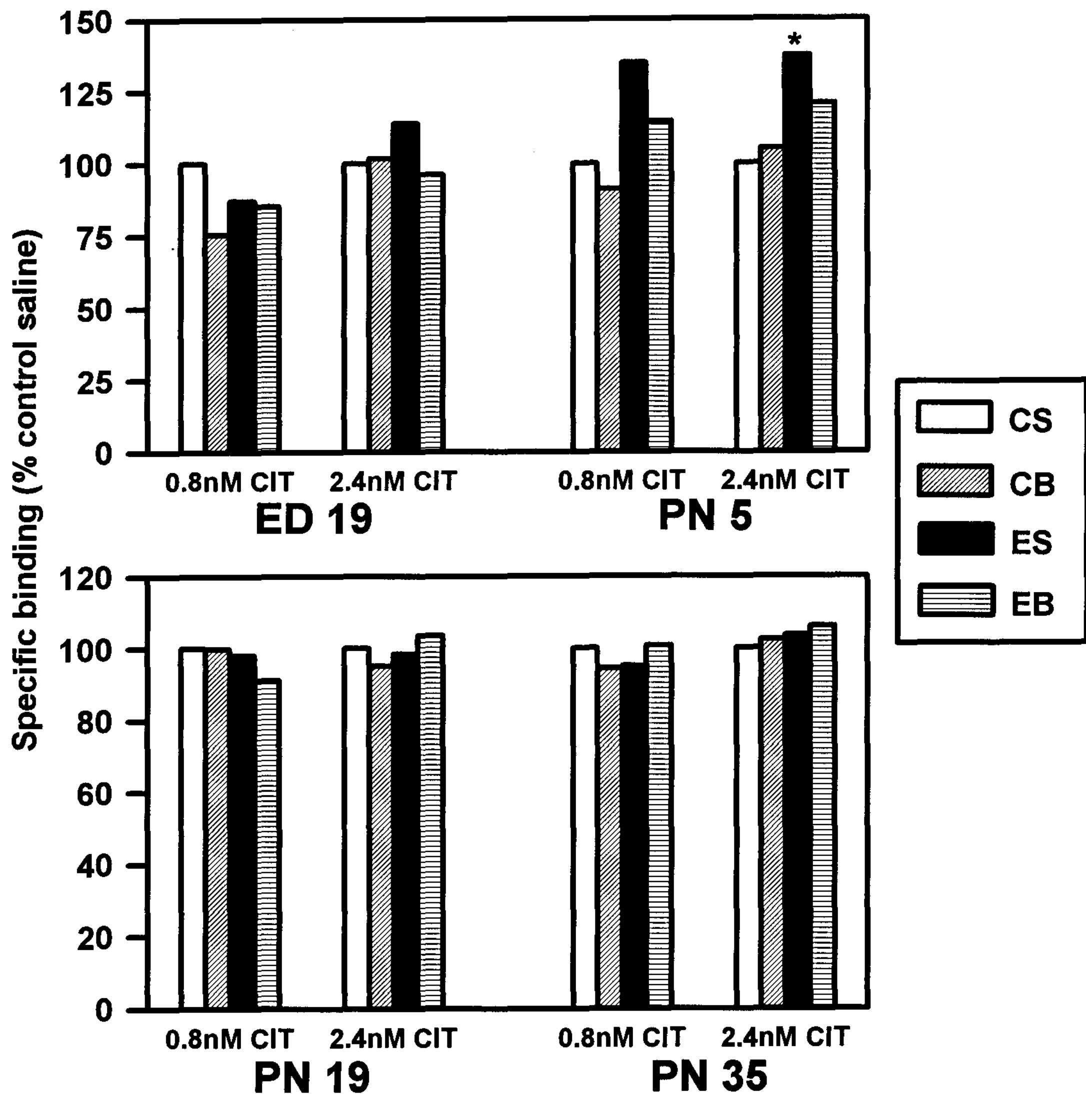


Fig. 25. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the dorsal raphe. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group ($F = 6.4$, $p = 0.045$). Abbreviations are the same as those in the Fig. 19.

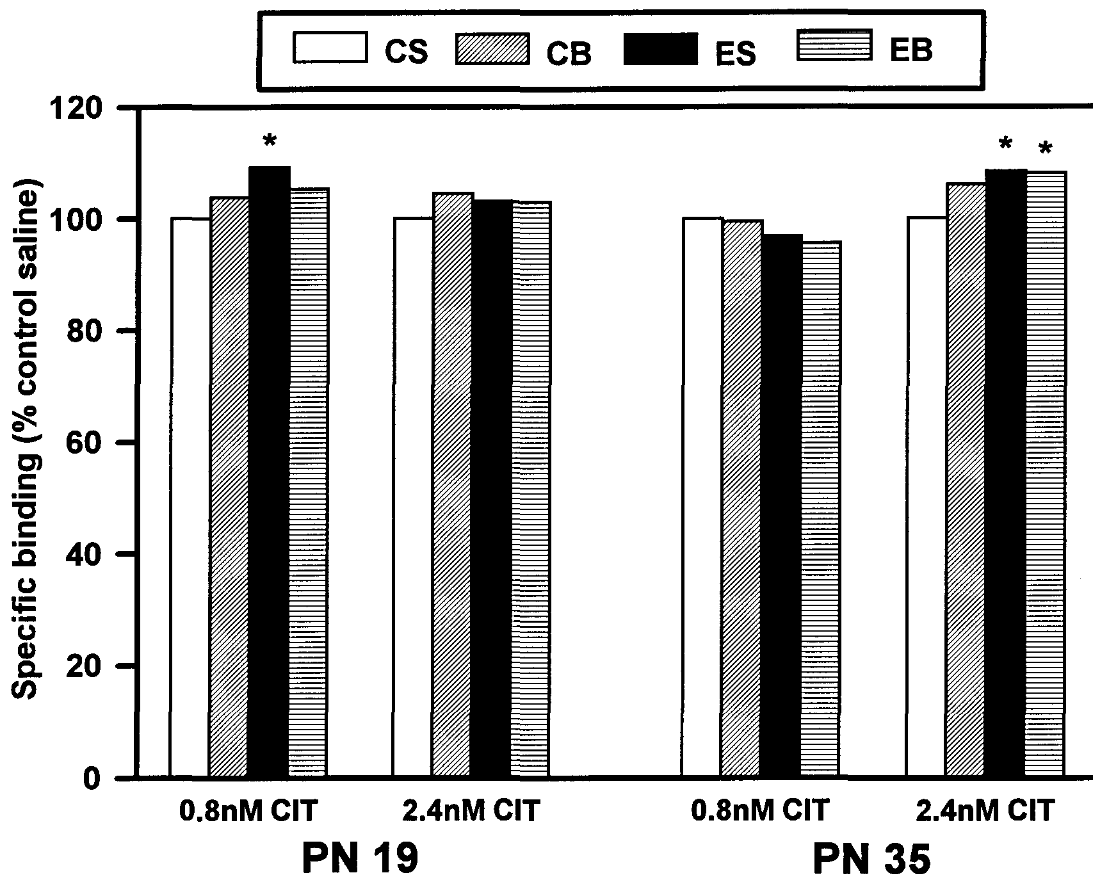


Fig. 26. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the median raphe. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (PN19, 0.8 nM [^{3}H]citalopram: $F = 30.2$, $p = 0.027$; PN35, 2.4 nM [^{3}H]citalopram: $F = 9.3$, $p = 0.02$). Abbreviations are the same as those in the Fig. 19.

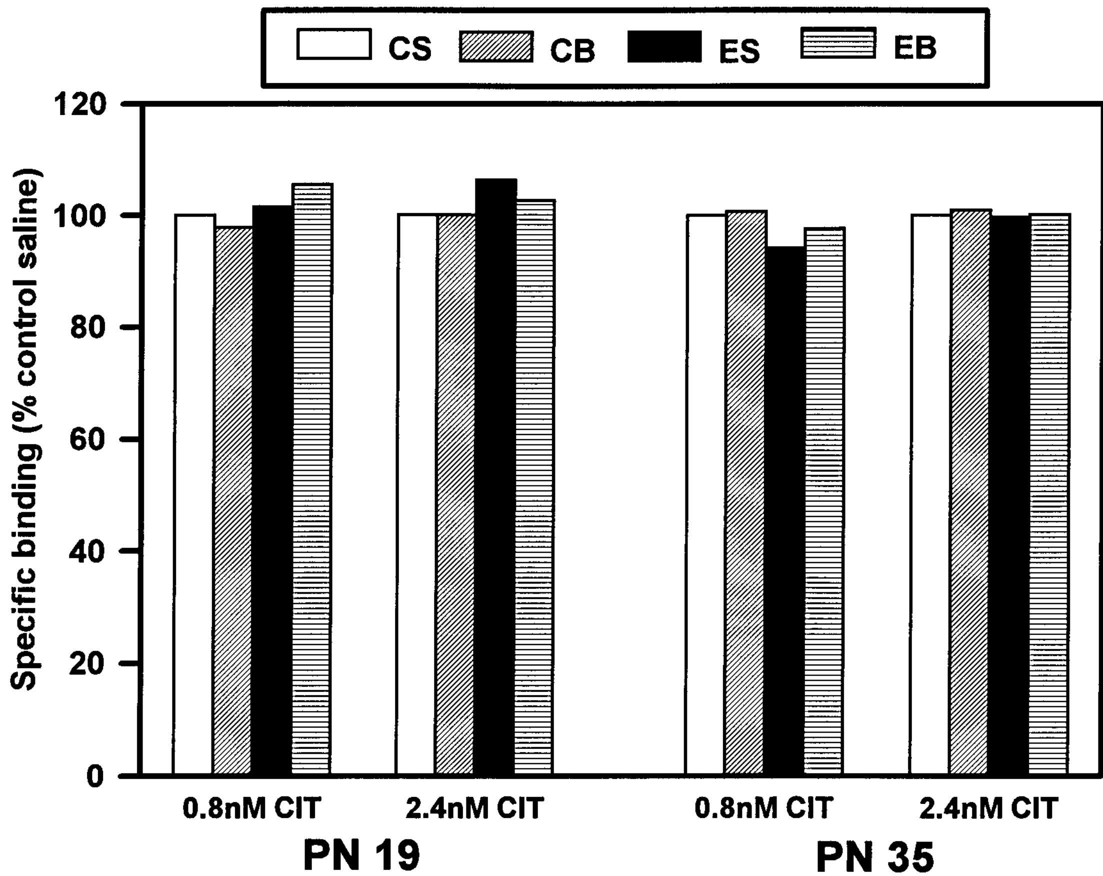


Fig. 27. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the CA3. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. Abbreviations are same as those in the Fig. 19.

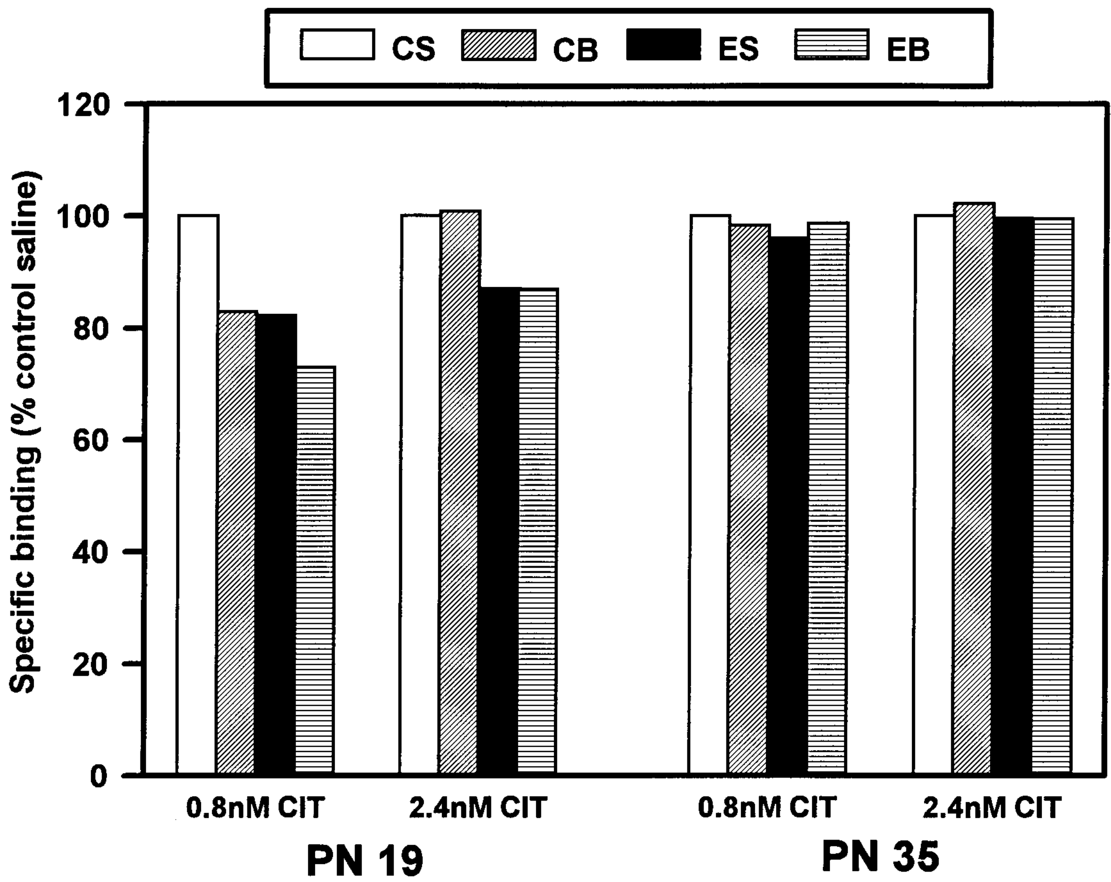


Fig. 28. Effects of buspirone treatment on the binding of [^{3}H]citalopram in the amygdala. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 5. Statistical analysis was performed on the specific binding values. Abbreviations are same as those in the Fig. 19.

The [^{3}H]-8-OH-DPAT binding study was also conducted in order to see whether maternal treatment with a 5-HT$_{1A}$ agonist, buspirone, prevents or reverses ethanol-associated developmental abnormalities in 5-HT$_{1A}$ receptors. Table 6 contains the values for the specific binding of 1.0 nM and 2.7 nM [^{3}H]-8-OH-DPAT to brain sections obtained from the offspring of CS rats. The binding pattern of [^{3}H]-8-OH-DPAT at PN19 and PN35 was similar to that observed in earlier experiments (Table 3). The binding was high in the DG, DR, LS and low in the MnR, PCx and FCx.

Compared to the binding in the offspring of CS, ethanol-exposed offspring had a transient increase of [^{3}H]-8-OH-DPAT binding in the DG (Fig. 29) at PN19 and a decrease of binding in the PCx (Fig. 30) at PN35. These observations confirm the earlier findings. The binding in the DR (Fig. 31) and MnR (Fig. 32) was unaffected by *in utero* exposure in both the first and second experiments. The changes in the binding of [^{3}H]-8-OH-DPAT in the LS (Fig. 33) and FCx (Fig. 34) were significant in this experiment; a similar, though nonsignificant trend was found in the earlier investigation.

Maternal treatment with buspirone appeared to prevent ethanol-associated developmental abnormalities in 5-HT$_{1A}$ receptors. Buspirone seemed to reverse the ethanol-associated increase in the binding of [^{3}H]-8-OH-DPAT in the DG at PN19. Buspirone also appeared to prevent the decreased binding of either the 1.0 nM or 2.7 nM [^{3}H]-8-OH-DPAT in the PCx and LS of PN35 ethanol-exposed rats. Buspirone treatment during the embryonic period did not significantly affect the binding of [^{3}H]-8-OH-DPAT in most brain regions examined in the postnatal rats. The only exception was found in the LS on PN35.

TABLE 6

SPECIFIC BINDING OF [^{3}H]-8-OH-DPAT IN THE CONTROL SALINE GROUP

		Specific binding (pmol/mg protein equivalent)			
Region	Age	1.0nM [^{3}H]-8-OH-DPAT	(n)	2.7nM [^{3}H]-8-OH-DPAT	(n)
FCx	PN19	0.081±0.065	(6)	0.095±0.040	(6)
	PN35	0.186±0.109	(6)	0.223±0.100	(6)
PCx	PN19	0.102±0.062	(6)	0.129±0.035	(6)
	PN35	0.214±0.096	(6)	0.212±0.058	(6)
LS	PN19	0.354±0.080	(7)	0.448±0.183	(7)
	PN35	0.472±0.182	(7)	0.634±0.200	(7)
DG	PN5	0.135±0.036	(5)	0.162±0.034	(8)
	PN19	0.485±0.135	(7)	0.512±0.170	(7)
	PN35	0.618±0.111	(7)	0.722±0.132	(7)
DR	PN19	0.592±0.134	(5)	0.659±0.054	(5)
	PN35	0.659±0.145	(5)	0.745±0.265	(5)
MnR	PN19	0.464±0.104	(4)	0.429±0.074	(4)
	PN35	0.246±0.025	(4)	0.333±0.126	(4)

All the data are presented as the mean ± SD.
Abbreviations are same as in Table 3.

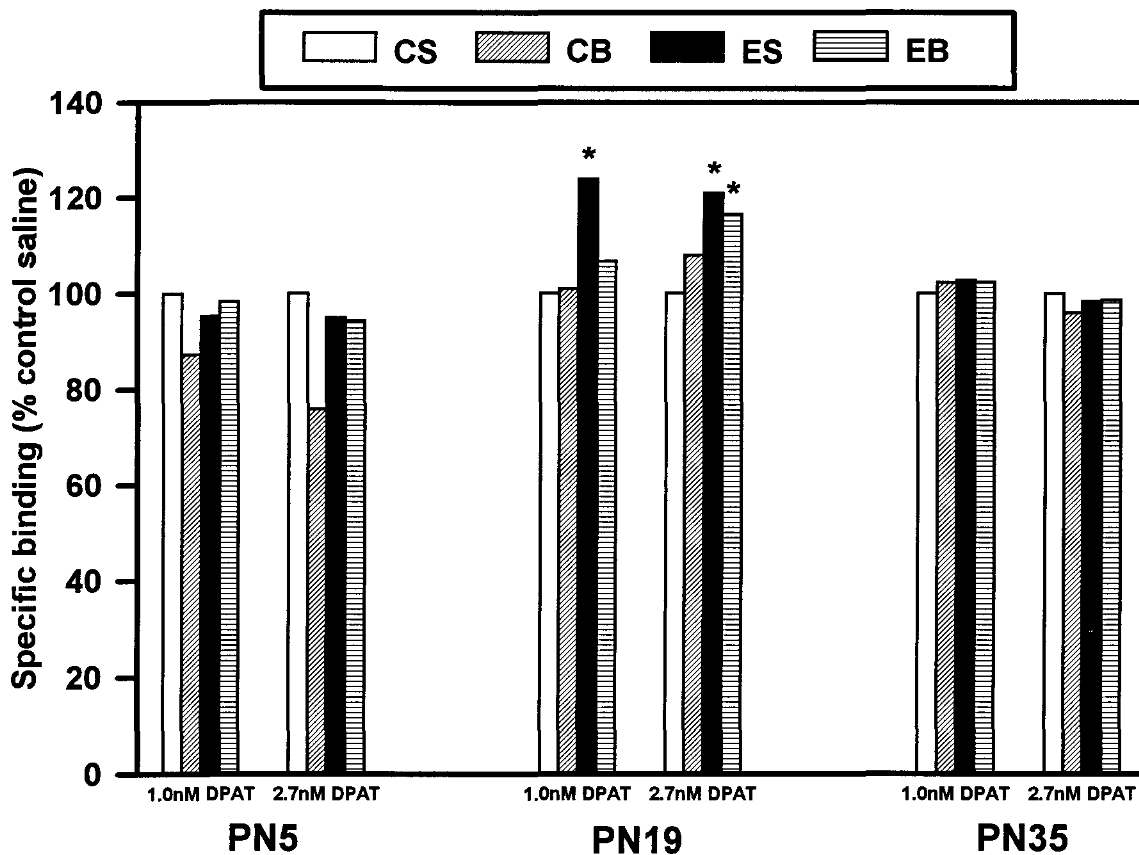


Fig. 29. Effects of buspirone treatment on the binding of [^{3}H]-8-OH-DPAT in the dentate gyrus. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 6. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (PN19, 2.7 nM [^{3}H]-8-OH-DPAT: $F = 9.4$, $p = 0.02$). Abbreviations are the same as those in the Fig. 19.

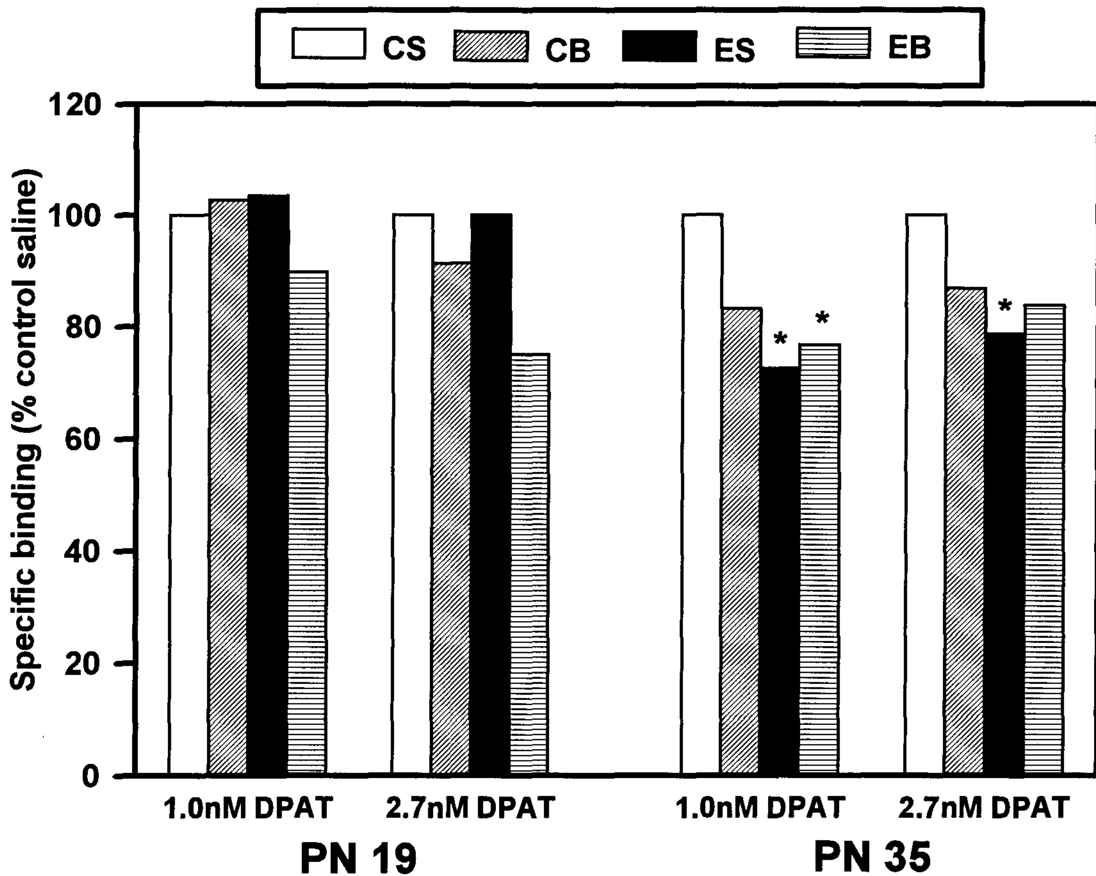


Fig. 30. Effects of buspirone treatment on the binding of [^{3}H]-8-OH-DPAT in the parietal cortex. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 6. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (PN35, 1.0 nM [^{3}H]-8-OH-DPAT: $F = 8.9$, $p = 0.03$; PN35, 2.7 nM [^{3}H]-8-OH-DPAT: $F = 8.5$, $p = 0.03$). Abbreviations are the same as those in the Fig. 19.

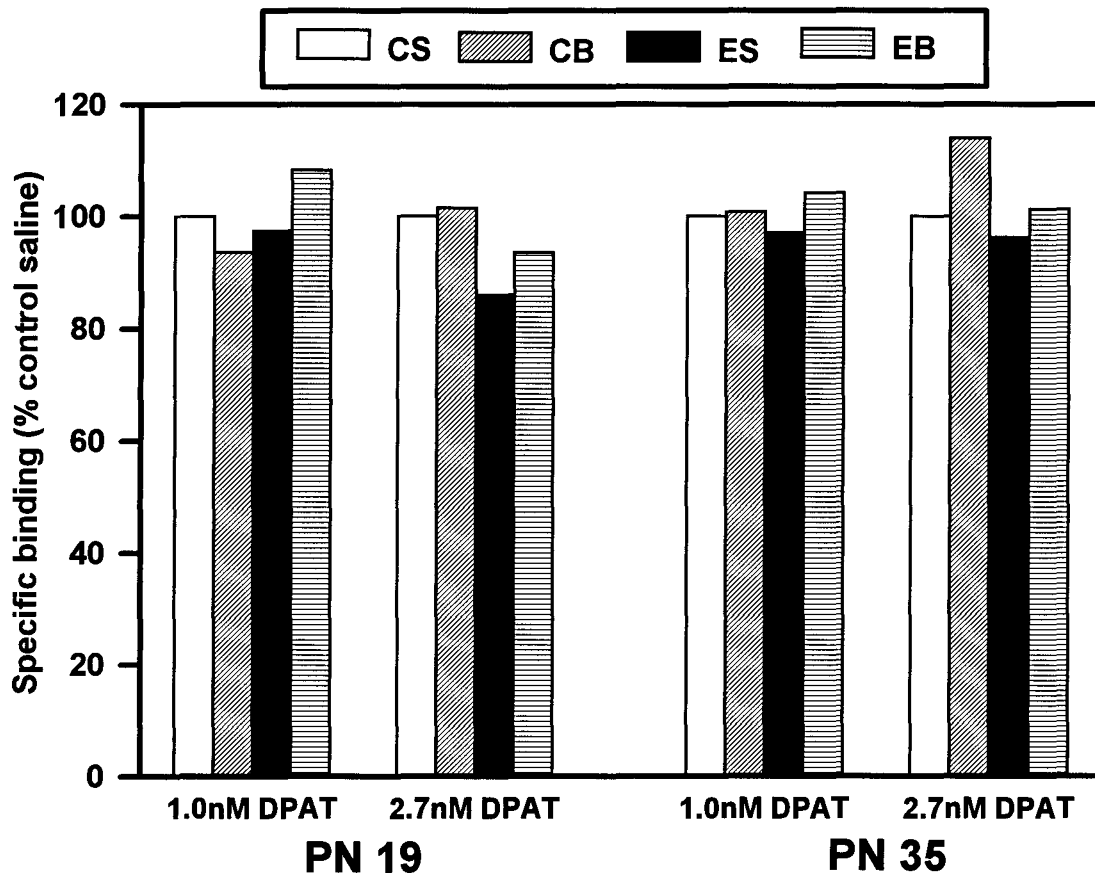


Fig. 31. Effects of buspirone treatment on the binding of [^{3}H]-8-OH-DPAT in the dorsal raphe. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 6. Statistical analysis was performed on the specific binding values. Abbreviations are the same as those in the Fig. 19.

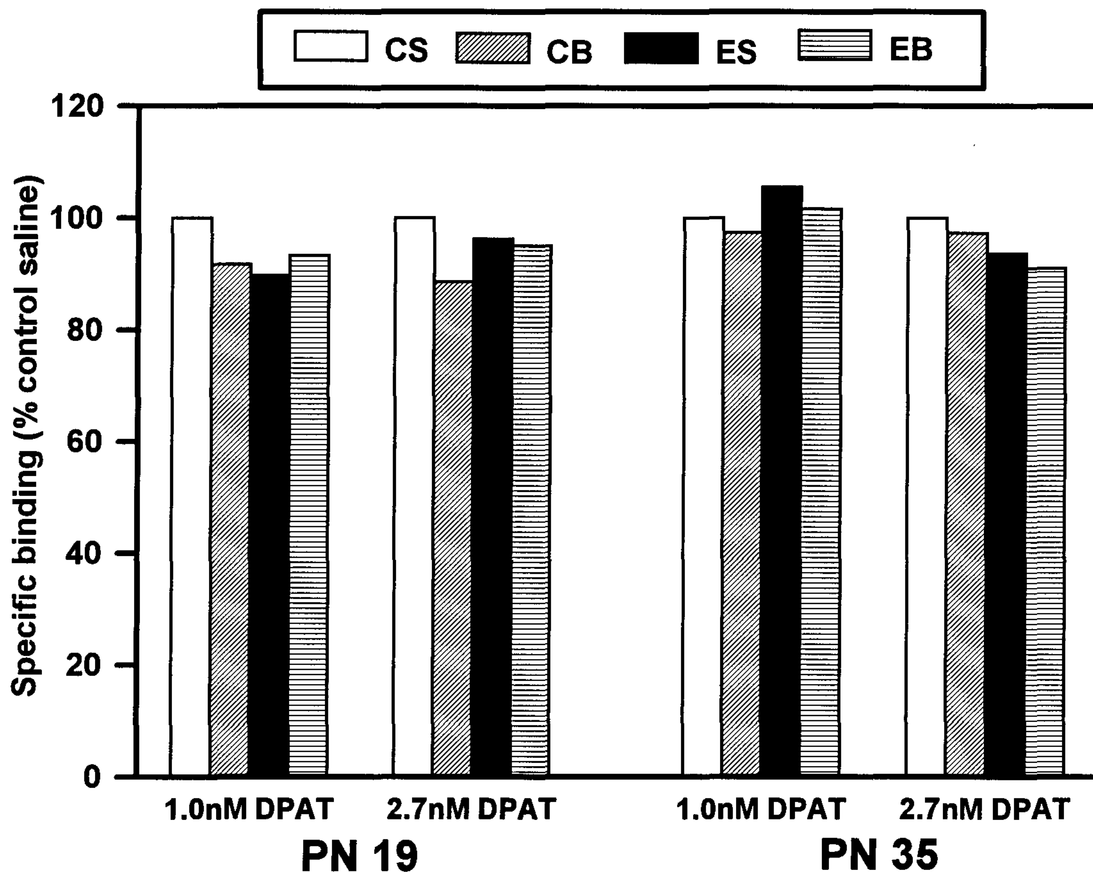


Fig. 32. Effects of buspirone treatment on the binding of [^{3}H]-8-OH-DPAT in the median raphe. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 6. Statistical analysis was performed on the specific binding values. Abbreviations are the same as those in the Fig. 19.

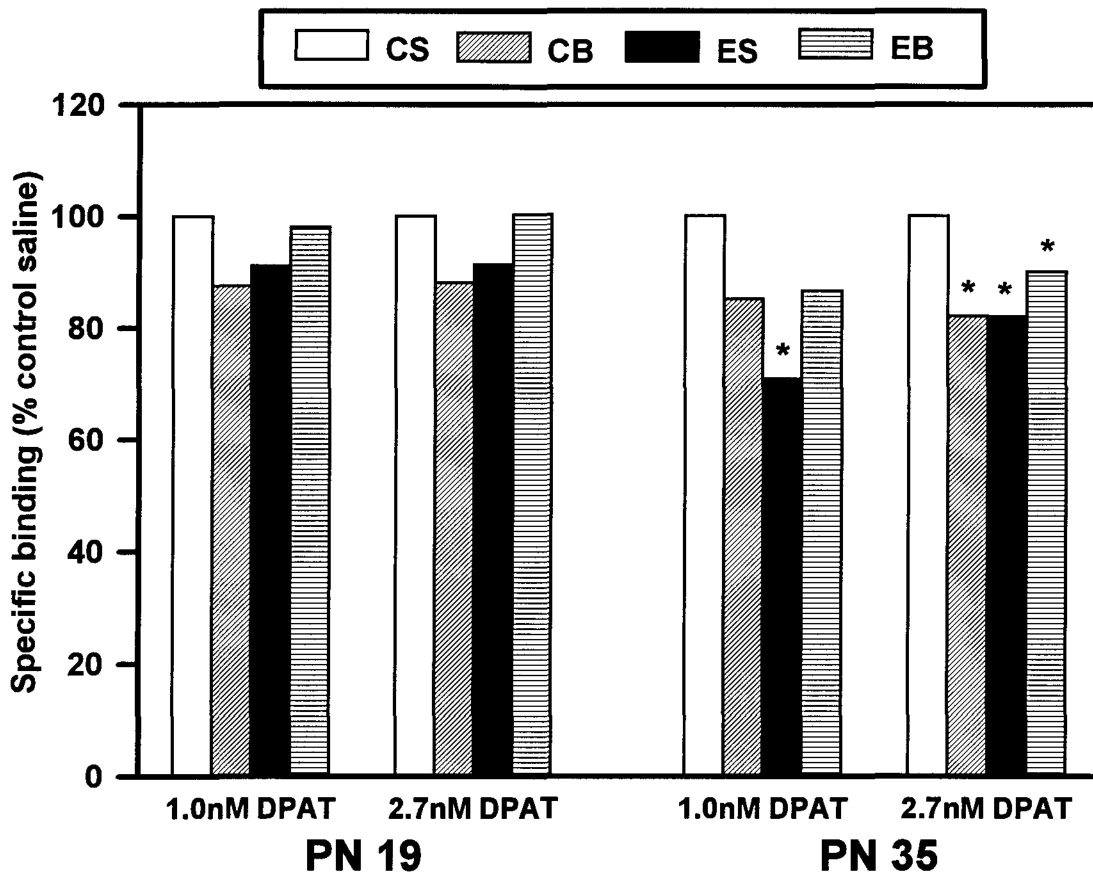


Fig. 33. Effects of buspirone treatment on the binding of [^{3}H]-8-OH-DPAT in the lateral septum. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 6. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (PN35, 1.0 nM [^{3}H]-8-OH-DPAT: $F = 9.6$, $p = 0.02$). Abbreviations are the same as those in the Fig. 19.

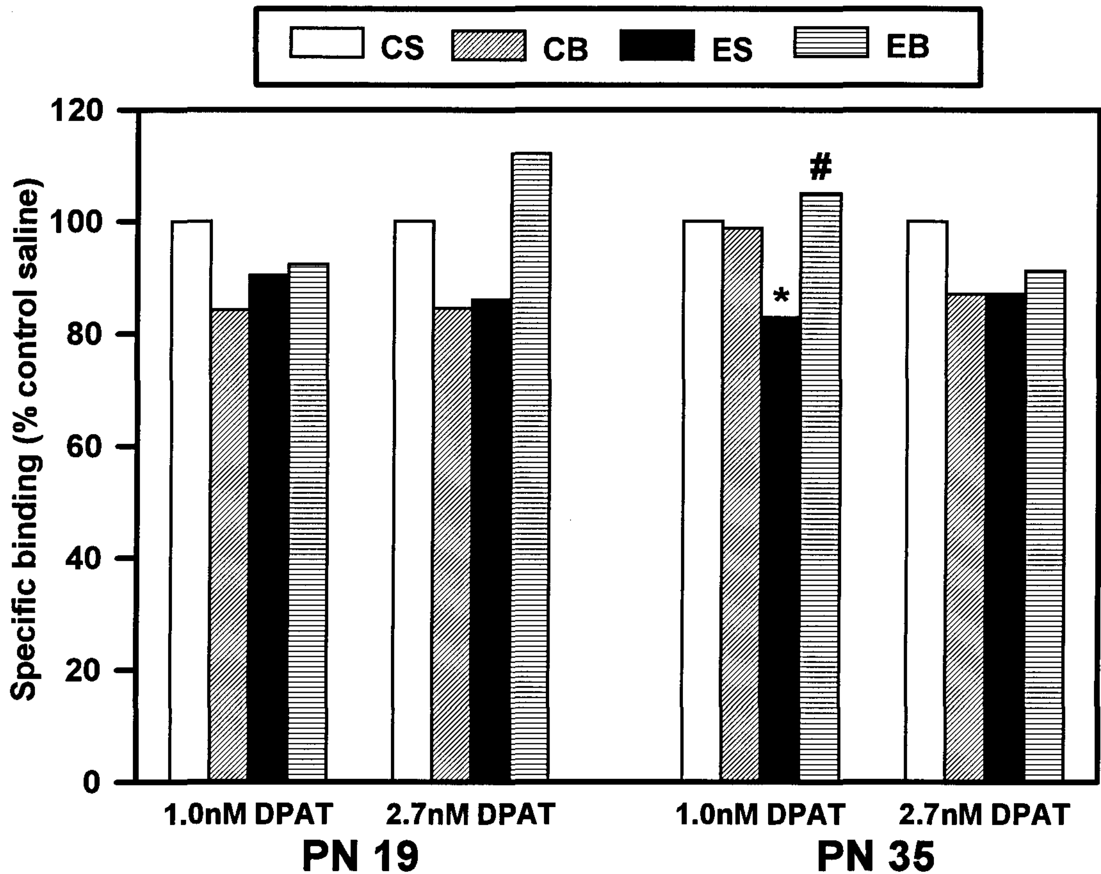
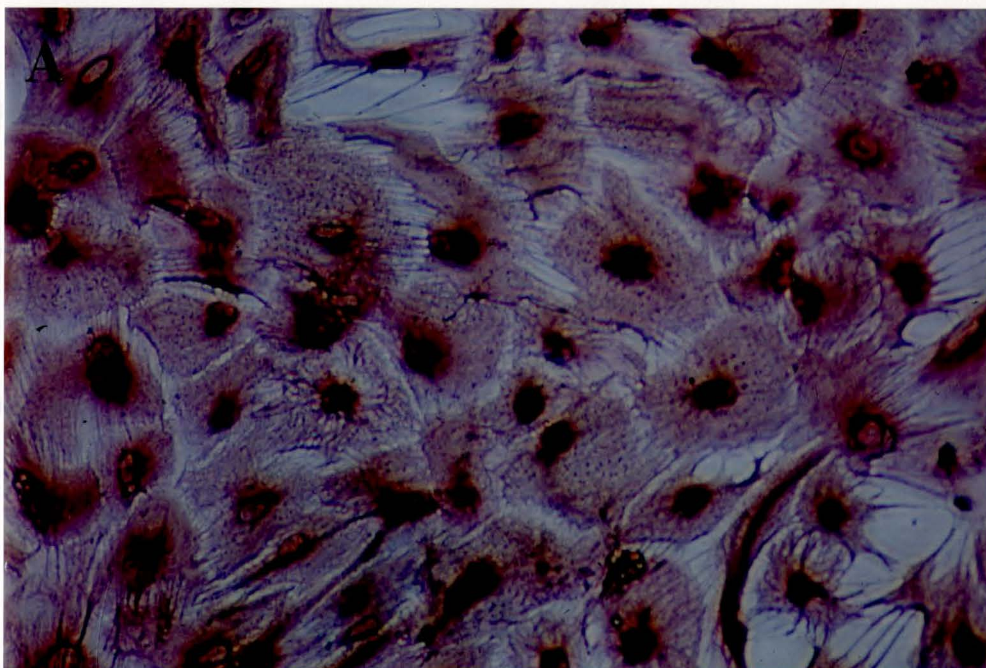


Fig. 34. Effects of buspirone treatment on the binding of [^{3}H]-8-OH-DPAT in the frontal cortex. Results are expressed as percent of the specific binding obtained from the control saline group at each age. The control saline values are given in Table 6. Statistical analysis was performed on the specific binding values. The asterisk (*) denotes values which are significantly different from those in the age-matched control saline group (PN35, 1.0 nM [^{3}H]-8-OH-DPAT: $F = 9.6$, $p = 0.02$). The pound (#) denotes values which are significantly different from those in the age-matched ethanol saline. There was a significant ethanol x buspirone interaction (PN35, 1.0 nM [^{3}H]-8-OH-DPAT: $F = 9.4$, $p = 0.03$). Abbreviations are the same as those in the Fig. 19.

In Vitro Cell Culture Studies

It was hypothesized that ethanol exerts some of its damaging effects on the developing serotonergic neurons by decreasing the concentration of serotonin and other essential trophic factors. The first portion of this dissertation discussed ethanol's effects on serotonergic neurons and the role of 5-HT_{1A} receptors. This section concentrates on the effects of ethanol on trophic factors produced by astroglia. Astroglia from a target region (e.g. the cortex) were cultured in the presence or absence of 100 mM ethanol. The purity of the astroglial cultures was assessed by the presence of GFAP as shown Fig. 35-A. Media from these astroglial cultures was collected. This media was assumed to contain trophic factors that were synthesized and secreted by astroglia. This conditioned media (CM) was then used at a concentration of 30% or 60% to culture control fetal rhombencephalic neurons, which contain the developing serotonergic neurons. Serotonergic neurons stained with 5-HT antibody are shown in Fig. 35-B. The effects of added CM (CCM or ECM) was noted on the development of several measurements of total neuronal development (cell number, DNA content, protein content, neurite outgrowth) and 5-HT neuronal development (5-HT immunopositive cells, 5-HT reuptake).

The results of these studies demonstrate that addition of CM increased the 5-HT uptake (Fig. 36), total DNA content (Fig. 37), total number of surviving neurons (Fig. 38) and the number of 5-HT immunopositive neurons (Fig. 39). CM also increased the number of neurons which had longer neurites (Fig. 40). These observations support the



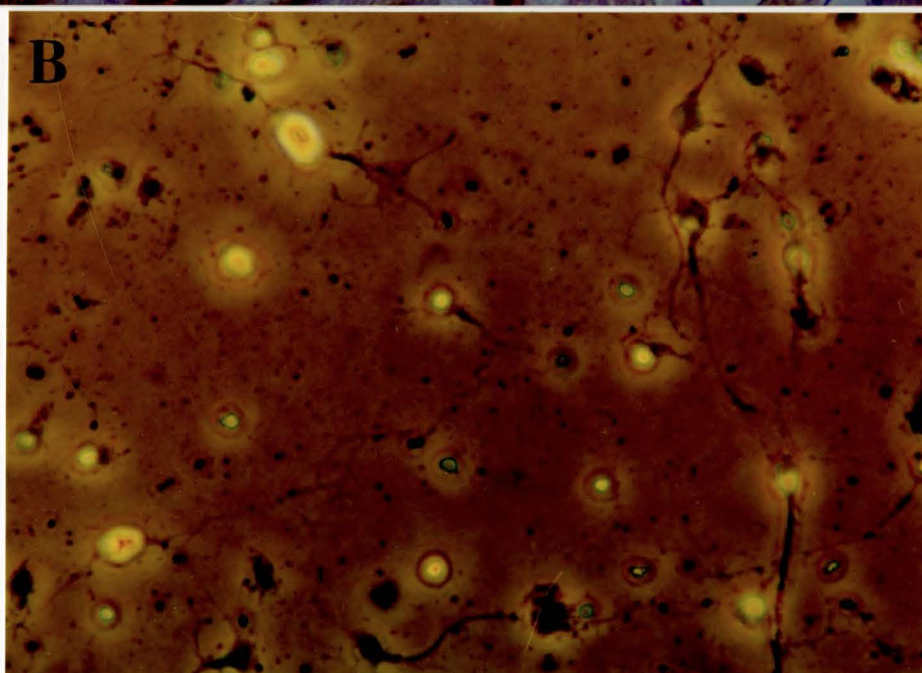


Fig. 35. Photographs of astroglial cells stained with GFAP antibody (A) and neuronal cells stained with 5-HT antibody (B). Cultures were stained with antibody as described in the Methods section. Cultures were photographed using a phase contrast microscope.

hypothesis that astroglia, derived from a target region, produce trophic factors which are necessary for normal neuronal development.

When neuronal cultures maintained in CCM were compared with those maintained in ECM, several significant differences were noted. That is, neuronal cultures maintained in ECM have a decreased total DNA content (Fig. 37), total number of surviving neurons (Fig. 38) and number of neurons with longer neurites (Fig. 40). More importantly, the number of 5-HT immunopositive neurons (Fig. 39) and 5-HT reuptake (Fig. 40) were decreased in the cultures maintained in the ECM. In contrast, total protein content was not changed (Fig. 42).

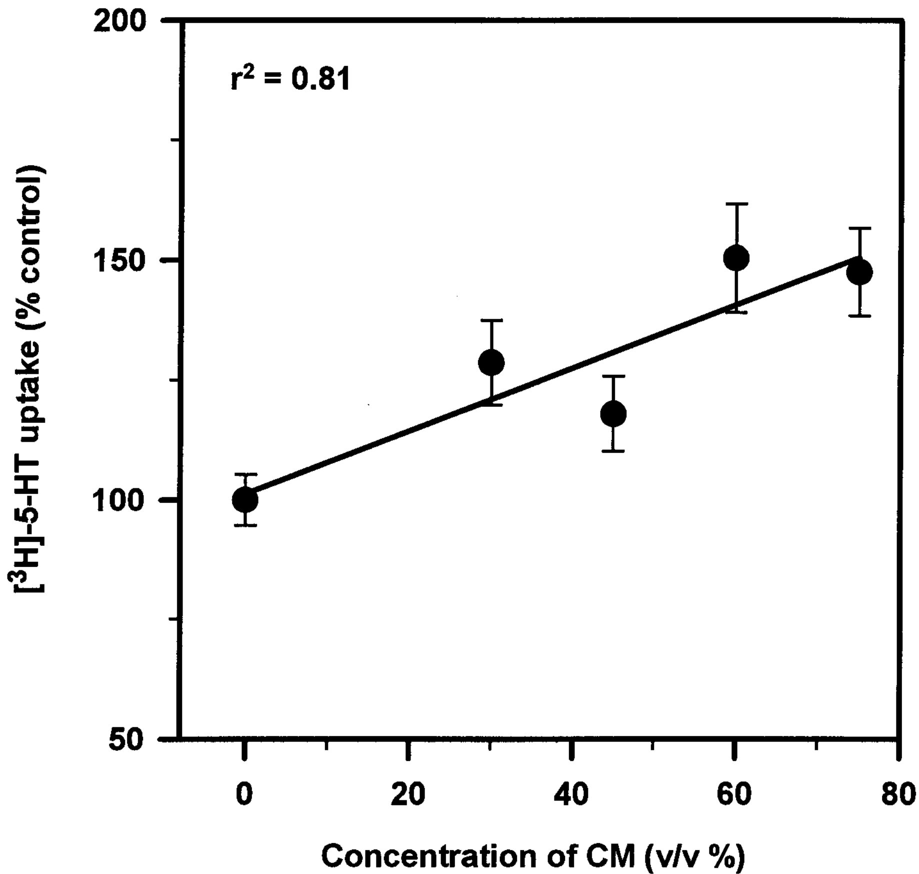


Fig. 36. Dose-dependent effect of astroglial-derived conditioned media (CM) on neuronal [^{3}H]5-HT uptake. Fetal rhombencephalic neurons were cultured in the presence of conditioned media obtained from astroglial cultures. Results are expressed as the percentage of the value in control cultures grown in chemically defined media. In the control cultures the mean uptake of [^{3}H]5-HT was 0.226 pmol/well/20 min. The error bars represent the SD (n = 3 experiments. In each experiment 4 different wells were averaged to get an n of 1.).

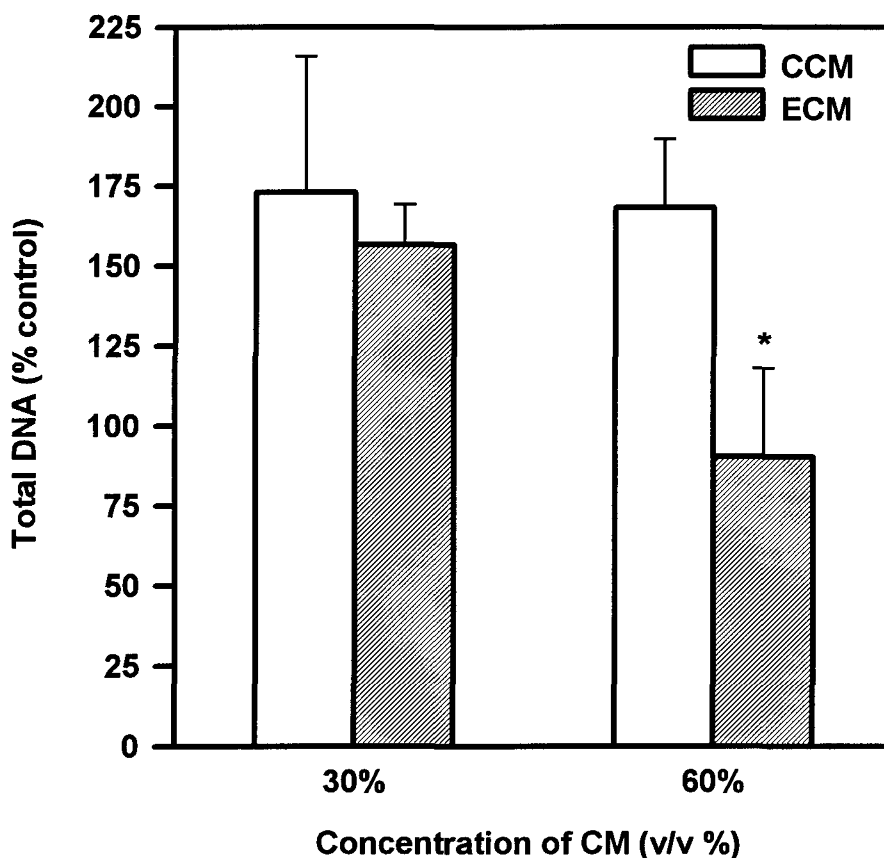


Fig. 37. Effects of CCM and ECM on total DNA content. Fetal rhombencephalic neurons were cultured in the presence of conditioned media obtained from astroglial cultures. CCM and ECM were derived from astroglial cultures grown for 4 days in the absence or presence of 100 mM ethanol. Results are expressed as the percentage of DNA in control cultures grown in chemically defined media (CDM). In the CDM cultures the mean DNA content was 2.99 μg/well. Each column and the error bar represent the mean ± SD in one experiment. The findings were replicated in a second experiment. *$p<0.05$ compared to CCM.

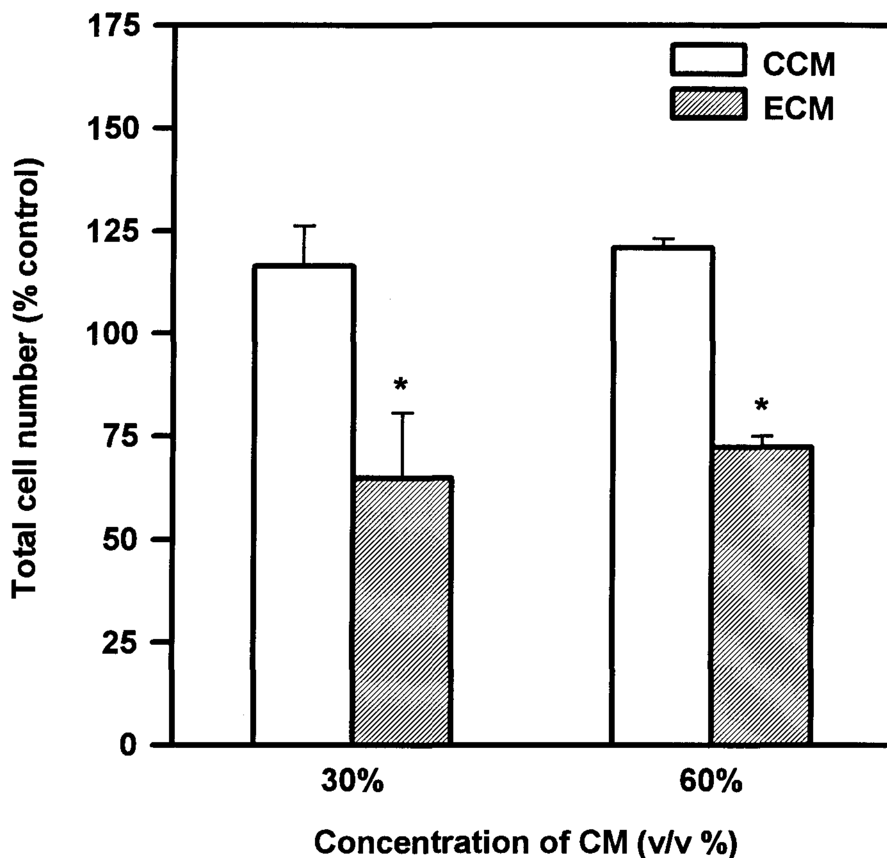


Fig. 38. Effects of CCM and ECM on total cell number. Fetal rhombencephalic neurons were cultured in the presence of conditioned media obtained from astroglial cultures. CCM and ECM were derived from astroglial cultures grown for 4 days in the absence or presence of 100 mM ethanol. Results are expressed as the percentage of surviving cell number in the control cultures grown in chemically defined media (CDM). In the CDM cultures the mean was 4.47 x 10^4 cells/well. Each column and the error bar represent the mean $\pm$ SD in one experiment. The findings were replicated in a second experiment. *$p<0.05$ compared to CCM.

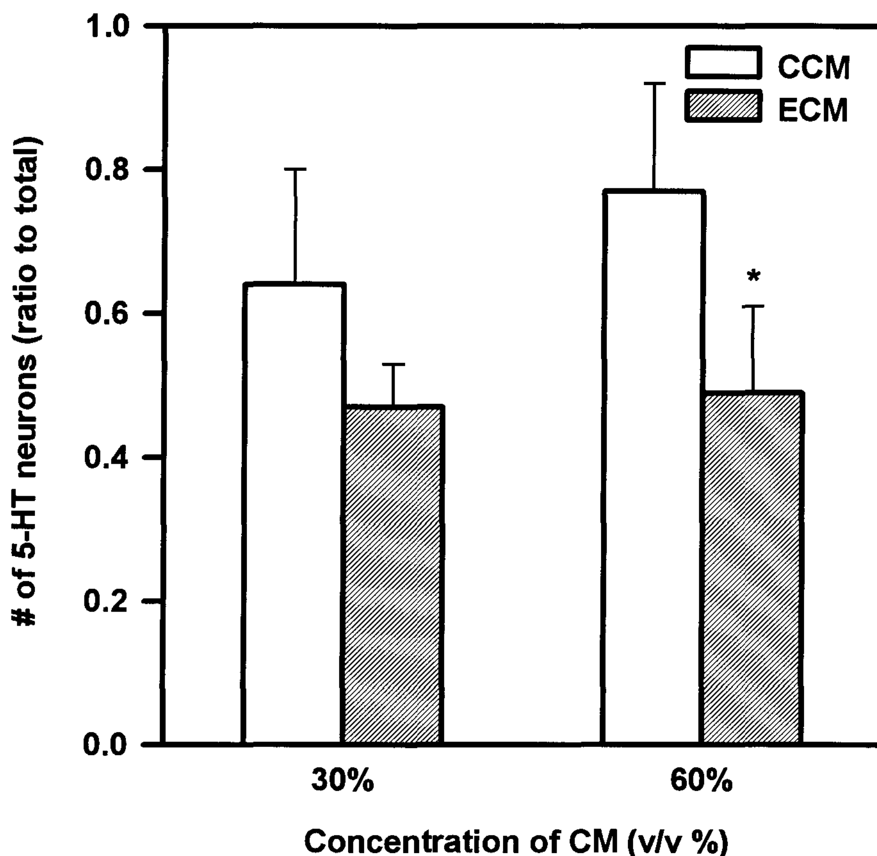


Fig. 39. Effects of CCM and ECM on the number of 5-HT immunopositive cells. Fetal rhombencephalic neurons were cultured in the presence of conditioned media obtained from astroglial cultures. CCM and ECM were derived from astroglial cultures grown for 4 days in the absence or presence of 100 mM ethanol. Results are expressed as the ratio of the number of 5-HT immunopositive cells to the total number of neurons. Each column and the error bar represent the mean ± SD in one experiment. The findings were replicated in a second experiment. *$p<0.05$ compared to CCM.

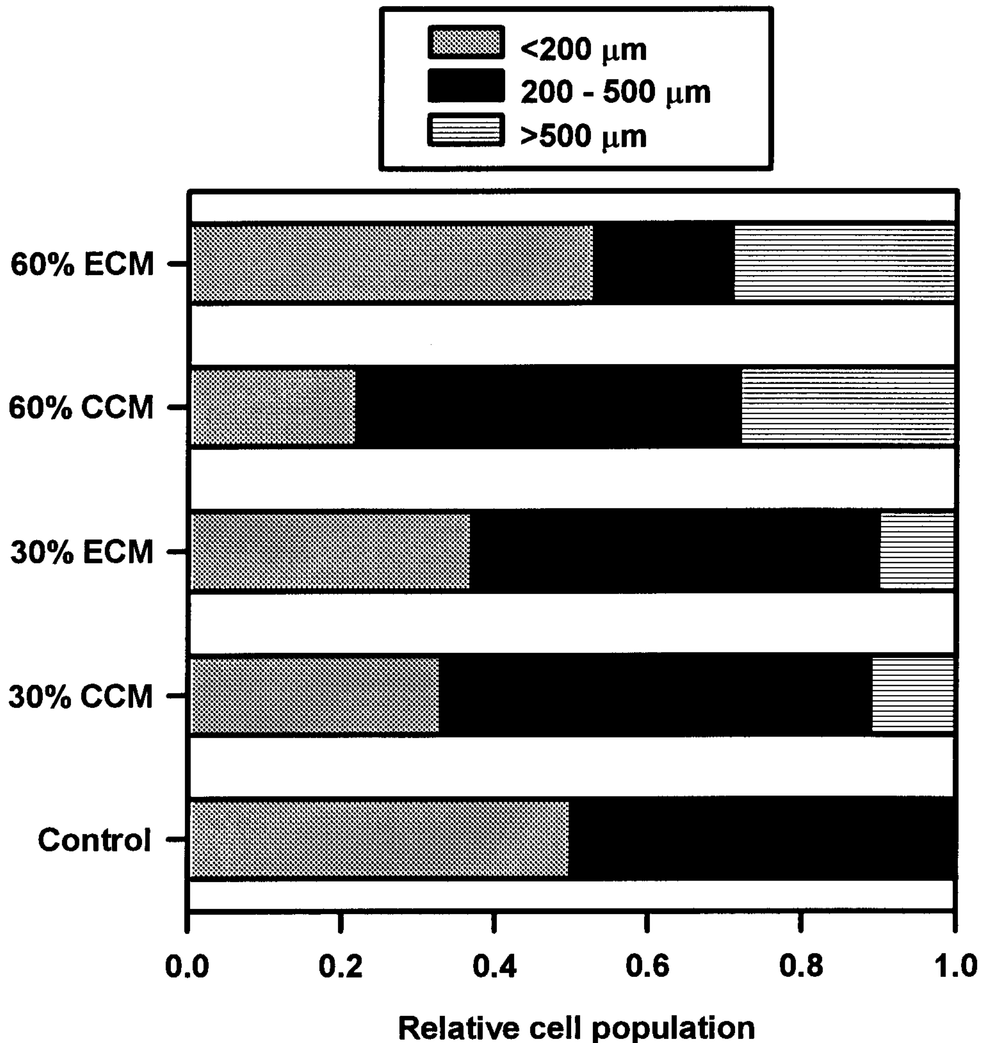


Fig. 40. Effects of CCM and ECM on neurite length. Fetal rhombencephalic neurons were cultured in the presence of conditioned media obtained from astroglial cultures. CCM and ECM were derived from astroglial cultures grown for 4 days in the absence or presence of 100 mM ethanol. The longest neurite on each neuron was measured from cell body to the end of neurite. The results are expressed as the relative number of neurons which had a neurite length in the categories, less than 250 μm, 250 - 500 μm or greater than 500 μm.

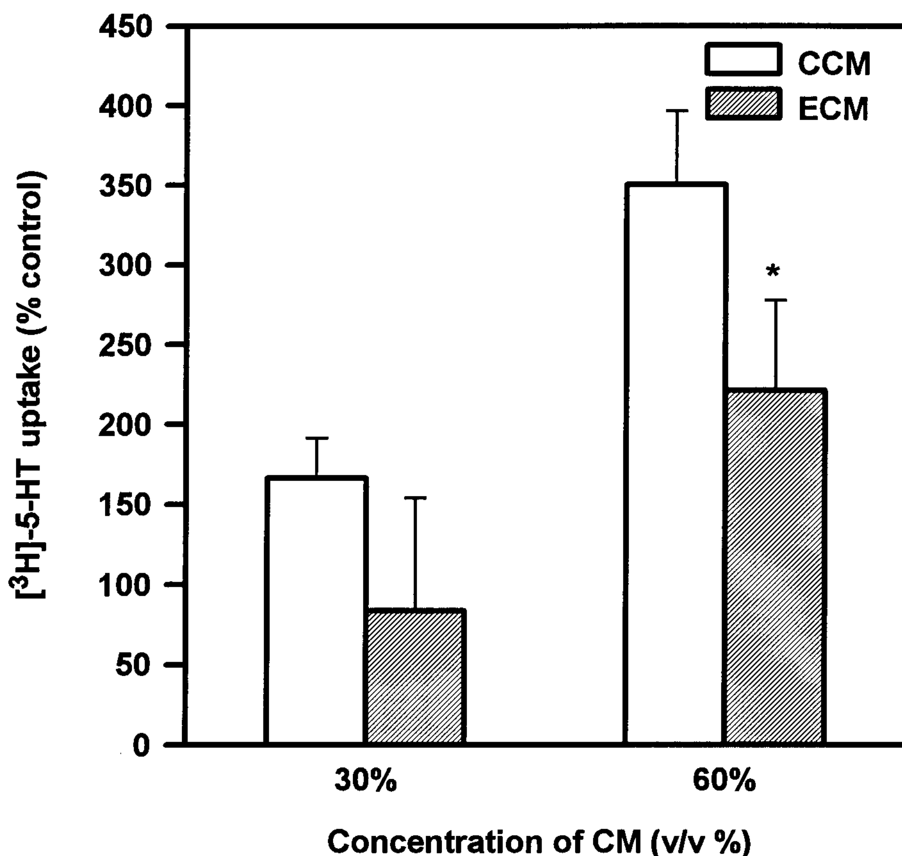


Fig. 41. Effects of CCM and ECM on neuronal [^{3}H]5-HT uptake. Fetal rhombencephalic neurons were cultured in the presence of conditioned media obtained from astroglial cultures. CCM and ECM were derived from astroglial cultures grown for 4 days in the absence or presence of 100 mM ethanol. Results are expressed as the percentage of the [^{3}H]5-HT uptake in control cultures grown in chemically defined media (CDM). In the CDM cultures the mean [^{3}H]5-HT uptake was 6.32 pmol/mg protein/20 min. Each column and the error bar represent the mean $\pm$ SD in one experiment. The findings were replicated in a second experiment. *$p<0.05$ compared to CCM.

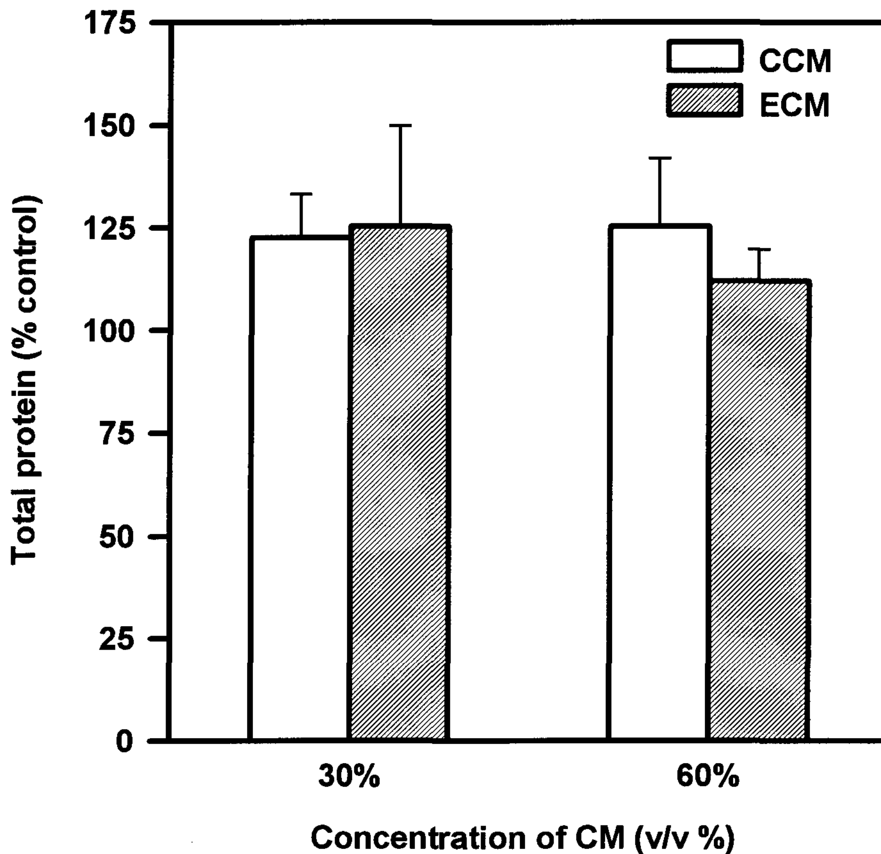


Fig. 42. Effects of CCM and ECM on neuronal protein content. Fetal rhombencephalic neurons were cultured in the presence of conditioned media obtained from astroglial cultures. CCM and ECM were derived from astroglial cultures grown for 4 days in the absence or presence of 100 mM ethanol. Results are expressed as the percentage of protein in control cultures grown in chemically defined media (CDM). In the CDM cultures the mean protein content was 17.49 μg/well. Each column and the error bar represent the mean ± SD in one experiment. The finding was replicated in the second experiment.

CHAPTER V

DISCUSSION

Animals

The FAS model used in this dissertation included rats which were fed alcohol chronically before conception and throughout the gestation. Since maternal malnutrition, especially protein deficiency, is known to affect brain development (Druse et al., 1980; Smith and Druse, 1982), protein-rich, and nutritionally well-balanced diets (Norohona and Druse, 1982) were pair-fed to control and ethanol consuming rats. The comparable weight gains of mothers and of offspring in the pair-fed groups indicate that these two groups of rats were comparably nourished. Although the ad lib control rats consumed more diet and had a greater apparent weight gain during pregnancy, the weights of their offspring were not significantly different from those of the offspring of pair-fed control rats. Moreover, we rarely found any significant differences in terms of 5-HT reuptake and 5-HT_{1A} binding sites between the offspring of pair-fed and ad lib control rats.

Buspirone treatment did not affect diet consumption. This is consistent with a previous report in which subcutaneous injections of low doses of buspirone (up to 1.25 mg/kg) increased food intake, but a higher dose (2.5 - 5 mg/kg) such as used in this study did not affect food consumption (Fletcher and Davies, 1990). Even though

buspirone-treated saline rats had a decreased maternal weight gain, the number of pups/litter and the pup weight were not different.

Blood alcohol levels were in the physiological range for humans. Our values ranged from 75 to 120 mg/dl. In most states one is arrested for drunken driving with a BAL of 100 mg/dl or less. Since it has been recognized that heavier ethanol exposure leads to more severe effects in human FAS (Streissguth et al., 1988), one might expect higher BALs to cause more damage than that seen in our studies.

5-HT Reuptake Sites and 5-HT$_{1A}$ Receptor Sites

In order to quantitate the 5-HT nerve terminals, quantitative autoradiography (QAR) of [^{3}H]citalopram-labeled serotonin reuptake sites was used. 5-HT reuptake sites are highly localized on the 5-HT nerve terminals (Kuhar and Aghajanian, 1973) and citalopram selectively binds to the 5-HT reuptake sites (D'Amato et al., 1987a; Duncan et al., 1992). Compared to binding assays using membrane homogenates, QAR provides improved localization and quantitation of reuptake sites in small, anatomically defined regions of the brain (Young and Kuhar, 1979; Palacios et al., 1981; Pazos and Palacios, 1985).

The 5-HT reuptake sites in the dorsal raphe seem to reach a peak density at about 3 weeks after birth, and at this age the dorsal raphe contained the highest concentration of 5-HT reuptake sites among the regions (Pranzatelli and Martens, 1992). A dense patch of 5-HT reuptake sites was found in the parietal cortex at PN5 and PN19. This observation resembles the innervation pattern of 5-HT immunopositive fibers observed

by D'Amato et al. (1987b).

Ligand binding was assessed at 2 concentrations; one concentration approximates the K_D and the second concentration was three times the K_D. Any changes of ligand binding at the concentration approximating K_D can potentially detect changes in the both affinity and density of receptors, whereas abnormalities detected at the higher concentration can detect changes only in the density of receptors. In some regions ethanol-associated differences are found at one but not both concentrations. However, generally a similar trend was found at the other ligand concentration, suggesting that perhaps a greater n was needed to demonstrate significance at other concentration.

The results of quantitation of 5-HT reuptake sites showed that in the offspring of ethanol-fed rats 5-HT reuptake sites were increased in the dorsal raphe at PN5, and in the median raphe at PN19 and PN35. In contrast to those cell body regions, the reuptake sites were decreased in the frontal cortex, parietal cortex, lateral hypothalamus, substantia nigra, medial septum and striatum at PN19. The decreased 5-HT reuptake sites were also found in the lateral hypothalamus and substantia nigra at PN35. Ethanol-associated changes of 5-HT reuptake sites in dorsal raphe, lateral hypothalamus and substantia nigra were consistently found in both the first and second investigation. The changes in the frontal cortex were significant in the second study; similar trends were found in the first study. In contrast, significant changes in the parietal cortex and median raphe may not be meaningful because the changes were only significant in one study and at only one concentration. The results of the first and second studies on 5-HT reuptake sites were summarized in Table 7. In a previous study from this laboratory, 5-HT

reuptake sites were measured in few, gross brain regions of the rat offspring (Druse and Paul, 1989). In that study, 5-HT uptake was decreased in the motor cortex of ethanol-exposed rats at both PN19 and PN35. Similarly, decreased 5-HT reuptake sites in the frontal cortex were found in the present study. In addition, the present study considerably expands the earlier study by including many more regions and even including fetal brain tissue.

5-HT reuptake sites were increased in the dorsal raphe of the ethanol-exposed rats. This finding could indicate 5-HT hyperinnervation (sprouting) due to the ethanol-associated deficit of serotonin, because a similar hyperinnervation of 5-HT fibers has been observed in the brainstem of neonatal rats that have been treated with 5,7-DHT, a 5-HT neurotoxin (Pranzatelli and Martens, 1992).

In contrast to the raphe region, 5-HT reuptake sites were decreased in the terminal regions (e.g. frontal cortex, lateral hypothalamus, substantia nigra, medial septum and striatum). Decreased reuptake sites may indicate the delayed development of serotonergic fibers in ethanol-exposed rats. This is supported by the observations that the catch-up growth of 5-HT fibers was found in some regions at later ages. Delayed development may be caused by either decreased number of raphe 5-HT neurons, or decreased projections from raphe to target areas or both. *In utero* ethanol exposure has been demonstrated to decrease cell number in many regions such as somatosensory cortex, the hippocampal CA3 region and the cerebellum (Barnes and Walker, 1981; Bonthius and West, 1990; Miller and Potempa, 1990). Decreased 5-HT projections could be caused by either alterations in cell adhesion molecules (CAM) or altered levels of

trophic factors (e.g. 5-HT) or chemotactic factors which guide projecting nerve fibers. Interestingly, in the cell line (neurolastoma x glioma hybrid) ethanol inhibited the cell-cell adhesion by altering the level of N-CAM and L1 (Charness et al., 1994).

In utero ethanol may indirectly affect the development of 5-HT neurons possibly through the modulation of trophic factors, such as 5-HT. Previous studies from this laboratory as well as others demonstrated that serotonin content was decreased in the offspring of ethanol-exposed animals (Druse et al., 1991; Elis et al., 1976, 1978; Rathbun and Druse, 1985). More importantly, the decreased serotonin content was observed in the fetuses as early as ED15 in the brainstem and ED19 in the cortex (Druse et al., 1991). Fetal 5-HT acts as a developmental signal (Lauder, 1990). In addition to influencing growth cone elongation and neurite outgrowth to target areas (Haydon et al., 1984, 1987), 5-HT has been demonstrated to regulate the normal development and maturation of serotonergic neurons in the fetal brain (Budnik et al., 1989; De Vitry et al., 1986; Whitaker-Azmitia and Azmitia, 1986; Whitaker-Azmitia et al., 1987). The effects of serotonin on its own neurons seem to be mediated through interaction with 5-HT_{1A} receptors (Whitaker-Azmitia and Azmitia, 1986; Whitaker-Azmitia et al., 1987). Treatment of pregnant rats with buspirone, a 5-HT_{1A} receptor agonist, prevented the abnormal development of 5-HT terminals in the dorsal raphe (PN5), medial septum (PN19), frontal cortex (PN19), substantia nigra (PN35) and lateral hypothalamus (PN19 and PN35) in the offspring of ethanol-exposed rats. This suggests that at least part of the abnormalities in the 5-HT terminal density induced by *in utero* ethanol exposure were due to decreased stimulation of 5-HT_{1A} receptors. The effects of ethanol and buspirone

on citalopram binding to 5-HT reuptake sites were summarized in Table 7. These results are consistent with a previous report that the cortical deficit of 5-HT and its metabolite, 5-HIAA, in ethanol-exposed offspring was prevented by maternal treatment with buspirone (Tajuddin and Druse, 1993).

There could be possible mechanisms by which *in utero* ethanol alters 5-HT content. Serotonin synthesis depends on the concentration of tryptophan, the substrate for tryptophan hydroxylase (Tong and Kaufman, 1975). The amount of tryptophan transport may be decreased, since ethanol impairs placental nutrient transport (Schenker, 1989). *In utero* ethanol exposure may indirectly affect 5-HT content by affecting other systems such as the production of corticosterone. Pregnant rats fed with alcohol-containing diet increased plasma corticosterone (Weinberg and Bezio 1987). It has been also shown that rats exposed to ethanol *in utero* have elevated plasma and brain corticosterone levels at birth (Kakihana et al., 1980; Taylor et al., 1982a). Alteration of corticosterone level in fetus as well as mother may affect the normal development of tryptophan hydroxylase, a serotonin synthesizing enzyme since corticosterone is required for the normal development of tryptophan hydroxylase (Sze, 1980).

The results of the present experiments also indicate that *in utero* ethanol exposure has region- and age-specific effects on the development of 5-HT fibers. Similar region-specific effects of *in utero* ethanol exposure have been observed in the hippocampus. Hippocampal pyramidal cells in the CA1 region are decreased by prenatal ethanol exposure, whereas the cells in the CA3 region were not affected (Barnes and Walker, 1981). The present studies also indicate that many of the detected developmental

abnormalities are transient. Transient, age-specific ethanol effects have also been demonstrated in the cerebellum (Volk, 1977).

In some regions (e.g. the parietal cortex and striatum) buspirone did not prevent or reverse the abnormalities of 5-HT terminal density. This may be due to differences between the timing of the buspirone treatment (ED13 to ED21) and key developmental processes in the unaffected regions. Alternatively, ethanol-associated 5-HT terminal abnormalities in these regions may be due to factors other than the fetal deficit of serotonin. *In utero* ethanol exposure may affect the production of trophic factors, which consequently affect the development of 5-HT terminals. This effect may be mediated by ethanol's action on astroglial cells. Astroglial cells are known to produce growth factors which are essential for the neuronal survival and differentiation during brain development (Manthorpe et al., 1986).

The development of postsynaptic 5-HT_{1A} receptors were increased in the dentate gyrus at PN19 and decreased in the parietal cortex at PN35 by ethanol exposure *in utero*. In contrast, the development of 5-HT_{1A} receptors in the raphe at both PN19 and PN35 was not affected. Although the binding of [^{3}H]-8-OH-DPAT in the raphe regions of the fetal brain was not measurable in this study, membrane homogenate binding studies from this laboratory demonstrated that the binding of 10 nM [^{3}H]-8-OH-DPAT was significantly increased in ethanol-exposed rat fetuses on ED19 (Druse et al., 1991). Thus, *in utero* ethanol exposure adversely affects the developmental pattern of 5-HT_{1A} receptors.

Decreased fetal serotonin may contribute to the developmental abnormalities in 5-HT_{1A} receptors. This hypothesis is supported by studies that found delayed 5-HT target

differentiation in the offspring brain, when pregnant rats were treated with *p*CPA, a 5-HT synthesis inhibitor (Lauder and Krebs, 1978). The expression of 5-HT_1 receptors in those offspring was affected in both brainstem and forebrain regions (Whitaker-Azmitia et al., 1987). However, buspirone treatment during the embryonic period did not always prevent the effects of *in utero* ethanol exposure on 5-HT_{1A} receptors. Table 8 summarized the effects of ethanol and buspirone on 8-OH-DPAT binding to 5-HT_{1A} receptors. This suggests that the abnormal development of 5-HT_{1A} receptors in ethanol-exposed rats may be due to more than the decreased stimulation of 5-HT_{1A} receptors on developing raphe neurons. There may also be altered levels of other trophic factors such as astroglial-derived growth factors or decreased stimulation of other fetal brain 5-HT receptors (e. g. 5-HT_2 receptors).

The mechanism by which buspirone exerts its effects on the fetal brain is presently unknown. However, the effects of buspirone in the adult brain are mediated through its interaction with 5-HT_{1A} receptors. Buspirone binds to 5-HT_{1A} receptors with high affinity as indicated by an IC_{50} of 9.5 nM for displacing [^{3}H]-8-OH-DPAT (Peroutka, 1985). In addition, the binding pattern of *in vivo* [^{3}H]buspirone autoradiography is remarkably similar to the distribution pattern of 5-HT_{1A} receptors (Matheson and Tunnicliff, 1991). Electrophysiological, biochemical and pharmacological studies demonstrated that buspirone acts as a full agonist on the 5-HT_{1A} receptors of serotonergic dorsal raphe neurons (Meller et al., 1990; VanderMaelen et al., 1986). In the hippocampus buspirone acts as a partial agonist on the postsynaptic 5-HT_{1A} receptors (Andrade and Nicoll, 1987; Yocca et al., 1986). Since 5-HT_{1A} receptors and mRNA are

present in the fetal rat brain (Daval et al., 1987; Hillion et al., 1990), the effects of maternal buspirone treatment are presumably mediated through its action on fetal 5-HT_{1A} receptors. Buspirone not only acts on the neuronal receptors, but may also act on the astroglial 5-HT_{1A} receptors, whose activation stimulates the production of serotonergic growth factors, such as S100β (Azmitia et al., 1990; Whitaker-Azmitia and Azmitia, 1989; Whitaker-Azmitia et al., 1990).

There is also the possibility that the effects of buspirone could be mediated through other neurotransmitter systems. Although its binding affinity to D_2 receptors is low as indicated by an IC_{50} of 260 nM for displacement of [^{3}H]spiperone from striatal membranes (Riblet et al., 1982), buspirone can act on dopamine D_2 receptors as an antagonist. The latter effect consequently increases dopaminergic neurotransmission (Riblet et al., 1984). One also can not exclude the possibility that buspirone treatment elevates maternal ACTH, corticosterone and prolactin (Gilbert et al., 1988; Montefrancesco et al., 1990; Meltzer et al., 1991), and that these changes contribute to the protective effects of buspirone. In fact, ACTH stimulates the development of serotonergic neurons in culture (Davila et al., 1986).

Effects of Astroglial Cells on the Survival and Differentiation of 5-HT Neurons *In Vitro*

During brain development astroglial cells play an important role in the proper maturation of the nervous system. For example, radial glial cells guide the migration of immature neurons (Rakic, 1972). Astroglial cells secrete soluble molecules which are

essential for neuronal growth and survival (Banker, 1980; Kadle et al., 1988; Lieth et al., 1989; Manthorpe et al., 1986; Muller and Seifert, 1982). Prenatal exposure to ethanol induces premature degradation of the network of radial glial fibers in the cortex (Miller and Robertson, 1993).

Ethanol exposure has been shown to alter the function of astroglial cells. For example, ethanol decreases glutamine synthetase activity in astroglial cells (Babu et al., 1994; Davies and Vernadakis, 1984) as well as hexose uptake (Singh et al., 1990). It appears that astroglial cells are more susceptible to ethanol than neurons in terms of their function (Babu et al., 1994; Davies and Vernadakis, 1984; Guerri et al., 1990; Snyder et al., 1992). When rhombencephalic astroglial cells and neurons were cultured in a serum-containing media, ethanol decreased rhombencephalic astroglial protein content and astroglial 5-HT uptake (Lokhorst and Druse, 1993b), while having no effect on the rhombencephalic neuronal protein and 5-HT uptake (Lokhorst and Druse, 1993b). In addition, glutamine synthetase activity was decreased in the astroglial cells derived from the ethanol-treated rats, whereas the neuron specific enolase activity was not affected in the neurons derived from the same animal (Babu et al., 1994). The ethanol-associated decrement in astroglial protein content and synthesis may be indicative of decreased astroglial production of neurotrophic factors.

The results of the present cell culture study indicate that conditioned media (CM), derived from target astroglia, contained neuronal growth factors since CM enhanced the number of neurons and 5-HT immunopositive neurons, total DNA content, and 5-HT uptake. The effect of CM on 5-HT immunopositive neurons and serotonin uptake suggest

that target astroglial cells produce growth factors essential for serotonergic neurons as well as other neurons.

Total number of neurons, total DNA content and the number of 5-HT immunopositive neurons were decreased in the cultures grown in the conditioned media derived from astroglial cells treated with 100 mM ethanol for 4 days (ECM). Serotonin uptake was also decreased in the cultures grown in the ECM. The cultures showed a disturbed pattern of neurite outgrowth; the relative number of neurons having medium length neurites was decreased. This demonstrates that astroglial exposure to ethanol decreases the astroglial production of growth factors which are involved with neuronal survival and growth. The possible neurotrophic factors which could be affected by ethanol include S100ß, fibroblast growth factor (FGF), epidermal growth factor (EGF) and insulin-like growth factors I and II (IGF-I & IGF-II). S100ß is synthesized and secreted mainly by astrocytes and acts on CNS neurons (reviewed by Walicke, 1989). Serotonergic neurons are among those which respond to S100ß. FGF appears to be synthesized by astrocytes (Ferrara et al., 1988). Basic FGF (bFGF) has been shown to promote neuron survival and neurite extension in hippocampal (Walicke et al., 1986), cortical (Morrison et al., 1986) and mesencephalic neurons (Ferrari et al., 1989) *in vitro*. EGF immunoreactivity and EGF receptors have been demonstrated in the frontal and parietal cortex, and striatum of developing rats (Fallon et al., 1984; Quirion et al., 1988). EGF promotes the maturation of telencephalic neurons (Monnet-Tschudi and Honegger, 1989). The receptors for IGF-I and IGF-II are found in fetal and adult mammalian brain (reviewed by Baskin et al., 1988). IGF-I promotes survival and neurite

outgrowth of fetal hypothalamic neurons (Torres-Aleman et al., 1990).

There are several possible mechanisms by which ethanol affects astroglial function, including the production of growth factors. Chronic ethanol exposure has been shown to alter phosphoinositide (PI) hydrolysis in astrocytes (Ritchie et al., 1988). PI hydrolysis generates diacylglycerol which functions as an essential cofactor for protein kinase C, and inositol triphosphate which regulates Ca^{+2} mobilization. Thus, altered PI hydrolysis may lead to the alteration of Ca^{+2} and protein kinase C levels. Both Ca^{+2} and protein kinase C are important mediators of cell proliferation and differentiation (reviewed by Clapham, 1995; Clemens et al., 1992; Nishizuka, 1992; Rosen et al., 1995; Whitaker, 1995). Cytosolic Ca^{+2} levels seem to be quantitatively correlated with transcription factor expression (Negulescu et al., 1994). Intracellular Ca^{+2} levels are also involved in cell death such as programmed cell death (Nicotera et al., 1994) and excitotoxicity (Schanne et al., 1979; Dugan and Choi, 1994). Therefore, effects of ethanol on the alteration of PI hydrolysis may be ultimately involved in gene regulation and/or cell death. Ethanol may alter gene regulation by acting through alterations of second messengers, and by disturbing gene transcription. In fact, ethanol exposure has been shown to increase the transcription rate of the stress protein Hsc70 and tyrosine hydroxylase in neural cell lines in culture (Miles et al., 1991; Gayer et al., 1991). In primary cultures of cortical astrocytes ethanol has been also shown to increase the GFAP mRNA levels (Fletcher and Shain, 1993).

Concluding Remarks

The present study demonstrates that *in utero* ethanol exposure adversely affects the development of 5-HT reuptake sites and 5-HT_{1A} receptors. This study also demonstrates that ethanol exposure decreases astroglial production of neurotrophic factors. Maternal treatment with buspirone, a 5-HT_{1A} agonist, has been shown in this and a prior study to overcome or prevent some of the damaging effects of ethanol. Therefore, the mechanism by which ethanol causes these abnormalities may be due partly to the decreased stimulation of 5-HT_{1A} receptors and decreased production of astroglial cell-derived growth factors that are essential for normal development of 5-HT and other neurons. Decreased stimulation of raphe 5-HT_{1A} receptors may result in altered development of 5-HT terminals as seen in altered 5-HT reuptake sites. Decreased stimulation of astroglial 5-HT_{1A} receptors potentiates the ethanol effect on the decreased production of astroglial cells.

However, additional studies are needed to assess buspirone's effects on other neurotransmitter systems which were affected by *in utero* ethanol exposure. In addition, more studies are needed to address the mechanism by which *in utero* ethanol exposure decreases the serotonin content and astroglial growth factors in the fetal brain. A schematic diagram summarizing the postulated action mechanism of ethanol and buspirone is presented in Fig. 43.

TABLE 7

EFFECTS OF ETHANOL AND BUSPIRONE ON CITALOPRAM BINDING TO 5-HT REUPTAKE SITES

Region	Age	0.8 nM			2.4nM		
		Study 1	Study 2	Buspirone	Study 1	Study 2	Buspirone
FCx	PN19	-↓-	↓	---	-↓-	↓	---
	PN35	-↓-	---	---	---	---	---
PCx	PN5	---	---	---	---	---	---
	PN19	---	↓	↓	---	---	---
	PN35	---	---	---	---	---	---
MS	PN19	↓	↓		↓	↓	
	PN35	---	---		---	---	
Striatum	PN19	↓	↓		-↓-	-↓-	
	PN35	---	---		---	↓	
CA3	PN19	---	---	---	---	---	---
	PN35	---	---	---	---	---	---
LH	PN19	↓	↓	---	↓	↓	↓
	PN35	↓	↓	---	↓	-↓-	---
AMG	PN19	---	---	---	---	---	---
	PN35	---	---	---	---	---	---
SN	PN19	-↓-	↓	↓	↓	↓	↓
	PN35	↓	↓	---	-↓-	---	---
DR	ED19		---	---		-↑-	↑
	PN5	↑	-↑-	---	-↑-	↑	---
	PN19	---	---	---	---	---	---
	PN35	---	---	---	---	---	---
MnR	PN19	---	↑	---	---	---	---
	PN35	↑	---	---	---	↑	↑

The solid arrows (↑, ↓) indicate that values from ethanol-exposed offspring were significantly different from those of control saline-treated offspring ($P < 0.05$). The broken arrows (-↑-, -↓-) indicate nonsignficant trends ($P > 0.05$). The dotted lines (---) are used to demonstrate that *in utero* ethanol exposure had no effect on 5-HT reuptake sites. Abbreviations are same as in Table 5.

TABLE 8

EFFECTS OF ETHANOL AND BUSPIRONE ON 8-OH-DPAT BINDING TO 5-HT_{1A} RECEPTORS

		1.1 nM			2.75 nM		
Region	Age	Study 1	Study 2	Buspirone	Study 1	Study 2	Buspirone
FCx	PN19	---	---	---	---	---	---
	PN35	---	---	---	---	---	---
PCx	PN19	---	---	---	---	---	---
	PN35	↓	↓	↓	↓	↓	---
DG	PN5	---	---	---	---	---	---
	PN19	-↑-	↑	---	↑	↑	↑
	PN35	---	---	---	---	---	---
LS	PN19	---	---	---	---	---	---
	PN35	---	↓	---	---	↓	↓
DR	PN19	---	---	---	---	---	---
	PN35	---	---	---	---	---	---
MnR	PN19	---	---	---	---	---	---
	PN35	---	---	---	---	---	---

This table summarizes the results obtained in two experiments in which [^{3}H]-8-OH-DPAT was used to radiolabel 5-HT_{1A} receptors. The solid arrows (↑, ↓) indicate that values from ethanol-exposed offspring were significantly different from those of control saline-treated offspring ($P < 0.05$). The broken arrows (-↑-, -↓-) indicate nonsignficant trends ($P > 0.05$). The dotted lines (---) are used to demonstrate that *in utero* ethanol exposure had no effect on 5-HT_{1A} receptors. Abbreviations are same as in Table 3.

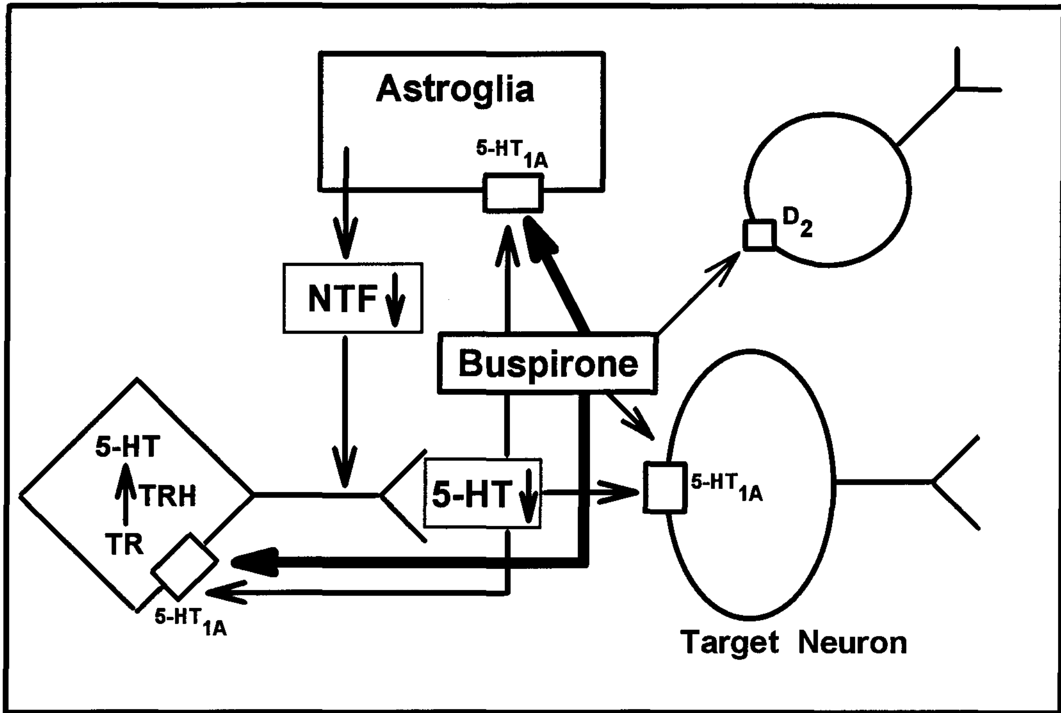


Fig. 43. Proposed action mechanisms of ethanol and buspirone on the serotonergic system. Ethanol decreases serotonin content and astroglial production of neurotrophic factors. Decreased serotonin less stimulates raphe 5-HT_{1A} receptors, which results in altered development of serotonergic fibers. Decreased serotonin also less stimulates astroglial 5-HT_{1A} receptors, which potentiates ethanol's effect on the decreased production of neurotrophic factors. Buspirone prevents ethanol-associated abnormal development of serotonergic fibers by stimulating both raphe and astroglial 5-HT_{1A} receptors. However, further studies are required for the effects of buspirone acting on D_2 receptors as an antagonist, which, in turn, affects the development of 5-HT fibers. Abbreviations are as follows: D_2, dopamine D_2 receptor; 5-HT_{1A}, 5-HT_{1A} receptor; NTF, neurotrophic factor; TR, tryptophan.

APPENDIX

STATISTICAL SIGNIFICANCE LEVEL OF BINDING ASSAY RESULTS

Figure	Region	Age	[Ligand]	F-ratio	P value
		$[^3H]$Citalopram			
3	DR	PN 5	0.8 nM	19.4	0.0004
			2.4 nM	2.6	0.12
		PN19	0.8 nM	0.1	0.9
			2.4 nM	2.6	0.1
		PN35	0.8 nM	1.4	0.3
			2.4 nM	0.4	0.7
4	MnR	PN19	0.8 nM	1.2	0.3
			2.4 nM	0.6	0.6
		PN35	0.8 nM	7.7	0.0071
			2.4 nM	0.4	0.7
5	LH	PN19	0.8 nM	12.2	0.001
			2.4 nM	8.2	0.006
		PN35	0.8 nM	5.3	0.02
			2.4 nM	12.1	0.0013
6	SN	PN19	0.8 nM	2.4	0.1
			2.4 nM	6.6	0.01
		PN35	0.8 nM	17.8	0.0005
			2.4 nM	2.9	0.09
7	Amygdala	PN19	0.8 nM	0.2	0.8
			2.4 nM	1.3	0.3
		PN35	0.8 nM	1.2	0.3
			2.4 nM	1.4	0.3
8	CA3	PN19	0.8 nM	0.1	0.9
			2.4 nM	0.7	0.5
		PN35	0.8 nM	0.8	0.5
			2.4 nM	0.2	0.8
9	PCx	PN5	0.8 nM	0.1	0.9
			2.4 nM	0.3	0.8
		PN19	0.8 nM	0.3	0.8
			2.4 nM	1.5	0.3

Figure	Region	Age	[Ligand]	F-ratio	P value
		PN35	0.8 nM	1.4	0.3
			2.4 nM	0.1	0.9
10	FCx	PN19	0.8 nM	0.9	0.43
			2.4 nM	1.3	0.3
		PN35	0.8 nM	0.6	0.6
			2.4 nM	1.5	0.3
		[^{3}H]-8OH-DPAT			
12	DG	PN5	1.1 nM	3.8	0.6
			2.75 nM	2.0	0.2
		PN19	1.1 nM	1.0	0.4
			2.75 nM	4.0	0.05
		PN35	1.1 nM	0.4	0.7
			2.75 nM	0.1	0.9
13	PCx	PN19	1.1 nM	1.0	0.4
			2.75 nM	1.4	0.3
		PN35	1.1 nM	4.2	0.04
			2.75 nM	4.3	0.04
14	FCx	PN19	1.1 nM	0.2	0.8
			2.75 nM	1.7	0.2
		PN35	1.1 nM	1.9	0.2
			2.75 nM	1.2	0.3
15	LS	PN19	1.1 nM	1.3	0.3
			2.75 nM	1.0	0.4
		PN35	1.1 nM	0.4	0.7
			2.75 nM	2.0	0.2
16	DR	PN19	1.1 nM	2.3	0.2
			2.75 nM	1.3	0.3
		PN35	1.1 nM	0.1	0.9
			2.75 nM	0.3	0.8
17	MnR	PN19	1.1 nM	0.9	0.5
			2.75 nM	0.6	0.6
		PN35	1.1 nM	0.7	0.5
			2.75 nM	1.9	0.2

Figure	Region	Age	[Ligand]	Group	F-ratio	P value
			[³H]Citalopram			
19	FCx	PN19	0.8 nM	Ethanol	4.2	0.09
				Buspirone	0.3	0.6
				Interaction	2.8	0.1
			2.4 nM	Ethanol	1.3	0.3
				Buspirone	0.002	1.0
				Interaction	7.0	0.045
		PN35	0.8 nM	Ethanol	0.5	0.5
				Buspirone	2.0	0.2
				Interaction	2.3	0.2
			2.4 nM	Ethanol	0.5	0.5
				Buspirone	0.03	0.9
				Interaction	0.9	0.4
20	PCx	PN5	0.8 nM	Ethanol	0.5	0.5
				Buspirone	0.3	0.6
				Interaction	1.5	0.3
			2.4 nM	Ethanol	0.4	0.6
				Buspirone	0.4	0.6
				Interaction	0.2	0.6
		PN19	0.8 nM	Ethanol	8.7	0.03
				Buspirone	0.6	0.5
				Interaction	0.1	0.8
			2.4 nM	Ethanol	0.04	0.8
				Buspirone	0.2	0.7
				Interaction	0.03	0.9
		PN35	0.8 nM	Ethanol	0.03	0.9
				Buspirone	1.4	0.3
				Interaction	2.2	0.2
			2.4 nM	Ethanol	0.3	0.6
				Buspirone	0.9	0.4
				Interaction	1.8	0.2
21	LH	PN19	0.8 nM	Ethanol	13.7	0.01
				Buspiron	2.3	0.2
				Interaction	9.8	0.02

Figure	Region	Age	[Ligand]	Group	F-ratio	P value
			2.4 nM	Ethanol	7.4	0.03
				Buspirone	0.1	0.7
				Interaction	0.1	0.7
		PN35	0.8 nM	Ethanol	1.7	0.2
				Buspirone	12.5	0.01
				Interaction	11.6	0.01
			2.4 nM	Ethanol	1.83	0.2
				Buspirone	0.01	0.9
				Interaction	2.2	0.2
22	SN	PN19	0.8 nM	Ethanol	27.0	0.002
				Buspirone	0.2	0.6
				Interaction	0.99	0.4
			2.4 nM	Ethanol	12.1	0.01
				Buspirone	0.2	0.7
				Interaction	3.8	0.1
		PN35	0.8 nM	Ethanol	5.9	0.05
				Buspirone	3.8	0.1
				Interaction	1.0	0.4
			2.4 nM	Ethanol	20.4	0.004
				Buspirone	0.5	0.4
				Interaction	0.17	0.7
23	MS	PN19	0.8 nM	Ethanol	8.0	0.03
				Buspirone	0.3	0.6
				Interaction	6.4	0.04
			2.4 nM	Ethanol	5.7	0.05
				Buspirone	7.1	0.04
				Interaction	2.3	0.2
		PN35	0.8 nM	Ethanol	0.05	0.8
				Buspirone	0.7	0.4
				Interaction	0.5	0.5
			2.4 nM	Ethanol	0.4	0.5
				Buspirone	1.1	0.3
				Interaction	0.1	0.8

Figure	Region	Age	[Ligand]	Group	F-ratio	P value
24	Striatum	PN19	0.8 nM	Ethanol	8.7	0.03
				Buspirone	1.3	0.3
				Interaction	0.5	0.5
			2.4 nM	Ethanol	38.7	0.0008
				Buspirone	0.003	0.96
				Interaction	0.1	0.8
		PN35	0.8 nM	Ethanol	0.03	0.9
				Buspirone	0.3	0.6
				Interaction	0.4	0.6
			2.4 nM	Ethanol	7.3	0.04
				Buspirone	3.3	0.1
				Interaction	0.3	0.6
25	DR	ED19	0.8 nM	Ethanol	0.01	0.9
				Buspirone	0.9	0.4
				Interaction	0.6	0.5
			2.4 nM	Ethanol	0.05	0.8
				Buspirone	0.3	0.6
				Interaction	0.4	0.5
		PN5	0.8 nM	Ethanol	4.3	0.1
				Buspirone	1.9	0.2
				Interaction	0.3	0.6
			2.4 nM	Ethanol	6.4	0.045
				Buspirone	0.2	0.7
				Interaction	1.4	0.3
		PN19	0.8 nM	Ethanol	0.8	0.4
				Buspirone	0.5	0.5
				Interaction	0.5	0.5
			2.4 nM	Ethanol	0.3	0.6
				Buspirone	0.0002	0.99
				Interaction	1.8	0.2
		PN35	0.8 nM	Ethanol	0.08	0.8
				Buspirone	0.001	0.98
				Interaction	5.0	0.07
			2.4 nM	Ethanol	3.0	0.1
				Buspirone	0.9	0.4
				Interaction	0.002	0.97

Figure	Region	Age	[Ligand]	Group	F-ratio	P value
26	MnR	PN19	0.8 nM	Ethanol	0.2	0.7
				Buspirone	2.2	0.2
				Interaction	2.2	0.2
			2.4 nM	Ethanol	30.2	0.003
				Buspirone	0.00	0.999
				Interaction	4.6	0.09
		PN35	0.8 nM	Ethanol	9.3	0.02
				Buspirone	1.9	0.2
				Interaction	2.2	0.2
			2.4 nM	Ethanol	0.4	0.5
				Buspirone	0.2	0.7
				Interaction	0.007	0.9
27	CA3	PN19	0.8 nM	Ethanol	2.5	0.2
				Buspirone	0.2	0.7
				Interaction	0.1	0.7
			2.4 nM	Ethanol	0.06	0.8
				Buspirone	0.08	0.8
				Interaction	0.0097	0.9
		PN35	0.8 nM	Ethanol	0.3	0.6
				Buspirone	0.02	0.9
				Interaction	0.5	0.5
			2.4 nM	Ethanol	0.6	0.5
				Buspirone	0.2	0.7
				Interaction	0.3	0.6
28	Amygdala	PN19	0.8 nM	Ethanol	4.4	0.08
				Buspirone	8.6	0.03
				Interaction	0.2	0.7
			2.4 nM	Ethanol	32.0	0.001
				Buspirone	0.0053	0.9
				Interaction	0.0083	0.9
		PN35	0.8 nM	Ethanol	0.2	0.7
				Buspirone	0.03	0.9
				Interaction	0.9	0.4
			2.4 nM	Ethanol	0.4	0.5
				Buspirone	0.07	0.8
				Interaction	0.09	0.8

Figure	Region	Age	[Ligand]	Group	F-ratio	P value
			[^{3}H]-8-OH-DPAT			
29	DG	PN5	1.0 nM	Ethanol	0.1	0.7
				Buspirone	1.8	0.2
				Interaction	0.6	0.5
			2.7 nM	Ethanol	0.09	0.8
				Buspirone	0.5	0.5
				Interaction	0.5	0.5
		PN19	1.0 nM	Ethanol	5.0	0.07
				Buspirone	0.8	0.4
				Interaction	2.5	0.2
			2.7 nM	Ethanol	9.4	0.02
				Buspirone	0.07	0.8
				Interaction	2.02	0.2
		PN35	1.0 nM	Ethanol	0.2	0.7
				Buspirone	0.04	0.8
				Interaction	0.2	0.7
			2.7 nM	Ethanol	0.03	0.9
				Buspirone	0.4	0.6
				Interaction	0.2	0.7
30	PCx	PN19	1.0 nM	Ethanol	0.3	0.6
				Buspirone	0.2	0.7
				Interaction	0.3	0.6
			2.7 nM	Ethanol	1.3	0.3
				Buspirone	6.7	0.05
				Interaction	0.8	0.4
		PN35	1.0 nM	Ethanol	8.9	0.03
				Buspirone	1.4	0.3
				Interaction	3.0	0.1
			2.7 nM	Ethanol	8.5	0.03
				Buspirone	3.4	0.1
				Interaction	2.8	0.2
31	DR	PN19	1.0 nM	Ethanol	0.3	0.6
				Buspirone	0.04	0.8
				Interaction	0.6	0.5

Figure	Region	Age	[Ligand]	Group	F-ratio	P value
			2.7 nM	Ethanol	2.8	0.1
				Buspirone	0.5	0.5
				Interaction	0.2	0.7
		PN35	1.0 nM	Ethanol	0.0004	0.98
				Buspirone	0.2	0.6
				Interaction	0.1	0.7
			2.7 nM	Ethanol	0.4	0.5
				Buspirone	0.6	0.5
				Interaction	0.1	0.7
32	MnR	PN19	1.0 nM	Ethanol	0.32	0.58
				Buspirone	0.119	0.74
				Interaction	0.7	0.4
			2.7 nM	Ethanol	0.006	0.9
				Buspirone	0.1	0.7
				Interaction	0.1	0.8
		PN35	1.0 nM	Ethanol	0.4	0.5
				Buspirone	0.2	0.7
				Interaction	0.007	0.9
			2.7 nM	Ethanol	0.2	0.7
				Buspirone	0.03	0.9
				Interaction	0.00	0.998
33	LS	PN19	1.0 nM	Ethanol	0.02	0.9
				Buspirone	0.4	0.6
				Interaction	1.7	0.2
			2.7 nM	Ethanol	0.02	0.9
				Buspirone	0.03	0.9
				Interaction	3.9	0.1
		PN35	1.0 nM	Ethanol	9.6	0.02
				Buspirone	0.003	0.96
				Interaction	6.8	0.04
			2.7 nM	Ethanol	0.9	0.4
				Buspirone	2.4	0.2
				Interaction	20.0	0.0042

Figure	Region	Age	[Ligand]	Group	F-ratio	P value
34	FCx	PN19	1.0 nM	Ethanol	0.004	0.96
				Buspirone	0.3	0.6
				Interaction	2.9	0.2
			2.7 nM	Ethanol	0.4	0.5
				Buspirone	0.2	0.6
				Interaction	4.4	0.09
		PN35	1.0 nM	Ethanol	0.9	0.4
				Buspirone	4.2	0.1
				Interaction	9.4	0.03
			2.7 nM	Ethanol	0.4	0.5
				Buspirone	1.7	0.2
				Interaction	1.8	0.2

REFERENCES

Abel, E.L. (1979) Prenatal effects of alcohol on adult learning in rats. *Pharmacol. Biochem. Behav.* 10:239-243.

Abel, E.L. (1984) *Fetal Alcohol Syndrome and Fetal Alcohol Effects*. Plenum Press, New York.

Abel, E.L. and Sokol, R.J. (1991) A revised conservative estimate of the incidence of FAS and its economic impact. *Alcohol. Clin. Exp. Res.* 15:514-524.

Abel, E.L., Jacobson, S. and Sherwin, B.T. (1983) In utero acohol exposure: Functional and structural brain damage. *Neurobehav. Toxicol. Tetratol.* 5:363-366.

Acsady, L., Halasy, K. and Freund, T.F. (1993) Calretinin is present in non-pyramidal cells of the rat hippocampus-III. Their inputs from the median raphe and medial septal nuclei. *Neuroscience* 52:829-841.

Adell, A., Carceller, A. and Artigas, F. (1993) In vivo brain dialysis study of the somatodendritic release of serotonin in the raphe neuclei of the rat. Effects of 8-hydroxy-2-(di-n-propylamino)tetralin. *J. Neurochem.* 60:1673-1681.

Aghajanian, G.K., Rosecrans, J.A. and Sheard, M.H. (1967) Serotonin: release in the forebrain by stimulation of midbrain raphe. *Science* 156:402-403.

Aitken, A.R. and Tork, I. (1988) Early development of serotonin-containing neurons and pathways as seen in wholemount preparations of the fetal rat brain. *J. Comp. Neurol.* 274:32-47.

Altaman, H.J., Normile, H.J., Galloway, M.P., Ramirez, A. and Azmitia, E.C. (1990) Enhanced spatial discrimination learning in rats following 5,7-DHT-induced serotonergic deafferentation of the hippocampus. *Brain Res.* 518:61-66.

Altura, R.M., Altura B.T., Carella, A., Chatterjee, M., Halvey, S. and Tejani, N. (1983) Alcohol produces spasms of human umbilical blood vessels: relationship to fetal alcohol syndrome (FAS). *Eur. J. Pharmacol.* 86:311-312.

Andrade, R. and Nicoll, R.A. (1987) Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurons

of the rat hippocampus. *Naunyn Schmiedebergs Arch. Pharmacol.* 336:5-10.

Angevine, J.B. and Sidman, R.L. (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* 192:766-768.

Apostol, G. and Creutzfeldt, O.D. (1974) Cross-correlation between the activity of septal units and hippocampal EEG during arousal. *Brain Res.* 67:65-75.

Aronson, M. and Olegard, R. (1987) Children of alcoholic mothers. *Pediatrician* 14:57-61.

Aronson, M., Kyllerman, M., Sabel, K.-G., Sandin, B. and Olegard, R. (1985) Children of alcoholic mothers: Developmental, perceptual and behavioural characteristics as compared to matched controls. *Acta. Paediatr. Scand.* 74:27-35.

Assaf, S.Y. and Miller, J.J. (1978) The role of a raphe serotonin system in the control of septal unit activity and hippocampal desynchronization. *Neuroscience* 3:539-550.

Auer, R.N., Jensen, M.L. and Whishaw, I.Q. (1989) Neurobehavioral deficit due to ischemic brain damage limited to half of the CA1 sector of the hippocampus. *J. Neurosci.* 9:1641-1647.

Azmitia, E.C. and Segal, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.* 179:641-668.

Azmitia, E.C. and Gannon, P.J. (1986) Anatomy of the serotonergic system in the primate and sub-primate brain. *Adv. Neurol.* 43:407-468.

Azmitia, E.C., Dolan, K. and Whitaker-Azmitia, P.M. (1990) S-100_B but not NGF, EGF, insulin or calmodulin is a CNS serotonergic growth factor. *Brain Res.* 516:354-356.

Babu, P.P., Kumari, L.R. and Vemuri, M.C. (1994) Differential changes in cell morphology, macromolecular composition and membrane protein profiles of neurons and astrocytes in chronic ethanol treated rats. *Mol. Cell. Biochem.* 130:29-40.

Baillie, P. and Morrison, S.D. (1963) The nature of the suppression of food intake by lateral hypothalamic lesions in rats. *J. Physiol.* 165:227-245.

Baker, P.C. and Quay, W.B. (1969) Serotonin metabolism in early embryogenesis, and the development of brain retinal tissues: a review. *Brain Res.* 12:273-295.

Balagura, S., Wilcox, R.H. and Coscina, D.V. (1969) The effect of diencephalic lesions on food intake and motor activity. *Physiol. Behav.* 4:229-233.

Banker, G.A. (1980) Trophic interactions between astroglial cells and hippocampal neurons in culture. *Science* 209:809-810.

Bar-Peled, O., Gross-Isseroff, R., Ben-Hur, H., Hoskins, I., Groner, Y. and Biegon, A. (1991) Fetal human brain exhibits a prenatal peak in the density of serotonin 5-HT_{1A} receptors. *Neurosci. Lett.* 127:173-176.

Barnes, D.E. and Walker, D.W. (1981) Prenatal ethanol exposure permanently reduces the number of pyramidal neurons in rat hippocampus. *Dev. Brain Res.* 1:333-340.

Barron, S., Tieman, S.B. and Riley, E.P. (1988) Effects of prenatal alcohol exposure on the sexually dimorphic nucleus of the preoptic area of the hypothalamus in male and female rats. *Alcohol. Clin. Exp. Res.* 12:59-64.

Baskin, D.G., Wilcox, B.J., Figlewicz, D.P. and Dorsa, D.M. (1988) Insulin and insulin-like growth factors in the CNS. *Trends Neurosci.* 11:107-111.

Bauer-Moffett, C. and Altman, J. (1975) Ethanol-induced reductions in the growth of infant rats. *Exp. Neurol.* 48:378-382.

Beck, S.G. and Choi, K.C. (1991) 5-Hydroxytryptamine hyperpolarizes CA3 hippocampal pyramidal cells through an increase in potassium conductance. *Neurosci. Lett.* 133:93-96.

Bianchi, C., Siniscalchi, A. and Beani, L. (1989) Effect of 5-HT on [^{3}H]-acetylcholine release from guinea pig striatal slices. *Br. J. Pharmacol.* 97:213-221.

Biegon, A., Kargman, S. and Snyder, L. (1986) Chaterization and localization of serotonin receptors in human brain post-mortem. *Brain Res.* 363:91-98.

Bennett, J.P. Jr., Enna, S.J., Bylund, D.B., Gillin, J.C. Wyatt, R.J. and Snyder, S.H. (1979) Neurotransmitter receptors in frontal cortex of schizophrenics. *Arch. Gen. Psychiatry* 36:927-934.

Bliss, T.V.P. (1979) Synaptic plasticity in the hippocampus. *Trends Neurosci.* 2:42-45.

Bliss, T.V.P., Goddard, G.V. and Riives, M. (1983) Reduction of long-term potentiation in the dentate gyrus of the rat following selective depletion of Monoamines. *J. Physiol.* 334:475-491.

Blue, M.E. and Molliver, M.E. (1985) Postnatal development of the serotonergic

innervation of the serotonergic of the cerebral cortex of the rat. *Soc. Neurosci. Abstr.* 11:1128.

Blundell, J.E. (1979) Serotonin and feeding. In: *Serotonin in Health and Disease*, vol. 5, (ed. W. B. Essman), pp. 403-450. Spectrum, New York.

Bond, N.W. and DiGiusto, E.L. (1976) Effects of prenatal alcohol consumption on open-field behavior and alcohol preference in rats. *Psychopharmacology* 46:163-165.

Bond, N.W. and DiGiusto, E.L. (1977) Prenatal alcohol consumption and open-field behavior in rats: Effects of age at time of testing. *Psychopharmacology* 52:311-312.

Bonthius, D.J. and West, J.R. (1990) Alcohol-induced neuronal loss in developing rats: increased brain damage with binge exposure. *Alcohol. Clin. Exp. Res.* 14:107-118.

Borges, S. and Lewis, P.D. (1982) A study of alcohol effects on brain during gestation and lactation. Teratology 25:283-289.

Borges, S. and Lewis, P.D. (1983) Effects of ethanol on postnatal cell acquisition in the rat cerebellum. *Brain Res.* 271:388-391.

Bourgoin S., Faivre-Bauman, A. Benda, P. Glowinski J. and Hamon M. (1974) Plasma tryptophan and 5-HT metabolism in the CNS of the newborn rat. *J. Neurochem.* 23:319-327.

Bourgoin, S., Artaud, F., Adrien, J., Hery, F., Glowinski, J. and Hamon, M. (1977) 5-hydroxytryptamine catabolism in the rat brain during ontogenesis. *J. Neurochem.* 28:415-422.

Branchey, L. and Friedhoff, A.J. (1976) Biochemical and behavioral changes in rats exposed to ethanol in utero. *Ann. N.Y. Acad. Sci.* 273:328-330.

Brien, J.F., Loomis, C.W., Tranmer, J. and McGath, M. (1983) Diposition of ethanol in human maternal venous blood and amniotic fluid. *Am. J. Obstet. Gynecol.* 146:181-186.

Brown, N.A., Goulding, E.H. and Fabro, S. (1979) Ethanol embryotoxicity: Direct effects on mammalian embryos in vitro. *Science* 206:573-575.

Budnik, V., Wu, C.F. and White, K. (1989) Altered branching of serotonin-containg neurons in *Drosophila* mutants unable to synthesize serotonin and dopamine. *J. Neurosci.* 9:2866-2877.

Bunny, B.S. and Aghajanian, G.K. (1978) d-Amphetamine-induced depression of central dopamine neurons: evidence for mediation by both autoreceptors and a striatonigral feedback pathway. *Naunyn Schmiedebergs Arch. Pharmacol.* 307:255-261.

Burd, L. and Martsolf, J.T. (1989) Fetal alcohol syndrome: diagnosis and syndrome variability. *Physiol. Behav.* 46:39-43.

Burton, K. (1956) A study of the conditions and mechanisms of the dephenylamine reaction for the colorimetric estimation of DNA. *Biochem. J.* 62:315-323.

Carter, C.J. and Pycock, C.J. (1979) The effects of 5,7-dihydroxytryptamine lesions of extrapyramidal and mesolimbic sites on spontaneous motor behavior and amphetamine-induced stereotypy. *Naunyn Schmiedebergs Arch. Pharmacol.* 308:51-54.

Cedar, H. (1988) DNA methylation and gene activity. *Cell* 53:3-4.

Chafetz, M.D., Thomson, R.G., Evans, S.H. and Gage, F.H. (1981) Biochemical specificity of septal hyperactivity: a behavioral discrimination. *Behav. Brain Res.* 2:409-420.

Charness, M.E., Safran, R.M. and Perides, G. (1994) Ethanol inhibits neural cell-cell adhesion. *J. Biol. Chem.* 269:9304-9309.

Chernoff, G.S. (1977) The fetal alcohol syndrome in mice: an animal model. *Teratology* 15:223-230.

Chubakov, A.R., Gromova, E.A., Konovalov, G.V., Sarkisova, E.F. and Chumasov, E.I. (1986) The effects of serotonin on the morpho-functional development of rat cerebral neocortex in tissue culture. *Brain Res.* 369:285-297.

Chumasov, E.I., Chubakov, A.R., Konovalov, G.V., and Gromova, E.A. (1980) Effect of serotonin on growth and differentiation of hippocampal cells in culture. *Neurosci. Behav. Physiol.* 10:125-131.

Clapham, D.E. (1995) Calcium signaling. *Cell* 80:259-268.

Clarren, S.K. and Smith, D.W. (1978) The fetal alcohol syndrome. *N. Engl. J. Med.* 298:1063-1067.

Clarren, S.K., Alvord, E.C., Jr., Sumi, S.M., Streissguth, A.P. and Smith, D.W. (1978) Brain malformations related to prenatal exposure to ethanol. *J. Pediatr.* 92:64-67.

Claustre, Y., Rouquier, L. and Scatton, B. (1988) Pharmacological characterization of serotonin-stimulated phosphoinositide turnover in brain regions of the immature rat. *J. Pharmacol. Exp. Ther.* 244:1051-1056.

Clemens, M.J., Trayner, I. and Menaya, J. (1992) The role of protein kinase C isoenzymes in the regulation of cell proliferation and differentiation. *J. Cell Sci.* 103:881-887.

Conry, J. (1990) Neuropsychological deficits in fetal alcohol syndrome and fetal alcohol effects. *Alcohol. Clin. Exp. Res.* 14:650-655.

Coop, C.F. and McNaughton, N. (1991) Buspirone affects hippocampal rhythmical slow activity through serotonin$_{1A}$ rather than dopamine D_2 receptors. *Neuroscience* 40:169-174.

Coscina, D.V., Grant L.D., Balagura, S. and Grossman, S.P. (1972) Hyperdipsia after serotonin-depleting midbrain lesions. *Nature* 235:63-74.

Costall, B., Hui, S.C.G. and Naylor, R.J. (1979) The importance of serotonergic mechanisms for the induction of hyperactivity by amphetamine and its antagonism by intra-accumbens (3,4-dihydroxy-phenyl amino)-2-imidazoline (DPI). *Neuropharmacology* 18:605-609.

Cowan, P.J., Anderson, I.M. and Grahame-Smith, D.G. (1990) Neuroendocrine effects of azapirones. *J. Clin. Psychopharmacol.* 10:21S-25S.

D'Amato, R.J., Largent, B.L., Snowman, A.M. and Snyder, S.H. (1987a) Selective labeling of serotonin uptake sites in rat brain by [^{3}H]citalopram contrasted to labeling of multiple sites by [^{3}H]imipramine. *J. Pharmacol. Exp. Ther.* 242:364-371.

D'Amato, R.J., Blue, M.E., Largent, B.L., Lynch, D.R., Ledbetter, D.J., Molliver, M. E. and Snyder, S.H. (1987b) Ontogeny of the serotonergic projection to rat neocortex: Transient expression of a dense innervation to primary sensory areas. *Proc. Natl. Acad. Sci.* 84:4322-4326.

Daval, G., Verge, D., Becerril, A., Gozlan, H., Sampinato, U. and Hamon, M. (1987) Transient expression of 5-HT$_{1A}$ receptor binding sites in some areas of rat CNS during postnatal development. *Int. J. Dev. Neurosci.* 5:171-180.

Davies, D.L. and Cox, W.E. (1991) Delayed growth and maturation of astrocytic cultures following exposure to ethanol: electron microscopic observations. Brain Res. 547:53-61.

Davies, D.L. and Vernadakis, A. (1984) Effects of ethanol on cultured glial cells: proliferation and glutamine synthetase activity. *Dev. Brain Res.* 16:27-35.

Davila, M.I., de Kloet, R. and Azmitia, E.C. (1986) Effects of neuropeptides on the maturation of serotonergic neurons in tissue culture. *Soc. Neurosci. Abstr.* 12:152.

Davila-Garcia, M.I. and Azmitia, E.C. (1990) Neuropeptides as positive neuronal growth regulatory factors: effects of ACTH and leu-enkephalin on cultured serotonergic neurons. In: *Molecular Aspects of Development and Aging of the Nervous System*, (ed. J.M. Lauder), pp. 75-92. Plenum Press, New York.

Davison, A. N. and Dobbing, J. (1968) The developing brain. In: *Applied Neurochemistry* (eds. A.N. Davison and J. Dobbing), pp. 253-286. Blackwell Scientific Publications, Oxford.

De France, J.F. (1976) *The Septal Nuclei.* Plenum Press, New York.

Deguchi, T. and Barchas, J. (1972) Regional distribution and developmental change of tryptophan hydroxylase activity in rat brain. *J. Neurochem.* 19:927-929.

De Vitry, F., Hamon, M., Catelon, J., Dubois, M. and Thibault, J. (1986) Serotonin initiates and autoamplifies its own synthesis during mouse central nervous system development. *Proc. Natl. Acad. Sci.* 83:8629-8633.

Dewey, S.L. and West, J.R. (1984) Evidence for altered lesion-induced sprouting in the dentate gyrus of adult rats exposed to ethanol in utero. *Alcohol.* 1:81-88.

Diaz, J. and Samson, H.H. (1980) Impaired brain growth in neonatal rat pups exposed to ethanol. *Science* 208:751-753.

Di Chiara, G., Morelli, M., Porceddu, M.L., Mulas, M. and Del Fiacco, M. (1980) Effect of discrete kainic acid-induced lesions of corpus caudatus and globus pallidus on glutamic acid decarboxylase of rat substantia nigra. *Brain Res.* 189:193-208.

Dillon, K.A., Gross-Isseroff, R., Israeli, M. and Biegon, A. (1991) Autoradiographic analysis of serotonin 5-HT_{1A} receptor binding in the human brain postmortem: effects of age and alcohol. *Brain Res.* 554:56-64.

Dinopoulos, A., Dori, I. and Parnavelas, J.G. (1993) Serotonergic innervation of the mature and developing lateral septum of the rat: A light and electron microscopic immunocytochemical analysis. *Neuroscience* 55:209-222.

Dobbing, J. and Sands, J. (1979) Comparative aspects of the brain growth spurt. *Early Hum. Dev.* 3:79-83.

Dohler, K.-D., Coquelin, A., Davis, F., Hines, M., Shryne, J.E. and Gorski, R.A. (1984) Pre- and postnatal influence of testosterone and diethylstilbesterol on differentiation of the sexually dimorphic nucleus of the preoptic area in male and female rats. *Brain Res.* 302:291-295.

Dohler, K.-D., Coquelin, A., Davis, F., Hines, M., Shryne, J.E., Sickmoller, P.M., Jarzab, B. and Gorski, R.A. (1986) Pre- and postnatal influence of an estrogen antagonist and an androgen antagonist on differentiation of the sexually dimorphic nucleus of the preoptic area in male and female rats. *Neuroendocrinology* 42:443-448.

Dow, K.E. and Riopelle, R.J. (1985) Ethanol neurotoxicity: Effects on neurite formation and neurotrophic factor production in vitro. *Science* 228:591-593.

Dray, A., Gonye, T.J., Oakley, N.R. and Tanner, T. (1976) Evidence for the existence of a raphe projection to the substantia nigra in rat. *Brain Res.* 113:45-57.

Druse, M.J. (1992) Effects of in utero ethanol exposure on the development of CNS neurotransmitter systems. In: *Development of the Central Nervous System: Effects of Alcohol and Opiates.* (ed. M.W. Miller), pp. 139-167. Wiley-Liss, New York.

Druse, M.J. and Paul, L.H. (1988) Effects of in utero ethanol exposure on serotonin uptake in cortical regions. *Alcohol* 5:455-459.

Druse, M.J., Noronha, A.B., Gnaedinger, J.M. and Smith, D.M. (1980) Effects of maternal alcohol consumption and maternal malnutrition on CNS membranes in offspring. *Teratology* 21:36A.

Druse, M.J., Kuo, A. and Tajuddin, N. (1991) Effects of in utero ethanol exposure on the developing serotonergic system. *Alcohol. Clin. Exp. Res.* 15:678-684.

Dugan, L.L. and Choi, D.W. (1994) Excitotoxicity, free radicals, and cell membrane changes. *Ann. Neurol.* 35:S17-S21.

Duncan, G.E., Little, K.Y., Kirkman, J.A., Kaldas, R.S., Stumpf, W.E. and Breese, G.R. (1992) Autoradiographic characterization of [^{3}H]imipramine and [^{3}H]citalopram binding in rat and human brain: species differences and relationships to serotonin innervation patterns. *Brain Res.* 591:181-197.

Ellis, J., Krsiak, M., Poschlova, N. and Masek, K. (1976) The effect of alcohol administration during pregnancy on the concentration of noradrenaline, dopamine

and 5-hydroxytryptamine in the brain of offspring of mice. *Act Nerv. Super. (Praha)* 18:220-221.

Ellis, J., Krsiak, M. and Poschlova, N. (1978) Effect of alcohol given at different periods of gestation on brain serotonin in offspring. *Act Nerv. Super. (Praha)* 20:287-288.

Engel, G., Gothert, M., Hoyer, D., Schlicker, E. and Hillenbrand, K. (1986) Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT1B binding sites. *Naunyn Schmiedebergs Arch. Pharmacol.* 332:1-7.

Fallon, J.H., Seroogy, K.B., Loughlin, S.E., Morrison, R.S., Bradshaw, R.A., Knauer, D.J. and Cunningham, D.D. (1984) Epidermal growth factor immunoreactive material in the central nervous system: location and development. *Science* 224:1107-1109.

Feighner, J.P., Merideth, C.H. and Hendrickson, G.A. (1982) A double-blind comparison of buspirone and diazepam in outpatients with generalized anxiety disorder. *J. Clin. Psychiatry* 43:103.

Ferguson, J., Henriken, S., Cohen, H., Mitchell, G., Barchas, J. and Dement, W. (1970) 'Hypersexuality' and behavioral changes caused by administration of p-chlorphenylalanine. *Science* 168:499-501.

Ferrara, N., Ousley, F. and Gospodarowicz, D. (1988) Bovine brain astrocytes express basic fibroblast growth factor, a neurotrophic and angiogenic mitogen. *Brain Res.* 462:223-232.

Ferrari, G., Minozzi, M.-C., Toffano, G., Leon, A. and Skaper, S.D. (1989) Basic fibroblast growth factor promotes the survival and development of mesencephalic neurons in culture. *Dev. Biol.* 133:140-147.

Ferre, S. and Artigas, F. (1993) Dopamine D_2 receptor-mediated regulation of serotonin extracellular concentration in the dorsal raphe nucleus of freely moving rats. *J. Neurochem.* 61:772-775.

Fibiger, H.C. and Miller, J.J. (1977) An anatomical and electrophysiological investigation of the serotonergic progection from the dorsal raphe nucleus to the substantia nigra in the rat. *Neuroscience* 2:975-987.

Fillion, G., Beaudoin, D., Rousselle, J.C. and Jacobs, J. (1980) [^{3}H]5-HT binding sites and 5-HT-sensitive adenylate cyclase in glial cell membrane fraction. *Brain Res.* 198:361-374.

Fillion, G., Beaudoin, D., Fillion, M.-P., Rousselle, J.-C., Robaut, C. and Netter, Y. (1983) 5-Hydroxytryptamine receptors in neurons and glia. *J. Neural Transm. Suppl.* 18:307-317.

Fletcher, P.J. and Davies, M. (1990) The involvement of 5-hydroxytryptaminergic and dopaminergic mechanisms in the eating induced by buspirone, gepirone and ipsapirone. *Br. J. Pharmacol.* 99:519-525.

Fletcher, T.L. and Shain, W. (1993) Ethanol-induced changes in astrocyte gene expression during rat central nervous system development. *Alcohol. Clin. Exp. Res.* 17:993-1001.

Freidman, J.M. (1982) Can maternal alcohol ingestion cause neural tube defects? *J. Pediatr.* 101:232-234.

Freund, T.F., Gulyas, A.I., Acsady, L., Gorcs, T. and Toth, K. (1990) Serotonergic control of the hippocampus via local inhibitory interneurons. *Proc. Natl. Acad. Sci. USA* 87:8501-8505.

Gale, K. (1979) GABA receptors in rat substantia nigra: changes in response to lesions and chronic drug treatment. *Soc. Neuroscience. Abstr.* 9:71P.

Gallager, D.W. (1982) Spontaneous unit activity of neurons within the dorsal raphe nucleus of the neonatal rat. *Life Sci.* 30:2109-2113.

Garro, A.J., McBeth, D.L., Lima, V. and Lieber, C.S. (1991) Ethanol consumption inhibits fetal DNA methylation in mice: implications for the fetal alcohol syndrome. *Alcohol. Clin. Exp. Res.* 15:395-398.

Gayer, G.G., Gordon, A. and Miles, M.F. (1991) Ethanol increases tyrosine hydroxylase gene expression in N1E-115 neuroblastoma cells. *J. Biol. Chem.* 266:22279-22284.

Giambalvo, C.T. and Snodgrass, S.T. (1978) Biochemical and behavioral effects of serotonin neurotoxins on the nigro-striatal dopamine system: comparison of injection sites. *Brain Res.* 152:555-566.

Gilbert, F., Brazell, C., Tricklebank, M.D. and Atahl, S.M. (1988) Activation of 5-HT_{1A} receptor subtype increases rat plasma ACTH concentration. *Eur. J. Pharmacol.* 147:431-439.

Gladfelter, W.E. and Brobeck, J.R. (1962) Decreased spontaneous locomotor activity in the rat induced by hypothalamic lesions. *Am. J. Physiol.* 203:811-817.

Goddard, G.V. (1964) Functions of the amygdala. *Psychol. Bull.* 62:89-109.

Goldberg, J.I. and Kater, S.B. (1989) Expression and function of the neurotransmitter serotonin during development of Helisoma nervous system. *Dev. Biol.* 131:483-495.

Golden, N.L., Sokol, R.J., Kuhnert, B.R. and Bottoms, S. (1982) Maternal alcohol use and infant development. *Pediatrics* 70:931-934.

Gozlan, H., Daval, G., Verge, D., Stampinato, U., Fattaccini, C.M., Gallissot, M.C. and El Mestikawy, S. (1990) Aging associated changes in serotonergic and dopaminergic pre- and post-synaptic neurochemical markers in rat brain. *Neurobiol. Aging* 11:437-449.

Grant, K.A., Choi, E.Y. and Samson, H.H. (1983) Neonatal ethanol exposure: effects on adult behavior and brain growth parameters. *Pharmacol. Biochem. Behav.* 18 (suppl. 1):331-336.

Greene, T., Ernhart, C.B., Martier, S., Sokol, R. and Ager, J. (1990) Prenatal alcohol exposure and language development. *Alcohol. Clin. Exp. Res.* 14:937-945.

Groves, P.M., Wilson, P.J., Young S.J. and Rebec, G.V. (1975) Self-inhibition by dopaminergic neurons. *Science* 190:522-529.

Guerri, C., Saez, R., Sancho-Tello, M., Martin de Aquilera, E. and Renau-Piqueras, J. (1990) Ethanol alters astrocyte development: a study of critical periods using primary cultures. *Neurochem. Res.* 15:559-565.

Hablitz, J.J. (1986) Prenatal exposure to alcohol alters short-term plasticity in hippocampus. *Exp. Neurol.* 93:423-427.

Hagan, M.D., Jones, B.J., Jordan, C.C. and Tyers, M.B. (1990) Effect of 5-HT_3 receptor antagonists on responses to selective activation of mesolimbic dopaminergic pathways in the rat. *Br. J. Pharmacol.* 99:227-232.

Hall, M.D., El Mestikawy, S., Emerit, M.B., Pichat, L., Hamon, M. and Gozlan, H. (1985) [^{3}H]8-Hydroxy-2-(di-n-propylamino)tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the brain. *J. Neurochem.* 44:1685-1696.

Hammer, R.P. Jr. and Scheibel, A.B. (1981) Morphologic evidence for a delay of neuronal maturation in fetal alcohol exposure. *Exp. Neurol.* 74:587-596.

Hamon, M. and Bourgoin, S. (1979) Ontogenesis of tryptophan transport in rat brain.

J. Neural Transm. Suppl. 15:93-105.

Hamon, M. and Bourgoin, S. (1982) Characteristics of 5-HT metabolism and function in the developing brain. In: *Biology of Serotonergic Transmission* (ed. N.N. Osborne), pp. 197-220, John Wiley & Sons Ltd.

Hamon, M., Lanfumey, L., El Mestikawy, S., Boni, C., Miquel, M.C., Bolanos, F., Schechter, L. and Gozlan, H. (1990) The main features of central 5-HT_1 receptors. *Neuropsychopharmacology* 3:349-360.

Handa, R.J., Hines, M., Schoonmaker, J.N., Shryne, J.E. and Gorski, R.A. (1986) Evidence that serotonin is involved in the sexually dimorphic development of the preoptic area in the rat brain. *Dev. Brain Res.* 30:278-282.

Hanson, J.W., Jones, K.L. and Smith D.W. (1976) Fetal alcohol syndrome: experience with 41 patients. *JAMA* 235:1458-1460.

Hanson, J.W., Strissguth, A.P. and Smith, D.W. (1978) The effects of moderate alcohol consumption during pregnancy on fetal growth and morphogenesis. *J. Pediatr.* 92:457-460.

Hard, E., Musi, B., Dahlgren, I.L., Engel, J., Larsson, K., Liljequist, S. and Lindh, A.S. (1985a) Impaired maternal behavior and altered central serotonergic activity in the adult offspring of chronically ethanol treated dams. *Acta Pharmacol. Toxicol.* 56:347-353.

Hard, E., Engel, J., Larsson, K., Liljequist, S. and Musi, B. (1985b) Effects of maternal ethanol consumption on the offspring sensory-motor development, ultrasonic vocalization, audiogenic immobility reaction and brain monoamine synthesis. *Acta Pharmacol. Toxicol.* 56:354-363.

Harris, S.R., Osborn, J.A., Weinberg,J., Loock, C. and Junaid, K. (1993) Effects of prenatal alcohol exposure on neuromotor and cognitive development during early childhood: a series of case reports. *Phys. Ther.* 73:608-617.

Hartig, P.R., Hoffman, B.J., Kaufman, M.J. Hirata, F. (1990) The 5-HT_{1C} receptor. *Ann. N.Y. Acad. Sci.* 600:149-167.

Haydon, P.G., McCobb, D.P. and Kater, S.B. (1987) Serotonin selectively inhibits growth cone motility and synaptogenesis of specific identified neurons. *Science* 236:561-564.

Haydon, P.G., McCobb, D.P. and Kater, S.B. (1987) The regulation of neurite outgrowth, growth cone motility, and electrical synaptogenesis by serotonin. *J.*

Neurobiol. 18:197-215.

Heaton, M.B., Swanson, D.J., Paiva, M. and Walker, D.W. (1992) Ethanol exposure affects trophic factor activity and responsiveness in chick embryo. *Alcohol* 9:161-166.

Hellendall, R.P., Schambra, U.B., Liu, J.P. and Lauder, J.M. (1993) Prenatal expression of 5-HT_{1C} and 5-HT_2 receptors in the rat central nervous system. *Exp. Neurol.* 120:186-201.

Henderson, G.I., Turner, D., Patwardhan, R.V., Lumeng, L., Hoyumpa, A.M. and schenker, S. (1981) Inhibition of placental valine uptake after acute and chronic maternal ethanol consumption. *J. Pharmacol. Exp. Ther.* 216:465-472.

Herregodts, P., Velkeniers, B., Ebinger, G., Michotte, Y., Vanhaelst, L. and Hooghe-Peters E. (1990) Development of monoaminergic neurotransmitters in fetal and postnatal rat brain: analysis by HPLC with electrochemical detection. *J. Neurochem.* 55:774-779.

Hertz, L. and Tamir, H. (1981) Some properties of an astrocytic protein fraction that binds serotonin. *J. Neurochem.* 37:1331-1334.

Hery, F., Soubrie, P., Gourgoin, S., Montrastruc, J. L., Artaud, F. and Glowinski, J. (1980) Dopamine released from dendrites in the substantia nigra control the nigral and striatal release of serotonin. *Brain Res.* 193:143-151.

Heym, J. and Gladfelter, W.E. (1982) Serotonergic innervation of the lateral hypothalamus: evidence from synaptosomal uptake studies. *Brain Res. Bull.* 8:131-137.

Hillion, J., Dumas Milne-Edwards, J.B., Catelon, J., de Vitry, F., Gros, F. and Hamon, M. (1993) Prenatal developmental expression of rat brain 5-HT_{1A} receptor gene followed by PCR. *Biochem. Biophys. Res. Comm.* 191:991-997.

Hjorth, S. and Carlsson, A. (1982) Buspirone: effects on central monoaminergic transmission - possible relevance to animal experimental and clinical findings. *Eur. J. Pharmacol.* 83:299-303.

Hockl, P.F., Diaz, G.S. and Carlos, L. (1993) Prolactin-releasing effect of buspirone in developing and adult male and female rats. *Proc. Soc. Exp. Biol. Med.* 202:447-450.

Hoff, S.F. (1988) Synaptogenesis in the hippocampal dentate gyrus: Effects of in utero ethanol exposure. *Brain Res. Bull.* 21:47-54.

Hoyer, D. (1988) Molecular pharmacology and biology of 5-HT_{1C} receptors. *Trends Pharmacol. Sci.* 9:89-94.

Hoyer, D., Engel, G. and Kalkman, H.O. (1985) Characterization of the 5-HT_{1B} recognition site in rat brain: binding studies with [^{125}I]-iodocyanopindolol. *Eur. J. Pharmacol.* 118:1-12.

Hume, R.I., Role, L.W. and Fischbach, G.D. (1983) Acetylcholine release from growth cones detected with patches of acetylcholine receptor-rich membranes. *Nature* 305:632-634.

Humphrey, P.P.A., Hartig, P. and Hoyer, D. (1993) A proposed new nomenclature for 5-HT receptors. *Trends Pharmacol. Sci.* 14:233-236.

Idanpaan-Heikkila, J. E., Fritchie, G. E., Ho, B. T. and McIssac, W. M. (1971) Placental transfer of ^{14}C-ethanol. *Am. J. Obstet. Gynecol.* 110:4266-428.

Idanpaan-Heikkila, J.,Jouppila, P., Akerblom, H. K., Isoaho, R., Kauppila, E. and Koivisto, M. (1972) Elimination and metabolic effects of ethanol in mother, fetus and newborn infant. *Am. J. Obstet. Gynecol.* 112:387-393.

Imai, H., Steindler, D.A. and Kitai, S.T. (1986) The organization of divergent axonal projections from the midbrain raphe nuclei in the rat. *J. Comp. Neurol.* 243:363-380.

Imperato, A.N. and Angelucci, L. (1989) 5-HT_3 receptors control dopamine release in the nucleus accumbens of freely moving rats. *Neurosci. Lett.* 101:214-217.

Jacobs, B.L. and Azmitia, E.C. (1992) Structure and function of the serotonin system. *Physiol. Rev.* 72:165-229.

Jacobs, B.L., Simon, S., Ruimy, D.D. and Trulson, M.E. (1977) A quantitative rotational model for studying serotonergic function in the rat. *Brain Res.* 124:271-281.

Jones, K.L. and Smith, D.W. (1973) Recognition of the fetal alcohol syndrome in early infancy. *Lancet* 2:999-1001.

Jones, K.L., Smith, D.W., Ulleland, C.N. and Strissguth, A.P. (1973) Pattern of malformations in offspring of chronic alcoholic mothers. *Lancet* 1:1267-1271.

Jones, P.J.H., Leichter, J. and Lee, M. (1981) Placental blood flow in rats fed alcohol before and during gestation. *Life Sci.* 29:1153-1159.

Jorgensen, M.B. and Diemer, N.H. (1982) Selective neuron loss after cerebral ischemia in the rat: possible role of transmitter glutamate. *Acta Neurol. Scand.* 66:536-546.

Kadle, R., Suksang, C., Roberson, E.D. and Fellows, R.E. (1988) Identification of insulin-like factor in astrocyte conditioned medium. *Brain Res.* 460:60-67.

Kai, Y., Oomura, Y. and Shimizu, N. (1988) Responses of rat lateral hypothalamic neuron activity to dorsal raphe nuclei stimulation. *J. Neurophysiol.* 60:524-535.

Kakihana, R., Butte, J.C. and Moore, J.A. (1980) Endocrine effects of maternal alcoholization: plasma and brain testosterone, dihydrotestosterone, estradiol and corticosterone. *Alcohol. Clin. Exp. Res.* 4:57-61.

Katz, D.M. and Kimelberg, H.K. (1985) Kinetics and autoradiography of high affinity uptake of serotonin by primary astrocyte cultures. *J. Neurosci.* 5:1901-1908.

Kaufman, S. (1974) Properties of pterin-dependent aromatic amino acid hydroxylases. In: *Aromatic Amino Acids in the Brain* (eds. G.E.W. Wolstenholme and D.W. Fitzsimons), pp. 85-108, North-Holland Elsevier, Amsterdam.

Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1987) Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. *Nature* 330:746-748.

Kirksey, D.F. and Slotkin, T.A. (1979) Concomitant development of [^{3}H]-dopamine and [^{3}H]-5-hydroxytryptamine uptake systems in rat brain regions. *Br. J. Pharmacol.* 67:387-391.

Köhler, C., Chan-Palay, V. and Steinbusch, H. (1982) The distribution and origin of serotonin-containing fibers in the septal area: a combined immunohistochemical and fluorescent retrograde tracing study in the rat. *J. Comp. Neurol.* 209:91-111.

Köhler, C., Radesater, A.C., Lang, W. and Chan-Palay, V. (1986) Distribution of serotonin-1A receptors in the monkey and post-mortem human hippocampal region: a quantitative autoradiographic study using the selective agonist [^{3}H]8-OH-DPAT. *Neurosci. Lett.* 72:43-48.

Kojima, H., Mineta-Kitajima, R., Saitoh-Harada, N. Kurihara, T., Takahashi, Y., Furudate, S., Shiratake, M., Nakamura, K. and Tamai, Y. (1994) Prenatal ethanol exposure affects the activity and mRNA expression of neuronal membrane enzymes in rat offspring. *Life Sci.* 55:1433-1442.

Kolasa, K., Fusi, R., Garattini, S., Consolo, S. and Ladinsky, H. (1982) Neurochemical effects of buspirone, a novel psychotropic drug, on the central cholinergic system. *J. Pharm. Pharmacol.* 34:314-317.

Kornguth, S.E., Rutledge, J.J., Sunderland, E., Siegel, F., Carlson, I. Smollens, J., Juhl, U. and Young, B. (1979) Impeded cerebellar development and reduced serum thyroxine levels associated with fetal alcohol intoxication. *Brain Res.* 177:347-360.

Kosofsky, B.E. and Molliver, M.E. (1987) The serotonergic innervation of cerebral cortex: different classes of axon terminals arise from dorsal and median raphe nuclei. *Synapse* 1:153-168.

Krsiak, M., Elis, N., Poschlova, N. and Masek, K. (1977) Increased aggressiveness and lower brain serotonin levels in offspring of mice given alcohol during gestation. *J. Stud. Alcohol* 38:1696-1704.

Kuhar, M.J. and Aghajanian, G.K. (1973) Selective accumulation of ^{3}H-serotonin by nerve terminals of raphe neurons: an autoradiography study. *Nature* 241:187-189.

Kuriyama, K., Rauscher, G.E. and Sze P.Y. (1971) Effect of acute and chronic administration of ethanol on the 5-hydroxytryptamine turnover and tryptophan hydroxylase activity of the mouse brain. *Brain Res.* 26: 450-454.

Kyllerman, M., Aronson, M., Sabel, K.-G., Sandin, B. and Olegard, R. (1985) Children of alcoholic mothers: Growth and motor performance compared to matched control. *Acta. Paediatr. Scand.* 74:20-26.

Lanfumey, L. and Jacobs, B. (1982) Developmental analysis of raphe dorsalis unit activity in the rat. *Brain Res.* 242:317-320.

Lancaster, F.E., Mayur, P.N., Tsalos, T. Samorajski, T. and Wiggins, R.C. (1982) The synthesis of myelin and brain subcellular membrane proteins in the offspring of rats fed ethanol during pregnancy. *Brain Res.* 235:105-113.

Lankford, K., DeMello, F.G. and Klein, W.L. (1987) A transient embryonic dopamine receptor inhibits growth cone motility and neurite outgrowth in a subset of avian retina neurons. *Neurosci. Lett.* 75:169-174.

Lauder, J.M. and Bloom, F.E. (1974) Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. I. Cell differentiation. *J. Comp. Neurol.* 155:469-481.

Lauder, J.M. and Krebs, H. (1978) Serotonin as a differentiation signal in early neurogenesis. *Dev. Neurosci.* 1:15-30.

Lauder, J.M., Wallace, J.A., Krebs, H., Petrusz, P. and McCarthy, K. (1982) In vivo and in vitro development of serotonergic neurons. *Brain Res. Bull.* 9:605-625.

Lauder, J.M. (1990) Ontogeny of the serotonergic system in the rat: serotonin as a developmental signal. *Ann. N.Y. Academic Sci.* 600:297-313.

Lauder, J.M. (1993) Neurotransmitters as growth regulatory signals: role of receptors and second messengers. *Trends Neurosci.* 16:233-240.

Lee, E.H.Y., Lin, W.R., Chen, H.Y., Shiu, W.H. and Liang, K.C. (1992) Fluoxetine and 8-OH-DPAT in the lateral septum enhances and impairs retention of an inhibitory avoidance response in rats. *Physiol. Behav.* 51:681-688.

Le Prince, G., Copin, M.-C., Hardin, H., Belin, M.-F., Bouilloux, J.-P. and Tardy, M. (1990) Neuron-glia interactions: effect of serotonin on the astroglial expression of GFAP and its encoding message. *Dev. Brain Res.* 51:295-298.

Leslie, M.J., Bennett-Clarke, C.A. and Rhoades R.W. (1992) Serotonin 1B receptors form a transient vibrissa-related pattern in the primary somatosensory cortex of the developing rat. *Dev. Brain Res.* 69:143-148.

Levitt, P. and Moore, R.Y. (1978) Developmental organization of raphe serotonin neuron groups in the rat. *Anat. Embryol.* 154:241-251.

Lidov, H.W. and Molliver, M.E. (1982) Immunohistochemical study of the development of serotonergic neurons in the rat CNS. *Brain Res. Bull.* 9:559-604.

Lidov, H.W. and Molliver, M.E. (1982a) Immunohistochemical study of serotonin neuron development in the rat: ascending pathways and terminal fields. *Brain Res. Bull.* 8:389-430.

Lieth, E., Towle, A.C. and Lauder, J.M. (1989) Neuronal-glial interactions: Quantitation of astrocytic influences on development of catecholamine neurons. *Neurochem. Res.* 14:979-985.

Liu, J. and Lauder, J.M. (1991) Serotonin and nialamide differentially regulate survival and growth of cultured serotonin and catecholamine neurons. *Dev. Brain Res.* 60:59-67.

Liu, J. and Lauder, J.M. (1992) Serotonin promotes region-specific glial influences on cultured serotonin and dopamine neurons. *Glia* 5:306-317.

Liu, K.P., Tamir, H., Hsiung, S., Adlersberg, M. and Gershon, M.D. (1987) Prenatal development of serotonin binding protein in relation to other transmitter-related characteristics of central serotonergic neurons. *Dev. Brain Res.* 32:31-41.

Lochry, E.A. and Riley, E.P. (1980) Retention of passive avoidance and T-maze escape

in rats exposed to alcohol prenatally. *Neurobehav. Toxicol.* 2:107-115.

Lokhorst, D.K. and Druse, M.J. (1993a) Effects of ethanol on cultured fetal serotonergic neurons. *Alcohol. Clin. Exp. Res.* 17:86-93.

Lokhorst, D.K. and Druse, M.J. (1993b) Effects of ethanol on cultured fetal astroglia. *Alcohol. Clin. Exp. Res.* 17:810-815.

Lowry, O.H., Rosebrough, N.H., Farr, A.L. and Randall, R.H. (1951) Protein measurement with folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Maeda, T., Kaneko, S. and Satoh, M. (1994) Inhibitory influence via 5-HT3 receptors on the induction of LTP in mossy fiber-CA3 system of guinea-pig hippocampal slices. *Neurosci. Res.* 18:277-282.

Manthorpe, M., Rudge, J.S., Varon, S. (1986) Astroglial cell contributions to neuronal survival and neuritic growth. In: *Astrocytes* (eds. S. Fedoroff and A. Vernadakis) pp. 315-375. Academic Press, New York

Maricq, A.V., Peterson, A.S., Brake, A.J., Myers, R.M. and Julius, D. (1991) Primary structure and functional expression of the 5-HT_3 receptor, a serotonin-gated ion channel. *Science* 254:432-437.

Marlier, L., Teilhac, J.-R., Cerruti, C. and Privat, A. (1991) Autoradiographic mapping of 5-HT_1, 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptors in the rat spinal cord. *Brain Res.* 550:15-23.

Marshall, J.F., Turner, B.H. and Teitelbaum, P. (1971) Sensory neglect produced by lateral hypothalamic damage. *Science* 174:523-525.

Matheson, G.K. and Tunnicliff, G. (1991) Localization of [^{3}H]buspirone binding in rat brain by autoradiography. In: *Buspirone: Mechanisms and Clinical Aspects* (eds. G. Tunnicliff, A.S. Eison and D.P. Taylor), pp. 163-176. Academic Press, San Diego.

Maura, G. and Raiteri, M. (1986) Cholinergic terminals in rat hippocampus possess 5-HT_{1B} receptors mediating inhibition of acetylcholine release. *Eur. J. Pharmacol.* 129:333-337.

Mayordomo, F., Renau-Piqueras, J., Megias, L., Guerri, C., Iborra, F.J., Azorin, I. and Ledig, M. (1992) Cytochemical and stereological analysis of rat cortical astrocytes during development in primary culture. Effect of prenatal exposure to ethanol. *Int. J. Dev. Biol.* 36:311-321.

McCobb, D.P., Haydon, P.G. and Kater, S.B. (1988) Dopamine and serotonin inhibition of neurite elongation of different identified neurons. *J. Neurosci. Res.* 19:19-26.

McGivern, R.F., Clancy, A.N., Hill, M.A. and Nobel, E.P. (1984) Prenatal alcohol exposure alters adult expression of sexually dimorphic behavior in the rat. *Science* 224:896-898.

McGivern, R.F., Raum, W.J., Salido, E. and Redei, E. (1988) Lack of prenatal testosterone surge in fetal rats exposed to alcohol: Alteration in testicular morphology and physiology. *Alcohol. Clin. Exp. Res.* 12:243-247.

McGivern, R.F., Handa, R.J. and Redei, E. (1993) Decreased postnatal testosterone surge in male rats exposed to ethanol during the last week of gestation. *Alcohol. Clin. Exp. Res.* 17:1215-1222.

McMillen, B.A., Matthews, R.T., Sanghera, M.K., Shepard, P.D. and German, D.C. (1983) Dopamine receptor antagonism by the novel antianxiety drug, buspirone. *J. Neurosci.* 3:733-738.

Meller, E., Goldstein, M. and Bohmaker, K. (1990) Receptor reserve for 5-hydroxytryptamine$_{1A}$-mediated inhibition of serotonin synthesis: Possible relationship to anxiolytic properties of 5-hydroxytryptamine$_{1A}$ agonists. *Mol. Pharmacol.* 37:231-237.

Meltzer, H.Y., Gudelsky, G.A., Lowy, M.T., Nash, J.F. and Koenig, J.I. (1991) Neuroendocrine effects of buspirone: mediation by dopaminergic and serotonergic mechanisms. In: *Buspirone: Mechanisms and Clinical Aspects* (eds. G. Tunnicliff, A.S. Eison and D.P. Taylor), pp. 163-176. Academic Press, San Diego.

Metzer, H.Y., Simonovic, M., Fang, V.S. Gudelsky, G.A. (1982) Effect of buspirone on rat plasma prolactin levels and striatal dopamine turnover. *Psychopharmacology* 78:49-53.

Michaelis, E. (1990) Fetal alcohol exposure: cellular toxicity and molecular events involved in toxicity. *Alcohol. Clin. Exp. Res.* 14:819-826.

Middlemiss, D.N., Bremer, M.E. and Smith, S.M. (1988) A pharmacological analysis of the 5-HT receptor mediating inhibition of 5-HT release in the guniea pig frontal cortex. *Eur. J. Pharmacol.* 157:101-107.

Miles, M.F., Diaz J.E. and DeGuzman, V.S. (1991) Mechanisms of neuronal adaptation to ethanol. Ethanol induces Hsc70 gene transcription in NG108-15 neuroblstoma x glioma cells. *J. Biol. Chem.* 266:2409-2414.

Miller, M.W. (1987) Effect of prenatal exposure to alcohol on the distribution and time of origin of corticospinal neurons in the rat. *J. Comp. Neurol.* 257:372-382.

Miller, M.W. (1988) Effect of prenatal exposure to ethanol on the development of cerebral cortex: I. Neuronal generation. *Alcohol. Clin. Exp. Res.* 12:440-449.

Miller, M.W. (1989) Effects of prenatal exposure to ethanol on neocortical development: II. Cell proliferation in the ventricular and subventricular zones of the rat. *J. Comp. Neurol.* 287:326-338.

Miller, M.W. and Potempa, G. (1990) Numbers of neurons and glia in mature rat somatosensory cortex: Effects of prenatal exposure to ethanol. *J. Comp. Neurol.* 293:92-102.

Miller, M.W. and Robertson, S. (1993) Prenatal exposure to ethanol alters the postnatal development and transformation of radial glia to astrocytes in the cortex. *J. Comp. Neurol.* 337:253-266.

Millhouse, O.E. (1969) A Golgi study of the descending medial forebrain bundle. *Brain Res.* 15:341-363.

Milner, B. (1972) Disorders of learning and memory after temporal lobe lesions in man. *Clin. Neurosurg.* 19:421-446.

Monnet-Tschudi, F. and Honneger, P. (1989)Influence of epidermal growth factor on the maturation of fetal rat brain cells in aggregate culture. *Dev. Neurosci.* 11:30-40.

Montefrancesco, G., Giorgi, G., Dal Pra, P., Fiaschi A.I., Micheli, L. and Segre, G. (1990) Plasma ACTH levels in buspirone-treated rats. *Arch. Int. Pharmacodyn. Ther.* 305:183-188.

Moore, R.Y., Halaris, A.E. and Jones B.E. (1978) Serotonin neurons of the midbrain raphe: ascending projections. *J. Comp. Neurol.* 180:417-438.

Morilak, D.A. and Ciaranello, R.D. (1993) Ontogeny of 5-hydroxytryptamine$_2$ receptor immunoreactivity in the developing rat brain. *Neuroscience* 55:869-880.

Morris, P.L., Mayberg, H.S., Bolla, K., Wong, D.F., Dannals, R.F., Starkstein, S.E. and Robinson, R. G. (1993) A preliminary study of cortical S2 serotonin receptors and cognitive performance following stroke. *J. Neuropsychiatry & Clin. Neurosci.* 5:395-400.

Morrison, J.H., Foote, S.L., Molliver, M.E., Bloom, F.E. and Lidov, G.W. (1982) Noradrenergic and serotonergic fibers innervate complementary layers in monkey

visual cortex: an immunohistochemical study. *Proc. Natl. Acad. Sci. USA* 79:2401-2405.

Morrison, R.S., Sharma, A., de Vellis, J. and Bradshaw, R.A. (1986) Basic fibroblast growth factor supports the survival of cerebral cortical neurons in primary culture. *Proc. Natl. Acad. Sci. USA* 83:7537-7541.

Muller, H.W. and Seifert, W. (1982) A neurotrophic factor (NTF) released from primary glial cultures supports survival and fiber outgrowth of cultured hippocampal neurons. *J. Neurosci. Res.* 8:195-204.

Muramatsu, M., Tamaki-Ohashi, J., Usuki, C., Araki, H., Chaki, S. and Aihara, H. (1988) Serotonin-2 receptor-mediated regulation of release of acetylcholine by minaprine in cholinergic nerve terminal of hippocampus of rat. *Neuropharmacology* 27:603-609.

Muramatsu, M., Tamaki-Ohashi, J., Usuki, C., Araki, H., Chaki, S. and Aihara, H. (1988a) 5-HT2 antagonists and minaprine block the 5-HT-induced inhibition of dopamine release from rat brain striatal slices. *Eur. J. Pharmacol.* 153:89-95.

Nauta, W.J.H. and Haymaker, W. (1969) Hypothalamic nuclei and fiber connections. In: *The Hypothalamus* (eds. W. Haymaker, E. Anderson and W.J.H. Nauta), pp. 1366-209.

Nedergaard, S., Bolam, J.P. and Greenfield, S.A. (1988) Facilitation of a dendritic calcium conductance by 5-hydroxytryptamine in the substantia nigra. *Nature* 333:174-177.

Nedergaard, S., Flatman, J.A. and Engberg, I. (1991) Excitation of substantia nigra pars compacta neurons by 5-hydroxytryptamine *in vitro*. *Neuroreport* 2:239-332.

Negulescu, P.A., Shastri, N. and Cahalan, M.D. (1994) Intracellular calcium dependence of gene expression in single T lymphocytes. *Proc. Natl. Acad. Sci. USA* 91:2873-2877.

Neijt, H.C., Te Duis, I.J. and Vijverberg, H.P.M. (1988) Pharmacological characterisation of serotonin$_3$ receptor-mediated electrical response in cultured mouse neuroblastoma cells. *Neuropharmacology* 27:301-307.

Nelson, D.L., Herbet, A., Glowinski, J. and Hamon, M. (1979) [^{3}H]Harmaline as a specific ligand of MAO A-II. Measurement of the turnover rates of MAO A during ontogenesis in the rat brain. *J. Neurochem.* 32:1829-1836.

Nicotera, P., Zhivotovsky, B. and Orrenius, S. (1994) Nuclear calcium transport and the

role of calcium in apoptosis. *Cell Calcium* 16:279-288.

Nio, E., Kogure, K., Yae, T. and Onodera, H. (1991) The effects of maternal ethanol exposure on neurotransmission and second messenger systems: a quantitative autoradiographic study in the rat brain. *Dev. Brain Res.* 62:51-60.

Nishizuka, Y. (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* 258:607-614.

Nomura, Y., Naitoh, F. and Segawa, T. (1976) Regional changes in monoamine content and uptake of the rat brain during postnatal development. *Brain Res.* 101:305-315.

Noronha, A.B. and Druse, M.J. (1982) Maternal ethanol consumption and synaptic membrane glycoproteins in offspring. *J. Neurosci. Res.* 8:83-97.

O'Hearn, E. and Molliver, M.E. (1984) Organization of raphe cortical projections in rat: a quantitative retrograde study. *Brain Res. Bull.* 13:709-726.

Olegard, R., Sabel, K.G., Aronsson, M., Sandin, B., Johansson, P.R., Carlsson, C., Kyllerman, M., Iversen, K. and Hrbek, A. (1979) Effects on the child of alcohol abuse during pregnancy. *Acta. Paediatr. Scand. [Suppl]* 275:112-121.

Olpe, H.R. and Koella, W.P. (1977) The responses of striatal cells upon stimulation of the dorsal and median raphe nuclei. *Brain Res.* 122:357-360.

Ols, M.E. (1973) Short-term changes in the firing pattern of hypothalamic neurons during Pavlovian conditioning. *Brain Res.* 58:95-116.

Palacios, J.M., Niehoff, D.L. and Kuhar, M.J. (1981) Receptor autoradiography with tritum-sensitive film: potential for computerized densitometry. *Neurosci. Lett.* 25:101-105.

Palkovits, M., Brownstein, M. and Saavedra, J. M. (1974) Serotonin content of the brainstem nuclei in the rat. *Brain Res.* 80:237-249.

Park, D.H., Snyder, D.W. and Joh, T.H. (1986) Postnatal developmental changes of tryptophan hydroxylase activity in serotonergic cell bodies and terminals of rat brain. *Brain Res.* 378:183-185.

Parker, S., Udani, M., Gavaler, J.S. and Van Thiel, D.H. (1984) Adverse effects of ethanol upon the adult sexual behavior of male rats exposed to in utero. *Neurobehav. Toxicol. Teratol.* 6:289-293.

Pasini, A., Tortorella, A and Gale, K. (1992) Anticonvulsant effect of intranigral

fluoxetine. *Brain Res.* 593:287-290.

Paxinos, G. and Watson, C. (1986) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, Australia.

Pazos, A. and Palacios, J.M. (1985)Quantitative autoradiographic mapping of serotonergic receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.* 346:205-230.

Pazos, A., Cortes, R. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res.* 346:231-249.

Pazos, A., Probst, A. and Palacios, J.M. (1987) Serotonin receptors in the human brain. III. Autoradiographic mapping of serotonin-1 receptors. *Neuroscience* 21:97-121.

Pazos, A., Hoyer, D. and Palacios, J.M. (1985a) The binding of serotonergic ligands to the porcine choroid plexus: charaterization of a new type of serotonin recognition site. *Eur. J. Pharmacol.* 106:539-546.

Pedigo, N.W., Yamamura, H.I. and Nelson, D.L. (1981) Discrimination of multiple [^{3}H]5-HT binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.* 36:220-226.

Peiffer, J., Majaewski, F., Fishback, H., Bierich, J.R. and Volk, B. (1979) Alcohol embryo and fetopathy. *J. Neurol. Sci.* 41:125-137.

Pennington, S. (1988) Ethanol-induced growth inhibition: the role of cyclic AMP protein kinase. *Alcohol. Clin. Exp. Res.* 12:125-129.

Peroutka, S.J. (1985) Selective interaction of novel anxiolytic with 5-hydroxytryptamine$_{1A}$ receptors. *Biol. Psychiatry* 20:971-979.

Phillips, S.C. and Cragg, B.G. (1982) A change in susceptability of rat crebellar Purkinje cells to damage by alcohol during fetal, neonatal and adult life. *Neuropathol. Appl. Neurobiol.* 8:441-454.

Pikkarainen, P.H. (1971) Metabolism of ethanol and acetaldehyde in perfused human fetal liver. *Life Sci.* 10:1359-1364.

Pranzatelli, M.R. and Martens, J.M. (1992) Plasticity and ontogeny of the central 5-HT transporter: effect of neonatal 5,7-dihydroxytryptamine lesions in the rat. Dev. *Brain Res.* 70:191-195.

Pycock, C.J. (1980) Turning behavior in animals. *Neuroscience* 5:461-514.

Quirion, R., Araujo, D., Nair, N.P.V. and Chabot, J.G. (1988) Visualization of growth factor sites in rat forebrain. *Synapse* 2:212-218.

Raisman, G (1966) The connections of the septum. *Brain Res.* 89:317-349.

Raisman, G (1970) Some aspects of the neural connections of the hypothalamus. In: *The Hypothalamus* (eds. L. Martini, M. Motta, and F. Fraschini). Academic Press, New York.

Rajaofetra, N., Sandillon, F., Geffard, M. and Privat, A. (1989) Pre- and post-natal ontogeny of serotonergic projections to the rat spinal cord. *J. Neurosci. Res.* 22:305-321.

Rakic, P. (1972) Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. Comp. Neurol.* 145:61-83.

Randall, C.L., Taylor, W.J. and Walker, D.W. (1977) Ethanol-induced malformations in mice. *Alcohol. Clin. Exp. Res.* 1:219-224.

Rathbun, W. and Druse, M.J. (1985) Dopamine, serotonin, and acid metabolites in brain regions from the developing offspring of ethanol-treated rats. *J. Neurochem.* 44:57-62.

Rawat, A.K. (1974) Brain levels and turnover rates of presumptive neurotransmitters as withdrawl of ethanol in mice. *J. Neurochem.* 22:915-922.

Rawat, A.K. (1975) Ribosomal protein synthesis in the fetal and neonatal rat brain as influenced by maternal ethanol consumption. *Res. Commun. Chem. Pathol. Pharmacol.* 12:723-732.

Redei, E. and McGivern, R. (1988) Attenuation of postnatal testosterone surge and decreased response to LH in fetal alcohol exposed males. *Alcohol. Clin. Exp. Res.* 12:341.

Reisine, T., Soubrie, P., Artaud, F. and Glowinski, J. (1982) Application of glutamic acid and substance P to the substantia nigra modulates in vivo ^{3}H-serotonin release in the basal ganglia of the cat. *Brain Res.* 2366:317-327.

Renau-Piqeras, J., Zaragoza, R., De Paz, P., Baguena-Cervellera, R., Megias, L. and Guerri, C. (1989) Effects of prolonged ethanol exposure on the glial fibrillary acidic protein-containing intermediate filaments of astrocytes in primary culture: a quantitative immunofluorescence and immunogold electron microscopic study.

J. Histochem. Cytochem. 37:229-240.

Renson, J. (1973) Assays and properties of tryptophan 5-hydroxylase. In: *Serotonin and Behavior* (Eds. J. Barchas and E. Usdin), pp. 19-32, Academic Press, New York.

Ribak, C.E., Vaughn, J.E. and Roberts E. (1980) GABAergic nerve terminals decrease in the substantia nigra following hemitransections of the striatonigral and pallidonigral pathways. *Brain Res.* 192:413-420.

Ribary, U., Schlumpf, M. and Lichtensteiger, W. (1986) Analysis by HPLC-EC of metabolites of monoamines in fetal and postnatal rat brain. *Neuropharmacology* 25:981-986.

Riblet, L.A., Taylor, D.P., Eison, M.S. and Stanton, H.C. (1982) Pharmacology and neurochemistry of buspirone. *J. Clin. Psychiatry* 43(12, sect. 2):11-16.

Riblet, L.A., Eison, A.S., Eison, M.S., Taylor, D.P., Temple, D.L. and VanderMaelen, C.P. (1984) Neuropharmacology of buspirone. *Psychopathology* 17 (suppl. 3):69-78.

Richter-Levin, G., Greenberger, V. and Segal, M. (1994) The effects of general and restricted serotonergic lesions on hippocampal electrophysiology and behavior. *Brain Res.* 642:111-116.

Richter-Levin, G., Greenberger, V. and Segal, M. (1993) Regional specificity of raphe graft-induced recovery of behavioral functions impaired by combined serotonergic/cholinergic lesions. *Exp. Neurol.* 121:256-260.

Ritchie, T., Kim, H.-S., Cole, R., deVellis, J. and Noble, E.P. (1988) Alcohol-induced alterations in phosphoinositide hydrolysis in astrocytes. *Alcohol* 5:183-187.

Roivainen, R., Hundle, B. and Messing, R.O. (1994) Protein kinase C and adaptation to ethanol. *EXS* 71:29-38.

Rolls, E. (1975) *The Brain and Reward.* Pergamon Press, New York.

Rosen, L.B., Ginty, D.D. and Greenberg, M.E. (1995) Calcium regulation of gene expression. In: *Advances in Second Messenger and Phosphoprotein Reserch* Vol. 30 (ed. A.R. Means), pp. 225-253. Raven Press, New York.

Roth, B.L., Hamblin, M.W. and Ciaranello, R.D. (1991) Developmental regulation of 5-HT_2 and 5-HT_{1C} mRNA and receptor levels. *Dev. Brain Res.* 58:51-58.

Rudge, J.S., Manthorpe M. and Varon, S. (1985) The output of neuronotrophic and

neurite-promoting agents from rat brain astroglial cells: a microculture method for screening potential regulatory molecules. *Dev. Brain Res.* 19:161-172.

Sandor, G.G., Smith, D.F., MacLeod, P.M. (1981) Cardiac malformations in the fetal alcohol syndrome. *J. Pediatr.* 81:771-773.

Savoy-Moore, R.T., Dombrowski, M.P., Cheng, A., Abel, E.A. and Sokol, R.J. (1989) Low dose alcohol contracts the human umbilical artery in vitro. *Alcohol. Clin. Exp. Res.* 13:40-42.

Schanne, F., Kane, A.B., Young, E.E. and Farber, J.L. (1979) Calcium dependence of toxic cell death. *Science* 206:700-702.

Schapiro, M.B., Rosman, N.P. and Kemper, T.L. (1984) Effects of chronic exposure to alcohol on the developing brain. *Neurobehav. Toxicol. Teratol.* 6:351-356.

Schenker, S., Dicke, J.M., Johnson, F.R., Hays, S.E. and Henderson, G.I. (1989) Effect of ethanol on human placental transport of model amino acid and glucose. *Alcohol. Clin. Exp. Res.* 13:112-119.

Schenker, S., Becker, H.C., Randall, C.L., Phillips, D.K., Baskin, G.S. and Henderson, G.I. (1990) Fetal alcohol syndrome: current status of pathogenesis. *Alcohol. Clin. Exp. Res.* 14:635-647.

Schmechel, D.E. and Rakic, P. (1979) A Golgi study of radial glial cells in developing monkey telencephalon: Morphogenesis and transformation into astrocytes. *Anat. Embryol.* 156:115-152.

Schmidt, M.J. and Sanders-Bush, E. (1971) Tryptophan hydroxylase activity in developing rat brain. *J. Neurochem.* 18:2549-2551.

Segal, M. (1975) Physiological and pharmacological evidence for a serotonergic projection to the hippocampus. *Brain Res.* 94:115-131.

Segal, M. (1976) 5-HT antagonists in rat hippocampus. *Brain Res.* 103:161-166.

Sharma, A. and Rawat, A.K. (1989) Toxicological consequences of chloroquine and ethanol on the developing fetus. *Pharmacol. Biochem. Behav.* 34:77-82.

Shaywitz, B.A., Griffieth, G.G. and Warshaw, J.B. (1979) Hyperactivity and cognitive deficits in developing rat pups born to alcoholic mothers: An experimental model of the expanded fetal alcohol syndrome (EFAS). *Neurobehav. Toxicol.* 1:113-122.

Shemer, A.V., Azmitia, E.C. and Whitaker-Azmitia, P.M. (1991) Dose-related effects

of prenatal 5-methoxytryptamine (5-MT) on development of serotonin terminal density and behavior. *Dev. Brain Res.* 59:59-63.

Sikich, L., Hickok, J.M. and Todd, R.D. (1990) 5-HT_{1A} receptors control neurite branching during development. *Dev. Brain Res.* 56:269-274.

Singh, S.P., Snyder, A.K. and Eman, S. (1990) Effects of ethanol on hexose uptake by cultured rat brain cells. *Alcohol. Clin. Exp. Res.* 14:741-745.

Singh, S.P., Srivenugopal, K.S., Ehmann, S., Yuan, X.-H. and Snyder, A.K. (1994) Insulin-like growth factors (IGF-I and IGF-II), IGF-binding proteins, and IGF gene expression in the offspring of ethanol-fed rats. *J. Lab. Clin. Med.* 124:183-192.

Siniscalchi, A., Beani, L. and Bianchi, C. (1990) Different effects of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, on cortical acetylcholine release, electrocortigram and body temperature in guinea pigs and rats. *Eur. J. Pharmacol.* 175:219-223.

Sipe, J.C. and Moore, R.Y. (1977) The lateral hypothalamic area: an ultrasturucturel analysis. *Cell Tissue Res.* 179:177-196.

Slotkin, T.A., Schanberg, S.M. and Kuhn, C.M. (1980) Synaptic development in brains of rats exposed perinatally to ethanol. *Experientia* 36:1005-1007.

Smith, D.M. and Druse, M.J. (1982) Effects of maternal protein deficiency on synaptic plasma membranes in offspring. *Dev. Neurosci.* 5:403-411.

Smith, L.M. and Peroutka, S.J. (1986) Differential effects of 5-$hydroxytryptamine_{1A}$ selective drugs on the 5-HT behavioral syndrome. Pharmacol. Biochem. Behav. 24:1513-1519.

Snyder, A.K., Singh, S.P. and Pullen, G.L. (1986) Ethanol-induced intrauterine growth retardation correlation with placental glucose transfer. *Alcohol. Clin. Exp. Res.* 10:167-170.

Snyder, A.K., Singh, S.P. and Ehmann, S. (1992) Effects of ethanol on DNA, RNA, and protein synthesis in rat astrocyte cultures. *Alcohol. Clin. Exp. Res.* 16:295-300.

Sokol, R.J. and Clarren, S.K. (1989) Guidelines for use of terminology describing the impact of prenatal alcohol on the offspring. *Alcohol. Clin. Exp. Res.* 13:597-598.

Sonderegger, T.B., Calmes, H., Corbitt, S. and Zimmermann, E.G. (1982) Lack of persistent effects of low dose ethanol administered postnatally in rats.

Neurobehav. Toxicol. Teratol. 4:463-468.

Soubrie, P., Montastruc, J.L., Bourgoin, S., Reisine, T., Artaud, F. and Glowinski, J. (1981) In vivo wvidence for GABAergic control of serotonin release in the cat substantia nigra. *Eur. J. Pharmacol.* 69:483-488.

Sprouse, J.S. and Aghajanian, G.K. (1987) Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonists. *Synapse* 1:3-9.

Staiger, J.F. and Nurnberger, F. (1991) The efferent connections of the lateral septal nucleus in the guinea pig: projections to the diencephalon and brainstem. *Cell Tissue Res.* 264:391-413.

Stanley, M. Virgilio, J. and Gershon, S. (1982) Tririated imipramine binding sites are decreased in the frontal cortex of suicides. *Science* 216:1337-1339.

Stanley, M. and Mann, J.J. (1983) Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet* 1:214-216.

Steinbusch, H.W.M., Van der Kooy, D., Verhofstad, A.A.J. and Pellegrine, A. (1980) Serotonergic and non-serotonergic projections from the nucleus raphe dorsalis to caudate-putamen complex in rat, studied by a combined immunofluorescence and fluorescent retrograde axonal ladeling technique. *Neurosci. Lett.* 19:137-142.

Steinbusch, H.W.M. (1981a) Distribution of serotonin-immuno-reactivity in the central nervous system in the rat cell bodies and terminals. *Neuroscience* 6:557-618.

Steinbusch, H.W.M., Nieuwenhuys, R., Verhofstad, A. A. J. and Van der Kooy, D. (1981b) The nucleus raphe dorsalis of the rat and its projection upon the caudatoputamen. A combined cytoarchitectonic, immunohistochemical and retrograde transport study. *J. Physiol. (Paris)* 77:157-174.

Steinhausen, H.-C., Willms, J. and Spohr, H.-L. (1993) Long-term psychopathological and cognitive outcome of children with fetal alcohol syndrome. *J. Am. Acad. Child Adolesc. Psychiatry* 32:990-994.

Stoltenburg-Didinger, G. and Spohr, H.L. (1983) Fetal alcohol syndrome and mental retardation: spine distribution of pyramidal cells in prenatal alcohol-exposed rat cerebral cortex; a Golgi study. *Dev. Brain Res.* 11:119-123

Streissguth, A.P., Herman, C.S. and Smith, D.W. (1978) Intelligence, behavior and dysmorphogenesis in the fetal alcohol syndrome: a report on 20 patients. *J. Pediatr.* 92:363-367.

Streissguth, A.P.,Landesman-Dwyer, S.L., Martin, J.C. and Smith, D.W. (1980) Teratonic effects of alcohol in humans and laboratory animals. *Science* 209:353-361.

Streissguth, A.P., Clarren, S.K. and Jones, K.L. (1985) Natural history of the fetal alcohol syndrome: a ten-year follow-up of eleven patients. *Lancet* 4:85-92.

Streissguth, A.P., Sampson, P.D., Barr, H.M., Clarren, S.K. and Martin, D.C. (1988) Studying alcohol teratogenesis from the perspective of the fetal alcohol syndrome: methodological and statistical issues. *Ann. N.Y. Acad. Sci.* 477:63-86.

Streissguth, A.P., Barr, H.M. and Sampson, P.D. (1990) Moderate prenatal alcohol exposure:Effects on child IQ and learning problems at age 7.5 years. *Alcohol. Clin. Exp. Res.* 14:662-669.

Streissguth, A.P., Aase, I.M. Clarren, S.K., Randels, S.P., Ladue, R.A. and Smith D. F. (1991) Fetal alcohol syndrome in adolescents and adults. *JAMA* 265:1961-1967.

Swanson, L.W. and Cowan, W.M. (1979) The connections of the septal region in the rat. *J. Comp. Neurol.* 186:621-656.

Swartzwelder, H.S., Farr, K.L., Wilson, W.A. and Savage, D.D. (1988) Prenatal exposure to ethanol decreases physiological plasticity in the hippocampus of the adult rat. *Alcohol* 5:121-124.

Sze, P.Y. (1980) Glucocorticoids as a regulatory factor for brain tryptophan hydroxylase during development. *Dev. Neurosci.* 3:217-223.

Tajuddin, N. and Druse, M.J. (1989) Chronic maternal ethanol consumption results in decreased serotonergic 5-HT_1 sites in cerebral cortical reigons from offspring. *Alcohol* 5:465-470.

Tajuddin, N. and Druse, M.J. (1989a) Effects of in utero ethanol exposure on cortical 5-HT_2 binding sites. *Alcohol* 5:461-464.

Tajuddin, N.F. and Druse, M.J. (1993) Treatment of pregnant alcohol-consuming rats with buspirone: effects on serotonin and 5-hydroxyindoleacetic acid content in offspring. *Alcohol. Clin. Exp.Res.* 17:110-114.

Tan, S.E., Berman, R.F., Abel, E.L. and Zajac, C.S. (1990) Prenatal alcohol exposure alters hippocampal slice electrophysiology. *Alcohol* 7:507-511.

Taylor, A.N., Branch, B.J. Liu, S.H. and Kokka, N. (1982) Long-term effects of fetal

ethanol exposure on pituitary-adrenal responses to stress. *Pharmacol. Biochem. Behav.* 16:585-589.

Taylor, A.N., Branch, B.J. Cooley-Matthews, B. and Poland, R.E. (1982a) Effects of maternal ethanol consumption on basal and rhythmic pituitary-adrenal function in neonatal offspring. *Psychoneuroendocrinology* 7:49-58.

Taylor, A.N., Branch, B.J., Nelson, L.R., Lane, L.A. and Poland R.E. (1986) Prenatal ethanol and ontogeny of pituitary-adrenal responses to ethanol and morphine. Alcohol 3:255-259.

Teiltelbaum, P. and Epstein, A.N. (1962) The lateral hypothalamic syndrome: recovery of feeding and drinking after lateral hypothalamic lesions. *Psychol. Rev.* 69:74-90.

Tissari, A.H. (1973) Serotoninergic mechanisms in ontogenesis. In: *Fetal Pharmacology* (ed. L. Boreus), pp. 237-253. Raven Press, New York.

Tong, J.H. and Kaufman, S. (1975) Tryptophan hydroxylase: purification and some properties of the enzyme from rabbit hindbrain. *J. Biol. Chem.* 250:4152-4158.

Tork, I. (1990) Anatomy of the serotonergic system. *Ann. N.Y. Acad. Sci.* 600:9-34.

Torres-Aleman, I., Naftolin, F. and Robbins R.J. (1990) *Neuroscience* 35:601-608.

Trent, F. and Tepper, J.M. (1991) Dorsal raphe stimulation modifies striatal-evoked antidromic invasion of nigral dopaminergic neurons in vivo. *Exp. Brain Res.* 84:6620-630.

Turner, B.H. (1973) A sensorimotor syndrome produced by lesions of the amygdala and lateral hypothalamus. *J. Comp. Psychol.* 82:37-47.

Tze, W.J. and Lee, M. (1975) Adverse effects of maternal alcohol consumption on pregnancy and fetal growth in rats. *Nature* 257:479-480.

Urban, J.H., Van de Kar, L.D., Lorens, S.A. and Bethea, C.L. (1986) Effect of the anxiolytic drug buspirone on prolactin and corticosterone secretion in stressed and unstressed rats. *Pharmacol. Biochem. Behav.* 25:457-462.

Van de Kar, L.D. (1991) Neuroendocrine pharmacology of serotonergic neurons. *Annu. Rev. Pharmacol. Toxicol.* 31:289-320.

Van der Kooy, D. and Hattori, T. (1980) Dorsal raphe cells with collateral projections to the caudate-putamen and substantia nigra: a fluorescent retrograde double

labeling study in the rat. *Brain Res.* 186:1-7.

VanderMaelen, C.P., Matheson, G.K., Wilderman, R.C. and Patterson, L.A. (1986) Inhibition of serotonergic dorsal raphe neurons by systemic and iontophoretic administration of buspirone, a non-benzodiazepine anxiolytic drug. *Eur. J. Pharmacol.* 129:123-130.

Verge, D., Davl, G., Patey, A., Gozlan, H., El Mestikawy, S. and Hamon, M. (1985) Presynaptic 5-HT autoreceptors on serotonergic cell bodies and /or dendrities but not terminals are of 5-HT_{1A} subtype. *Eur. J. Pharmacol.* 113:463-464.

Verge, D., Davl, G., Marcinkiewicz, M., Patey, A., El Mestikawy, S., Gozlan, H. and Hamon, M. (1986) Quantitative autoradiography of multiple 5-HT_1 receptor subtypes in the brain of control and 5,7-DHT treated rats. *J. Neurosci.* 6:3474-3482.

Voigt, M.M., Laurie, D.J., Seeburg, P.H. and Bach, A. (1991) Molecular cloning and characterization of a rat brain cDNA encoding a 5-hydroxytryptamine-1B receptor. *EMBO J.* 10:4017-4023.

Volk, B. (1977) Delayed histogenesis in the 'embryofetal alcohol syndrome'. *Acta. Neuropathol. (Berl)* 39:157-163.

Volk, B. (1984) Cerebellar histogenesis and synaptic maturation following pre- and postnatal alcohol administration: an electron-microscopic investigation of the rat cerebellar cortex. *Acta Neuropathol. (Berl)* 63:57-65.

Volk, B., Maletz, J., Tiedemann, M., Mall, G., Klein, C. and Berlet, H.H. (1981) Impaired maturation of Purkinje cells in the fetal alcohol syndrome of the rat: light and electron microscopic investigation. *Acta Neuropathol. (Berl)* 54:19-29.

Vorhees, C.V. and Butcher, R.E. (1982) Behavioural terarogenicity. In: *Developmental Toxicology* (ed. K. Snell), pp. 249-297. Croom-Helm, London.

Waeber, C., Dietl, M.M., Hoyer, D., Probst, A. and Palacios, J.M. (1989) 5-HT_1 receptors in the vertebrate brain. Regional distribution examined by autoradiography. *Naunyn Schmiedebergs Arch. Pharmacol.* 340:486-494.

Waeber, C., Hoyer, D. and Palacios, J.M. (1989a) 5-HT_3 receptors in the human brain, autoradiographic visualisation using [^{3}H]ICS 205-930. *Neuroscience* 31:393-400.

Walicke, P.A. (1989) Novel neurotrophic factors, receptors and oncogenesis. *Annu. Rev. Neurosci.* 12:103-126.

Walicke, P., Cowan, W.M., Ueno, N., Baird, A. and Guillemin, R. (1986) Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension. *Proc. Natl. Acad. Sci. USA* 83:3012-3016.

Walker, D.W., Heaton, M.B., Smothers, C.T. and Hunter, B.E. (1990) Chronic ethanol ingestion reduces the trophic activity contained in rat hippocampus. *Alcohol. Clin. Exp. Res.* 14:350

Wallace, J.A. and Lauder, J.M. (1983) Development of the serotonergic system in the rat embryo: an immunocytochemical study. *Brain Res. Bull.* 9:550-604.

Walsh, M., Truitt, E. and Davis, V.E. (1970) Acetaldehyde mediation in the mechanisms of ethanol-induced changes in norepinephrine metabolism. *Mol. Pharmacol.* 6:416-424.

Waltman, R. and Iniquez E.S. (1972) Placental transfer of ethanol and its elimination at term. *Obstet. Gynecol.* 40:180-185.

Wang, R.Y. and Aghajanian, G.K.(1977) Inhibition of neurons in the amygdala by dorsal raphe stimulation: Mediation theough a direct serotonergic pathway. *Brain Res.* 120:85-102.

Weathersbee, P.S. and Lodge, J.R. (1978) A review of ethanol's effects on the reproductive process. *J. Reprod. Med.* 21:63-78.

Weinberg, J. (1988) Hyperresponsiveness to stress: Differential effects of prenatal ethanol on males and females. *Alcohol. Clin. Exp. Res.* 12:647-652.

Weinberg, J. (1989) Prenatal ethanol exposure alters adrenocortical development of offspring. *Alcohol. Clin. Exp. Res.* 13:73-83.

Weinberg, J. and Bezio, S. (1987) Alcohol-induced changes in pituitary-adrenal activity during pregnancy. *Alcohol. Clin. Exp. Res.* 11:274-280.

West, J.R., Hodges, C.A. and Black, A.C., Jr. (1981) Prenatal exposure to ethanol alters the organization of hippocampal mossy fibers in rats. *Science* 211:957-959.

West, J.R., Hamre, K.M. and Pierce, D.R. (1984) Delay in brain development induced by alcohol in artificially reared rat pups. *Alcohol* 1:213-222.

Whitaker, M. (1995) Regulation of the cell division cycle by inositol triphosphate and the calcium signaling pathway. In: *Advances in Second Messenger and Phosphoprotein Reserch* Vol. 30 (ed. A.R. Means), pp. 299-310. Raven Press, New York.

Whitaker-Azmitia, P.M. and Azmitia, E.C. (1986) Autoregulation of fetal serotonergic neuronal development: role of high affinity serotonin receptors. *Neurosci. Lett.* 67:307-312.

Whitaker-Azmitia, P.M. and Azmitia, E.C. (1986a) [^{3}H]5-hydroxytryptamine binding to brain astroglial cells: differences between intact and homogenized preparations and mature and immature cultures. *J. Neurochem.* 46:1186-1189.

Whitaker-Azmitia, P.M., Lauder, J.M., Shemmer, A. and Azmitia, E.C. (1987) Postnatal changes in serotonin$_1$ receptors following prenatal alterations in serotonin levels: further evidence for functional fetal serotonin$_1$ receptors. *Dev. Brain Res.* 33:285-289.

Whitaker-Azmitia, P.M., Murphy, R. and Azmitia, E.C. (1990) Stimulation of astroglial 5-HT$_{1A}$ receptors releases the serotonergic growth factor, protein S-100, and alters astroglial morphology. *Brain Res.* 528:155-158.

Whitaker-Azmitia, P.M., Clarke, C. and Azmitia, E.C. (1993) Localization of 5-HT$_{1A}$ receptors to astroglial cells in adult rats: implications for neuronal-glial interactions and psychoactive drug mechanism of action. *Synapse* 14:201-205.

Wilke, N., Sganga, M., Barhite, S. and Miles, M.F. (1994) Effects of alcohol on gene expression in neural cells. *EXS* 71:49-59.

Williams, J. and Davies J.A. (1983) The involvement of 5-hydroxytryptamine in the release of dendritic dopamine from slices of rat substantia nigra. *J. Pharm. Pharmacol.* 35:734-737.

Yakel, J.L. and Jackson, M.B. (1988) 5-HT3 receptors mediate rapid responses in cultured hippocampus and a clonal cell line. *Neuron* 1:615-621.

Yocca, F.D., Hyslop, D.K., Taylor, D.P. and Maayani, S. (1986) Buspirone and gepirone: partial agonists at the 5HT$_{1A}$ receptor linked to adenylate cyclase (AC) in rat and guinea pig hippocampal preparations. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* 45:436.

Young, W.S. III., and Kuhar, M.J. (1979) A new method for receptor autoradiography: [^{3}H]-opoid receptors in rat brain. *Brain Res.* 179:255-270.

Young, S.H. and Poo, M.M. (1983) Spontaneous release of transmitter from growth cones of embryonic neurons. *Nature* 305:634-637.

Zeisel, S.H., Mauron, C., Watkins, C.J. and Wurtman, R.J. (1981) Developmental changes in brain indols, serum tryptophan and other serum neutral amino acids

in the rat. *Dev. Brain Res.* 1:551-564.

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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