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Neural Grafting as a Tool for the Study and Reversal of Neurobehavioral Birth Defects

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YANAI, J., M. ABU-ROUMI, W. F. SILVERMAN AND R. A. STEINGART. *Neural grafting as a tool for the study and reversal of neurobehavioral birth defects.* PHARMACOL BIOCHEM BEHAV **55**(4) 673–681, 1996.—The transplantation of fetal neurons has gained notoriety in recent years for its perceived potential to reverse neurological deficits caused by loss of one or another neuronal population. The present paper describes a neural grafting approach employed by our laboratory to gain more insight into the drug-induced neurobehavioral teratogenicity. Mice were exposed prenatally to phenobarbital by feeding the barbiturate to the pregnant dam on gestation days 9–18. Heroin exposure was accomplished by injecting dams during the same gestational period. At maturity, the drug-exposed offspring displayed profound deficits in specific behavioral tasks, suggesting alterations in the septohippocampal cholinergic pathway. Biochemically, we observed increased presynaptic activity in the pathway, which was not accompanied by a corresponding reduction in postsynaptic activity. Rather, there was a general hyperactivation along the different postsynaptic phases. In contrast, we noted a desensitization of protein kinase C activity in response to the exposure of a cholinergic agonist to the drug-exposed offspring. Subsequent transplantation of embryonic cholinergic cells from normal mice to the impaired hippocampus reversed the behavioral deficits, whereas sham-operated controls exhibited no improvement. Concomitantly, all the biochemical alterations studied, both presynaptic and postsynaptic, were either partially or completely reversed following grafting.

Acetylcholine Behavior Heroin Hippocampus Mice Neural grafting Neurobehavioral teratology Phenobarbital Protein kinase C

Most early studies of behavioral teratology were descriptive only, fulfilling the necessary first requirement in a new field. The obvious next step is to ascertain the pathways from the early input to the behavioral alterations which will, hopefully, facilitate future studies on the prevention or reversal of the effect of early exposure to the substance, especially in those cases when exposure is deemed necessary or unavoidable (73).

THE ultimate objective of neurobehavioral teratology (or behavioral teratology, as it has been previously defined) is to understand the mechanism(s) underlying behavioral birth defects and to establish interventions that can reverse them. The increasing accessibility of these goals is due to the availability of models in which specific behavioral deficits and their related neural alterations are being investigated (81) and the development of new strategies for the reversal of behavioral deficits, including neural grafting. An important first step toward reaching this goal was the establishment of an animal model of neurobehavioral teratogenesis developed and refined in our laboratory in recent years. As expected, we encountered the inherent methodological obstacle that most drugs, including potential teratogens, act at numerous sites in the brain, simultaneously affecting many regions, neural pathways and transmitter systems and consequently causing multiple behavioral deficits. However, focusing on prenatal drug-induced deficits in defined behaviors, which can at least in part be related to specific brain regions and/or biochemical processes, has greatly reduced this problem. Our model describes the effects of early exposure to drugs on behaviors that, based on previous studies, may be partly mediated by the hippocampus and especially by its septohippocampal cholinergic innervation. The well-defined nature of the behavioral and biochemical changes observed in this model, therefore, provides the basis for obtaining a detailed understanding of distinct stages in the devel-

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opment of drug-induced behavioral deficits even while many other processes and behaviors are also probably altered (49,75,77,79). As a consequence, it becomes more feasible to attempt to reverse these deficits, at least partially. In the present study, heroin and phenobarbital were employed for their neuroteratogenic properties (14,51) and action in different brain regions and innervations, which are similar in that both act at the septohippocampal cholinergic synapse in the developing (81) and mature (43,58) brain.

In the studies described here, exposure of mouse embryos in utero to these drugs produced a reproducible, quantifiable behavioral deficit that is partly correlated with septohippocampal cholinergic innervations. Therefore, possible changes in these innervations were concomitantly ascertained. Moreover, the nature of the observed neurochemical alterations suggested that the deficit might be reversed by exposure of the affected hippocampus to normal cholinergic neurons. Although described for the first time in the late 19th century (67), neural transplantation as an approach for studying nervous system development and as a therapeutic strategy in neurological disorders did not gain broad scientific acceptance until nearly a century later. The key breakthrough in this field occurred in 1979, when two research groups simultaneously reported the reversal of parkinsonian motor deficits in a rat model of the disease by grafting dopamine-producing tissue (7,47). Neural grafting differs from somatic organ transplants in that, except in rare circumstances, the donor cells must be immature, i.e., embryonic or fetal, when the recipient is an adult. For reasons that are still not completely understood, mature central nervous system (CNS) neurons retain little of the plasticity exhibited by their embryonic predecessors. However, because the brain is relatively immunologically privileged, neural cells can be grafted without the need for immunosuppression to prevent rejection. In fact, embryonic neural tissues can be grafted between species with relatively little rejection (46,57). Human embryonic tissue has, for example, been successfully grafted into a rodent brain (78) and can even reverse functional deficits in the host brain (28). Because of the prodigious complexity of the brain, neural grafting is normally used to address specific well-defined deficits, for example, to replace one or another chemical transmitter. The treatment of Parkinson and Huntington diseases presents an applicable case (30). However, the increasing appreciation of the role of trophic factors in developing and maintaining appropriate neuronal interactions, particularly those involving cholinergic neurons (16), suggests that transplants could be used to address a broader range of problems.

In addition to its still-evolving clinical application in the treatment of neurological disease (30), the neural transplantation approach is widely used experimentally as a probe to explore issues of neural development and regulation (62,64). Transplantation of neural tissue has been shown to reverse genetic and experimentally induced behavioral deficits. Virtually all major types of neurons have been successfully transplanted. However, reversing prenatally induced neural birth defects with this technique has received limited attention, which may be related to a lack of models rather than to lack of feasibility. For this reason, the studies presented here focus on deficits in behaviors whose proper expression is greatly dependent on the integrity of the hippocampus and more specifically on the septohippocampal cholinergic innervations, thus providing a workable model of a drug-induced neural birth defect (31,39). Obviously, truly region-specific behaviors do not exist, and other loci in the brain have also been implicated in relation to our behaviors (15). However, the principal role of the hippocampal cholinergic innervations in these behaviors has been well established. As a result of this model, achieving a deeper understanding of the early drug-induced neurobehavioral alterations and generating strategies for reversing these became feasible. Similarly to neural grafting, the pharmacological approach is an important tool in the study of neurobehavioral birth defects. Applying this approach with neural grafting provides further understanding of the neurobehavioral deficits and of their reversal. However, although pharmacological treatment only provides transient reversal of the deficits, neural grafting permanently restores, partly or totally, a normal neurobehavioral state.

To reverse the drug-induced behavioral and biochemical alterations, septal cholinergic neurons from non-drug-exposed mice were implanted bilaterally into the hippocampus of mature mice exhibiting the behavioral deficits. Neural grafts of fetal tissue can ameliorate or reverse physiological and behavioral deficits (3,7,17) in a host of experimental paradigms. Not surprisingly, because of the typically comprehensive nature of the behavioral deficits observed, few studies have looked at the effect of neural grafting in substance-induced teratogenesis. However, the development of relatively "region- and process-specific" models of behavioral birth defects raises the possibility of reversing the deficits by neural grafting. These results and the findings of our experiments provide the rationale for further studies that would elucidate the underlying mechanism(s) of substance-induced neuroteratogenesis.

The present article presents our findings on behavioral deficits and concomitant alterations in the septohippocampal cholinergic innervations after prenatal exposure to heroin and phenobarbital. Previously published data will be reviewed and preliminary findings will be described.

MATERIALS AND METHODS

General

Genetically heterogeneous HS/Ibg mice (33) were used as parents because this strain reproduces well, even under drug exposure, and because the large gene pool possessed by this stock allows for the generalization of effects. Animal maintenance and animal experimentation were in compliance with the NIH Guide of Care and Use of Laboratory Animals. The mice were housed in mating groups of one male and four females. Females were checked daily and those that conceived, as shown by the presence of a vaginal plug (gestation day 1, or GD1), were housed with other pregnant females. The mouse offspring (the subjects of these experiments) received phenobarbital or heroin prenatally via the placenta. Drug administration commenced on GD9 and was continued until GD18.

Prenatal Drug Administration

Prenatal phenobarbital administration (54). Treated females received milled mouse food containing 3 g/kg phenobarbital in acid form as their only food source and water, both available ad libitum. Control females received milled food and water. Under this regimen, the animals consumed the drug at fairly regular intervals throughout most of the day and night, resulting in relatively consistent blood–phenobarbital concentrations during the drug administration period. The control groups, for both phenobarbital- and heroin-consuming dams, included a pair-fed control. This control did not differ from controls consuming food ad libitum because monitoring of food consumption revealed no phenobarbital or heroin effect on maternal food intake.

Prenatal heroin administration (80). Heroin (diacetylmorphine), the major "street" opiate, is far less commonly used in neuroteratological research than is morphine. It was prepared from morphine hydrochloride (10 g; Sigma, USA) dissolved in pyridine (30 ml) and acetic anhydride (30 ml). The solution was concentrated until dry under reduced pressure; the resultant oily syrup was dissolved in methanol and further evaporated. The subsequent solid was dissolved in chloroform, dried over magnesium sulfate, filtrated and evaporated. The oils were dissolved in carbon tetrachloride and cooled, and HCl gas was allowed to pass through. The solution was then evaporated and crystallized by cooling from a mixture of ethyl acetate and petrol ether (78:20). Both the infrared spectrum and the melting point obtained correspond with the literature (71). Pregnant females received a single, daily subcutaneous injection of 10 mg/kg heroin in saline solution (10 ml vehicle/kg). Control females received vehicle injections only. On GD18 the drugs were withdrawn, and the females were housed individually and returned to standard laboratory conditions with a regular feeding regimen.

Both control and treated offspring were fostered within 24 h after birth by control females. They were weaned at age 25 days, segregated according to sex and maintained in groups of five. Behavioral and/or biochemical testing usually started at 50 days of age. Animals employed in transplantation studies received the graft at 35 days of age and were studied approximately 2 months later. Female and male offspring were used in equal numbers.

Statistical analysis and considerations. Analysis of variance, single or multiple level with repeated or nonrepeated measures, was applied in most cases with ad hoc tests (72). When appropriate, the nonparametric chi-square and exact probability tests were used (59). No effect of sex could be determined from the statistical analysis; therefore, the scores were pooled across sexes. To prevent bias due to litter effects, the experiments were designed such that no two pups from the same litter were ever used for any one experiment. However, in some experiments in which homogenate comprised of several hippocampi was considered one unit (e.g., Scatchard plots), we did allow the employment of more than one offspring per litter within the same homogenate.

Cellular Procedures

The methods will be described for those variables that were subjected to neural grafting and whose findings, being still preliminary, have not been published previously. The methods will be briefly described to emphasize our modification of established procedure.

Neural grafting. Our method is a modification of the one described by Rogel-Fuchs et al. (55). Brains of 15-day-old embryos were rapidly removed and the septal–diagonal band extracted by dissecting the ventral forebrain from the rostral border of the hypothalamus caudal to the olfactory bulb. The 2-mm tissue pieces were pooled from 20 embryos and transferred to a small glass tube (6 × 12 mm) containing the volume of saline glucose (0.6%) necessary for implanting tissue from one embryo into each graft recipient. Tissues were then gently triturated by repeated pipetting in fire-polished pipettes. We found this procedure advantageous because of the functional outcome and increased viability of cell suspensions produced without trypsin. The number of viable cells in the suspensions were assessed periodically with the acridine orange-ethidium bromide method described by Brundin et al. (9).

For transplantation, recipient and control mice were anesthetized with pentobarbital, and the cell suspension or medium only (2–5 μl) was injected stereotaxically into the hippocampus on both sides. The following coordinates were used: 1.8 mm posterior to Bregma, ±1.5 mm lateral from the midline and 1.7 mm below the calvarium (21,76). Two months after surgery, a sample of mice from each group was perfused intracardially with 75 ml of 4% paraformaldehyde, 0.05% glutaraldehyde and saturated picric acid in 0.1 M phosphate buffer (pH 7.4).

Immunocytochemical and histochemical procedures. Survival and localization of grafted neurons were assessed by avidinbiotin–horseradish peroxidase (HRP) immunocytochemistry for choline acetyltransferase (ChAT) with a previously described protocol (10). After sectioning on a vibrating microtome, the 50-μm sections were incubated sequentially in a blocking solution containing 10% normal horse serum and 0.5% Triton X-100 in phosphate buffered saline (pH 7.2), the monoclonal antibody to ChAT (1:1000; Boehringer-Mannheim GmBH, Germany), biotinylated goat antimouse IgG, and the avidin-biotin-HRP solution. Visualization of labeled antisera was accomplished with 3,3′ diaminobenzidine and 0.05% nickel aluminum sulfate in 0.1 M Tris-imidazole buffer (pH 7.2) and 0.003% hydrogen peroxide.

Behavioral and Biochemical Tests

Eight-arm maze. Our radial eight-arm maze is a scaled down version (48) of the original maze developed by Olton and Samuelson (45) for rats. The major modification in our test procedure is the use of water deprivation and reward of 50 μl of water presented at the end of each arm. Water deprivation was achieved for 7 days by limiting water consumption to 30 min each day. The offspring were tested for 6 consecutive days. The animals were observed until they had made entries to all eight arms or until they had completed 16 entries (whichever came first). The following variables were recorded: the number of correct entries within the 16 visits (maximum = 8), the number of correct entries within the first 8 visits and the number of days needed to reach the "correct response," i.e., 8 correct entries out of the first 8 entries for 2 consecutive days.

Morris water maze test. This test is also our mouse adaptation (54) of the procedure developed by Morris for rats (35). The pool was scaled down to 80 cm in diameter and the platform (5 × 8 cm) was placed 1 cm under the water surface. The place test consisted of two blocks of four trials on each day for 4 consecutive days and one block of four trials on the fifth day (39). For each trial, the mouse was given 60 s to reach the platform and climb on it, and the latency was recorded. Four trials of the spatial probe test were applied on the fifth day.

[^{3}H]Hemicholinium-3 (HC-3) binding. This method was described in a previous report (83) and is based primarily on the method by Vickroy et al. (68). A tissue membrane preparation (approximately 0.15 –0.5 mg protein) in a final volume of 0.2 ml of ice cold 50 mM glycine-glycine buffer was incubated with a ligand in a concentration range of 0.125–5.00 nM. The nonspecific component is defined as radioligand binding in the presence of an excess concentration (0.01 mM) of hemicholinium-3 and is typically 30% of the total. Binding values are calculated as femtomole bound per milligram of protein.

Inositol Phosphate (IP) formation. This procedure (54) is based on that by Berridge et al. (5). The hippocampal slices were incubated in Krebs Ringer's bicarbonate buffer containing 0.3 mM [^{3}H]inositol. IP formation was induced by 20

mM KCl alone, KCl in the presence of 1 mM physostigmine, with the addition of 1 mM carbachol, or by carbachol alone.

Protein kinase C (PKC) activity. This assay (Steingart et al., unpublished observations) is based on one by Kikkawa et al. (24). Hippocampi were homogenized in a 0.25-M sucrose buffer and incubated for 60 min in sucrose buffer containing 0.2% Triton X-100. Cytosol and membrane fraction were separated by centrifugation. Histon type IIIs were added to the reaction mixture as an external substrate (24). For measuring specific PKC activity, phosphatidyl serine, diacyl glycerol and 500 mM $CaCl_2$ were added to the reaction mixture as PKC activators.

Stimulation of PKC activity by carbachol. Carbachol-induced PKC activation and translocation are commonly studied in tissue culture (44). We have recently adapted these procedures and established the salient parameters for assays on mice hippocampus slices (Steingart et al., unpublished observations). The slices were incubated in 1 mM carbachol at 32°C for different periods of time. Basal PKC activity was determined in the absence of carbachol for each time point. The slices were washed twice with fresh, ice-cold sucrose buffer and homogenized, and PKC activity was then determined.

RESULTS AND DISCUSSION

Some of the findings presented in this section concern the behavioral deficits and biochemical alterations at the septohippocampal cholinergic synapse induced by prenatal exposure to the two drugs (1,81). Preliminary findings (Abu-Roumi, Newman and Yanai, unpublished observations; Steingart, Barg, Vogel and Yanai, unpublished observations) will be presented here.

Behavioral Alterations

Offspring that were exposed to heroin or phenobarbital prenatally (drug offspring) showed deficits in their performance in the eight-arm and Morris mazes (55,76,80).

Presynaptic Cholinergic Alterations

The drug-exposed offspring exhibited an increase in hippocampal choline uptake as shown by the increase in HC-3 binding to the choline transporter (83). An increase was also found in acetylcholine (ACh) release assessed by measuring formation of IP elevated due to the addition to the incubation medium of high depolarizing concentration of K^+ either alone or in the presence of the cholinesterase inhibitor physostigmine (1). However, acetylcholinesterase (AChE) and ChAT (assessed only in the phenobarbital model) were not affected by the prenatal exposure (26,54).

Postsynaptic Cholinergic Alterations

Extensive evaluation of the major postsynaptic phases of the septohippocampal cholinergic transmission was performed; hyperactivity was demonstrated in all phases. The general number of cholinergic muscarinic receptors as assessed by QNB binding was increased in the drug offspring (54,80). More pertinently, M_1 subtype, assessed at the transcriptional and translation (i.e., pirenzepine binding) levels, was also increased (81). Downstream from the receptors, the activity of the general pool of G-proteins available for several neurotransmitters, as assessed by IP formation in response to NaF/AlCl3, was increased in the drug-exposed offspring (1). Furthermore, quantitative analysis of the subtypes of these proteins was performed on immunoblots by using specific antisera for each class of protein. An increase was observed in Gq and Gi in drug-treated offspring, whereas Go remained unchanged (81). Because G-proteins provide the link from the receptor to the second messenger, an experiment was done to assess carbachol-induced IP formation after early exposure to drugs. An increase was found in carbachol-induced IP formation in the drug-exposed offspring as opposed to the controls (1,81). Continuing along the nerve transduction cascade, the drug-exposed offspring displayed increased basal activity of membrane PKC in the hippocampus (81).

The results presented thus far demonstrate hyperactivity at the pre- and postsynaptic levels in the septohippocampal cholinergic innervations and corresponding behavioral deficits. This phenomenon is rather unusual, particularly when considering the typically widespread effects of insult to the adult brain. Our hypothesis to explain this phenomenon is that the observed hyperactivity at septohippocampal nerve terminals represents a futile attempt by pre- and postsynaptic elements to compensate for a perceived "bottleneck" in the nerve transduction cascade. This notion would imply that the primary deficit was induced by the prenatal insult in one of the phases along the nerve conduction cascade and that the increased activity of the other phases was a secondary, compensatory event. In testing this hypothesis, the subsequent search for the primary alteration demonstrated that the hippocampal cholinergic synapses displayed PKC activity that was completely unresponsive to stimulation by the cholinergic agonist carbachol. This finding suggests that a desensitization (either homologous or heterologous) had occurred (81).

This desensitization could, in fact, be the primary event, followed by a compensatory hyperactivation. Alternately, the hyperactivation could be the primary event and the desensitization a compensatory reaction. To determine which of these possibilities was correct, we grafted normal septal neurons into the hippocampus of affected subjects. According to our hypothesis, normal presynaptic components (from the grafts) would be unable to reverse the postsynaptic desensitization if it were the primary event. However, if the primary event were presynaptic hyperactivation, then the desensitization could be reversed by the grafting of unaffected fetal neurons. Thus, mouse offspring exposed to phenobarbital or heroin prenatally were given transplants of embryonic septal cholinergic minced tissue to their hippocampus at age 35 days, as previously described (76). Grafted ChAT-immunoreactive neurons were observed 2 months after implantation in the host hippocampus and exhibited a rich outgrowth of fibers (Fig. 1).

Approximately 2 months after grafting, the effects of the grafts on septohippocampal biochemistry and cholinergic-related behaviors were assessed. On the behavioral level, mouse offspring that were prenatally exposed to phenobarbital showed marked deficits in their performance of the eight-arm and Morris mazes, as was previously described. Transplantation of cholinergic cells nearly completely restored normal maze performance (55,76). This phenomenon was specific to cholinergic grafts because noradrenergic grafting show no effect on the behavioral deficits (76). Similar findings were demonstrated in the heroin model. For example, on the sixth test day, when observed differences were at their maximum, heroin offspring required 13.7 ± 0.8 entries to visit all eight arms, whereas control offspring needed only 9.6 ± 0.4 entries (mean ± SEM, $p < 0.001$, analysis of variance). However, transplantation of septal cholinergic cells into the hippocampus of

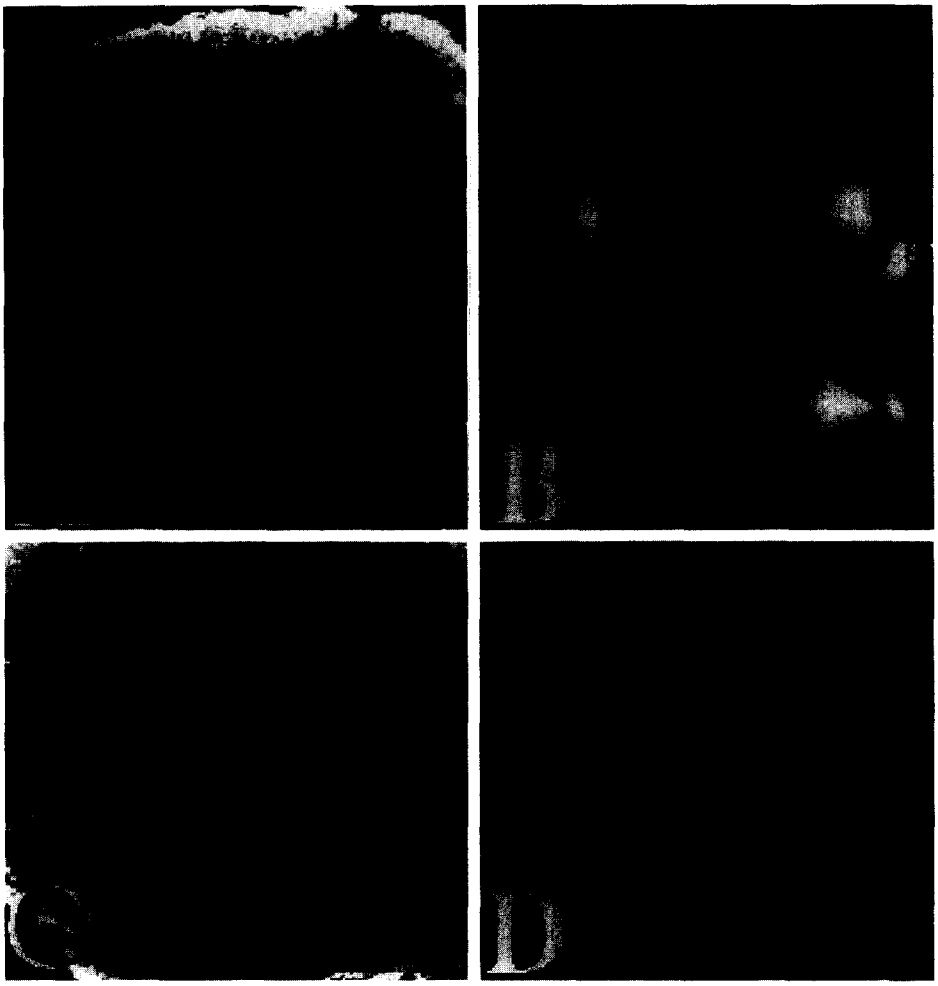

FIG. 1. Videomicrographs of unilateral graft of septal cholinergic neurons into the hippocampus of treated mice. (A) Transplanted ChAT immunoreactive neurons in the CA1 region. The tract of the needle through which the cells were implanted is shown by white dotted lines in cortex at top. ChAT-positive neurons are indicated by an arrow, and some are seen inside the box. (B) ChAT-immunoreactive profiles from boxed area in A shown at higher magnification. (C, D) Neuropil from the noninjected CA1 region exhibits no ChAT-positive cells, only fine fibers.

the heroin offspring restored their behavioral performance to 9.3 ± 0.6, which approximates the normal state ($p < 0.01$; Steingart et al., unpublished observations). Previous studies in which the septohippocampal cholinergic innervations were experimentally impaired have demonstrated behavioral deficits very similar to those displayed in the eight-arm and Morris mazes in the present model (31,39). These studies, like our own, have found that transplantation of normal embryonic septal cells reverse these behavioral deficits. It is tempting then to hypothesize that the deficits found in these behaviors after heroin or phenobarbital exposure are a manifestation of hippocampal cholinergic deficits. However, as will be discussed below, other cholinergic regions and even other noncholinergic innervations in the brain may be related in varying degrees to the behaviors studied (15). Although the role of hippocampal noradrenergic innervations in the drug-induced maze deficits was excluded (76), further transplantation studies on other brain regions and innervations are necessary to assess their possible contribution to the behavioral deficits in question. Learning deficits after early exposure to heroin and phenobarbital have been demonstrated (74,82); however, as in the present study, the tests were not sufficiently detailed to categorize the deficits finely and thus to pinpoint their possible association to additional brain loci. Further scrutiny of the drug-induced behavioral deficits should be the subject of future investigations.

Additional studies were carried out on the reversal of the major pre- and postsynaptic phases of septohippocampal cholinergic transmission. Presynaptically, direct measurement of ACh level and rate of turnover following neural grafting would appear to be the natural choice to evaluate the manner in which this intervention affects nerve impulse activity in the drug-exposed animals. However, the rapid degradation of ACh and the difficulty of measuring this transmitter makes such measurement impractical at best. Although in our studies on early phenobarbital exposure we measured ChAT (54), this enzyme is not the rate-limiting step in ACh biosynthesis, and its activity is not affected by changes in neuronal activity (13,22,60). A reduction of ChAT activity substantiates the loss of cholinergic nerve terminals but gives no indication of the

state of cholinergic function of the remaining terminals. It is essential to appreciate that the ratelimiting step in ACh synthesis is the uptake of choline from the synapse and that the activity of the high-affinity transporter, located on cholinergic nerve terminals, is directly affected by neuronal impulses (13,22,60). Upregulation of choline transport is readily obtained after manipulations that enhance cholinergic tone (11,22,37,60). However, measuring highaffinity choline uptake (HACU) is not without methodological difficulties, which greatly impair its dependability (27). Because impulse-induced upregulation of HACU involves increases in the amount of transporter (11,22,37,60,61,83), it is also possible to detect these changes with binding of the specific ligand HC-3, and we have demonstrated the ability to do so in the drug-perturbed developing brain (81,83) (Steingart et al., unpublished observations). Although sham-control (control) B_{max} was 79.4 ± 8.8 (fmol/mg protein), the sham heroin (heroin) offspring binding score was 128.4 ± 16.7, i.e., 62% above control levels. However, in heroin animals with cholinergic grafts, this increase was reduced to 105.9 ± 29.3, only 33% above the control level (Steingart et al., unpublished observations). The results are still too preliminary to allow for statistical analysis, yet in comparison with results of neural grafting in other phases of cholinergic transmission neural grafting did not reverse the alterations in HC-3 or, at best, was only intermediate between those of the control and heroin offspring.

Postsynaptically, we observed that although drug-exposed offspring had an increased muscarinic B_{max} cholinergic transplants partially restored B_{max} level (55). Because QNB was used as the ligand, the findings only apply to the general population of muscarinic receptors. Of the five muscarinic receptor subtypes, M_1 is the most relevant to the present model because of (1) its role as the predominant hippocampal muscarinic receptor subtype, (2) the coupling to cell signaling via G-proteins and the activation of PKC, (3) the availability of the highly selective radioligand [^{3}H]pirenzepine for characterization of the receptor site and (4) the probable involvement of this receptor subtype in altered behavior associated with the drug exposure (23,52,63,69). Therefore, similar studies are presently being conducted employing the M_1-specific ligand pirenzepine to assess the specific reversal in drug-exposed offspring.

In studying the reversal of drug-induced hyperactivity along the major phases of the nerve conduction cascade, we assessed the effect of neural grafting on carbachol-induced IP formation. This evaluation is especially pertinent to the present case in light of the established coupling of this second messenger to the M_1 receptor subtype (20,34). Furthermore, M_1 activates phospholipase C, which stimulates phosphoinositide hydrolysis in the two second messengers, IP and diacylglycerol. IP leads to Ca^{++} release from intracellular stores, which in turn acts synergistically with diacylglycerol to activate PKC (41).

In our studies, fetal cholinergic grafts completely restored the high level of carbachol-induced IP formation in the drug-exposed offspring to a normal level. Thus, in control offspring, carbachol-stimulated IP formation was 1.42 ± 0.09 (proportion of basal IP formation), whereas the phenobarbital-exposed offspring measured 2.58 ± 0.37 ($p < 0.05$). Transplanted phenobarbitalexposed mice exhibited an IP formation of 1.35 ± 0.11, which is similar to the control level and significantly lower ($p < 0.05$) than that of the drug-exposed untransplanted group. Likewise, in the heroin study, carbachol-stimulated IP formation was 1.42 ± 0.09 in the control offspring and 2.38 ± 0.36 in the exposed offspring ($p < 0.05$). Similarly, transplanted heroin-exposed offspring exhibited an IP formation of 1.42 ± 0.10, which is similar to the control level and significantly ($p < 0.05$) lower than the corresponding untransplanted group (n = 6–10 assays for each group; Abu-Roumi et al., unpublished observations).

IP and diacylglycerol formation activates PKC, a calcium-dependent protein kinase. PKC has a dual action: it stimulates intracellular processes and prevents overactivation by feedback inhibition control. This regulation is mediated by downregulation of the receptor and uncoupling between the receptor and G-protein, which is initiated by PKC activation. Moreover, inhibition of PKC activity is regulated directly by reducing the intracellular Ca^{++} level after PKC activation (24,42). Functionally, long-term potentiation (LTP) is associated with deficits in the Morris and eight-arm mazes (36,66). The cholinergic innervation of the hippocampus has been shown to participate in LTP (32). More specifically, PKC translocation from the cytosol to the membrane, normal basal PKC activity, and the integrity of PKC have all been shown to be necessary for both the maintenance of LTP and for hippocampus-related learning abilities, including normal performance in the Morris and eight-arm mazes (2,29,70).

Consequently, offspring prenatally exposed to phenobarbital or heroin and control offspring were either sham transplanted or given septal cholinergic grafts; basal and carbachol-induced hippocampal PKC activities were measured. Transplantation virtually restored the elevated basal PKC activity of the drug-exposed offspring to normal levels. Moreover, responsiveness to carbachol stimulation of hippocampal PKC activity was restored in the transplanted drug-exposed offspring. The carbachol-stimulated membrane PKC activity index of the control offspring was 1.44 ± 0.08 (where basal level is considered to be 1.00) (81), whereas it measured 0.81 ± 0.10 in the phenobarbital-exposed offspring ($p < 0.05$). Transplanted phenobarbital-exposed offspring exhibited a score of 1.42 ± 0.06, which is similar to the control level and significantly ($p < 0.05$) higher than that of the corresponding untransplanted group. Likewise, in the heroin study, the carbachol-stimulated membrane PKC activity index of the control offspring was 1.44 ± 0.08 (phenobarbital and heroin controls were pooled because they did not differ), whereas in the heroinexposed offspring it measured 0.83 ± 0.10 ($p < 0.01$). Again, transplanted heroin-exposed offspring exhibited a score of 1.36 ± 0.13, which is similar to the control level and significantly ($p < 0.01$) higher than that of the corresponding untransplanted group (n = 9–14 mice in each group; Steingart et al., unpublished observations).

The transplantation findings are strengthened by our recent studies in which the deficits in eight-arm maze performance, resulting from prenatal exposure to phenobarbital or heroin, were correlated within individual animals with the biochemical alterations. Significant correlation with the behavioral deficits (r = 0.5–0.7) were demonstrated in HC-3 binding, carbachol-induced IP formation and PKC basal level and sensitization to cholinergic agonist stimulation.

The present study has demonstrated that all observed alterations of the septohippocampal innervation in drug-exposed progeny, both pre- and postsynaptically, can be partly or totally reversed by transplanting normal fetal presynaptic components. Moreover, transplantation restored PKC sensitivity to the cholinergic agonist to nearly normal levels. The functional ramifications of this reversal are attested to by the fact that the related behavioral deficits were also restored. If postsynaptic desensitization can be reversed by rebuilding the presynaptic components through neural grafting, then the logi-

cal interpretation is that hyperactivity of the presynaptic (i.e., cholinergic) element is the primary alteration as opposed to PKC desensitization being the primary bottleneck for which other phases attempt to compensate. Increased basal PKC activity has been shown to be accompanied by PKC desensitization (25,40).

Presynaptic overstimulation present during the period of synaptic development may result in hyperactive postsynaptic receptors and responses possibly because the neurotransmitter exerts a positive trophic effect on receptors and responses, thus producing a permanently hyperactive biochemical postsynaptic state (19,38,50). Exposure to heroin and phenobarbital may have a similar effect. Therefore, a compelling hypothesis is that if presynaptic alterations are the primary events, then the druginduced presynaptic overstimulation, which was present during the period of synaptic development, caused a "turned-on" postsynaptic state, thereby resulting in desensitization of PKC to cholinergic agonist stimulation. However, other alternatives should be considered, including the possibility that the primary event was an early drug-induced alteration in the afferent pathways regulating the activity of the septal neurons involved in the septohippocampal pathway. One candidate might be the dopaminergic input from the mesencephalic A10 group (53,79), which acts on cholinergic cells via GABAergic intermediates.

It is unclear how grafts of fetal cholinergic neurons could restore the normal rhythmic fluctuations in pre- and postsynaptic activity at the septohippocampal synapse. It has not been determined yet whether prenatal exposure to phenobarbital or heroin decreases the number of cholinergic neurons projecting to the hippocampus. If the number is unchanged in the drug-exposed offspring, then opportunities for grafted neurons to establish synapses with host hippocampal cells would seem intuitively to be rare (6,12). Perhaps "normal" cholinergic cells, free from defectively hyperactive afferent influences, e.g., from A10 dopamine neurons, regulate the host septohippocampal afferents by means of tonic release of transmitter that would act at cholinergic autoreceptors on the nerve terminals. Relatively few cholinergic neurons were evident in the grafts, suggesting that restoration of normal activity may have also resulted from a more general mechanism. A diffusable factor or factors released by neurons or nonneuronal cells in the fetal grafts (65) might have mediated a "resetting" of pre- and/or postsynaptic cholinergic/muscarinic activity or induced sprouting of new projections and synapses by the host neurons and thereby reversed the functional impairments (4,8). Defective elements at the postsynaptic membrane would in this scenario be corrected by trophic factors released by grafts of fetal cholinergic cells (3). Introducing different agents into the brain in the absence of fetal grafts can reverse neurologic deficits (56). One approach to test this theory is the transplantation of non-ACh-producing septal neurons and glial cells into the drug-exposed hippocampus. Such an approach is possible today, thanks to advances in our ability to transfect genes that may prevent expression of ACh or one of the enzymes in its biosynthetic pathway and would presumably not affect other functions of the cell [for review, see (18)].

The data presented here provide new insights into the neurobehavioral teratogenicity of heroin and phenobarbital. The initial steps toward elucidating the mechanism(s) underlying the observed behavioral deficits induced by these drugs became feasible by focusing on behaviors that are largely related to a particular area and biochemical process in the brain. We noted, for instance, that pre- and postsynaptic hyperactivity, uncommon sequellae of insults to the mature brain, characterized the behavioral syndrome resulting from prenatal drug exposure. The behavioral and biochemical changes proved responsive to the introduction of fetal septal neurons, which also provided important new information regarding the site and nature of the precipitating deficit. This finding illustrates the power and utility of neural grafting as an approach in neurobiology in general and neurobehavioral teratology in particular. It is clear, however, that successful application of neural transplantation and interpretation of the outcome is dependent on the existence of a specific and well-defined deficit even when, as in the present model, it is accompanied by deficits in other neural processes. Thus, neural grafting was early in showing reverse motor deficits in animal models of Parkinson disease and seems promising for different clinical applications (17). For similar reasons, neural grafting could be applied to reverse the behavioral deficits caused by damage to the hippocampus and to other mechanism-specific models. Neural grafting also suggests a possible direction in which further studies designed to tie the behavioral deficits to the biochemical alterations observed in causal relationships should aim. Furthermore, neural grafting may aid in the interpretation of those alterations that are unique to the outcome of neural insult incurred during development.

Despite these benefits, the neural transplantation approach cannot by itself provide complete answers to the types of questions posed here. Nevertheless, when applied in concert with other approaches, it provides a unique substrate for assessing developmental and regulatory influences on the CNS. These approaches should come from diverse origins. An obvious choice in the present model is to attempt a reversal or worsening of the behavioral deficits by way of a cholinergic agonist or agonists, respectively. Other possible methodologies are the local administration of gene transfection or the correlation between behavior and biochemistry, either of which may move us closer to the determination of lineal relationships. For example, the hypothesized relationship between alterations in the septohippocampal cholinergic innervations and behavioral deficits observed following prenatal exposure to heroin (80) and to phenobarbital (54) gained important support from the results of the grafting studies presented here.

The present approach demonstrates that reversal of impaired processes, once understood, can be carried out even amidst multiple deficits, which remain undescribed. This approach also opens a way for the development of animal models for human congenital neurobehavioral anomalies, in which one of several central compromised processes may be restored to near normalcy even though the rest of the anomalies remain beyond reach, i.e., an improved functional state even without restoring complete normalcy. With each piece of the puzzle of the impaired neural network solved, the number of pieces left is reduced.

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REFERENCES

1. Abu-Roumi, M.; Newman, M. E.; Yanai, J. Muscarinic receptor related inositol phosphate formation in mice prenatally-exposed to drugs. Brain Res. Bull. in press.
2. Akers, R. P.; Lovin, D. M.; Colley, P.; Linden, D. J.; Routtenberg, A. Translocation of PKC activity may mediate hippocampal long-term potentiation. Science, 231:374–378; 1986.
3. Arendt, T.; Allen, Y.; Sindin, J.; Schugens, M. M.; Marchbanks, R. M.; Lantos, P. L; Gray, J. A. Cholinergic-rich brain transplants reverse alcohol-induced memory deficits. Nature 332:448–450; 1989.
4. Bankiewicz, S.; Plunkett, R.; Jacobowitz, D.; Kopin, I.; Oldfield, E. Fetal nondopaminergic neural implants in parkinsonian primates. J. Neurosurg. 74:97–104; 1991.
5. Berridge, M. J.; Dawson, R. M. C.; Downes, C. P.; Heslop, J. P.; Irvine, R. F. Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membranephosphoinositides. Biochem. J. 212:473–482; 1983.
6. Bjorklund, A.; Stenevi, U. Reformation of the severed septohippocampal cholinergic pathway in the adult rat by transplanted septal neurons. Cell Tissue Res. 185:289–302; 1977.
7. Bjorklund, A.; Stenevi, U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. Brain Res. 177:555–560; 1979.
8. Bohn, M. C.; Cupit, L.; Marciano, F. Adrenal medulla grafts enhance recovery of striatal dopaminergic fibers. Science 237:913–915; 1987.
9. Brundin, P.; Isacson, O.; Bjorklund, A. Monitoring of cell viability in suspensions of embryonic CNS tissue and its use as criterion for intracerebral graft survival. Brain Res. 331:251–259; 1985.
10. Chang, H. T. Dopamine–acetylcholine interaction in the rat striatum: A dual-labeling immunocytochemical study. Brain Res. Bull. 21:295–304; 1988.
11. Cheney, D. L.; Lehmann, J.; Cosi, C.; Wood, P. L. Determination of acetylcholine dynamics. Drugs Neurotransmit. Res. 12:443–495; 1989.
12. Clarke, D. J.; Gage, F. H.; Nilsson, O. G.; Bjorklund, A. Grafted septal neurons form cholinergic sytnaptic connections in the dentate gyrus of behaviorally impaired aged rats. J. Comp. Neurol. 252:483–492; 1986.
13. Cooper, J. R.; Bloom, F. E.; Roth, R. H. The biochemical basis of neuropharmacology, 5th ed. New York: Oxford University Press; 1986.
14. Doberczak, T. M., Kandall, S. R., Wilets, I. Neonatal opiate abstinence syndrome in term and preterm infants. J. Pediatr. 118(6):933–937; 1991.
15. Dunnet, S. B.; Everitt, B. J.; Robbins, T. W. The basal forebrain-cortical cholinergic system: Interpreting the functional consequences of exotoxic lesions. Trends Neurosci. 14:494–500; 1991.
16. Ebendal, T. NGF in CNS: Experimental data and clinical implications. Prog. Growth Factor Res. 1:143–159; 1989.
17. Freed, W. J. Substantia nigra grafts and Parkinson's disease: From animal experiments to human therapeutic trials, Restor. Neurol. Neurosci. 3:109–134; 1991.
18. Gage, F. H.; Ray, J.; Fisher, L. J. Isolation, characterization, and use of stem cells from the CNS. Annu. Rev. Neurosci. 18:159–192; 1995.
19. Giannuzzi, C. E.; Seidler F. J.; Slotkin, T. A.; βAdrenoceptor control of cardiac adenylyl cyclase during development: Agonist pretreatment in the neonate uniquely causes heterologous sensitization, not desensitization. Brain Res. in press.
20. Gil, D. G.,; Wolfe, B. B. Pirenzepine distinguishes between muscarinic receptor-mediated phosphoinositide breakdown and inhibition of adenylate cyclase. Therapeutics 232(3):608–616; 1985.
21. Jaffard, R.; Ebel, A.; Destade, C.; Durkin, T.; Mandel, P.; Cardo, B. Effects of hippocampal electrical stimulation on longterm memory and on cholinergic mechanisms in three inbred strains of mice. Brain Res. 133:277–290; 1977.
22. Jope, R. S. High affinity choline transport and acetylCoA production in brain and their roles in the regulation of acetylcholine synthesis. Brain Res. Rev. 1:314–344; 1979.
23. Kellar, K. J.; Martino, A. M.; Hall, D. P.; Schwartz, R. D.; Taylor, R. L. High affinity binding of [^{3}H]acetylcholine to muscarinic cholinergic receptors. J. Neurosci. 5:1577–1582; 1985.
24. Kikkawa, U.; Takai, Y.; Minakuchi, R.; Inohara, S.; Nishizoka, Y. Calcium activated phospholipid dependent protein kinase from rat brain: Subcellular distribution, purification and properties. J. Biol. Chem. 257:13341–13348; 1982.
25. Kikkawa, U.; Kishimoto, A.; Nishizuka, Y. The protein kinase C familiy: Heterogeneity and its impication. Annu. Rev. Biochem. 58:31–44; 1989.
26. Kleinberger, N.; Yanai, J. Early phenobarbital-induced alterations in hippocampal acetylcholinesterase activity and behavior. Dev. Brain Res. 22:113–123; 1985.
27. Klemm, N.; Kuhar, M. J. Post-mortem changes in high affinity choline. J. Neurochem. 32:1487–1494; 1979.
28. Kondoh, T.; Blount, J. P.; Conrad, J. A.; Pundt, L. L.; Low, W. C. Functional effects of transplanted human fetal ventral mesencephalic brain tissue from spontaneous abortions into a rodent model of Parkinson's disease. Transplant. Proc. 26(6):3335; 1994.
29. Linden, D. J.; Routtenberg, A. The role of protein kinase C in long term potentiation a stable model. Brain Res. Rev. 14:279–296; 1989.
30. Lindvall, O. Prospects of transplantation in human neurodegenerative diseases. Trends Neurosci. 14:376–388; 1991.
31. Low, W. C.; Lewis, P. R.; Bunch, S. T.; Dunnet, S. B.; Thomas, R.; Iversen, S. D.; Bjorklund, A.; Stenevi, U. Function recovery following neural transplantation of embryonic septal nuclei in adult rats with septohippocampal lesions. Nature 300:260–262; 1982.
32. Maeda, T.; Kaneko, S.; Satoh, M. Bidirectional modulation of long-term otentiation by carbachol via M_1 and M_2 muscarinic receptors in guinea pig hippocampal mossy fiber–CA3 synapses. Brain Res. 619:324–330; 1993.
33. McClearn, G. E.; Wilson, J. R.; Meredith, W. The use of isogenic and heterogenic mouse stocks in behavioral research. In: Lindzey, G.; Thiessen, D. D., eds. Contribution to behavioral–genetic analysis: The mouse as a prototype. New York: Appleton-Century-Crofts; 1970:3–22.
34. McKinney, M.; Danderson, D.; Vella-Rountree, L. Different agonist-receptor active conformations for rat brain M_1 and M_2 muscarinic receptors that are separately coupled to two biochemical effector systems. Mol. Pharmacol. 35(1):39–47; 1989.
35. Morris, R. Development of water maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11:47–60; 1984.
36. Morris, R. M. G.; Anderson, E.; Lynch, G. S.; Baudry, M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-aspartate receptors agonist, AP5. Nature 319:774–776; 1986.
37. Murrin, L. C. High-affinity transport of choline in neuronal tissue. Pharmacology 21:132–140; 1980.
38. Nathnson, N. M. Regulation and development of muscarinic receptor number and function. Muscarin. Recept. 419–454; 1989.
39. Nilsson, O. G.; Shapiro, M. L.; Gage, F. H.; Olton, D. S.; Bjorklund, A. Spatial learning and memory following fimbria-fornix lesion and grafting of fetal septal neurons to the hippocampus. Exp. Brain Res. 67:195–215; 1987.
40. Nishizuka, Y. Three multifunctional protein kinase systems in transmembrane control. Mol. Biol. Biochem. Biophys. Mol. Biol. Biochem. Biophys. 32:113–135; 1980.
41. Nishizuka, Y. The role of protein kinase c in cell surface signal transduction and tumour promotion. Nature 308:693–697; 1984.
42. Nishizuka, Y. Studies and perspectives of protein kinase C. Science 233:305–311; 1986.
43. Norberg, A.; Wahlstrom, G. Effect of long-term oral barbital administration on endogenous acetylcholine in different regions of the rat brain. Eur. J. Pharmacol. 43:237–242; 1977.
44. Ohkuma, S.; Kisi, M.; Fu-Hai, M. A.; Kuriyama, K. Association of functional alteration in intracellular signal transduction system with the occurrence of up-regulation of muscarinic receptors in

primary cultured neurons. Prog. NeuroPsychopharmacol. Biol. Psychiatr. 16:413–424; 1992.
45. Olton, D. S.; Samuelson, R. J. Remembrance of places passed: spatial memory in rats. J. Exp. Psychol. Animal Behav. Proc. 2:97–116; 1976.
46. Pakzalian, P.; Isaccson, O. Neural xenotransplantation: Reconstruction of neural circuitry across species barriers. Neuroscience 62:989–1001; 1994.
47. Perlow, M. J.; Freed, W. J.; Hoffer, B. J.; Seiger, A.; Olson, L; Wyatt, R. J. Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. Science 204:643–647; 1979.
48. Pick, C. G.; Yanai, J. Long-term reduction in eight arm maze performance after early exposure to phenobarbital. Int. J. Dev. Neurosci. 3(3):223–227; 1985.
49. Pick, C. G.; Weizman, A.; Fares, F.; Gavish, M.; Kanner, B. I.; Yanai, J. Hippocampal γ-aminobutyric acid and benzodiazepine receptors after early phenobarbital exposure. Dev. Brain Res. 74:111–116; 1993.
50. Pratt, R. M. Hormones, growth factors, and their receptors in normal and abnormal prenatal development. In: Kalter, H., eds. Issues and reviews in teratology. New York: Plenum Press; 1984:189–209.
51. Reinisch, M. C.; Sanders, S. A.; Mortensen, E. L.; Psych, C.; Rubin, D. B. In utero exposure to phenobarbital and intelligence deficits in adult men. JAMA 274(19):1518–1525; 1995.
52. Richards, M. H. Pharmacology and second messenger interactions of cloned muscarinic receptors. Biochem. Pharmacol. 42:1645–1653; 1991.
53. Robinson, S.; Malthe-Sorenssen, D.; Wood, P.; Commissiong, J. Dopaminergic control of the septal hippocampal cholinergic pathway. J. Pharmacol. Exp. Ther. 208:476; 1979.
54. Rogel-Fuchs, Y.; Newman, M. E.; Trombka, D.; Zahalka, E. A.; Yanai J. Hippocampal cholinergic alterations and related behavioral deficits after early exposure to phenobarbital. Brain Res. Bull. 29:1–6; 1992.
55. Rogel-Fuchs, Y.; Zahalka, E.; Yanai, J. Reversal of early phenobarbital-induced cholinergic and related behavioral deficits by neuronal grafting. Brain Res. Bull. 33:273–279; 1994.
56. Sabel, B. A.; Labbe, R.; Stein, D. G. The saline effect: Minimizing the severity of brain damage if secondery degeneration. Exp. Neurol. 881:95–107; 1985.
57. Saitoh, Y.; Matsui, Y.; Nihonmatsu, I.; Kawamura, H. Crossspecies transplantation of the suprachiasmatic nuclei from rats to Siberian chipmunks (*Eutamias sibiricus*) with suprachiasmatic lesions. Neurosci. Lett. 123:77–81; 1991.
58. Sharkawi, M.; Schulman, M. P. Inhibition by morphine of the release of [^{14}C] acetylcholine from rat brain cortex slices. J. Pharmacol. Pharm. 21:546; 1969.
59. Siegel, S. Non-parametric statistics for the behavioral sciences. New York: McGraw-Hill; 1956.
60. Simon, J. R.; Atweh, S.; Kuhar, M. J. Sodium-dependent high affinity choline uptake: A regulatory step in the synthesis of acetylcholine. J. Neurochem. 26:909–922; 1976.
61. Slotkin, T. A.; Nemeroff, C. B.; Bissette, G.; Seidler, F. J. Overexpression of the high affinity choline transporter in cortical regions affected by Alzheimer's disease: Evidence from rapid autopsy studies. J. Clin. Invest. 94:696–702; 1994.
62. Snyder, E. Y. Grafting immortalized neurons to the CNS. Curr. Opin. Neurobiol. 4:742–751; 1994.
63. Sokolovsky, M. Muscarinic cholinergic receptors and their inter actions with drugs. Adv. Drug Res. 18:431–509; 1989.
64. Solberg, Y.; Pollack, Y.; Silverman, W. F. Expression of tyrosine hydroxylase mRNA in transplanted dopamine neurons, Neurosci. Lett. 106:36–42.; 1989.
65. Springer, J. E.; Collier, T. J.; Sladek, J. R. Jr.; Loy, R. Transplantation of male mouse submaxillary gland increases survival of axotomized basal forebrain neurons. J. Neurosci. Res. 19:291–296; 1988.
66. Staubli, U.; Rogers, G.; Lynch, G. Facilitation of glutamate receptors enhances memory, Proc. Natl. Acad. Sci. USA 91:777–781; 1994.
67. Thompsos, G. W. Successful brain grafting. N.Y. Med. 2:701–702; 1980.
68. Vickroy, T.; Roeske, W.; Yamamura, H. Sodium-dependent high-affinity binding of [^{3}H]hemicholinium-3 in the rat brain: A potentially selective marker for presynaptic cholinergic sites. Life Sci. 35:2335–2343; 1984.
69. Watson, M.; Roeske, W. R.; Amamura, H. I. [^{3}H]Pirenzepine selectively identifies a high affinity population of muscarinic cholinergic receptors in the rat cerebral cortex. Life Sci. 31:2019–2023; 1982.
70. Wehner, J. M.; Sleight, S.; Upchurch, M. Hippocampal protein kinase C activity is reduced in poor spatial lerners. Brain Res. 523:181–187; 1990.
71. Welsh, L. H. O^3-monoacetyl-morphine. J. Organ. Chem. 19:1409–1415; 1954.
72. Weiner, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1971.
73. Yanai, J; Preface. In: Yanai, J., ed. Neurobehavioral teratology. Amsterdam: Elsevier; 1984:V–X.
74. Yanai, J. An animal model for the effect of barbiturate on the development of the central nervous system. In: Yanai, J., ed. Neurobehavioral teratology. Amsterdam: Elsevier, Amsterdam; 1984:111–132.
75. Yanai, J.; Pick, C. G. Studies on noradrenergic alternations in relation to early phenobarbital-induced behavioral changes. Int. J. Dev. Neurosci. 5:337–344; 1987.
76. Yanai, J.; Pick, C. G. Neuron transplantation reverses phenobarbital-induced behavioral birth defects in mice. Int. J. Dev. Neurosci. 6:409–416; 1988.
77. Yanai, J.; Sze, P. Y.; Iser, C.; Melamed, E. Studies on brain monoamine neurotransmitters in mice after prenatal exposure to barbiturate. Pharmacol. Biochem. Behav. 23:215–219; 1985.
78. Yanai, J.; Greenfeld, Z.; Laxer, U.; Pick, C. G.; Trombka, D.; Weinstein, D. CNS changes after early barbiturate exposure: Mechanisms and reversal. In: Fujii, T.; Adams, P. M., eds. Functional teratogenesis. Tokyo: Tokyo University Press; 1987: 121–130.
79. Yanai, J.; Laxer, U.; Pick, C. G.; Trombka, D. Dopaminergic denervation reverses behavioral deficits induced by prenatal exposure to phenobarbital. Dev. Brain Res. 48:255–261; 1989.
80. Yanai, J.; Avraham, J.; Maslaton, J.; Rogel-Fuchs, Y.; Zahalka, E. A. Alterations in septohippocampal cholinergic innervations and related behaviors after early exposure to heroin and phencyclidine. Dev. Brain Res. 69:207–214; 1992.
81. Yanai, J.; Abu-Roumi, M.; Avraham, Y.; Steingart, R. A. Behavioral deficits and both pre-and postsynaptic cholinergic hyperactivity after early drug-exposure. Proceedings of the DrugAbuse Conference. Madeira, Portugal. 1995: in press.
82. Zagon, I. S.; McLaughlin, P. J. An overview of the neurobehavioral sequelae of prenatal opioid exposure. In: Yanai, J., ed. Neurobehavioral teratology. Amsterdam: Elsevier; 1984:197–234.
83. Zahalka, E.; Seidler, F. J.; Lappi, S. E.; Yanai, J.; Slotkin, T. A. Deficits in the development of central cholinergic pathways caused by fetal nicotine exposure: Differential effects on choline acetyltransferase activity and [^{3}H]hemicholinium-3 binding. Neurobehav. Toxicol. Teratol. 14:375–382; 1992.