The 1998 ASPET Otto Krayer Award Lecture

Fetal Nicotine or Cocaine Exposure: Which One is Worse?[1]

THEODORE A. SLOTKIN

Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina

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ABSTRACT

Despite extensive adverse publicity, tobacco use continues in approximately 25% of all pregnancies in the United States, overshadowing illicit drugs of abuse, including cocaine. The societal cost of maternal smoking is seen most readily in underweight newborns, in high rates of perinatal morbidity, mortality and Sudden Infant Death Syndrome and in persistent deficits in learning and behavior. We have designed animal models of nicotine exposure to prove that nicotine itself is a neuroteratogen, thus providing a causative link between tobacco exposure and adverse perinatal outcomes. In particular, nicotine infusion paradigms that, like the transdermal patch used in man, produce drug exposure without the confounds of other components of tobacco or of episodic hypoxic-ischemic insult, have enabled a mechanistic dissection of the role played by nicotine in fetal brain damage. Nicotine targets specific neurotransmitter receptors in the fetal brain, eliciting abnormalities of cell proliferation and differentiation, leading to shortfalls in the number of cells and eventually to altered synaptic activity. Because of the close regulatory association of cholinergic and catecholaminergic systems, adverse effects of nicotine involve multiple transmitter pathways and influence not only the immediate developmental events in fetal brain, but also the eventual programming of synaptic competence. Accordingly, defects may appear after a prolonged period of apparent normality, leading to cognitive and learning defects that appear in childhood or adolescence. Comparable alterations occur in peripheral autonomic pathways, leading to increased susceptibility to hypoxia-induced brain damage, perinatal mortality and Sudden Infant Death. Identifying the receptor-driven mechanisms that underlie the neurobehavioral damage caused by fetal nicotine exposure provides a rational basis for decisions about nicotine substitution therapy for smoking cessation in pregnancy. In contrast to the effects of nicotine, animal models of crack cocaine use in pregnancy indicate a more restricted spectrum of effects, a reflection of differences both in pharmacokinetics and pharmacodynamics of the two drugs. Notably, although cocaine, like nicotine, also targets cell replication, its effects are short-lived, permitting recovery to occur in between doses, so that the eventual consequences are much less severe. To some extent, the effects of cocaine on brain development resemble those of nicotine because the two share cardiovascular actions (vasoconstriction) that, under some circumstances, elicit fetal hypoxia-ischemia. In light of the fact that nearly all crack cocaine users smoke cigarettes, the identification of specific developmental effects of cocaine may prove difficult to detect. Although scientists and the public continue to pay far more attention to fetal cocaine effects than to those of nicotine or tobacco use, a change of focus to concentrate on tobacco could have a disproportionately larger impact on human health.

A Tribute to Otto Krayer

Throughout the course of 40 years, Otto Krayer led a distinguished scientific career on three continents. Perhaps more importantly, he combined his scientific accomplishments with exemplary strength of character, at great cost to his professional and personal life. Awarded the M.D. degree from the University of Freiburg in 1926, Krayer became Professor Extraordinarius at the University of Berlin in 1932. Despite his noteworthy contributions to the German scientific community, the subsequent years found him in London and Beirut, before he was appointed to the faculty at Harvard University in 1937. He then remained at Harvard, serving as department chairman from 1939 to 1966, and as ASPET president in 1957. His ground-breaking research in cardiovascular pharmacology led to his being named the first recipient of the Torald Sollman Award in 1961. However, it is worth examining the curious turn of events that led him to this country. In modest fashion, Krayer himself described the events in his Sollman Award Address as follows: "In 1933, when the German universities had ceased to tolerate freedom

ABBREVIATION: SIDS, Sudden Infant Death Syndrome; CNS, central nervous system.

of expression, an invitation from the Department of Pharmacology of University College, London, brought me the privilege of working with one of Starling's pupils, E.B. Verney." What he did not say was that his departure from Germany was triggered by his refusal to accept the Chair of Pharmacology in Düsseldorf, a position that had been opened by the forced expulsion of a Jewish scientist. At that time, Krayer, at considerable personal risk, wrote a letter to the Minister of Education, saying, "The exclusion of Jewish scientists [is] an injustice, the necessity of which I cannot understand. . . The work to which I have heretofore dedicated all my strength, means so much to me that I could not compromise it with the least bit of dishonesty. I therefore prefer to forego this appointment, rather than having to betray my convictions." As a consequence, he was dismissed from his professorship and banned from accepting any positions at German universities.

In light of these events, as well as his scientific accomplishments, I feel deeply honored to receive the award named for Otto Krayer, and I thank ASPET, the award selection committee and Zeneca Pharmaceuticals for the privilege of delivering this address. It must be remembered that any scientific effort requires the participation of many individuals, and I am indebted to the many outstanding students and fellows who have worked with me during the past three decades, and especially to Dr. Frederic Seidler, who has assisted me throughout most of that period. Our research has focused on the pharmacology and toxicology of nervous system development, most especially on the trophic roles of neurotransmitters and neuroactive drugs, the development of autonomic function and its role in fetal and neonatal homeostasis and the adverse effects of environmental contaminants and drugs of abuse. For this presentation, I will concentrate on the aspect of our work that has had the most visible impact on human health, the characterization of fetal nicotine exposure and its relationship to smoking in pregnancy.

Unique Developmental Roles of Neurotransmitters

It is more than 30 years since Buznikov first reported that a wide variety of neurotransmitters could be identified and found in high concentration in developing sea urchin embryos (Buznikov et al., 1964, 1970). Since that time, numerous investigations have characterized the transient appearance of these substances in developing organisms and most prominently during ontogeny of the mammalian nervous system. The phenotypic expression of the ability to manufacture neurotransmitters or their receptors is a common feature of developing cells and even occurs in nonneuronal cells, in which it is now clear that transmitter chemicals play roles outside the realm of classical synaptic communication (Lauder, 1985; Whitaker-Azmitia, 1991). The basic difference between developing and nondeveloping systems is that, in the latter, input to a target cell elicits a short-term response and continued stimulation elicits compensatory adjustments that offset stimulation (desensitization or receptor downregulation). During development, however, receptor stimulation uniquely communicates with the genes that control cell differentiation, changing the ultimate fate of the cell, so that there are permanent alterations in responsiveness (fig. 1). Because these types of changes are not characteristic of the mature nervous system, it is evident that the ontogenetic
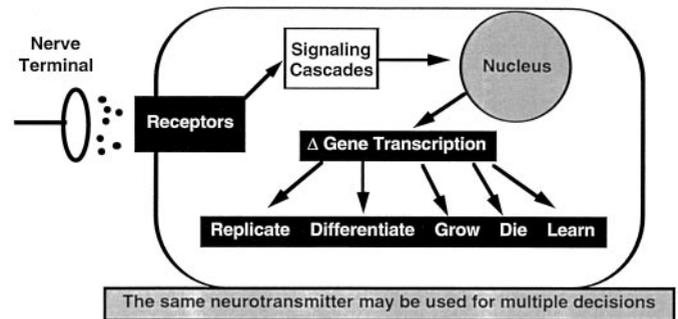
Fig. 1. Schematic representation of the linkage of neurotransmitters to cell development. During specified critical periods, neurotransmitter receptors and their associated signaling cascades can elicit changes in gene expression that influence differentiation. Depending on the context in which stimulation occurs, the same neurotransmitter, operating through the same receptors, can promote cell replication, can elicit a switch from replication to differentiation, can promote or arrest cell growth, can evoke apoptosis or can program the genes that determine the future responsiveness of the cell to external stimulation (cell learning).

state of the target cells is pivotal in determining the outcome. Accordingly, the same transmitter or receptor may be involved in cell replication, in termination of replication and initiation of differentiation, in cell growth, in cell death (apoptosis) or in determination of the ability of the cell to respond to future stimulation (cell learning). As just one example, small amounts of norepinephrine, acting at *beta* adrenergic receptors in fetal and early neonatal rat tissues, serve to promote cell acquisition (Slotkin et al., 1988a, b; Duncan et al., 1990; Renick et al., 1997). Later on, much higher levels of stimulation, operating through the same *beta* receptors, serve to terminate cell replication and to determine the set-point for adrenergic reactivity that will persist into adulthood (Slotkin et al., 1987d, 1988c; Hou et al., 1989a, b; Duncan et al., 1990; Wagner et al., 1991, 1994, 1995). It is thus not just the transmitter and receptor, but also the developmental context in which stimulation occurs, that determine the net effect on the fate of the target cell.

The case of fetal nicotine exposure thus occupies two different frameworks. First, nicotine stimulates a specific population of cholinergic target sites, the nicotinic cholinergic receptors, and thus can be expected to recapitulate many of the roles of acetylcholine as a neurotrophic factor (Hohmann et al., 1988; Navarro et al., 1989a; Spitzer, 1991; Wessler et al., 1998). However, nicotine is a drug of abuse and thus fetal exposures can be expected to involve levels of stimulation beyond those experienced in the course of normal development, and equally important, will involve exposure outside of the proper ontogenetic context. Before describing our work on these effects and their underlying mechanisms, it is worthwhile to review the societal impact of nicotine as a drug of abuse in pregnancy.

Smoking and Pregnancy

Despite decades of adverse publicity, approximately one-fourth of all women in the United States continue to smoke during pregnancy (Bardy et al., 1993; DiFranza and Lew, 1995). Potentially encouraging news, such as a recent lay press account that smoking during pregnancy had been cut

in half, is deceptive because of reliance on self-reportage of cigarette use, which is notoriously unreliable. Controlled examinations of plasma, urine or hair markers of smoking demonstrate clearly that most pregnant smokers do not quit (Bardy *et al.*, 1993). The consequences have been well identified in epidemiological studies: tens of thousands of spontaneous abortions and neonatal intensive care unit admissions annually, thousands of perinatal deaths and deaths from Sudden Infant Death Syndrome (SIDS) and substantially increased risk of learning disabilities, behavioral problems and attention deficit and hyperactivity disorder (Butler and Goldstein, 1973; Dunn and McBurney, 1977; Naeye, 1978, 1992; Naeye and Peters, 1984; Bell and Lau, 1995; DiFranza and Lew, 1995). Nevertheless, both the press and the medical community continue to regard tobacco as separate from, and less serious than, illicit drugs of abuse. A survey of the most prominent medical pharmacology texts in use in the United States showed that more than 80% of the pages devoted to substance abuse concerned illicit drugs, just more than 10% concerned alcohol and less than 5% concerned tobacco (Ginzel, 1985). In contrast, illicit drugs account for only a handful of deaths annually, alcohol 50,000 and tobacco 400,000. Public and medical bias against considering tobacco as equivalent in importance to illicit drugs continues into the realm of studies of development. Headlines concentrate on "crack baby" syndrome, a condition for which there is no current medical consensus of opinion (Coles, 1993; Day and Richardson, 1993), whereas tobacco use during pregnancy receives little or no attention. The same bias can be demonstrated simply by examining the annual publication rate for papers on fetal or neonatal development, comparing cocaine with nicotine (fig. 2). Cocaine is the subject of three to four times the number of papers as nicotine. Indeed, a recent study showed that public perception of the adverse effects of cocaine in pregnancy goes far beyond the actual potential for fetal damage; many physicians think that cocaine is an outright teratogen akin to thalidomide and have counseled abortion even when the patient does not seek one (Koren *et al.*, 1992). In actuality, cocaine is less likely to cause malformations than is cigarette smoking (Koren, 1993; Neuspiel, 1993). We also have been barraged by alarming figures for cocaine use in pregnancy, typically listed as high as 20% of all pregnancies for urban hospitals (Spear and Heyser, 1992). However, when blind screenings are conducted at private

clinics, the rate becomes vanishingly small (Burke and Roth, 1993). The realities are that cigarette smoking continues to involve a much larger population than does cocaine, and furthermore, that nearly all crack users smoke cigarettes (Budney *et al.*, 1993; Higgins *et al.*, 1994), so that purported effects of cocaine must be examined in comparison with smokers, not with substance-free subjects. It is thus necessary to perform controlled studies to answer the essential question of whether the effects of fetal nicotine exposure are worse than those of cocaine.

Designing an Animal Model of Fetal Nicotine Exposure

Given the adverse effects of smoking in pregnancy, why do we need to perform animal studies to isolate the specific effects of nicotine? There are three important issues that can be resolved only with animal research. First, what is it about smoking that injures the developing organism? As shown in figure 3, maternal smoking produces three different families of potential effects: actions of nicotine in the fetus, whether on brain development or general development; actions on the maternal-fetal unit, including episodic hypoxia-ischemia, exposure to other toxic smoke products (notably CO and HCN) (Mactutus and Fechter, 1986) and dietary restriction from the anorexic effects on the mother; and the epiphenomena of smoking, namely risky behaviors, co-abuse of other substances, poor prenatal care and low socioeconomic status. To assign a specific, deleterious role for smoking itself, studies must be designed that eliminate the potential covariables of smoking that can themselves influence perinatal outcome. The second reason to study the specific effects of nicotine concerns the now-common use of this drug for smoking cessation. If nicotine itself is injurious to the developing organism, then issues of dose, pharmacokinetics and critical period all become essential elements in smoking cessation strategies during pregnancy. Finally, from the basic scientist's viewpoint, a demonstration of nicotine effects on specific processes of cell development, in turn, can help identify how acetylcholine, acting as a neurotrophic chemical during development, controls the fate of its target cells.

In our initial work on this problem, we injected nicotine in pregnant rats throughout gestation, a strategy that had been used in behavioral studies to establish empirically that nicotine could disrupt development (Martin and Becker, 1970, 1971; Nasrat *et al.*, 1986). Although we obtained clear evidence of cell damage in the fetal brain, including compromised development of neurotransmitter systems that could account for the behavioral deficits (Slotkin *et al.*, 1986b,
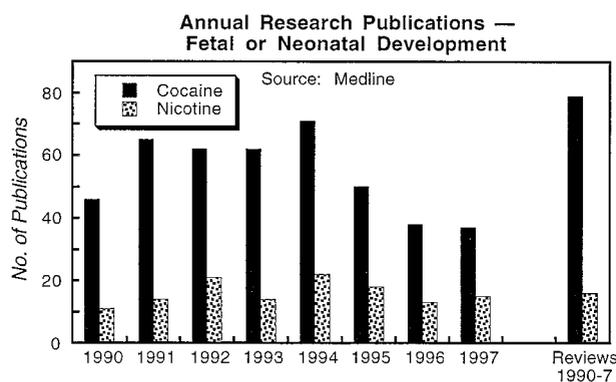
Fig. 2. Annual research publications on cocaine or nicotine and fetal or neonatal development. Yearly totals show primary papers and review articles and the right-hand bars reflect review articles published in the 1990s to date.
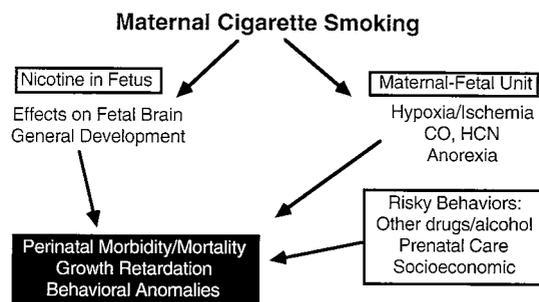

Fig. 3. Variables contributing to adverse perinatal outcomes from maternal cigarette smoking.

1987a, b), it quickly became evident that many of these effects resembled those of episodic hypoxia (Jonsson and Hallman, 1980; Slotkin *et al.*, 1986a; Seidler and Slotkin, 1990; Carlos *et al.*, 1991). This is not surprising, given that nicotine injections produce high peak plasma levels of drug, which induce obvious ischemic episodes (blanching, cyanosis) with each dose (McFarland *et al.*, 1991; Slotkin, 1992). In designing animal models of cigarette smoking, this may not be an unreasonable approach, given that the fetus also experiences hypoxia-ischemia during each cigarette. However, to identify the effects of nicotine itself in the fetus, such models are confounded ineluctably by hypoxia-ischemia (fig. 4). Accordingly, in the mid-1980s we developed the first animal model of fetal nicotine exposure to make use of continuous infusions *via* implantable osmotic minipumps (Slotkin *et al.*, 1987b, c; Navarro *et al.*, 1988, 1989a, b; Slotkin, 1992). By administering nicotine on a continuous basis, we can avoid peak levels that elicit hypoxia-ischemia and can deliver a clearly identifiable, fixed dose of drug akin to that provided by the transdermal nicotine patches used in humans (Murrin *et al.*, 1987; Lichtensteiger *et al.*, 1988). Another advantage of the infusion method is that steady-state plasma levels can be assessed and the dose rate adjusted to simulate human exposure levels. A typical infusion rate of 6 mg/kg/day in rats produces plasma levels at the upper limit of those achieved in heavy smokers, whereas 2 mg/kg/day simulates moderate smoking (Murrin *et al.*, 1987; Lichtensteiger *et al.*, 1988). It also should be noted that there are pharmacodynamic differences between rats and man, with higher doses generally required to elicit the same effects in the rat (Barnes and Eltherington, 1973; Lichtensteiger *et al.*, 1988). Accordingly, levels at the upper range of human exposures are probably the most appropriate in simulating fetal nicotine effects.

With the nicotine infusion model, we performed several studies to demonstrate conclusively that nicotine, without the participation of other confounding factors of smoking, causes fetal resorption and brain cell damage in the offspring (Slotkin *et al.*, 1987b, c; Navarro *et al.*, 1988, 1989b; Slotkin, 1992). A key indicator of cell damage, ornithine decarboxyl-

ase activity, is elevated in the postnatal period throughout neurogenesis and synaptogenesis (fig. 5). Equally important, the effects are present in early-developing regions (cerebral cortex) as well as in regions that undergo neurogenesis much later (cerebellum). Coincidentally with the activation of cell damage markers, deficits appear in regional DNA content; because each brain cell has a single nucleus, reductions in DNA content indicate a diminished cell number. Although cell deficits are apparent in the immediate postpartum period, an unusual feature of nicotine's effects is that cell numbers actually continue to decline during the first 2 weeks postpartum, well after the termination of nicotine exposure. This suggests that nicotine initiates a change in the program for cell development, leading ultimately to cell loss. Recently (Slotkin *et al.*, 1997a), we found that the protooncogene, c-*fos*, is constitutively activated in postnatal brain regions after prenatal nicotine exposure (fig. 6). Constitutive overexpression of c-*fos*, even in an otherwise healthy cell, elicits apoptosis, an effect which stands in distinction from the short-term activation that accompanies increased cell metabolism (Smeyne *et al.*, 1993; Miao and Curran, 1994; Curran and Morgan, 1995; Preston *et al.*, 1996). Indeed, the chronic elevation of c-*fos* caused by fetal nicotine exposure occurs at a time when acute nicotine is unable to increase c-*fos* activity (Slotkin *et al.*, 1997a). It is therefore likely that apoptosis contributes to brain cell loss caused by fetal nicotine exposure. DNA content eventually recovers in some brain regions (Slotkin *et al.*, 1987c), but the catch-up phase occupies the time frame after the closure of neurogenesis and during gliogenesis, which suggests that neurons have been replaced with glia, a typical pattern for neurotoxic compounds (Norton *et al.*, 1992; O'Callaghan, 1993; Guerri and Renau-Piqueras, 1997). The neuronal damage caused by fetal nicotine is thus likely to be irreversible.

The outright loss of cells does not in itself substantiate the existence of neurobehavioral abnormalities, which instead require that alterations ultimately influence synaptic function. Because nicotine works on a specific subset of cholinergic receptors, the most obvious starting point is to examine the effects on cholinergic synaptic function. We and others have found that fetal nicotine exposure up-regulates nicotinic receptor binding sites in the brain, with effects persisting into the early neonatal period (Hagino and Lee, 1985; Slotkin *et al.*, 1987b; Van de Kamp and Collins, 1994). During this phase, cholinergic trophic responses of the targeted cells are correspondingly supersensitive (Slotkin *et al.*, 1991), an effect which continues through early synaptogenesis, when cholinergic input programs structural and functional developmental events (Hohmann *et al.*, 1988; Navarro *et al.*, 1989a). This may provide an underlying mechanism for cell loss as well as synaptic abnormalities. In addition to changing reactivity to cholinergic stimulation, fetal nicotine exposure alters the ontogeny of cholinergic tone as assessed with choline acetyltransferase activity and high-affinity choline uptake (Navarro *et al.*, 1989a; Zahalka *et al.*, 1992). These two biomarkers measure different properties of cholinergic neurons. Choline acetyltransferase is a constitutive component of the nerve terminal, but its activity is not rate-limiting in transmitter synthesis and is not regulated by impulse activity (Cooper *et al.*, 1986). In contrast, the presynaptic, high-affinity choline transporter actually controls acetylcholine biosynthesis and is directly responsive to the rate of
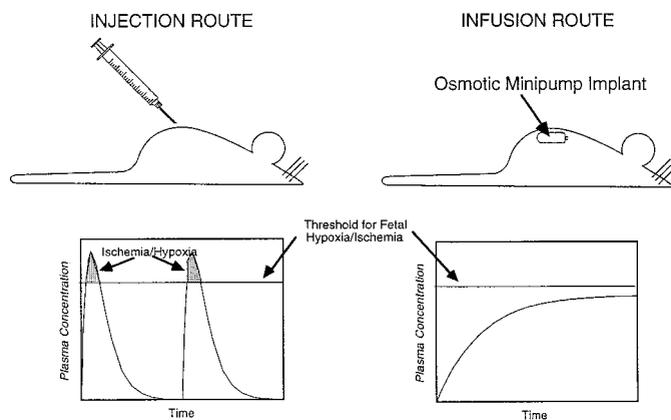
Fig. 4. Two different approaches to animal models of nicotine administration. The injection route produces peak plasma levels that exceed the threshold for fetal hypoxia-ischemia, with episodic recovery in between doses. Similar events occur in cigarette smoking, which superimposes the effects of nicotine on episodic hypoxia-ischemia. The infusion route, *via* minipump implants, produces a steady-state plasma level that can be adjusted to fall below the threshold for fetal hypoxia-ischemia and that can maintain levels found in smokers. Infusions closely resemble human exposures *via* transdermal nicotine patches.
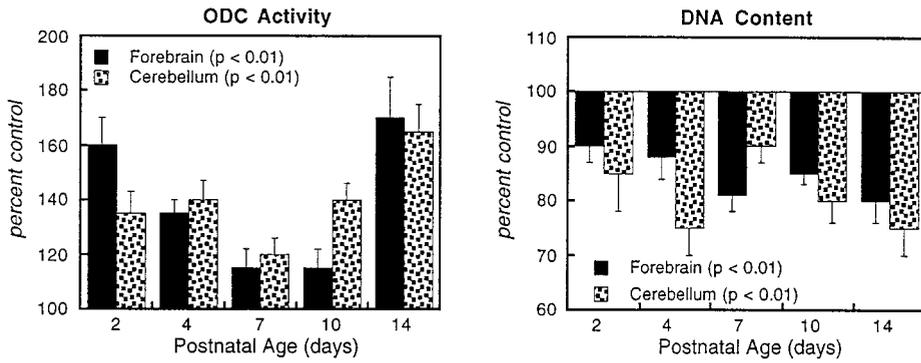
Fig. 5. Effects of nicotine infusions throughout gestation on biomarkers of cell damage (ornithine decarboxylase activity, ODC) and cell number (DNA content) evaluated in postnatal rat brain (Slotkin *et al.*, 1987c). Nicotine exposure elicits persistent damage and cell loss despite discontinuation of nicotine exposure at birth. Effects are discernible in both an early-developing region (forebrain) and late-developing region (cerebellum).
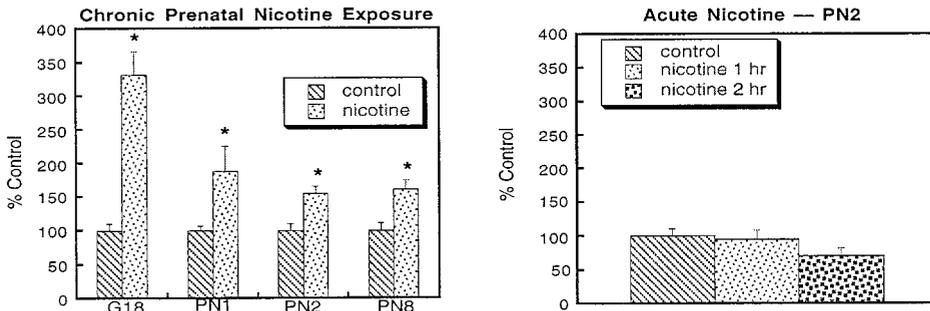

Fig. 6. Persistent elevation of c-*fos* mRNA in the forebrain during and after gestational nicotine exposure, measured on gestational day 18 and during the first postnatal week (Slotkin *et al.*, 1997a). Constitutive c-*fos* overexpression, suggestive of apoptosis, can be detected more than a week after discontinuing nicotine. The persistent effect is distinct from the ability of a single dose of nicotine to elicit short-term metabolic activation of c-*fos*, which has not yet developed by postnatal day 2.

neuronal stimulation (Simon *et al.*, 1976; Klemm and Kuhar, 1979; Murrin, 1980). Taking the ratio of these two markers thus provides an index of the net impulse activity per nerve terminal, an approach that has proved successful in evaluating cholinergic function in developing brain and in neurodegenerative disorders such as Alzheimer's Disease (Shelton *et al.*, 1979; Navarro *et al.*, 1989a; Slotkin *et al.*, 1990b; Zahalka *et al.*, 1992) .
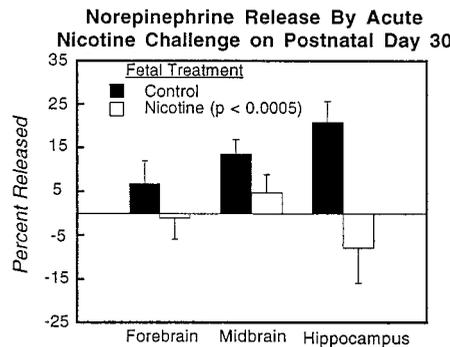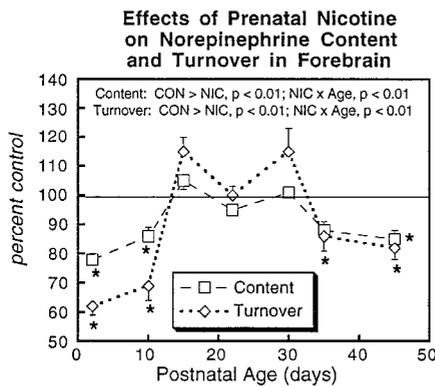
With the ratio method (Navarro *et al.*, 1989a) we found that cholinergic tone does not develop monotonically from low to high activity, but rather that there is a peak of activity centered around postnatal day 10 in the forebrain (fig. 7). In animals exposed to nicotine prenatally, this peak of activity is blunted. Similarly, in the hippocampus, prenatal nicotine elicits lasting deficiencies in choline transporter expression, assessed by binding of the specific transporter radioligand [³H]hemicholinium-3, again indicative of suppressed synaptic activity (Zahalka *et al.*, 1992). These presynaptic defects are compounded further by decreases in postsynaptic cholinoceptive mechanisms (Zahalka *et al.*, 1993). It is thus evident that underlying defects in synaptic function can be identified readily to account for behavioral disruption by fetal nicotine exposure.

Nicotinic cholinergic receptors are involved intimately in the regulation of catecholaminergic function in the CNS, so it is not surprising that noradrenergic and dopaminergic synaptic transmissions also are affected adversely by fetal nicotine exposure (Navarro *et al.*, 1988, 1989b; Seidler *et al.*, 1992b). Just as for cholinergic systems, we found hypoactivity in noradrenergic and dopaminergic projections and although apparent recovery occurs by 3 weeks of age, there is a subsequent, persistent deficit (fig. 8). Just before the reappearance of subnormal tonic activity, acute challenge with nicotine fails to release any neurotransmitter in the prenatal nicotine group, which confirms functional subsensitivity to exogenous stimulation.

We have identified a host of defects in postsynaptic signaling mechanisms evoked by prenatal nicotine exposure, all of which are likely to contribute to adverse behavioral outcomes. These include lasting up-regulation of the expression of adenylyl cyclase, leading to heterologous sensitization of some cell populations to a wide variety of neural, hormonal and trophic inputs (Slotkin *et al.*, 1990a, 1992); specific uncoupling of G-protein mechanisms linking muscarinic and *beta* adrenergic receptors to downstream cellular events (Navarro *et al.*, 1990a; Zahalka *et al.*, 1993); and alterations in
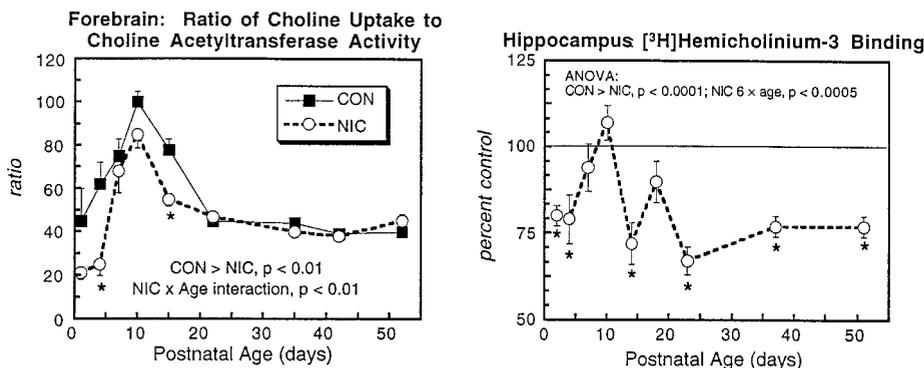
Fig. 7. Cholinergic hypoactivity elicited by prenatal nicotine exposure. In the forebrain, the ratio of choline uptake to choline acetyltransferase activity (a biochemical marker of impulse activity) shows a naturally occurring peak at postnatal day 10; nicotine blunts activity before and during the developmental spike (Navarro *et al.*, 1989a). In the hippocampus, [³H]hemicholinium-3 binding to the high-affinity choline transporter, which is regulated by nerve impulse activity, shows both initial postnatal deficits and a later-emerging, permanent deficit in the nicotine group (Zahalka *et al.*, 1992). CON, control; NIC, nicotine; ANOVA, analysis of variance.

Effects of Prenatal Nicotine on Norepinephrine Content and Turnover in Forebrain

Content: CON > NIC, p < 0.01; NIC x Age, p < 0.01
Turnover: CON > NIC, p < 0.01; NIC x Age, p < 0.01

Norepinephrine Release By Acute Nicotine Challenge on Postnatal Day 30

Fetal Treatment
■ Control
□ Nicotine (p < 0.0005)

Fig. 8. Noradrenergic hypoactivity elicited by prenatal nicotine exposure. Norepinephrine content and turnover are suppressed in the nicotine group, involving both the initial postnatal period, and after a transient recovery to normal, a secondary period of persistent hypoactivity (Navarro *et al.*, 1988). Before the reemergence of deficits in the measures of basal activity, the nicotine group shows a subnormal responsiveness to acute challenges. A single injection of nicotine, which releases norepinephrine in the control group, fails to do so in the nicotine group (Seidler *et al.*, 1992b). CON, control; NIC, nicotine.
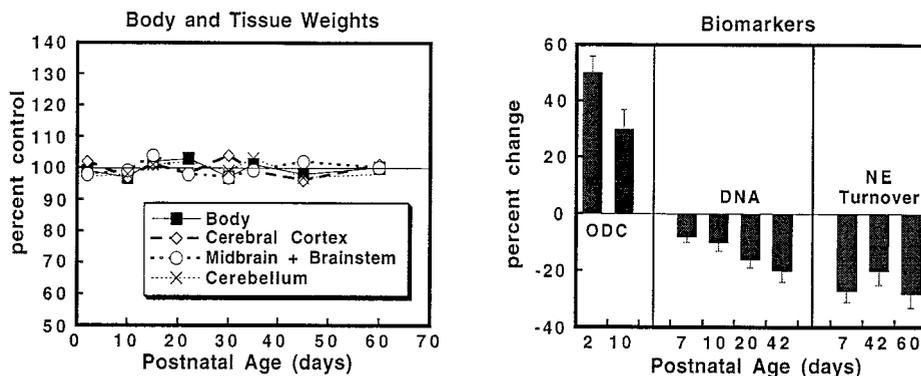
the ontogenetic patterns of cholinergic and catecholaminergic neurotransmitter receptors (Slotkin *et al.*, 1987b, 1990a; Navarro *et al.*, 1990a, b; Zahalka *et al.*, 1993). Developmental disruption by nicotine may thus occupy several different planes, ranging from outright cell loss to specific alterations of neural activity, to misprogramming of signaling molecules. Although it might be feasible to continue describing all these alterations, a more important set of issues concerns whether the basic origin of disrupted development lies in inappropriate nicotinic receptor stimulation in the fetal brain, and whether such effects occur at exposure levels that are otherwise thought to be safe.

Developmental Targeting of Nicotinic Cholinergic Receptors

In sorting out the variables of maternal smoking that contribute to adverse perinatal outcomes, an underlying, receptor-mediated mechanism stands out as an essential feature for the involvement of nicotine. For most standard fetotoxins, brain maturation is maintained at all possible cost to other ontogenetic processes ("brain sparing") (Dodge *et al.*, 1975; de Grauw *et al.*, 1986; Bell *et al.*, 1987). Accordingly, low birth weight is usually considered to be an adequate predictor of potential neural damage, a concept reflected by the Surgeon General's warning that smoking can produce "fetal injury, premature birth, and low birth weight." However, our animal studies indicate otherwise: doses of nicotine that simulate plasma levels found in moderate smokers and that do not cause growth retardation are nevertheless fully capable of affecting brain development as indicated by markers for cell damage and loss and synaptic dysfunction (Navarro *et al.*, 1989b; Seidler *et al.*, 1992b; Zahalka *et al.*, 1992, 1993; Slotkin *et al.*, 1997a) (fig. 9). Behavioral studies confirm that

neurobehavioral teratogenesis by nicotine occurs at doses that are not growth-impairing (Lichtensteiger and Schlumpf, 1985; Ribary and Lichtensteiger, 1989; Levin *et al.*, 1993, 1996; Cutler *et al.*, 1996). There are two corollaries to these findings. First, the standard marker of birth weight is inadequate to predict the presence or absence of brain damage from nicotine, and second, an extremely sensitive process must underlie the exquisite vulnerability of the developing brain to nicotine. This mechanism is obviously the existence of specific receptors for nicotine that respond to pharmacologically relevant concentrations of the drug that are not otherwise fetotoxic. The presence of nicotinic receptors in fetal brain has been well established both biochemically and morphologically (Hagino and Lee, 1985; Larsson *et al.*, 1985; Lichtensteiger *et al.*, 1987; Slotkin *et al.*, 1987b; Cairns and Wonnacott, 1988). Moreover, we have shown that these receptors are tonically stimulated by fetal nicotine exposure as evidenced by receptor up-regulation (Slotkin *et al.*, 1987b). Importantly, dose regimens of nicotine that do not impair growth still fully up-regulate nicotinic receptors in fetal brain, which explains why neuronal effects exhibit a lower threshold than low birth weight (Navarro *et al.*, 1989b) .

To understand how stimulation of nicotinic receptors disrupts target cell development we need to return to the general scheme relating neurotransmitter input to cell fate (fig. 1). In fetal development, one of the most prominent features is cell replication, which generally ceases as differentiation proceeds, events that are controlled partly by neurotransmitter-induced stimulation (Claycomb, 1976; Slotkin *et al.*, 1987a, 1988c; Duncan *et al.*, 1990; McFarland *et al.*, 1991). In our model, then, fetal nicotine exposure results in the premature elicitation of the natural trophic functions of acetylcholine at its target cells, namely the switch from replication

Body and Tissue Weights

—■— Body
— ◇ — Cerebral Cortex
··○·· Midbrain + Brainstem
····✕···· Cerebellum

Biomarkers

ODC
DNA
NE Turnover

Fig. 9. Demonstration that nicotine damages the developing brain at dose levels that do not compromise growth or general development (Navarro *et al.*, 1989b; Seidler *et al.*, 1992b; Slotkin, 1992). Administration of 2 mg/kg/day to pregnant rats, which simulates plasma levels of nicotine found in moderate smokers, results in normal body and brain region weights in the offspring. Nevertheless, cell damage (elevated ornithine decarboxylase activity [ODC]), cell loss (reduced DNA content) and synaptic hypoactivity (subnormal norepinephrine [NE] turnover) are still fully evident.

to differentiation (McFarland *et al.*, 1991). Within 30 min of exposure to a single dose of nicotine, and persisting for several hours, DNA synthesis is inhibited in fetal and neonatal brain regions, with a rank order corresponding to the concentration of nicotinic receptors, namely brainstem ≥ forebrain > cerebellum (fig. 10). The effects are mediated directly by nicotine acting within the brain, as confirmed by direct injection of minute amounts of nicotine directly into the CNS. Furthermore, effects are restricted to the macromolecule synthesis associated with cell replication, because there are no comparable changes in protein synthesis, which is common to both mitotic and postmitotic cells. In keeping with this view, other manipulations that promote cholinergic activity in the fetus, such as dietary choline supplementation, produce similar defects (Bell and Lundberg, 1985; Bell and Slotkin, 1985; Bell *et al.*, 1986a, b). These findings thus reveal a major reason for disruption of brain development by low doses of nicotine, namely the premature stimulation of a receptor-mediated process that normally controls the timing of cell replication and differentiation. Accordingly, there is little doubt that nicotine is a potent neuroteratogen whose actions account in large measure for the adverse effects of maternal cigarette smoking on subsequent behavior and neural performance in the offspring. The conclusion is inescapable that smoking itself, and not the ancillary epiphenomena of smoking, is responsible for tens of thousands of perinatal deaths and for like numbers of infants whose debilities may range from outright brain damage to subtle cognitive defects.

## Nicotine as a Factor in Perinatal Mortality and SIDS

The targeting of nicotinic receptors has consequences for fetal development that extend beyond the central nervous system. Autonomic ganglia and the adrenal medulla also contain nicotinic receptors and in these cells receptor-induced depolarization plays the same role in replication and differentiation as in brain (Black and Geen, 1973; Black *et al.*, 1976; Black and Mytilneou, 1976; Rosenthal and Slotkin, 1977; Bareis and Slotkin, 1978; Cochard *et al.*, 1979; Black, 1980; Lawrence *et al.*, 1981; Seidler and Slotkin, 1986b). We
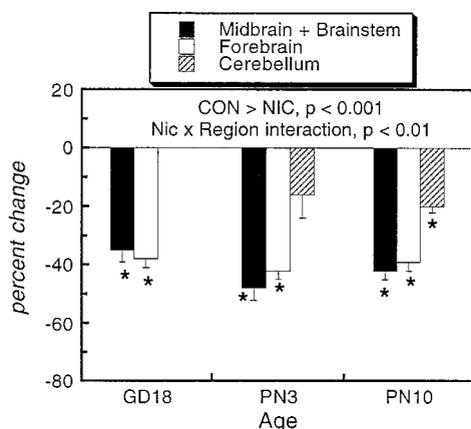
Fig. 10. Inhibition of DNA synthesis in rat brain regions after a single dose of nicotine (McFarland *et al.*, 1991; Slotkin, 1992; Tolson *et al.*, 1995). Measurements of [³H]thymidine incorporation into DNA were made in the first 30 min after nicotine administration. Susceptibility is directly related to the concentration of nicotinic cholinergic receptors in each region, namely brainstem ≥ forebrain > cerebellum. CON, control; NIC, nicotine. ANOVA, analysis of variance; CON, control; NIC, nicotine.

have used this relationship to develop an animal model of perinatal mortality and SIDS that accounts for their strong statistical association with maternal smoking (Slotkin *et al.*, 1995). Defective neonatal cardiovascular and/or respiratory control are postulated to underlie SIDS, with the agonal event precipitated by an acute episode of hypoxia, either from airway obstruction or from an excessive period of sleep apnea (Hunt and Brouillette, 1987; Stramba-Badiale *et al.*, 1992; Poets *et al.*, 1993; Southall *et al.*, 1993). The physiological processes that are called into play during hypoxia are the same as those that accompany the hypoxia experienced during parturition (Seidler and Slotkin, 1985, 1986a, b; Lagercrantz and Slotkin, 1986; Sylvia *et al.*, 1989; Kauffman *et al.*, 1994; Slotkin *et al.*, 1995). In terms of the maintenance of cardiac function, survival during fetal or neonatal hypoxia relies on an unique series of mechanisms centered around autonomous secretion of adrenal catecholamines (Seidler and Slotkin, 1985, 1986a, b; Lagercrantz and Slotkin, 1986; Kauffman *et al.*, 1994; Slotkin *et al.*, 1995), the presence of atypical adrenergic receptor populations in the myocardium (Drugge *et al.*, 1985; Lin *et al.*, 1992; Kauffman *et al.*, 1994) and the presence of isoforms of myosin that are adapted to contraction with low oxygen supplies (Bian *et al.*, 1992; Schachat *et al.*, 1995). It is therefore of critical importance that animals exposed prenatally to nicotine lack the autonomous adrenomedullary secretory response (Slotkin *et al.*, 1995), and that this occurs in conjunction with a reduction in stimulatory *beta* adrenergic receptors in the myocardium and a reduced cardiac response to adrenergic stimulation (Navarro *et al.*, 1990a). Upon exposure to an hypoxic environment normal rats can secrete nearly 40% of the entire catecholamine content of the adrenal, whereas nicotine-exposed offspring secrete virtually no catecholamines (fig. 11). The consequences for cardiac regulation can be seen immediately: control rats show a slight cardioacceleration during hypoxia, followed by a slight decline in heart rate; with prenatal nicotine treatment, heart rate declines immediately and precipitously in a low-oxygen environment (Slotkin *et al.*, 1997b). Consequently, the nicotine group experiences increased mortality during an extended period of hypoxia (Slotkin *et al.*, 1995) .

The absence of an adrenomedullary response to hypoxia is likely to be the most important deficiency in autonomic function after nicotine exposure. The fetal and neonatal heart do not possess fully competent neural connections and thus are dependent on circulating catecholamines for stimulation (Slotkin, 1986). The loss of adrenal responsiveness does not represent an inability to release catecholamines in general because direct depolarization evokes secretion in both control and nicotine groups (Slotkin *et al.*, 1995). It is thus necessary to understand how the immature adrenal operates to explain why prenatal nicotine exposure interferes with its response. As shown in figure 12, the immature adrenal is not innervated functionally but nevertheless responds to specific stimuli, notably hypoxia, with a response that far exceeds the adrenomedullary secretory capabilities of the adult. This direct mechanism for catecholamine release disappears upon differentiation of the chromaffin cells, triggered normally by the development of splanchnic nerve function and the consequent nicotinic cholinergic depolarization of the cells (Seidler and Slotkin, 1986b). In the normal course of development, then, the organism is protected from hypoxia because secre-

Adrenal Catecholamine Secretion Caused by 5% O₂ for 75 minutes
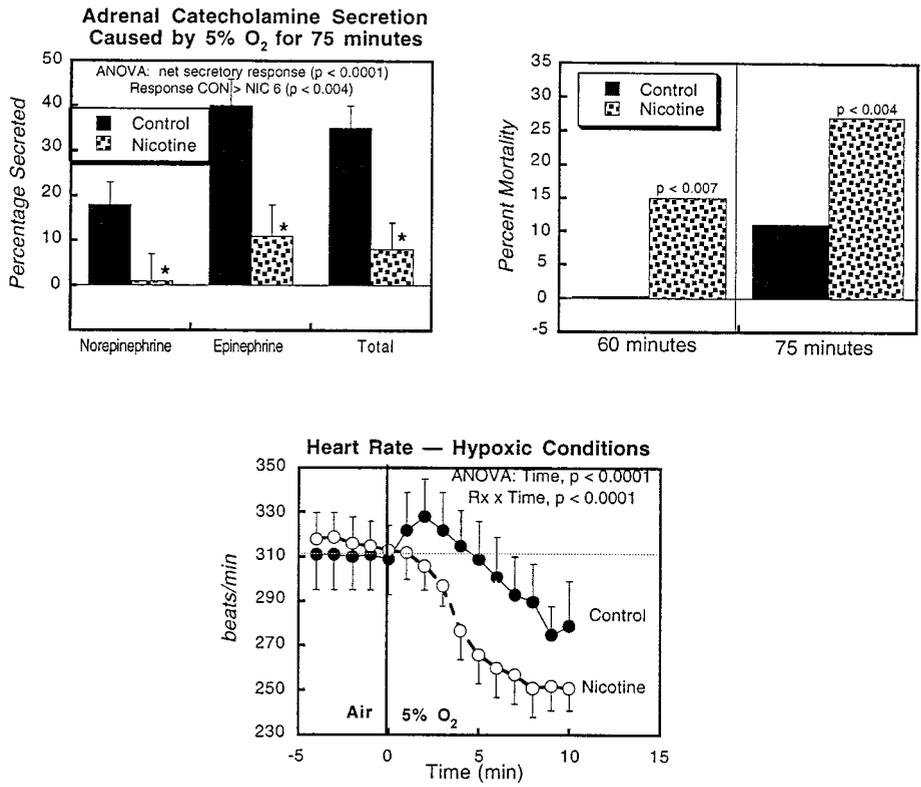
Fig. 11. Peripheral defects evoked by prenatal nicotine exposure that contribute to hypoxia intolerance (Slotkin *et al.*, 1995, 1997b). Low oxygen conditions elicit massive adrenal catecholamine release in 1-day-old control rats but no release occurs in animals exposed to nicotine prenatally. As a result, the nicotine group shows elevated mortality during hypoxia. The effects on cardiac function are evident within the first few minutes after initiation of low oxygen conditions: control rats show an initial increase in heart rate followed by a gradual decline to stable levels, whereas the nicotine group shows an immediate, precipitous decline in heart rate.

tory responses can occur by a direct response mechanism before the onset of innervation, and this ability is not lost until reflex control takes over (Seidler and Slotkin, 1985, 1986b; Mojet *et al.*, 1997). With fetal nicotine exposure, however, the cells receive cholinergic stimulation triggered by the drug itself, well in advance of the development of reflex autonomic control of the adrenal (Rosenthal and Slotkin, 1977; Bareis and Slotkin, 1978). Stimulation promotes the differentiation of the chromaffin cells, resulting in a premature loss of the direct secretory mechanism, opening a window of vulnerability in which there is no protection from hypoxia whatsoever. Thus, the same receptor-driven mechanism that targets specific sites within the CNS also serves to account for alterations in the periphery that compromise physiological competence.

These studies, along with additional work we have conducted on nicotine-induced changes in central respiratory control mechanisms (Slotkin *et al.*, 1995, 1997b), provides the first mechanistic proof of the epidemiological association between maternal smoking and perinatal morbidity and mortality associated with birth trauma. In light of the role of similar physiological adaptations required for surviving hypoxia during the first year of postnatal life, they also account for the relationship of smoking to SIDS (Kandall and Gaines, 1991; Milerad and Sundell, 1993; DiFranza and Lew, 1995; Cnattingius and Nordstrom, 1996), including the reasons why the critical window for SIDS eventually closes, namely when reflex control finally develops. Because the fetal environment is ordinarily relatively hypoxic (Nijland *et al.*, 1995), these events may account for the high incidence of fetal loss as a result of smoking during pregnancy.

Fetal Cocaine Exposure: A Comparison with Nicotine

Cocaine shares several essential characteristics with nicotine. Both are vasoconstrictors that converge on adrenergic neurotransmission as their underlying mechanism, nicotine by evoking catecholamine release and cocaine by preventing presynaptic uptake of catecholamines, thus intensifying their actions. Consequently, cocaine, like nicotine, is capable of evoking acute episodes of fetal hypoxia-ischemia (Mahalik *et al.*, 1984). Moreover, both cocaine and nicotine are anorexic drugs and thus influence the maternal nutritional state. A schematic for cocaine's impact on fetal development would resemble that of nicotine (fig. 3), without the participation of tobacco byproducts, but with much heavier emphasis on risky behaviors, poor prenatal care and socioeconomic status. Perhaps most importantly, co-abuse of tobacco is an invariable component in the use of crack cocaine (Budney *et al.*, 1993; Higgins *et al.*, 1994). Cocaine use differs from that of tobacco/nicotine, however, in that it tends to be episodic rather than continuous. An appropriate animal model for cocaine use therefore should involve repeated, acute exposure rather than continuous infusions (Siegel, 1982; Spear *et al.*, 1989a). Accordingly, we have used daily injections of cocaine to pregnant rats at a dose that simulates plasma levels found in crack cocaine users. This regimen causes CNS functional and behavioral alterations in the offspring (Dow-Edwards, 1989; Spear *et al.*, 1989a, b; Heyser *et al.*, 1992; Spear and Heyser, 1992; Goodwin *et al.*, 1993; Molina *et al.*, 1994).

Fetal cocaine exposure, like nicotine, elicits postnatal elevations in CNS ornithine decarboxylase activity, which is indicative of cell damage (Koegler *et al.*, 1991; Seidler and
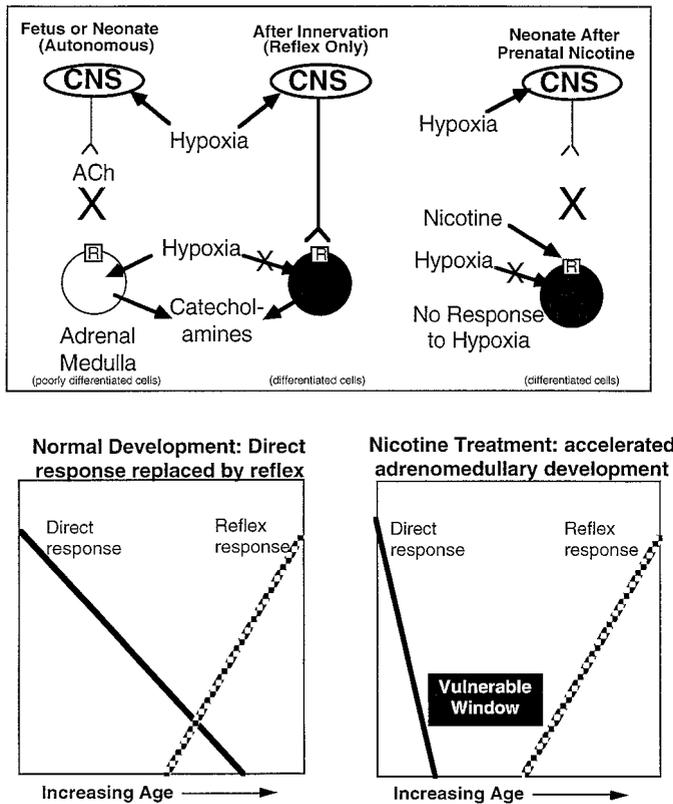
Fig. 12. Mechanisms underlying the adverse effects of prenatal nicotine on neonatal adrenomedullary function and their potential role in SIDS. In the fetus or neonate, the tissue responds autonomously to hypoxia so that catecholamines are released even though innervation is not functional. Ordinarily, the onset of innervation replaces the autonomous response with reflexly mediated release. With prenatal nicotine exposure, chronic, drug-induced depolarization of the chromaffin cells forces them into terminal differentiation prematurely so that autonomous responsiveness is lost *before* the onset of innervation, leading to an inability to respond to hypoxia. As shown at the bottom, normal rats are protected from the effects of hypoxia because they maintain adrenal catecholamine secretion at all times by autonomous or reflex mechanisms; the loss of autonomous response occurs only with the onset of reflex responses. In the nicotine group, the premature loss of the autonomous response opens a window of vulnerability in which neither process can contribute to catecholamine release. With further development, this window closes with the onset of functional innervation.

Slotkin, 1993; Slotkin *et al.*, 1993; Spraggins *et al.*, 1994) (fig. 13). However, the effects are smaller in magnitude than those of nicotine and do not persist into the second postnatal week. More strikingly, cocaine exposure does not lead to irrevocable cell loss, as shown by maintenance of normal

DNA content (Seidler and Slotkin, 1993). We were surprised therefore to find that a single injection of cocaine to neonatal rats does inhibit DNA synthesis acutely (Anderson-Brown *et al.*, 1990) (fig. 14). The effects differ from those of a single injection of nicotine in two regards. First, the effects of cocaine are not regionally selective, whereas the effect of nicotine follows the distribution of nicotinic cholinergic receptors. Second, the effects of cocaine are extremely short-lived, disappearing within 4 hr of administration, whereas the effect of nicotine persists.

These findings suggest a likely explanation for the greater impact of nicotine than cocaine on cellular development. Exposure to nicotine *via* continuous infusions depresses DNA synthesis for an extended period. Episodic cocaine exposure permits the DNA synthetic rate to recover in between injections, thus avoiding cell loss, although there is still evidence for cell damage (albeit less than for nicotine). The differing animal models used here are realistic because they mimic the patterns of tobacco and cocaine use. Smokers tend to maintain plasma nicotine levels at a constant value, a factor which also operates in transdermal nicotine patch administration. Cocaine is used in "jags" with extended periods of non-use. Given the much shorter plasma half-life of cocaine, there is a much greater opportunity for fetal CNS recovery from mitotic suppression.

These findings do not imply that cocaine is without effect on the developing brain, but rather that the effects are likely to be more subtle than those of nicotine. When "crack baby syndrome" first appeared in the scientific literature, the initial findings suggested extremely adverse effects (Chasnoff *et al.*, 1985, 1987, 1989). However, more carefully controlled human studies were unable to replicate the original findings and animal studies revealed only subtle behavioral differences; this led to a period in which the existence of an identifiable perinatal cocaine syndrome was called into question (Coles, 1993; Day and Richardson, 1993; Koren, 1993; Neuspiel, 1993; Konkol, 1994; Snodgrass, 1994). With further study, the pendulum has swung back somewhat because it is now evident that cocaine does indeed alter synaptic and behavioral performance, but without the frank damage found with nicotine or smoking (Spear and Heyser, 1992; Zuckerman and Frank, 1992, 1994; Meyer *et al.*, 1996; Levitt *et al.*, 1997) .

In keeping with the view that cocaine can alter neurobehavioral development, we have modeled some of the aspects of prenatal cocaine exposure on the development of synaptic activity and, with others, have found both transient and
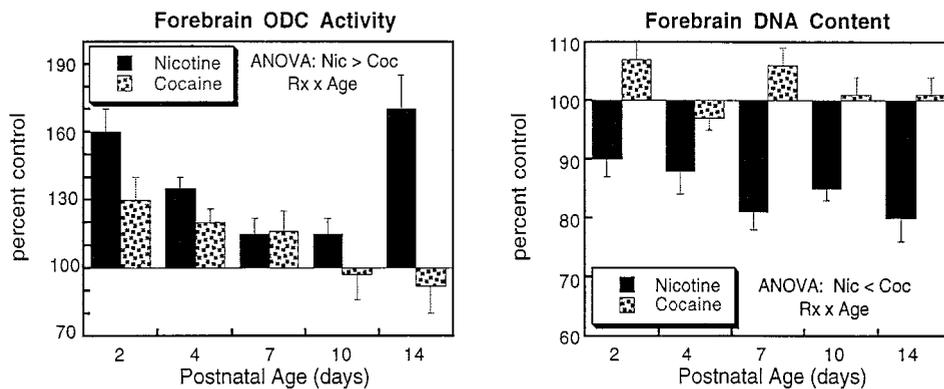
Fig. 13. Comparative effects of prenatal exposure to nicotine by infusion, or cocaine by injection (Slotkin *et al.*, 1987c; Seidler and Slotkin, 1993). Although both nicotine and cocaine elicit evidence of cell damage (elevated ornithine decarboxylase, ODC), the effect of nicotine is more persistent. Nicotine (Nic), but not cocaine (Coc), produces deficits in cell number, as evidenced by reduced DNA content. ANOVA, analysis of variance.
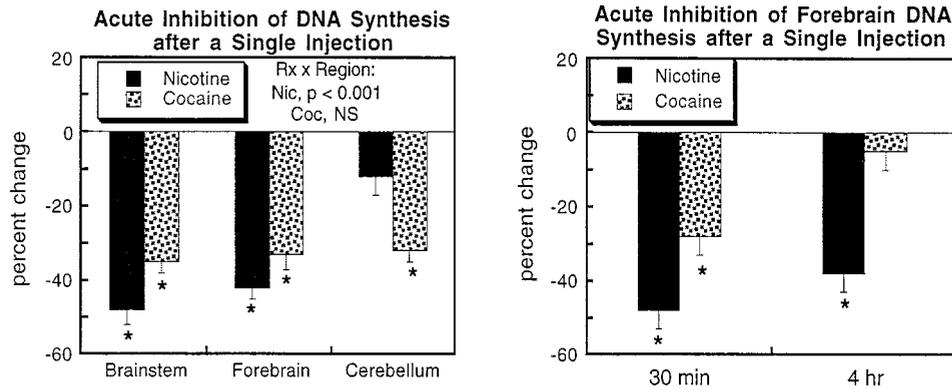
Fig. 14. Regional selectivity and persistence of effect of a single injection of nicotine (Nic) or cocaine (Coc) on DNA synthesis in neonatal rat brain (Anderson-Brown *et al.*, 1990; McFarland *et al.*, 1991). Both drugs inhibit DNA synthesis within the first 30 min after injection, but only the effect of nicotine is regionally selective, favoring regions that have high nicotinic receptor concentrations. Cocaine elicits uniform inhibition across all regions. The effect of nicotine persists for 4 hr after drug administration, whereas the effect of cocaine does not.

permanent alterations in presynaptic input and postsynaptic signal transduction (Nye *et al.*, 1991; Seidler *et al.*, 1992a, 1995; Seidler and Slotkin, 1992; Spear and Heyser, 1992; Miller *et al.*, 1995; Wang *et al.*, 1995; Choi and Ronnekleiv, 1996; Collins and Meyer, 1996; Friedman *et al.*, 1996; Keller *et al.*, 1996; Levitt *et al.*, 1997; McGrath *et al.*, 1997; Murphy *et al.*, 1997). However, in light of the fact that animal models of cocaine exposure involve repeated individual doses (similar to crack cocaine use), each of which elicits an hypoxic-ischemic episode, it is useful to compare some of the features of the effects of cocaine with those of hypoxia, or with maternally injected nicotine, which also evokes fetal hypoxia-ischemia. The same outcome is obtained with all three treatments; namely persistent noradrenergic hyperactivity, as evidenced by increased transmitter turnover (fig. 15). We have also found that blocking the acute hypoxic-ischemic reaction to cocaine can prevent some of the CNS developmental damage (Koegler *et al.*, 1991), and that interactions between cocaine and hypoxia that compromise cardiovascular function, and hence oxygen delivery to the brain, also contribute to the apparent CNS vulnerability to cocaine (Slotkin *et al.*, 1993; Spraggins *et al.*, 1994). The effects of fetal cocaine exposure on serotonergic systems can be duplicated by administering glucocorticoids (McGrath *et al.*, 1997), which are released during hypoxic episodes (Seidler and Slotkin, 1985). There is every reason to believe, then, that the repeated episodes of fetal hypoxia-ischemia accompanying crack co-

caine use (Mactutus and Fechter, 1986) play a key role in the effects on brain development and function, and that to the extent that both cigarette smoking and cocaine elicit hypoxia the outcomes associated with "crack baby syndrome" represent a common underlying mechanism. As a corollary, the high rate of cigarette smoking in cocaine users may make it extremely difficult to define long-term adverse effects that are attributable to cocaine *per se*.

The basic difference, then, is that the effects of injected nicotine or cocaine resemble each other strongly, but diverge substantially from the effects of infused nicotine. Thus, pharmacokinetic differences in drug delivery play an extremely important role in fetal outcome. Maintaining a fixed plasma level of nicotine, as with minipump infusions or transdermal patches, produces effects that are characteristic of the developmental neurotoxicity of nicotine. Repeated injections of nicotine superimpose the effects of hypoxic damage, leading to potentially different outcomes that resemble those of cocaine. As discussed below, these differences point the way to appropriate and inappropriate use of nicotine substitutes in smoking cessation strategies.

Implications for Human Effects of Cigarette Smoking, Nicotine Replacement Therapy and Crack Cocaine Use

Our findings indicate conclusively that nicotine is a neuroteratogen, acting to cause cell damage and reduced cell number, to impair synaptic activity and to evoke these changes at thresholds below those necessary for growth impairment. The underlying mechanisms are receptor-mediated, accounting for the low thresholds and for the involvement of brain regions and transmitter systems that have prominent cholinergic inputs. Receptor stimulation leads to two distinct errors in the program of cell development, a premature change from cell replication to differentiation and, after a delay, initiation of the program for apoptosis. But of what use is this information regarding smoking in pregnancy? First, our results indicate that cigarette smoking is directly injurious to fetal development because of the delivery of nicotine itself; the documented adverse effects of maternal smoking are not simply a correlation of associated epiphenomena, but represent a true, cause-and-effect relationship. Second, the low threshold for effects of nicotine indicates that the use of intrauterine growth retardation as an index of damage is totally inappropriate. For the same reason, the "safe" level for nicotine exposure *via* maternal smoking is
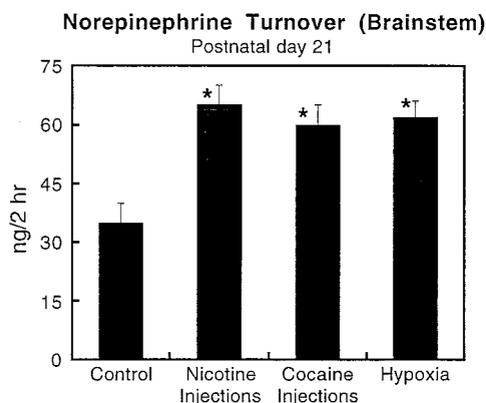
Fig. 15. Persistent effects of prenatal nicotine or cocaine exposure *via* maternal injections, or an episode of neonatal hypoxia (Slotkin *et al.*, 1987a; Seidler and Slotkin, 1990, 1992). All three treatments evoke a lasting elevation of norepinephrine turnover, which indicates synaptic hyperactivity. These results suggest that a common factor (hypoxia-ischemia) underlies some of the similarities between the effects of nicotine and cocaine on neurodevelopment.

probably lower than suspected heretofore. Third, we need to be more informed about how best to use nicotine for smoking cessation. Currently, nicotine substitutes are used freely, without regard to critical developmental periods or pharmacokinetic issues. It is therefore worthwhile to examine how our results can influence the use of nicotine replacement to minimize fetal effects.

The identification of nicotinic receptors as the specific target for the adverse effects of nicotine on the developing brain means that fetal susceptibility can be predicted by the ontogenetic emergence and increase of receptor sites. Contrary to current views of classical teratogenesis, the second and third trimesters should be more sensitive to adverse effects of nicotine than the first trimester, which is ordinarily considered to be the critical period for developmental disruptors. Indeed, the fact that receptors emerge after the major phase of systemic organogenesis is completed (Hagino and Lee, 1985; Larsson *et al.*, 1985; Lichtensteiger *et al.*, 1987; Slotkin *et al.*, 1987b; Cairns and Wonnacott, 1988) can provide an advantage. The first trimester is a window of opportunity in which to intervene to help a prospective mother discontinue smoking. Currently, however, the most common procedure is to counsel and cajole during the first trimester and only then to intervene pharmacologically. It obviously would be better to introduce nicotine replacement as early as possible and to try to reduce the exposure levels by the second to third trimester, rather than initiating and intensifying pharmacotherapy just at the point when receptors emerge.

The next issue raised by our findings is how to optimize nicotine delivery and pharmacokinetics in pregnancy. For many drugs, including nicotine and cocaine, the placenta provides significant fetal protection by metabolizing a portion of the drug and by introducing a phase delay between the maternal and fetal circulations (Goldstein *et al.*, 1974; Sastry, 1991). Thus, episodic drug delivery produces less penetration into the fetal compartment than does continuous exposure. In contrast, maintenance of a steady-state maternal plasma drug level enables the equilibration of all fluid compartments to the same final concentration, thus compromising the protective role of the placenta (Goldstein *et al.*, 1974). Indeed, in our hands, injections of even acutely toxic doses of nicotine to pregnant rats produce less fetal resorption than do otherwise less toxic infusion paradigms (Slotkin *et al.*, 1986b, 1987a, b; Navarro *et al.*, 1988; Slotkin, 1992). In a recent human study, nicotine transdermal patch use was associated with greater fetal cardiovascular effects than was cigarette smoking (Oncken *et al.*, 1997). How does this influence the potential use of nicotine replacement therapy during pregnancy? For smokers with unrestricted access to cigarettes there may be few adverse fetal consequences to substituting a transdermal patch for smoking because most tobacco users maintain their smoking to achieve a steady-state plasma level of nicotine. Replacement therapy would have the benefit of removing the additional injurious substances found in cigarette smoke. However, for smokers who spend their day in an environment where smoking is restricted nicotine intake may resemble an episodic exposure model, with relative protection of the fetus. In these smokers, especially if they smoke only small amounts, it is possible that greater fetal injury may result from the use of high-dose nicotine patches; accordingly, for this population, the total daily nicotine dose, whether delivered by smoking or by re-

placement therapy, should be considered carefully. In any case, some obvious rules can be applied, based solely on the recognition that nicotine injures the fetal brain through receptor-mediated mechanisms. Then, if patches are used they should be introduced early in pregnancy, with an attempt to discontinue use by the second trimester, assuming, of course, that compliance with smoking cessation will be maintained. Lower doses should be preferred and women should be encouraged to remove the patch overnight to permit plasma levels to decay from the steady-state. Perhaps most importantly, severe warnings should be given concerning smoking while the patch is applied, over and above the standard warnings given to nonpregnant patients; the combination of smoking and a high-dose nicotine patch can be expected to have significantly worse fetal consequences than either smoking or patch alone. It is uncertain, however, how these educational roles can be achieved when transdermal patch delivery systems for nicotine are available over the counter and thus without verbal instruction from physician or pharmacist. Finally, we can take even further advantage of pharmacokinetic differences in nicotine dosage forms because of the availability of nicotine inhalers and gum (Oncken *et al.*, 1996). Used as episodic delivery devices that elicit much lower plasma levels of nicotine, these are a better means of smoking cessation therapy during pregnancy, because they involve less fetal risk than with steady-state delivery systems.

Considering the comparative effects of fetal nicotine or cocaine exposure, a remaining issue is whether the reported adverse effects of maternal crack cocaine abuse represent actions of cocaine alone, cocaine combined with nicotine, or cocaine, nicotine and hypoxia all acting simultaneously. Although animal studies permit the individual contributing variables to be examined separately, the reality of crack cocaine use during pregnancy is that all three are superimposed in virtually all exposures (Budney *et al.*, 1993; Higgins *et al.*, 1994). Given the known consequences of maternal cigarette smoking for fetal outcome (DiFranza and Lew, 1995), it may prove difficult to identify in humans a specific, adverse consequence attributable solely to maternal cocaine abuse. From the public health perspective, cigarette smoking, and hence nicotine exposure, remains by far the larger problem and is much more likely to represent an issue where increased public awareness and education can have an impact. It is difficult to envision how comparable efforts can be undertaken successfully for prevention of cocaine abuse in pregnancy. Thus, the definitive proof that tobacco contains a substance that directly injures the fetal brain should help redirect our efforts at preventive measures and interventions that can reduce or avoid the public health consequences of maternal smoking.

Whereas epidemiological studies often provide the initial observations of adverse perinatal consequences of drug exposures, animal models can establish, once and for all, that these agents damage the developing brain. Relating the neurobehavioral disturbances found in animal studies to changes in human behavioral performance is far more difficult. Numerous factors, both genetic and environmental, contribute to intelligence, learning, cognitive performance, or other behaviors that may be the ultimate cost of maternal smoking or cocaine use. Consequently, regression toward the mean magnifies the difficulty of demonstrating a difference

in average performance scores in the human population, even when such effects might be robust in animal studies. Another way of stating this is that fetal exposure to drugs like nicotine evokes adverse changes whose consequences in human populations may be obscured by other variables. Nevertheless, it is feasible, at least in theory, to demonstrate such changes by concentrating on characteristics that appear only rarely in the normal population. At the physiological level, high rates of SIDS are therefore demonstrable as a definitive consequence of maternal smoking (Haglund and Cnattingius, 1990; DiFranza and Lew, 1995; Poets *et al.*, 1995). At the behavioral level, learning disabilities, disruptive behavior and attention deficit and hyperactivity disorder are all much more common in the offspring of women who smoke (Eskenazi and Trupin, 1995); there are no studies available yet that demonstrate comparable effects of crack cocaine use, nor is it likely that such studies can separate any effects of cocaine from the overriding consequence of concurrent tobacco use. However, the success of finding increased odds of these behavioral abnormalities suggests that similar approaches can be taken to characterize nicotine-induced fetal damage as they affect intelligence or cognition. As a purely theoretical experiment, we can imagine a situation in which the entire U.S. population is exposed to a subtle neuroteratogen, such that the average IQ is reduced by five points (fig. 16), a change that would be virtually undetectable in any reasonably sized experimental cohort. Indeed, the proportion of people exhibiting "normal" IQ values between −3 and +3 standard deviations (IQ of 55 to 145), would be virtually unchanged. In contrast, the proportion of individuals with severe retardation would triple and that of extremely gifted people would fall by two-thirds, effects that should be readily measurable. Given the smoking rate of 25% in pregnant women in the United States, these more subtle consequences of neurobehavioral teratogenesis by nicotine may thus have very real consequences within the population at large.

	Retarded	Normal	Extremely Gifted
mean = 100	0.26%	99.5%	0.26%
mean = 95	0.76%	99.2%	0.08%
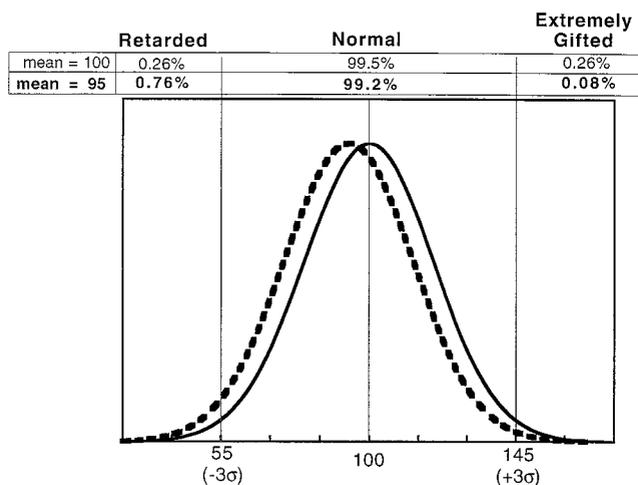
Fig. 16. An imaginary experiment in which a population is exposed to an agent that reduces the IQ by 5 points. If assessments are limited to people falling within the "normal" range (55–145), there is virtually no change in outcome. If, however, assessments are made in the tails of the distribution, focusing on rarely occurring values, the small change in average IQ produces disproportionately large changes, with a 3-fold increase in retardation and a loss of two-thirds of the extremely gifted group. Neurobehavioral teratogenesis by drugs of abuse or environmental factors might be studied better in human populations by concentrating on these small subpopulations, rather than by focusing on changes in average scores.

Nicotine exposure is likely to be the single most widespread prenatal chemical insult in the world, continuing unabated despite decades of educational and medical intervention. It is likely to become of even greater significance with the increasing use of tobacco in Third World countries that have typically high pregnancy rates, with the disappearance of societal prohibitions on women's smoking and with the covert encouragement of smoking by corporations or even governments that have a financial stake in the continued use of tobacco (World Health Organization, 1997). The attendant increase in smoking by women of childbearing age and the consequent rise in the incidence of fetal brain damage will continue to play a pivotal and tragic role in our society.

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