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Neurodevelopmental Liabilities of Substance Abuse

TOMAS PALOMO[a], TREVOR ARCHER[b], RICHARD J. BENINGER[c] and RICHARD M. KOSTRZEWA[d,*]

[a]*Servicio de Psiquiatria, Hospital 12 de Octobre, Ctra. Andalucia Km. 5,400, 28041 Madrid, Spain;* [b]*Department of Psychology, University of Göteborg, Box 500, SE-40530 Göteborg, Sweden;* [c]*Departments of Psychology and Psychiatry, Queen's University, Kingston, Canada K7L 3N6;* [d]*Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, P.O. Box 70577, VA Building #1 on Dogwood Lane, Room 1-44, Johnson City, TN 37614-1708, USA*

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The perinate is particularly risk-prone to chemical species which have the potential of inducing neuronal apoptosis or necrosis and thereby adversely altering development of the brain, to produce life-long functional and behavioral deficits. This paper is an overview for many substances of abuse, but the purview is much more broadened by the realization that even elevated levels of estrogens and corticosteroids in the pregnant mother can act as neuroteratogens, by passing via the placenta and altering neural development or inducing apoptosis in the perinate. Finally, therapeutic risks of anesthetics are highlighted, as these too induce neuronal apoptosis in the neonate by either blocking N-methyl-D-aspartate receptors or by acting as gamma-aminobutyric acid agonists. By understanding the mechanisms involved it may ultimately be possible to interrupt the mechanistic scheme and thereby prevent neuroteratological processes.

Keywords: Amphetamine; Cannabinoid; Cocaine; Corticosteroids; Estrogens; Ethanol; Nicotine; Opiates; Tetrahydrocannabinol

INTRODUCTION

The *Fundacion Cerebro y Mente* International Meeting was held over a three-day period in October 2001 in Mojacar, Spain. The meeting consisted predominately of 30–40 oral presentations, with additional poster sessions. Three over-riding themes were neurodevelopmental and neuroteratologic aspects of (1) Drug Abuse, (2) Schizophrenia and Affective Disorders, and (3) Movement Disorders. Papers in this journal edition, with some papers appearing in the previous edition of *Neurotoxicity Research*, are derived largely from the first of these themes—Drug Abuse.

In this Introductory paper, we highlight some of the described neurodevelopmental liabilities of drug abuse. As indicated by the first two papers, elevated hormone levels in the mother can produce changes in ontogenetic development of specific brain regions. Cortisol is one such hormone, elevated in the mother's serum by stress, then passing via the placenta to the fetus or via milk to the newborn, and altering receptor number or second messenger production that can result in life-long changes in brain. Estrogen is another hormone, produced by the mother and passed via the placenta or milk to the neonate, to exert neuroprotective and developmental changes. The paper by Archer *et al.* describes an animal model of hyperactivity, produced by ontogenetic destruction of dopaminergic innervation of forebrain of rats, by intracerebroventricular administration of the neurotoxin 6-hydroxydopamine. Different treatment modalities validate the model, and implicate serotoninergic drugs as viable alternative treatments to D-amphetamine or methylphenidate. Other papers summarize the neuroteratologic effects of specific substances of abuse, and indicate the life-long consequences to such ontogenetic exposure. It will be evidently clear that there is not only anatomic disorganization, but also introduction of a liability to substance abuse and for affective disorders in the offspring. Therefore, early exposure to substances of abuse, as well as elevated hormone levels in the mother during pregnancy or lactation, can produce immediate changes in cell survival and neuronal organization, which can adversely influence affective mood, ordered thinking, learning, and memory throughout life.

*Corresponding author. Tel.: +1-423-439-6321. Fax: +1-423-439-8773. E-mail: kostrzew@etsu.edu

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VULNERABILITY TO SUBSTANCE ABUSE: ONTOGENETIC STRESS

Early environmental influences, even during the prenatal period, can have long-lasting effects on development and behavioral patterning in adulthood. In a paper by Koehl *et al.* (2002) this theme is well defined for stress, either to the mother while pregnant or to the neonate after birth. During pregnancy, the link between mother and child for this effect would be an elevation in the mother's plasma corticosteroid level, which would cross the placenta, enter the circulation of the fetus, and act on corticosteroid receptors in the developing brain. After birth, stress in the neonate would produce an elevation in the neonate corticosteroid level, which in the case of frequent or prolonged stress, would produce excessive stimulation at corticosteroid receptors in brain and in the hypothalamic-pituitary-adrenal axis (HPA). Both scenarios are capable of producing alterations in ontogeny of corticosteroid receptors, or their sensitivity, for the remainder of the life span. In this way early stress (or other environmental factors) can have a life-long effect on an individual. An extension of this theme relates to the ontogenetically stressed individual's vulnerability to substance abuse.

Several recent studies establish that prenatal stress or anxiety in humans is linked to low birth weight (Pagel *et al.*, 1990; Cooper *et al.*, 1996), reduced circulation in the fetal middle cerebral artery (Sjöstrom *et al.*, 1997), increased HPA responsiveness (Barden *et al.*, 1995), and long-term neurodevelopmental effects (Glover, 1997).

Experimentally, prenatal stress is produced by restraining pregnant rats three times a day, from gestation day 14 (GD14) to GD21 (Koehl *et al.*, 2002), resulting in elevation of the dam's corticosterone level (Barbazanges *et al.*, 1996). Postnatal stress is produced by handling pups daily, from postnatal day 1 (P1) to P21 and keeping them separated from the mother for 15 min each time. Corticosteroid level would be elevated in the lactating dam and secreted into her milk and thereby would pass to the pup (Barbazanges *et al.*, 1996). Prenatal stress was shown to reduce type I (mineralocorticoid) and type II (glucocorticoid) corticosteroid receptors in hippocampus at P21 and P90 (Maccari *et al.*, 1995), and alter the pattern of daily corticosterone secretion (Koehl *et al.*, 1997; 1999).

Prenatally stressed rats were more rapidly sensitized to amphetamine (Henry *et al.*, 1995) and nicotine (Koehl *et al.*, 2000) in adulthood. These rats also self-administered amphetamine at a higher rate (Deminiere *et al.*, 1992). In the nucleus accumbens of these rats both dopamine level (Piazza *et al.*, 1991) and D_2 receptor density were increased, while D_3 receptor density was decreased in both the shell and core of the accumbens (Henry *et al.*, 1995).

In summary, there is an overwhelming amount of data indicating that early stresses in life can have life-long consequences, relating to functioning of the HPA axis, behavioral responses to stressful situations, vulnerability to substance abuse, and identifiable changes in brain that link to the behavioral patterning.

ESTROGEN AND BRAIN VULNERABILITY

Here, estrogen is contrasted with corticosteroids by assessing the capacity of estrogen to be neuroprotective—an effect that is apparently partly dependent on its endocrine status and partly independent of it (Garcia-Segura *et al.*, 2001; Azcoitia *et al.*, 2002). Simply by virtue of the C3-hydroxy moiety on the A ring, estrogen is imbued with antioxidant properties (Behl and Holsboer, 1999). By binding to estrogen receptors (ERs) in membranes, estrogens also initiate 2nd messenger cascades that tend to be neuroprotective in both glia and neurons. Also, by action at ERs in the nucleus of cells, estrogens promote synthesis of a variety of factors that tend to impede apoptotic processes. Finally, estrogens are actually synthesized by brain cells, demonstrating their role as neurosteroids. These multi-mechanistic processes evoked by estrogens lend credence to the postulate that estrogens are intricately involved in neuronal protection, particularly after damaging or cellularly stressful events.

It is now evident that estrogens protect neurons from cell death *in vitro*, following serum- or growth factor-deprivation (Chowen *et al.*, 1992), anoxia (Zaulyanov *et al.*, 1999), excitotoxicity (Singer *et al.*, 1996) or oxidative damage (Regan and Guo, 1997). *In vivo*, estrogens are neuroprotective in forebrain ischemia after middle cerebral artery occlusion in rats (Simpkins *et al.*, 1997).

Neurodegenerative insults promote neuronal formation of aromatase, a key enzyme in estrogen biosynthesis (Garcia-Segura *et al.*, 1999a,b). Also, ER number is increased after focal ischemia.

Some of the neuroprotection afforded by estrogens is mediated by estrogen action on neuronal ERs, and this effect is prevented by antagonists of ERs (Chowen *et al.*, 1992), particularly estrogen receptor alpha (Dueñas *et al.*, 1996; Gollapudi and Oblinger, 1999a). In neurons, estrogens promote formation of the antiapoptotic factor Bcl-2 (Singer *et al.*, 1998), and Bcl-XL (Gollapudi and Oblinger, 1999a), while inhibiting expression of the pro-apoptotic factor Bad (Gollapudi and Oblinger, 1999b). In glia, estrogens inhibit activation of NF-kappa B and

thereby attenuate the inflammatory response to insults (Dodel *et al.*, 1999). Simultaneously, estrogens enhance expression of ApoE and insulin-like growth factor-I (IGF-I), which are associated with repair processes (Stone *et al.*, 1997; Garcia-Segura *et al.*, 2000).

Non-endocrine effects of hormones can influence both neurons and glia, acting as stressors (e.g. glucocorticoids) or neuroprotectants (e.g. estradiol). Exposure in ontogeny can have life-long alterations in behavioral functions and reactions and even in the vulnerability to substance abuse. The lesson is that endogenous substances are multi-factorial in their effects, and even non-chemical challenges like stress can alter the endogenous milieu to modify neuronal balance and exert long-lived effects that compromise cellular interplay and alter person's reactions to life events.

SUBSTANCE ABUSE AND DEPRESSIVE SYMPTOMATOLOGY

The introductory and novel theme attending drugs of abuse is that their depressive symptomatology, particularly during the withdrawal phase, is phenomenonologically similar to the mood state attending clinical depression and schizophrenia (Markou and Kenny, 2002). This is a usual feature during withdrawal from psychostimulants (Gawin and Kleber, 1986; Waddington *et al.*, 1990), opiates (Haertzen and Hooks, 1969; Henningfield *et al.*, 1987), ethanol (Jaffe, 1990; Edwards, 1990), and nicotine (West *et al.*, 1984; West and Gossop, 1994). Accordingly, if the neurobiological substrate is common for depression and the depressive mood during drug withdrawal, then it might be useful to use these animal models to explore the neural basis of depression and to screen for antidepressants.

Antidepressants Reduce Cocaine Use by Cocaine Abusers

It is noteworthy that classical antidepressants appear to be useful in treating drug dependence. In depressed cocaine users, desmethylimipramine reduced cocaine use by 90%; in non-depressed users, by 50% (Ziedonis and Kosten, 1991). Imipramine, although less efficacious than desmethylimipramine, was also beneficial (Nunes *et al.*, 1995).

Schizophrenics Prefer Mood Enhancing Drugs Over Mood Depressants

Treated and untreated schizophrenics prefer psychostimulants over sedating drugs (Schneier and Siris, 1987). Reasons for this may be related to the ability of psychostimulants to ameliorate adverse effects of schizophrenia. This relates to mood depression (Schneier and Siris, 1987; Robinson *et al.*, 1991), cognitive deficits (Cesarec and Nyman, 1985; Krystal *et al.*, 1999), and negative symptoms of schizophrenia (Khantzian, 1985; 1997; Krystal *et al.*, 1999). It is notable that clozapine reduces drug abuse, including psychostimulant abuse, in >85% of schizophrenics, with the effect often persisting for months (Zimmet *et al.*, 2000).

Nicotine Dependence is Prevalent in Schizophrenics

There is a two- to three-fold higher incidence of smoking among schizophrenics, and heavy smoking (>30 cigarettes per day) is usual (Masterson and O'Shea, 1984; Olincy *et al.*, 1997). More than 90% of schizophrenics smoke vs. 25–30% of the general population (Masterson and O'Shea, 1984; Diwan *et al.*, 1998). Postulated reasons for this relate to nicotine's transiently normalizing auditory gating deficits (Freedman *et al.*, 1997) and improving prepulse inhibition of a startle response (Geyer and Braff, 1987).

Antidepressants (e.g. bupropion) were advantageous in reducing cigarette use and in reducing the relapse to smoking after cessation (Hurt *et al.*, 1997; Jorenby *et al.*, 1999). Clozapine similarly reduced smoking in schizophrenics by 25–30% (George *et al.*, 1995; Marcus and Snyder, 1995).

Mesolimbic Dopamine and Serotonin as Neural Substrates of Drug Reward

Many studies over the past one or two decades have established the extraneuronal (i.e. synaptic) dopamine is an essential mediator of reward including the rewards or in-part euphoric sensation of drugs of abuse. During cocaine withdrawal extraneuronal dopamine level is reduced (Weiss *et al.*, 1992), as well as 5-HT level (Parsons *et al.*, 1995). The changes in mesolimbic dopamine during nicotine withdrawal (and withdrawal from psychostimulants) are similar to that during cocaine withdrawal (Carboni *et al.*, 2000). The selective 5-HT transport inhibitors fluoxetine and paroxetine, in combination with a 5-HT_{1A} receptor antagonist, rapidly reversed the threshold elevation associated with amphetamine or nicotine withdrawal (Harrison *et al.*, 2001).

Therapeutic Potential for Antidepressants as Adjuncts During Drug Withdrawal

The above studies demonstrate many of the commonalities between clinical depression and the depressed mood during drug withdrawal.

Furthermore, there is clear demonstration that classical antidepressants are also beneficial in relieving the depressive signs and thereby diminishing symptoms attending withdrawal from drugs of abuse. Neural substrates and therefore neural mechanisms underlying these phenomena may be similar or identical at least in part. Additional studies may lead to widespread use of antidepressants as adjuncts during withdrawal from drug abuse.

MODELS OF HYPER/HYPOACTIVITY AND TREATMENT IMPLICATIONS

Destruction of DA neurons in newborn rats through intracisternal or intracerebroventricular microinjection of the catecholamine neurotoxin, 6-hydroxydopamine (6-OHDA), produces behavioral alterations in the adult animal characterized by: (a) a hyperactive syndrome observed over several types of tests and parameters (Luthman *et al.*, 1989; Archer and Fredriksson, 1992), (b) some hypoactivity in tests demanding exploratory–investigatory behavior (Archer *et al.*, 1990), (c) deficits in instrumental learning acquisitive performance (Archer *et al.*, 1988; Luthman *et al.*, 1997), (d) altered responding to low doses of psychostimulant compounds that reverse the hyperactivity transiently (Luthman *et al.*, 1989), (e) altered responding to 5-hydroxytryptamine antagonist ligands which also reversed transiently the hyperactive state (Luthman *et al.*, 1991). In general, (f) the severity of the behavioral alterations were associated a more severe depletion of DA (Luthman *et al.*, 1997). Also, (g) the more severe the depletion of DA and degree of hyperactivity, the greater the regional increase in 5-HT concentrations (Archer *et al.*, 2002). Archer *et al.* (2002) found that the selective DA reuptake inhibitors, amphonelic acid and GBR 12909, injected before 6-OHDA, blocked completely both the behavioral alterations and neurochemical changes. A "Drug effect quotient" was derived to examine more closely the acute effects of doses of D-amphetamine: for example, it was indicated that the hyperactive Sal-OHDA rats injected with the low (0.25 mg/kg) dose of D-amphetamine showed a pronounced reduction of motor activity (in particular rearing, 99% reduction, and lesser so locomotion, 90% reduction) whereas the "intact" groups [Sal-Veh, GBR-Veh and GBR-6-OHDA] all showed marked increases (locomotion: 3.50–5.60-fold increases during the 1st 12-min period; rearing: 2.40–13.00-fold increases). The differential responses of the hyperactive 6-OHDA and "intact" groups to the higher (1.0 mg/kg) dose of D-amphetamine also bear consideration (Archer *et al.*, 2002).

ONTOGENETIC EFFECTS OF SPECIFIC DRUGS OF ABUSE: ALCOHOL, CANNABINOIDS, AND OPIATES

With the above framework relating to general aspects of substances of abuse, we now move to the neurotertological aspects of some of the most common substances of abuse, namely alcohol, cannabinoids, and opiates.

ALCOHOL

At the start of this millennium the mechanisms underlying neuronal deficits in the fetal alcohol syndrome—first described nearly 30 years ago (Jones *et al.*, 1973; Jones and Smith, 1975)—were only starting to be understood. In a series of studies described in this edition by Olney *et al.* (2002), his group found that the window of selective vulnerability of neurons to undergo an apoptotic physiological cell death (PCD) coincides with the period of synaptogenesis known as the brain growth spurt (Dikranian *et al.*, 2001a; Ikonomidou *et al.*, 2001). Moreover, ethanol was shown to exert its fetotoxic effects predominately in this period, both (1) by blocking *N*-methyl-D-aspartate (NMDA) glutamate receptors and (2) by an agonist action at gamma γ-aminobutyric acid-A ($GABA_A$) receptors (Ikonomidou *et al.*, 1999; 2000b). Specific cell groups are most susceptible to the deleterious effects of ethanol and related drugs during this period: layer II non-pyramidal and layers IV and V pyramidal neurons in the cingulate cortex, frontal cortex, parietal cortex, temporal cortex, occipital cortex, hippocampus, subiculum, cerebellum, thalamus, hypothalamus, amygdala and caudate nucleus. Different of these cell groups become sensitive (susceptible) at different phases of the brain growth spurt. Whereas only about 1% of a neuronal population undergoes apoptotic neurodegeneration after migrating to its final destination, up to 30% of the neurons become apoptotic after NMDA receptor block or $GABA_A$ receptor activation during the brain growth spurt (Ikonomidou *et al.*, 1999; 2000b).

DEVELOPMENTAL STAGES OF ETHANOL NEUROTOXICITY

In the fetal rat exposed to ethanol between GD6 and GD11, there is increased neural crest cell death and neural tube defects that may result in anencephaly, hydrocephaly, and craniofacial malformation (Webster *et al.*, 1983; Kotch and Sulik, 1992).

Between GD11 and GD21, ethanol is toxic to radial glia, resulting in neural and astroglia deficits, and

additional abnormal cell migration and neuronal cell loss (Miller, 1992; 1995; Gressens *et al.*, 1992). From GD21 to P30, ethanol produces alterations in astroglial development, increased natural cell death and cell necrosis, alterations in neural adhesion molecules (NCAMs), and impaired cerebellar development.

MECHANISMS OF PERINATAL ETHANOL NEUROTOXICITY

Ethanol is preferentially toxic to different groups of neurons and glia, according to the stage of development at the time of ethanol exposure, and by a variety of mechanisms as described below and in the paper by Guerri (2002).

Attenuation of Neurotrophin Actions

When developing rat brain is exposed to ethanol, there is a reduction in brain-derived neurotrophic factor (BDNF) as well as its receptor TrkB and intracellular signaling pathways (MAPK/ERK and PI-3-K/Akt pathways) (Guerri *et al.*, 2001).

Impairment of Astroglia Cell Development

During development and in embryogenesis, ethanol impairs radial glia cell development, their production of glial fibrillary acidic protein (GFAP) (Vallés *et al.*, 1996), and release of nerve growth factor (Vallés *et al.*, 1994; Luo and Miller, 1999). Ethanol also impairs astroglial proliferation and maturation (see Guerri and Renau-Piqueras, 1997).

Reduction in Cell Adhesion Molecules (CAMs)

During the period of synaptogenesis ethanol disrupts cell–cell adhesion in cell cultures (Ramanathan *et al.*, 1996) and the expression pattern of neuronal CAMs (NCAMs), increasing the polysialyllated (PSA-NCAM) form (Miñana *et al.*, 2000).

Ethanol Promotes Free Radical Formation

As in the adult, ethanol produces oxidative stress and depletion of antioxidant defenses, particularly reduced glutathione (Lieber, 1988). This effect occurs during synaptogenesis in neural crest cells (Chen and Sulik, 1996) and in cultured astrocytes (Montoliu *et al.*, 1995).

Ethanol Reduces Retinoic Acid Levels

Ethanol in high amount reduces the amount of retinoic acid in neural cells (Duester *et al.*, 1996). As retinoic acid is an important regulator of Hox gene expression (Ross *et al.*, 2000), it is significant that ethanol suppresses expression of the homeobox gene msx2 in mouse embryos (Rifas *et al.*, 1997)—although there is some question on this point (Cartwright *et al.*, 1998).

Ethanol Impairs 5-HT Neurodevelopment

At GD21, alcohol has neuroteratological effects on serotoninergic raphe and other midline nuclei in brain (Zhou *et al.*, 2002). In rats exposed prenatally to ethanol, both dorsal raphe and medial raphe nuclei have reduced numbers of perikarya (Sari *et al.*, 2001; Zhou *et al.*, 2001), and there is a relative denervation of periventricular brain regions by non-varicose 5-HT axonal fibers, as determined by 5-HT content, 5-HT immunoreactivity, 5-HT_{1A} receptor number, and 5-HT transport number (Druse *et al.*, 1991; Maier *et al.*, 1996; Zafar *et al.*, 2000; Zhou *et al.*, 2002). This effect occurs prior to widespread expression of NMDA and $GABA_A$ receptors in brain at GD14–GD15 (Poulter *et al.*, 1993; Monyer *et al.*, 1994). Evidently, there are multiple mechanisms by which ethanol exerts its toxicological effects in ontogeny.

As discussed in the paper by Naranjo *et al.* (2002), alcohol preference and alcohol dependence are closely linked to 5-HT neuronal dysfunction. The 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA) reduces alcohol preference in rats (Myers and Veale, 1968). In alcohol-preferring mice or rats there is reduced 5-HT innervation and reduced 5-HT and 5-HIAA turnover in several brain regions (Morinan, 1987; Gongwer *et al.*, 1989; Zhou *et al.*, 1991). In alcohol-preferring rats as well as in chronic alcoholics (Halliday *et al.*, 1993), alcohol produces degeneration of 5-HT neurons and axonal projections (Halliday *et al.*, 1995). In C57Bl mice treated perinatally with ethanol (20–25% of calories), there was a loss of 5-HT immunoreactive (ir) perikarya in the dorsal and medial raphe nuclei, and in the number of 5-HT-ir axons in the medial forebrain bundle (Sari *et al.*, 2001; Zhou *et al.*, 2001). Also, destruction of 5-HT neurons in Sprague–Dawley rats which normally have low alcohol preference, increased voluntary ethanol consumption (Wang *et al.*, 1996). This body of evidence provides a clear link between ethanol consumption and 5-HT dysfunction.

Ethanol and DA in Brain

Acutely, ethanol dose-dependently increases the firing rate of DA fibers deriving from the ventral tegmental nucleus (Brodie and Appel, 2000) and enhances DA release in the nucleus accumbens of rats

(Olive *et al.*, 2000). Increased ethanol evoked DA release in nucleus accumbens appears to be a predictor of ethanol preference in rats (Katner and Weiss, 2001); and DA D_2 receptor number in brain was inversely correlated with ethanol preference and intake (Thanos *et al.*, 2001). However, there is debate as to whether alterations in DA neuronal markers are specific to alcohol, or rather, a general measure of the reward system *per se*.

Ethanol Alters Development of GABA-glutamate Systems

During brain development, ethanol reduces the number of functional NMDA receptors in neocortex and hippocampus (Vallés *et al.*, 1995; Diaz-Granados *et al.*, 1997), and during the growth spurt ethanol promotes apoptosis (above).

CLINICAL IMPLICATIONS OF PERINATAL ETHANOL

Ethanol Preference in Alcoholics

There have been many prospective studies in rodents and many retrospective studies in humans, seeking to determine a neurochemical basis for (1) alcohol-preference or (2) the alcohol dependency state. These studies have been reviewed in the paper by Naranjo *et al.* (2002). Because of frequent conflicting findings in humans, the reader is directed to the Naranjo paper to delve through the complexities and confounding aspects of specific studies. However, the following findings seem to be unequivocal. Children of alcoholics had a greater 5-HT uptake capacity by platelets (Rausch *et al.*, 1991), reduced CSF level of 5-HIAA (Rosenthal *et al.*, 1980), and reduced cortisol- or prolactin-response to fenfluramine (Schuckit *et al.*, 1987a,b). In Type II alcoholics (early onset, rapid and severe course of alcoholism) there is up-regulated 5-HT_2 receptors in brain (Virkkunen *et al.*, 1994), increased platelet 5-HT uptake (Javors *et al.*, 2000), and reduced cerebral 5-HIAA content (Fils-Aime *et al.*, 1996). There are ongoing studies to determine if changes in 5-HT function precede or follow ethanol abuse.

Fetal Alcohol Spectrum Disorders

Ethanol produces a spectrum of neuropathological/behavioral deficits in humans, including hyperactivity, learning deficits, and psychiatric disorders in adulthood. The term fetal alcohol spectrum disorder (FASD) was recently introduced to refer to the spectrum of neuropathological alterations produced by ethanol in humans (Barr and Streissguth, 2001). In human adults, the excitotoxic effects of ethanol, as antagonist at NMDA receptors, is counteracted by its inhibitory action of $GABA_A$ receptors (Olney *et al.*, 1991). In human perinates, the apoptotic effect of ethanol at NMDA receptors is enhanced by its inhibitory effect at $GABA_A$ receptors (Ikonomidou *et al.*, 2000b). The perinatal cortex and specific subcortical regions are uniquely sensitive to neuronal apoptotic effects of ethanol, which account for subsequent learning deficits, behavioral disorders, and psychiatric states. Nearly three of four FASD patients manifest major depression, psychosis or other psychiatric condition in adulthood (Famy *et al.*, 1998).

General Anesthesia in Children

As general anesthesia typically involves use of NMDA receptor antagonists (e.g. ketamine, nitrous oxide) and/or $GABA_A$ agonists (e.g. barbiturates, benzodiazepines), there is high risk for induction of neuronal apoptosis in children, particularly if anesthesia is maintained for hours. In rats with surgical plane anesthesia, maintained for 6 h with combined isoflurane–midazolam–nitrous oxide on postnatal day 7, there was notable apoptosis in cortical and subcortical regions of brain; and this was associated with later and seemingly permanent learning deficits (Hartman *et al.*, 2001; Jevtovic-Todorovic *et al.*, 2001).

Perinatal Use of Anti-seizure Drugs

As commonly used anti-seizure drugs inhibit neuronal activity by blocking sodium channels, they share in the net effect (i.e. neuronal inhibition) produced by NMDA antagonists and $GABA_A$ agonists in perinates. Phenytoin and valproate have been shown to produce neuronal apoptosis in the perinate-akin to the ethanol effect (Dikranian *et al.*, 2001b; Ikonomidou *et al.*, 2000a,c). Use of anti-seizure drugs by pregnant mothers or children, runs the risk of induction of neuronal apoptosis in the brain of the fetus or child, respectively. Learning deficits are reported for children exposed to these drugs *in utero* or after birth (Dessens *et al.*, 2000).

Perinatal Exposure to Drugs of Abuse

When pregnant women abuse NMDA blockers (e.g. ketamine, phencyclidine, nitrous oxide) or GABA-mimetics (e.g. benzodiazepines, barbiturates), there is increased risk of induction of neuronal apoptosis in the fetus—an action analogous to that of ethanol (Ikonomidou *et al.*, 1999).

Psychiatric Disorders

Because 5-HT has a regulatory role in cell division, differentiation, migration, growth and synaptogenesis (Lauder, 1990; Whitaker-Azmitia *et al.*, 1996), development and function of multiple neuronal systems can be altered including the somatosensory map (Blue *et al.*, 1991; Persico *et al.*, 2001). Because 5-HT neurons have a modulatory role in mood regulation, learning and memory, eating, temperature-regulation, sleep and circadian rhythm, reproductive processes and motor activities, a variety of additional life-long manifestations are possible. These relate to anxiety disorders, eating disorders, insomnia, mood and personality disorders including obsessive compulsive disorder (see Coccaro and Murphy, 1990).

CANNABINOIDS

Perinatal treatment with cannabinoids produces alterations in development not only of the endocannabinoid system *per se* (Ramos *et al.*, 2002), but in opioid and monoaminergic systems as well (Perez-Rosado *et al.*, 2002). Although changes in markers for these neurochemical systems are often small in magnitude, numerous behavioral alterations are observed in adulthood.

Development of the Endocannabinoid System in Brain

Cannabinoid CB_1 receptors and mRNA levels are definable by GD14 in rats, coincident with the onset of phenotypic expression of the endocannabinoids anandamide and 2-arachidonylglycerol (2-AG) (see Insel, 1995). CB_1 receptors are associated with commisural tracts such as the corpus callosum, stria terminalis, and anterior commissure and are thought to be localized to sprouting axons and/or astrocytes and oligodendroglia (see Shivachar *et al.*, 1996). As the pattern of distribution of CB_1 receptors is dramatically different at this time than in adulthood (Mailleux and Vanderhaeghen, 1992a,b), it is hypothesized that endocannabinoids may have a nurturing or neurotrophic role prenatally, apart from its later modulatory role.

Neuroteratological Effect of Cannabinoids on the Endocannabinoid System in Brain

When anandamide is administered to pregnant rats, the offspring in adulthood display reduced open field activity, catalepsy, hypothermia, hypoalgesia and tolerance to cannabinoid challenge (Fride and Mechoulam, 1996a,b). Perinatal treatment with the CB_1 selective agonist HU-210 produced an elevation in the adult basal level of luteinizing hormone (LH). A single low perinatal dose of HU-210 (1 μg/kg) sensitized to a corticosterone response while a single high perinatal dose of HU-210 (20 μg/kg) reduced the adult corticosterone level in offspring in adulthood (Del Arco *et al.*, 2000).

Neuroteratological Effect of Cannabinoids on the Opioid System in Brain

In rats exposed prenatally to delta-9-tetrahydrocannabinol (Δ^9-THC), there was a sexually dimorphic effect on opiod systems in brain. In the arcuate nucleus, cerebral cortex and habenula, pro-opiomelanocortin (POMC) mRNA levels were slightly elevated in females and slightly reduced in males at GD21, following daily oral administration of Δ^9-THC (5 mg/kg/day) from GD5. In females only, prodynorphin mRNA was slightly increased in the cerebral cortex, hippocampus and paraventricular nucleus of the hypothalamus. When assessments were made three days earlier, at GD18, the only significant change was a 6–10% elevation in POMC mRNA in arcuate nucleus and cerebral cortex in males and females combined (Perez-Rosado *et al.*, 2002).

In similarly treated rats that were behaviorally tested in adulthood, males copulated less frequently (Dalterio and Barke, 1979). Males also were less active in the open field (Navarro *et al.*, 1994), had impaired learning (Dalterio, 1986), and had a reduced stress-response (Mokler *et al.*, 1987), with altered nociceptive response (Vela *et al.*, 1995). Female offspring self-administered more morphine and had increased mu receptor binding and decreased proenkephalin mRNA in brain regions associated with drug-seeking behavior (Corchero *et al.*, 1998; Vela *et al.*, 1998).

Neuroteratological Effect of Cannabinoids on Neurochemistry of Brain

In rats perinatally exposed to Δ^9-THC for GD5, there was an abrupt and marked decrease in tyrosine hydroxylase (T-OH) mRNA, T-OH activity, and T-OH-ir in brain (Bonnin *et al.*, 1996), but this alteration was absent in adulthood (Rodríguez de Fonseca *et al.*, 1991). However, the effects of DA agonists or amphetamine were altered in these rats (García-Gil *et al.*, 1996; 1998).

Perinatal Δ^9-THC treatment reduced 5-HT content of the diencephalon of male perinates (Molina-Holgado *et al.*, 1996), and in adulthood the reduction in 5-HT with elevation in 5-HIAA extended to other brain areas (Molina-Holgado *et al.*, 1997). Although perinatal cannabinoids did not alter GABA

content and glutamic acid decarboxylase activity in the brain of offspring in adulthood, there was a greater behavioral response to treatment with the $GABA_B$ receptor agonist baclofen (García-Gil *et al.*, 1999).

Behavioral Alterations in Rats Treated Perinatally with Cannabinoids

Endocannabinoids are associated with modulatory effects on motor activity, antinociception, learning and memory (Mallet and Beninger, 1996). The seemingly small changes in the endocannabinoid and opioid systems in brain are nonetheless associated with demonstrable behavioral alterations later in life. While effects on self-administration of opiates is likely to be related to some of the above changes in the opioid systems in brain, it is quite evident that some other behaviors may be due, at least in part, to perinatal effects of cannabinoids on other neurochemical systems.

OPIATES

In a major review article, Trujillo (2002) summarizes findings and lays hypotheses relative to mechanisms of opiate tolerance, dependence, and sensitization. Somewhat surprisingly, opiate tolerance for one effect (e.g. analgesia) can develop simultaneously with opiate sensitization to another effect (e.g. locomotor depression). As discussed by these authors, NMDA receptors appear to be intricately involved in the above phenomena. By blocking long-term potentiation (LTP) or long-term depression (LTD), NMDA receptor antagonists interfere with the acquisition but not the expression of opiate effects; and interfere with adaptive responses to opiates.

A role for NMDA receptors in the depressant effects of opiates was indicated by MK-801-, memantine- and LY235959-suppression of tolerance to the locomotor depressant effect of morphine (Trujillo, 2002) and NMDA antagonist-suppression of operant responding (Bespalov *et al.*, 1999). Involvement of NMDA receptors in the development of opiate tolerance is indicated by MK-801 inhibition of the development of tolerance to morphine analgesia (Mao *et al.*, 1995).

A role for NMDA receptors in the development of opiate physical dependence is evidenced by MK-801 inhibition of the acquisition of physical dependence on morphine (Trujillo and Akil, 1990; 1991); similarly, by an antisense oligonucleotide against a key NMDA receptor subunit (Zhu and Ho, 1998). Finally, sensitization to opiates apparently is also NMDA receptor-dependent, as the effect is blocked by MK-801, CGS 19755 (Wolf and Jeziorski, 1993; Jeziorski *et al.*, 1994), memantine, and LY235959 (Peterson and Trujillo, 2001; Trujillo *et al.*, 2001).

Opioid tolerance and physical dependence is thought to involve a decoupling of opioid receptors from second messenger systems, and could sequentially involve protein kinase C (PKC), phospholipase C (PLC), activation of calcium calmodulin kinase II (CaMKII) and nitric oxide (NO) synthase, NO production, and guanylyl cyclase.

CLINICAL IMPLICATIONS RELATING TO OPIATES AND NMDA RECEPTORS

NMDA receptor antagonists may be useful in reversing opiate addiction, and able to accelerate the loss of tolerance, dependence and sensitization during opiate detoxification. Better understanding of second messenger systems in these phenomena may lead to better treatment for chronic pain.

SUMMARY

These series of papers deriving from the Cerebre y Mente meeting, on the theme *Neurodevelopmental Liabilities of Substance Abuse*, illustrate how substances of abuse and even ordinarily benign environmental situations can adversely affect neonate brain development, and produce life-long changes that potentially predispose to affective disorders or added risks in the face of substances of abuse. Because neural deficits can be reproducibly achieved in animal studies, much can be gained by educating women about substance abuse risk to the unborn child.

CONCLUSIONS

From a scientific perspective the series of papers on substance abuse, deriving from the Cerebro y Mente meeting, demonstrate that much progress has been made in understanding these mechanisms of action in producing neuroteratology. Abstinence is the best means for averting risk posed by substances of abuse. However, in the situation where a meonate must be anesthetized, there is no single anesthetic that is free of risk. Today all anesthetics either block NMDA receptors or enhance GABA activity—both effects being capable of producing damage to the developing brain. More research is needed to develop more satisfactory anesthetics and more studies are needed to develop drugs that can effectively prevent adverse effects of these substances on the developing brain.

References

Archer, T. and Fredriksson, A. (1992) "Functional changes implicating dopaminergic systems following perinatal treatments", *Dev. Pharmacol. Ther.* **18**, 201–222.

Archer, T., Danysz, W., Fredriksson, A., Jonsson, G., Luthman, J., Sundström, E. and Teiling, A. (1988) "Neonatal 6-hydroxydopamine-induced dopamine depletions: motor activity and performance in maze learning", *Pharmacol. Biochem. Behav.* **31**, 357–364.

Archer, T., Beninger, R.J., Järbe, T.U.C. and Seiden, L.S. (1990) "Latent learning in a radial arm maze following neonatal dopamine depletion", *Behav. Pharmacol.* **1**, 191–199.

Archer, T., Palomo, T. and Fredriksson, A. (2002) "Neonatal 6-hydroxydopamine-induced hypo/hyperactivity: blockade by dopamine reuptake inhibitors and effect of acute D-amphetamine", *Neurotoxicity Res.* **4**, 247–266.

Azcoitia, I., Doncarlos, L.L. and Garcia-Segura, L.M. (2002) "Estrogen and brain vulnerability", *Neurotoxicity Res.* **4**, 235–245.

Barbazanges, A., Piazza, P.V., Le Moal, M. and Maccari, S. (1996) "Maternal glucocorticoid secretion mediates long-term effects of prenatal stress", *J. Neurosci.* **16**, 3943–3949.

Barden, N., Reul, J.M. and Holsboer, F. (1995) "Do antidepressants stabilize mood through actions on the hypothalamic–pituitary–adrenocortical system?", *Trends Neurosci.* **18**, 6–11.

Barr, H.M. and Streissguth, A.P. (2001) "Identifying maternal self-reported alcohol use associated with fetal alcohol spectrum disorders", *Alcohol. Clin. Exp. Res.* **25**, 283–287.

Behl, C. and Holsboer, F. (1999) "The female sex hormone oestrogen as a neuroprotectant", *Trends Pharmacol. Sci.* **20**, 441–444.

Bespalov, A.Y., Balster, R.L. and Beardsley, P.M. (1999) "*N*-methyl-D-aspartate receptor antagonists and the development of tolerance to the discriminative stimulus effects of morphine in rats", *J. Pharmacol. Exp. Ther.* **290**, 20–27.

Blue, M.E., Erzurumlu, R.S. and Jhaveri, S. (1991) "A comparison of pattern formation by the thalamocortical and serotonergic afferents in the rat barrel field cortex", *Cereb. Cortex* **1**, 380–389.

Bonnin, A., de Miguel, R., Castro, J.G., Ramos, J.A. and Fernández-Ruiz, J.J. (1996) "Effects of perinatal exposure to Δ^9-tetrahydrocannabinol on the fetal and early postnatal development of tyrosine hydroxylase containing neurons in rat brain", *J. Mol. Neurosci.* **7**, 291–308.

Brodie, M.S. and Appel, S.B. (2000) "Dopaminergic neurons in the ventral tegmental area of C57BL/6J and DBA/2J mice differ in sensitivity to ethanol excitation", *Alcohol. Clin. Exp. Res.* **24**, 1120–1124.

Carboni, E., Bortone, L., Giua, C. and DiChiara, G. (2000) "Dissociation of physical abstinence signs from changes in extracellular dopamine in the nucleus accumbens and in the prefrontal cortex of nicotine dependent rats", *Drug Alcohol Depend.* **58**, 93–102.

Cartwright, M.M., Tessmer, L.L. and Smith, S.M. (1998) "Ethanol-induced neural crest apoptosis is coincident with their endogenous death, but is mechanistically distinct", *Alcohol. Clin. Exp. Res.* **22**, 142–149.

Cesarec, Z. and Nyman, A.K. (1985) "Differential response to amphetamine in schizophrenia", *Acta Psychiatr. Scand.* **71**, 523–538.

Chen, S.Y. and Sulik, K.K. (1996) "Free radicals and ethanol-induced cytotoxicity in neural crest cells", *Alcohol. Clin. Exp. Res.* **20**, 1071–1076.

Chowen, J.A., Torres-Aleman, I. and Garcia-Segura, L.M. (1992) "Trophic effects of estradiol on fetal rat hypothalamic neurons", *Neuroendocrinology* **56**, 895–901.

Coccaro, E.F. and Murphy, D.L. (1990) Serotonin in major psychiatric disorders (American Psych. Press, Washington, DC).

Cooper, R.L., Goldenberg, R.L., Das, A., Elder, N., Swain, M., Norman, G., Ramsey, R., Cotroneo, P., Collins, B.A., Johnson, F., Jones, P. and Meier, A.M. (1996) "The preterm prediction study: maternal stress is associated with spontaneous preterm brith at less than thirty-five weeks' gestation. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network", *Am. J. Obstet. Gynecol.* **175**, 1286–1292.

Corchero, J., García-Gil, L., Manzanares, J., Fernández-Ruiz, J.J., Fuentes, J.A. and Ramos, J.A. (1998) "Perinatal Δ^9-tetrahydrocannabinol exposure reduces proenkephalin gene expresion in the caudate-putamen of adult female rats", *Life Sci.* **63**, 843–850.

Dalterio, S.L. (1986) "Cannabinoid exposure: effects on development", *Neurobehav. Toxicol. Teratol.* **8**, 345–352.

Dalterio, S.L. and Barke, A. (1979) "Perinatal exposure to cannabinoids alter male reproductive functions in mice", *Science* **205**, 1420–1422.

Del Arco, I., Muñoz, R., Rodríguez, F., Escudero, L., Martin, J.L., Navarro, M. and Villanúa, M.A. (2000) "Maternal exposure to the synthetic cannabinoid HU-210: effects on the endocrine and immune systems of the adult male offspring", *Neuroimmunomodulation* **7**, 16–26.

Deminiere, J.M., Piazza, P.V., Guegan, G., Abrous, N., Maccari, S., Le Moal, M. and Simon, H. (1992) "Increased locomotor response to novelty and propensity to intravenous amphetamine self-administration in adult offspring of stressed mothers", *Brain Res.* **586**, 135–139.

Dessens, A.B., Cohen-Kettenis, P.T., Mellenbergh, G.J., Koppe, J.G., van de Poll, N.E. and Boer, K. (2000) "Association of prenatal phenobarbital and phenytoin exposure with small head size and with learning problems", *Acta Paediatr.* **89**, 533–541.

Diaz-Granados, J.L., Spuhler-Phillips, K., Lilliquist, M.W., Amsel, A. and Leslie, S.W. (1997) "Effects of prenatal and early postnatal ethanol exposure on (^{3}H).MK-801 binding in rat cortex and hippocampus", *Alcohol. Clin. Exp. Res.* **21**, 874–881.

Dikranian, K., Ishimaru, M.J., Tenkova, T., Labruyere, J., Qin, Y.Q., Ikonomidou, C. and Olney, J.W. (2001a) "Apoptosis in the *in vivo* mammalian forebrain", *Neurobiol. Dis.* **8**, 359–379.

Dikranian, K., Tenkova, T., Bittigau, P., Ikonomidou, C. and Olney, J.W. (2001b) "Histological characterization of apoptotic neurodegeneration induced in the developing rat brain by drugs that block sodium channels", *Soc. Neurosci. Abstr.* **26**, 323.

Diwan, A., Castine, M., Pomerleau, C.S., Meador-Woodruff, J.H. and Dalack, G.W. (1998) "Differential prevalence of cigarette smoking in patients with schizophrenic vs. mood disorders", *Schizophr. Res.* **33**, 113–118.

Dodel, R.C., Du, Y., Bales, K.R., Gao, F. and Paul, S.M. (1999) "Sodium salicylate and 17beta-estradiol attenuate nuclear transcription factor NF-kappaB translocation in cultured rat astroglial cultures following exposure to amyloid A beta(1–40) and lipopolysaccharides", *J. Neurochem.* **73**, 1453–1460.

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.

Dueñas, M., Torres-Aleman, I., Naftolin, F. and Garcia-Segura, L.M. (1996) "Interaction of insulin-like growth factor-1 and estradiol signaling pathways on hypothalamic neuronal differentiation", *Neuroscience* **74**, 531–539.

Duester, G., Deltour, L. and Ang, H.L. (1996) "Evidence that class IV alcohol dehydrogenase may function in embryonic retinoic acid synthesis", In: Weiner, H., ed, Enzymology and Molecular Biology of Carbonyl Metabolism (Plenum Press, New York, NY), pp 357–364.

Edwards, G. (1990) "Withdrawal symptoms and alcohol dependence: fruitful mysteries", *Br. J. Addict.* **85**, 447–461.

Famy, C., Streissguth, A.P. and Unis, A.S. (1998) "Mental illness in adults with fetal alcohol syndrome or fetal alcohol effects", *Am. J. Psychiatry* **155**, 552–554.

Fils-Aime, M.L., Eckardt, M.J., George, D.T., Brown, G.L., Mefford, I. and Linnoila, M. (1996) "Early-onset alcoholics have lower cerebrospinal fluid 5-hydroxyindoleacetic acid levels than late-onset alcoholics", *Arch. Gen. Psychiatry* **53**, 211–216.

Freedman, R., Coon, H., Myles-Worsley, M., Orr-Urtreger, A., Olincy, A., Davis, A., Polymeropoulos, M., Holik, J., Hopkins, J., Hoff, M., Rosenthal, J., Waldo, M.C., Reimherr, F., Wender, P., Yaw, J., Young, D.A., Breese, C.R., Adams, C., Patterson, D., Adler, L.E., Kruglyak, L., Leonard, S. and Byerley, W. (1997) "Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus", *Proc. Natl Acad. Sci. USA* **94**, 587–592.

Fride, E. and Mechoulam, R. (1996a) "Developmental aspects of anandamide: ontogeny of response and prenatal exposure", *Psychoneuroendocrinology* **21**, 157–172.

Fride, E. and Mechoulam, R. (1996b) "Ontogenic development of the response to anandamide and Δ^9-tetrahydrocannabinol in mice", *Dev. Brain Res.* **95**, 131–134.

García-Gil, L., de Miguel, R., Ramos, J.A. and Fernández-Ruiz, J.J. (1996) "Perinatal Δ^9-tetrahydrocannabinol exposure in rats modifies the responsiveness of midbrain dopaminergic neurons

in adulthood to a variety of challenges with dopaminergic drugs", *Drug Alcohol Depend.* **42**, 155–166.

García-Gil, L., Ramos, J.A., Rubino, T., Parolaro, D. and Fernández-Ruiz, J.J. (1998) "Perinatal Δ^9-tetrahydrocannabinol exposure did not alter dopamine transporter and tyrosine hydroxylase mRNA levels in midbrain dopaminergic neurons of adult male and female rats", *Neurotoxicol. Teratol.* **20**, 549–553.

García-Gil, L., de Miguel, R., Romero, J., Pérez, A., Ramos, J.A. and Fernández-Ruiz, J.J. (1999) "Perinatal Δ^9-tetrahydrocannabinol exposure augmented the magnitude of motor inhibition caused by GABA-B but not GABA-A, receptor agonists in adult rats", *Neurotoxicol. Teratol.* **21**, 277–283.

Garcia-Segura, L.M., Naftolin, F., Hutchison, J.B., Azcoitia, I. and Chowen, J.A. (1999a) "Role of astroglia in estrogen regulation of synaptic plasticity and brain repair", *J. Neurobiol.* **40**, 574–584.

Garcia-Segura, L.M., Wozniack, A., Azcoitia, I., Rodriguez, J.R., Hutchison, R.E. and Hutchison, J.B. (1999b) "Aromatase expression by astrocytes after brain injury: implications for local estrogen formation in brain repair", *Neuroscience* **89**, 567–578.

Garcia-Segura, L.M., Cardona-Gomez, G.P., Chowen, J.A. and Azcoitia, I. (2000) "Insulin-like growth factor-I receptors and estrogen receptors interact in the promotion of neuronal survival and neuroprotection", *J. Neurocytol.* **29**, 425–437.

Garcia-Segura, L.M., Azcoitia, I. and DonCarlos, L.L. (2001) "Neuroprotection by estradiol", *Prog. Neurobiol.* **63**, 29–60.

Gawin, F.H. and Kleber, H.D. (1986) "Abstinence symptomatology and psychiatric diagnosis in cocaine abusers: clinical observations", *Arch. Gen. Psychiatry* **43**, 107–113.

George, T.P., Sernyak, M.J., Ziedonis, D.M. and Woods, S.W. (1995) "Effects of clozapine on smoking in chronic schizophrenic outpatients", *J. Clin. Psychiatry* **56**, 344–346.

Geyer, M.A. and Braff, D.L. (1987) "Startle habituation and sensorimotor gating in schizophrenia and related animal models", *Schizophr. Bull.* **13**, 643–668.

Glover, V. (1997) "Maternal stress or anxiety in pregnancy and emotional development of the child", *Br. J. Psychiatry* **171**, 105–106.

Gollapudi, L. and Oblinger, M.M. (1999a) "Stable transfection of PC12 cells with estrogen receptor (ERα).: protective effects of estrogen on cell survival after serum deprivation", *J. Neurosci. Res.* **56**, 99–108.

Gollapudi, L. and Oblinger, M.M. (1999b) "Estrogen and NGF synergistically protect terminally differentiated ERα-transfected PC12 cells from apoptosis", *J. Neurosci. Res.* **56**, 471–481.

Gongwer, M.A., Murphy, J.M., McBride, W.J., Lumeng, L. and Li, T.K. (1989) "Regional brain contents of serotonin, dopamine and their metabolites in the selectively bred high- and low-alcohol drinking lines of rats", *Alcohol Alcohol.* **6**, 317–320.

Gressens, P., Lammens, M., Picard, J.J. and Evrard, P. (1992) "Ethanol-induced disturbances of gliogenesis and neurogenesis in the developing murine brain: an *in vitro* and *in vivo* immunohistochemical and ultrastructural study", *Alcohol Alcohol.* **27**, 219–226.

Guerri, C. (2002) "Mechanisms involved in central nervous system dysfunctions induced by prenatal ethanol exposure", *Neurotoxicity Res.* **4**, 327–335.

Guerri, C. and Renau-Piqueras, J. (1997) "Alcohol, astroglia, and brain development", *Mol. Neurobiol.* **15**, 65–81.

Guerri, C., Climent, E. and Pascual, M. (2001) "Ethanol exposure enhances apoptosis during brain development and affects brain-derived neurotrophic factor and its TrkB receptors", *Alcohol Alcohol.* **36**, 437.

Haertzen, C.A. and Hooks, Jr, N.T. (1969) "Changes in personality as subjective experience associated with the chronic administration and withdrawal of opiates", *J. Nerv. Ment. Dis.* **148**, 606–614.

Halliday, G., Ellis, J., Heard, R., Caine, D. and Harper, C. (1993) "Brainstem serotonergic neurons in chronic alcoholics with and without the memory impairment of Korsakoff's psychosis", *J. Neuropathol. Exp. Neurol.* **52**, 567–579.

Halliday, G., Baker, K. and Harper, C. (1995) "Serotonin and alcohol-related brain damage", *Metab. Brain Dis.* **10**, 25–30.

Harrison, A.A., Liem, Y.T. and Markou, A. (2001) "Fluoxetine combined with a serotonin-1A receptor antagonist reversed reward deficits observed during nicotine and amphetamine withdrawal in rats", *Neuropsychopharmacology* **25**, 55–71.

Hartman, R.E., Jevtovic-Todorovic, V., Olney, J.W. and Wozniak, D.F. (2001) "Neonatal exposure to common anesthetics leads to spatial learning deficits in juvenile rats", *Soc. Neurosci. Abstr.* **27**, 772.5.

Henningfield, J.E., Johnson, R.E. and Jasinski, D.R. (1987) "Clinical procedures for the assessment of abuse potential", In: Bozarth, M.A., ed, Methods of Assessing the Reinforcing Properties of Abused Drugs (Springer-Verlag, New York), pp 573–590.

Henry, C., Guegant, G., Cador, M., Arnauld, E., Arsaut, J., Le Moal, M. and Demotes-Mainard, J. (1995) "Prenatal stress in rats facilitates amphetamine-induced sensitization and induces long-lasting changes in dopamine receptors in the nucleus accumbens", *Brain Res.* **685**, 179–186.

Hurt, R.D., Sachs, D.P.L., Glover, E.D., Offord, K.P., Johnston, J.A., Dale, L.C., Khayrallah, M.A., Schroeder, D.R., Glover, P.N., Sullivan, C.R., Croghan, I.T. and Sullivan, P.M. (1997) "A comparison of sustained-release buproprion and placebo for smoking cessation", *N. Engl. J. Med.* **337**, 1195–1202.

Ikonomidou, C., Bosch, F., Miksa, M., Bittigau, P., Vockler, J., Dikranian, K., Tenkova, T., Stevoska, V., Turski, L. and Olney, J.W. (1999) "Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain", *Science* **283**, 70–74.

Ikonomidou, C., Bittigau, P., Ishimaru, M.J., Wozniak, D.F., Koch, C., Genz, K., Price, M.T., Stefovska, V., Horster, F., Tenkova, T., Dikranian, K. and Olney, J.W. (2000a) "Drug-induced damage in the developing brain", *Science* **288**, 976–977.

Ikonomidou, C., Bittigau, P., Ishimaru, M.J., Wozniak, D.F., Koch, C., Genz, K., Price, M.T., Stefovska, V., Horster, F., Tenkova, T., Dikranian, K. and Olney, J.W. (2000b) "Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome", *Science* **287**, 1056–1060.

Ikonomidou, C., Genz, K., Engelbrechten, S., Dikranian, K., Olney, J.W. and Bittigau, P. (2000c) "Antiepileptic drugs which block sodium channels cause neuronal apoptosis in the developing rat brain", *Soc. Neurosci. Abstr.* **26**, 323.

Ikonomidou, C., Bittigau, P., Koch, C., Genz, K., Hoerster, F., Felderhoff-Mueser, U., Tenkova, T., Dikranian, K. and Olney, J.W. (2001) "Neurotransmitters and apoptosis in the developing brain", *Biochem. Pharmacol.* **62**, 401–405.

Insel, T.R. (1995) "The development of brain and behavior", In: Bloom, F.E. and Kupfer, D.J., eds, Psychopharmacology: The Four Generation of Progress (Raven Press, New York), pp 683–694.

Jaffe, J.H. (1990) "Drug addiction and drug abuse", In: Rall, T.W., Nies, A.S. and Taylor, P., eds, Goodman & Gilman's Pharmacological Basis of Therapeutics, 8th edition (Pergamon Press, Elmsford, NY), pp 522–573.

Javors, M., Tiouririne, M. and Prihoda, T. (2000) "Platelet serotonin uptake is higher in early-onset than in late-onset alcoholics", *Alcohol Alcohol.* **35**, 390–393.

Jevtovic-Todorovic, V., Wozniak, D.F., Benshoff, N. and Olney, J.W. (2001) "Commonly used anesthesia protocol causes neuronal suicide in the immature rat brain", *Soc. Neurosci. Abstr.* **27**, 772.4.

Jeziorski, M., White, F.J. and Wolf, M.E. (1994) "MK-801 prevents the development of behavioral sensitization during repeated morphine administration", *Synapse* **16**, 137–147.

Jones, K.L. and Smith, D.W. (1975) "The fetal alcohol syndrome", *Teratology* **12**, 1–10.

Jones, K.L., Smith, D.W., Ulleland, C.N. and Streissguth, A.P. (1973) "Pattern of malformation in offspring of chronic alcoholic mothers", *Lancet* **I**, 1267–1271.

Jorenby, D.E., Leischow, S.J., Nides, M.A., Rennard, S.I., Johnston, J.A., Hughes, A.R., Smith, S.S., Muramoto, M.L., Daughton, D.M., Doan, K., Fiore, M.C. and Baker, T.B. (1999) "A controlled trial of sustained-release buproprion, a nicotine patch, or both for smoking cessation", *N. Engl. J. Med.* **340**, 685–691.

Katner, S.N. and Weiss, F. (2001) "Neurochemical characteristics associated with ethanol preference in selected alcohol-preferring and -nonpreferring rats: a quantitative microdialysis study", *Alcohol. Clin. Exp. Res.* **25**, 198–205.

Khantzian, E.J. (1985) "The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence", *Am. J. Psychiatry* **142**, 1259–1264.

Khantzian, E.J. (1997) "The self-medication hypothesis of substance use disorders: a reconsideration and recent applications", *Harvard Rev. Psychiatry* **4**, 231–244.

Koehl, M., Barbazanges, A., Le Moal, M. and Maccari, S. (1997) "Prenatal stress induces a phase advance of circadian

corticosterone rhythm in adult rats which is prevented by postnatal stress", *Brain Res.* **759**, 317–320.

Koehl, M., Darnaudery, M., Dulluc, J., Van Reeth, O., Le Moal, M. and Maccari, S. (1999) "Prenatal stress alters circadian activity of hypothalamo-pituitary- adrenal axis and hippocampal corticosteroid receptors in adult rats of both gender", *J. Neurobiol.* **40**, 302–315.

Koehl, M., Bjijou, Y., Le Moal, M. and Cador, M. (2000) "Nicotine-induced locomotor activity is increased by preexposure of rats to prenatal stress", *Brain Res.* **882**, 196–200.

Koehl, M., Lemaire, V., Mayo, W., Abrous, D.N., Maccari, S., Piazza, P.V., Le Moal, M. and Vallee, M. (2002) "Individual vulnerability to substance abuse and affective disorders: role of early environmental influences", *Neurotoxicity Res.* **4**, 281–296.

Kotch, L.A. and Sulik, K.K. (1992) "Experimental fetal alcohol syndrome: proposed pathogenic basis for a variety of associated facial and brain anomalies", *Am. J. Med. Genet.* **44**, 168–176.

Krystal, J.H., D'Souza, D.C., Madonick, S. and Petrakis, I.L. (1999) "Toward a rational pharmacotherapy of comorbid substance abuse in schizophrenic patients", *Schizophr. Res.* **35**, S35–S49.

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

Lieber, C.S. (1988) "Biochemical and molecular basis of alcohol-induced injury to liver and other tissues", *N. Engl. J. Med.* **319**, 1639–1650.

Luo, J. and Miller, M.W. (1999) "Platelet-derived growth factor-mediated signal transduction underlying astrocyte proliferation: site of ethanol action", *J. Neurosci.* **19**, 10014–10025.

Luthman, J., Fredriksson, A., Lewander, T., Jonsson, G. and Archer, T. (1989) "Effects of *d*-amphetamine and methylphenidate on hyperactivity produced by neonatal 6-hydroxydopamine treatment", *Psychopharmacology* **99**, 550–557.

Luthman, J., Fredriksson, A., Plaznik, A. and Archer, T. (1991) "Ketanserin or mianserin treatment reverses hyperactivity in neonatally dopamine lesioned rats", *J. Psychopharmacol.* **5**, 418–425.

Luthman, J., Bassen, M., Fredriksson, A. and Archer, T. (1997) "Functional changes induced by neonatal 6-hydroxydopamine lesions: effects of dose levels on behavioural parameters", *Behav. Brain Res.* **82**, 213–221.

Maccari, S., Piazza, P.V., Kabbaj, M., Barbazanges, A., Simon, H. and Le Moal, M. (1995) "Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress", *J. Neurosci.* **15**, 110–116.

Maier, S.E., Chen, W.J. and West, J.R. (1996) "Prenatal binge-like alcohol exposure alters neurochemical profiles in fetal rat brain", *Pharmacol. Biochem. Behav.* **55**, 521–529.

Mailleux, P. and Vanderhaeghen, J.J. (1992a) "Localization of cannabinoid receptor in the human developing and adult basal ganglia. Higher levels in the striatonigral neurons", *Neurosci. Lett.* **148**, 173–176.

Mailleux, P. and Vanderhaeghen, J.J. (1992b) "Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and *in situ* hybridation histochemistry", *Neuroscience* **48**, 655–668.

Mallet, P.E. and Beninger, R.J. (1996) "The endogenous cannabinoid receptor agonist anandamide impairs memory in rats", *Behav. Pharmacol.* **7**, 276–284.

Mao, J., Price, D.D., Phillips, L.L., Lu, J. and Mayer, D.J. (1995) "Increases in protein kinase C immunoreactivity in the spinal cord of rats associated with tolerance to the analgesic effects of morphine", *Brain Res.* **677**, 257–267.

Marcus, P. and Snyder, R. (1995) "Reduction of comorbid substance abuse with clozapine", *Am. J. Psychiatry* **152**, 959.

Markou, A. and Kenny, P.J. (2002) "Neuroadaptations to chronic exposure to drugs of abuse: relevance to depressive symptomatology seen across psychiatric diagnostic categories", *Neurotoxicity Res.* **4**, 297–313.

Masterson, E. and O'Shea, B. (1984) "Smoking and malignancy in schizophrenia", *Br. J. Psychiatry* **145**, 429–432.

Miller, M.W. (1992) "The effects of prenatal exposure to ethanol on cell proliferation and neuronal migration", In: Miller, M., ed, Developmental of the Central Nervous System: Effects of Alcohol and Opiates (Liss, New York), pp 47–69.

Miller, M.W. (1995) "Effect of pre- or postnatal exposure to ethanol on the total number of neurons in the principal sensory nucleus of the trigeminal nerve: cell proliferation and neuronal death", *Alcohol. Clin. Exp. Res.* **19**, 1359–1363.

Miñana, R., Climent, E., Barettino, D., Segui, J.M., Renau-Piqueras, J. and Guerri, C. (2000) "Alcohol exposure alters the expression pattern of neural cell adhesion molecules during brain development", *J. Neurochem.* **75**, 954–964.

Mokler, D.A., Robinson, S.E., Johnson, J.H., Hong, J.S. and Rosecrans, J.A. (1987) "Neonatal administration of Δ^9-tetrahydrocannabinol alters the neurochemical response to stress in the adult Fischer-344 rat", *Neurotoxicol. Teratol.* **9**, 321–326.

Molina-Holgado, F., Amaro, A., Gonzalez, M.I., Alvarez, F.J. and Leret, M.L. (1996) "Effect of maternal delta 9-tetrahydrocannabinol on developing serotonergic system", *Eur. J. Pharmacol.* **316**, 39–42.

Molina-Holgado, F., Alvarez, F.J., González, I., Antonio, M.T. and Leret, M.L. (1997) "Maternal exposure to Δ^9-tetrahydrocannabinol (Δ^9-THC) alters indolamine levels and turnover in adult male and female rat brain regions", *Brain Res. Bull.* **43**, 173–178.

Montoliu, C., Sancho-Tello, M., Azorin, I., Burgal, M., Valles, S., Renau-Piqueras, J. and Guerri, C. (1995) "Ethanol increases cytochrome P4502E1 and induces oxidative stress in astrocytes", *J. Neurochem.* **65**, 2561–2570.

Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B. and Seeburg, P.H. (1994) "Developmental and regional expression in the rat brain and functional properties of four NMDA receptors", *Neuron* **12**, 529–540.

Morinan, A. (1987) "Reduction in striatal 5-hydroxytryptamine turnover following chronic administration of ethanol to rats", *Alcohol Alcohol.* **22**, 53–60.

Myers, R.D. and Veale, W.L. (1968) "Alcohol preference in the rat: reduction following depletion of brain serotonin", *Science* **160**, 1469–1471.

Naranjo, C.A., Chu, A. and Tremblay, Y. (2002) "Neurodevelopmental liabilities in alcohol dependence: central serotonin and dopamine dysfunctions", *Neurotoxicity Res.* **4**, 343–361.

Navarro, M., Rodríguez, F., Hernández, M.L., Ramos, J.A. and Fernández, J.J. (1994) "Motor behavior and nigrostriatal dopaminergic activity in adult rats perinatally exposed to cannabinoids", *Pharmacol. Biochem. Behav.* **47**, 47–58.

Nunes, E.V., McGrath, P.J., Quitkin, F.M., Ocepek-Welikson, K., Stewart, J.W., Koenig, T., Wager, S. and Klein, D.F. (1995) "Imipramine treatment of cocaine abuse: possible boundaries of efficacy", *Drug Alcohol. Depend.* **39**, 185–195.

Olincy, A., Young, D.A. and Freedman, R. (1997) "Increased levels of the nicotine metabolite cotinine in schizophrenic smokers compared to other smokers", *Biol. Psychiatry* **42**, 1–5.

Olive, M.F., Mehmert, K.K., Messing, R.O. and Hodge, C.W. (2000) "Reduced operant ethanol self-administration and *in vivo* mesolimbic dopamine responses to ethanol in PKCepsilon-deficient mice", *Eur. J. Neurosci.* **12**, 4131–4140.

Olney, J.W., Labruyere, J., Wang, G., Wozniak, D.F., Price, M.T. and Sesma, M.A. (1991) "NMDA antagonist neurotoxicity: mechanism and prevention", *Science* **254**, 1515–1518.

Olney, J.W., Wozniak, D.F., Jevtovic-Todorovic, V., Farber, N.B. and Ikonomidou, C. (2002) "Glutamate and GABA receptor dysfunction in the fetal alcohol syndrome", *Neurotoxicity Res.* **4**, 315–325.

Pagel, M.D., Smilkstein, G., Regen, H. and Montano, D. (1990) "Psychosocial influences on new born outcomes: a controlled prospective study", *Soc. Sci. Med.* **30**, 597–604.

Parsons, L.H., Koob, G.F. and Weiss, F. (1995) "Serotonin dysfunction in the nucleus accumbens of rats during withdrawal after unlimited access to intravenous cocaine", *J. Pharmacol. Exp. Ther.* **274**, 1182–1191.

Perez-Rosado, A., Gomez, M., Manzanares, J., Ramos, J.A. and Fernandez-Ruiz, J. (2002) "Changes in prodynorphin and POMC gene expression in several brain regions of rat fetuses prenatally exposed to Δ^9-tetrahydrocannabinol", *Neurotoxicity Res.* **4**, 211–218.

Persico, A.M., Mengual, E., Moessner, R., Hall, F.S., Revay, R.S., Sora, I., Arellano, J., DeFelipe, J., Gimene-Amaya, J.M., Conciatori, M., Marino, R., Baldi, A., Cabib, S., Pascucci, T., Uhl, G.R., Murphy, D.L., Lesch, K.P., Keller, F. and Hall, S.F. (2001) "Barrel pattern formation requires serotonin uptake by thalamocortical afferents, and not vesicular monoamine releae", *J. Neurosci.* **21**, 6862–6873.

Peterson, D.J., Trujillo, K.A. (2001) The NMDA receptor antagonist memantine inhibits morphine-induced behavioral and neural plasticity. Presented by Trujillo's team at College on Problems of Drug Dependence Annual Meeting, Scottsdale, AZ.

Piazza, P.V., Rouge-Pont, F., Deminiere, J.M., Kharoubi, M., Le Moal, M. and Simon, H. (1991) "Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration", *Brain Res.* **567**, 169–174.

Poulter, M.O., Barker, J.L., O'Carroll, A.M., Lolait, S.J. and Mahan, L.C. (1993) "Co-existent expression of GABAA receptor beta 2, beta 3 and gamma 2 subunit messenger RNAs during embryogenesis and early postnatal development of the rat central nervous system", *Neuroscience* **53**, 1019–1033.

Ramanathan, R., Wilkemeyer, M.F., Mittal, B., Perides, G. and Charness, M.E. (1996) "Alcohol inhibits cell–cell adhesion mediated by human L1", *J. Cell Biol.* **133**, 381–390.

Ramos, J.A., de Miguel, R., Cebeira, M., Hernandez, M. and Fernandez-Ruiz, J. (2002) "Exposure to cannabinoids in the development of endogenous cannabinoid system", *Neurotoxicity Res.* **4**, 363–372.

Rausch, J.L., Monteiro, M.G. and Schuckit, M.A. (1991) "Platelet serotonin uptake in men with family histories of alcoholism", *Neuropsychopharmacology* **4**, 83–86.

Regan, R.F. and Guo, Y. (1997) "Estrogens attenuate neuronal injury due to hemoglobin, chemical hypoxia, and excitatory amino acids in murine cortical cultures", *Brain Res.* **764**, 133–140.

Rifas, L., Towler, D.A. and Avioli, L.V. (1997) "Gestational exposure to ethanol suppresses msx2 expression in developing mouse embryos", *Proc. Natl Acad. Sci. USA* **94**, 7549–7554.

Robinson, D., Mayerhoff, D., Alvir, J., Cooper, T. and Lieberman, J. (1991) "Mood responses of remitted schizophrenics to methylphenidate infusion", *Psychopharmacology* **105**, 247–252.

Rodríguez de Fonseca, F., Cebeira, M., Fernández-Ruiz, J.J., Navarro, M. and Ramos, J.A. (1991) "Effects of pre- and perinatal exposure to hashish extracts on the ontogeny of brain dopaminergic neurons", *Neuroscience* **43**, 713–723.

Rosenthal, N.E., Davenport, Y., Cowdry, R.W., Webster, M.H. and Goodwin, F.K. (1980) "Monoamine metabolites in cerebrospinal fluid of depressive subgroups", *Psychiatry Res.* **2**, 113–119.

Ross, S.A., McCaffery, P.J., Drager, U.C. and De Luca, L.M. (2000) "Retinoids in embryonal development", *Physiol. Rev.* **80**, 1021–1054.

Sari, Y., Powrozek, T. and Zhou, F.C. (2001) "Alcohol deters the outgrowth of serotonergic neurons at midgestation", *J. Biomed. Sci.* **8**, 119–125.

Schneier, F.R. and Siris, S.G. (1987) "A review of psychoactive substance use and abuse in schizophrenia: patterns of drug choice", *J. Nerv. Ment. Dis.* **175**, 641–652.

Schuckit, M.A., Gold, E. and Risch, C. (1987a) "Plasma cortisol levels following ethanol in sons of alcoholics and controls", *Arch. Gen. Psychiatry* **44**, 942–945.

Schuckit, M.A., Gold, E. and Risch, C. (1987b) "Serum prolactin levels in sons of alcoholics and control subjects", *Am. J. Psychiatry* **144**, 854–859.

Shivachar, A.C., Martin, B.R. and Ellis, E.F. (1996) "Anandamide and Δ^9-tetrahydrocannabinol-evoked arachidonic acid mobilization and blockade by SR141716A", *Biochem. Pharmacol.* **51**, 669–676.

Simpkins, J.W., Rajakumar, G., Zhang, Y.Q., Simpkins, C.E., Greenwald, D., Yu, C.J., Bodor, N. and Day, A.L. (1997) "Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat", *J. Neurosurg.* **87**, 724–730.

Singer, C.A., Rogers, K.L., Strickland, T.M. and Dorsa, D.M. (1996) "Estrogen protects primary cortical neurons from glutamate toxicity", *Neurosci. Lett.* **212**, 13–16.

Singer, C.A., Rogers, K.L. and Dorsa, D.M. (1998) "Modulation of Bcl-2 expression: a potential component of estrogen protection in NT2 neurons", *NeuroReport* **9**, 2565–2568.

Sjötrom, K., Valentin, L., Thelin, T. and Marsal, K. (1997) "Maternal anxiety in late pregnancy and fetal hemodynamics", *Eur. J. Obstet. Gynecol. Reprod. Biol.* **74**, 149–155.

Stone, D.J., Rozovsky, I., Morgan, T.E., Anderson, C.P., Hajain, H. and Finch, C.E. (1997) "Astrocytes and microglia respond to estrogen with increased apoE mRNA *in vivo* and *in vitro*", *Exp. Neurol.* **143**, 313–318.

Thanos, P.K., Volkow, N.D., Freimuth, P., Umegaki, H., Ikari, H., Roth, G., Ingram, D.K. and Hitzemann, R. (2001) "Overexpression of dopamine D_2 receptors reduces alcohol self-administration", *J. Neurochem.* **78**, 1094–1103.

Trujillo, K.A. (2002) "The neurobiology of opiate tolerance, dependence and sensitization: mechanisms of NMDA receptor-dependent synaptic plasticity", *Neurotoxicity Res.* **4**, 373–391.

Trujillo, K.A. and Akil, H. (1990) "Behavioral interactions between morphine and MK-801: analgesia, tolerance, dependence and lethality", *Soc. Neurosci. Abstr.* **16**, 211.

Trujillo, K.A. and Akil, H. (1991) "Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801", *Science* **251**, 85–87.

Trujillo, K.A., Warmoth, K.P., Peterson, D.J., Albertson, D.N., Watorski, K. and Swadley-Lewellen, R.M. (2001) "NMDA receptor antagonists inhibit the development of tolerance and sensitisation to the locomotor effects of opiates", *Soc. Neurosci. Abstr.*, 27.

Vallés, S., Lindo, L., Montoliu, C., Renau-Piqueras, J. and Guerri, C. (1994) "Prenatal exposure to ethanol induces changes in the nerve growth factor and its receptor in proliferating astrocytes in primary culture", *Brain Res.* **656**, 281–286.

Vallés, S., Felipo, V., Montoliu, C. and Guerri, C. (1995) "Alcohol exposure during brain development reduces ^{3}H-MK-801 binding and enhances metabotropic-glutamate receptor-stimulated phosphoinositide hydrolysis in rat hippocampus", *Life Sci.* **56**, 1373–1383.

Vallés, S., Sancho-Tello, M., Miñana, R., Climent, E., Renau-Piqueras, J. and Guerri, C. (1996) "Glial fibrillary acidic protein expression in rat brain and in radial glia culture is delayed by prenatal ethanol exposure", *J. Neurochem.* **67**, 2425–2433.

Vela, G., Fuentes, J.A., Bonnin, A., Fernández-Ruiz, J.J. and Ruiz-Gayo, M. (1995) "Perinatal exposure to Δ^9-tetrahydrocannabinol (Δ^9-THC). leads to changes in opioid-related behavioral patterns in rats", *Brain Res.* **680**, 142–147.

Vela, G., Martín, S., García-Gil, L., Crespo, J.A., Ruiz-Gayo, M., Fernández-Ruiz, J.J., García- Lecumberri, C., Pelaprat, D., Fuentes, J.A., Ramos, J.A. and Ambrosio, E. (1998) "Maternal exposure to Δ^9-tetrahydrocannabinol facilitates morphine self-administration behavior and changes μ-opioid receptor binding in adult offspring female rats", *Brain Res.* **807**, 101–109.

Virkkunen, M., Kallio, E., Rawlings, R., Tokola, R., Poland, R.E., Guidotti, A., Nemeroff, C., Bissette, G., Kalogeras, K., Karonen, S.L. and Linnoila, M. (1994) "Personality profiles and state aggressiveness in Finnish alcoholic, violent offenders, fire setters, and healthy volunteers", *Arch. Gen. Psychiatry* **51**, 28–33.

Waddington, W.W., Brown, B.S., Haertzen, C.A., Cone, E.J., Dax, E.M., Herning, R.I. and Michaelson, B.S. (1990) "Changes in mood, craving, and sleep during short-term abstinence reported by male cocaine addicts: a controlled, residential study", *Arch. Gen. Psychiatry* **47**, 861–868.

Wang, J.Y., Shum, A.Y., Lin, T.C. and Wang, Y. (1996) "Central serotonergic lesions increase voluntary alcohol consumption in Sprague Dawley rats: moderation by long-term ethanol administration", *Alcohol. Clin. Exp. Res.* **20**, 1252–1259.

Webster, W.S., Walsh, D.A., McEwen, S.E. and Lipson, A.H. (1983) "Some teratogenic properties of ethanol and acetaldehyde in C57BL/6J mice: Implications for the study of the fetal alcohol syndrome", *Teratology* **27**, 231–243.

Weiss, F., Markou, A., Lorang, M.T. and Koob, G.F. (1992) "Basal dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration", *Brain Res.* **593**, 314–318.

West, R. and Gossop, M. (1994) "Overview: a comparison of withdrawal symptoms from different drug classes", *Addiction* **89**, 1483–1489.

West, R.J., Jarvis, M.J., Russell, M.A.H., Carruthers, M.E. and Feyerabend, C. (1984) "Effect of nicotine replacement on the cigarette withdrawal syndrome", *Br. J. Addict.* **79**, 215–219.

Whitaker-Azmitia, P.M., Druse, M., Walker, P. and Lauder, J.M. (1996) "Serotonin as a developmental signal", *Behav. Brain Res.* **73**, 19–29.

Wolf, M.E. and Jeziorski, M. (1993) "Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than

transiently masks the development of behavioral sensitization", *Brain Res.* **613**, 291–294.

Zafar, H., Shelat, S.G., Redei, E. and Tejani-Butt, S. (2000) "Fetal alcohol exposure alters serotonin transporter sites in rat brain", *Brain Res.* **856**, 184–192.

Zaulyanov, L.L., Green, P.S. and Simpkins, J.W. (1999) "Glutamate receptor requirement for neuronal cell death from anoxia-reoxygenation: an *in vitro* model for assessment of the neuroprotective effects of estrogens", *Cell. Mol. Neurobiol.* **19**, 705–718.

Zhou, F.C., Bledsoe, S., Lumeng, L. and Li, T.K. (1991) "Immunostained serotonergic fibers are decreased in selected brain regions of alcohol-preferring rats", *Alcohol* **8**, 425–431.

Zhou, F.C., Sari, Y., Zhang, J.K., Goodlett, C.R. and Li, T.-K. (2001) "Prenatal alcohol exposure retards the migration and development of serotonin neurons in fetal C57BL mice", *Brain Res. Dev. Brain Res.* **126**, 147–155.

Zhou, F.C., Sari, Y., Li, T.-K., Goodlett, C. and Azmitia, E.C. (2002) "Deviations in brain early serotonergic deelopment as a result of fetal alcohol exposure", *Neurotoxicity Res.* **4**, 337–342.

Zhu, H. and Ho, I.K. (1998) "NMDA-R1 antisense oligonucleotide attenuates withdrawal signs from morphine", *Eur. J. Pharmacol.* **352**, 151–156.

Ziedonis, D.M. and Kosten, T.R. (1991) "Depression as a prognostic factor for pharmacological treatment of cocaine dependence", *Psychopharmacol. Bull.* **27**, 337–343.

Zimmet, S.V., Strous, R.D., Burgess, E.S., Kohnstamm, S. and Green, A.I. (2000) "Effects of clozapine on substance use in patients with schizophrenia and schizoaffective disorder: a retrospective survey", *J. Clin. Pharmacol.* **20**, 94–98.