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CHRONIC DRUG EXPOSURES DURING DEVELOPMENT IN NONHUMAN PRIMATES: MODELS OF BRAIN DYSFUNCTION IN HUMANS

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1. ABSTRACT

This review of our work presents three specific examples of how nonhuman primates (rhesus monkeys, *Macaca mulatta*) have been used to study the effects of chronic drug exposures on brain function during different stages of development. In all cases, exposure levels similar to those experienced by humans were employed and the focus was on long-term--not acute--effects. In the case of the marijuana studies, exposures occurred during the adolescent period; for the cocaine studies, exposures occurred in binge-like fashion entirely before birth (*in utero*); and for the remacemide studies, exposures occurred daily in juveniles, prior to adolescence. An automated battery of behavioral tasks, the National Center for Toxicological Research Operant Test Battery (NCTR OTB), designed to assess aspects of motivation, visual discrimination, time perception, short-term memory, and learning, was used to monitor treatment effects. Chronic marijuana smoke exposure resulted in an 'amotivational' syndrome--even in weekend-only smokers--that resolved within three months of exposure cessation. *In utero* cocaine exposure was shown to cause behavioral rigidity or lack of plasticity as evidenced by the difficulty of subjects to adjust to rules changes for some OTB tasks. These effects were seen in adult subjects suggesting that the effects of gestational cocaine exposure are long-term or permanent. In addition, animals exposed to cocaine *in utero* were less sensitive to the behaviorally-disrupting effects of cocaine as adults. Remacemide caused profound and long-lasting, perhaps permanent, changes in learning task performance and because performance of this same task by children is significantly correlated with traditional measures of intelligence (IQ), these data suggest that such treatment may provide a valuable model of chemically-induced mental retardation.

2. INTRODUCTION

A primary assumption of this review is that for many aspects of biomedical research the need for animal models is absolute. Animal models are required pre-clinically in drug-discovery processes and in studies of safety pharmacology and toxicology. Animal models are also used to study disease processes and interventions and to determine the safety of environmental chemicals. Sometimes conditions resulting from drug or chemical exposures in animals can form the basis for the development of important new animal models. We believe this especially to be the case in our studies of remacemide exposures during preadolescence.

In an ideal world, drug efficacy and toxicity and chemical safety would be well established using appropriate animal models under well-controlled experimental conditions before the occurrence of human exposures. This is not always possible, however, so studies on safety and toxicity are often conducted in animals in parallel with human use. The continued need for animal studies in the presence of human exposures results from the general impossibility of conducting definitive human studies. For example, the difficulties in demonstrating effects of *in utero* drug exposures in human offspring have been attributed—in the case of cocaine for example-- to difficulties in the techniques of measurement which preclude accurate determination of the type, dose, and pattern of drug use (1-5) and to difficulties in controlling for a wide range of potentially confounding variables, such as other drug use, race, socioeconomic status, and level of prenatal care (1, 3, 5). Such limitations can seriously undermine assessments in humans concerning the effects of chronic drug exposure on growth and development and more recent studies are taking these potential confounds into consideration (e.g., (6, 7)).

Computer models and *in vitro* systems simply do not approach the requisite complexity of whole organisms and therefore cannot replace them. If one were to generate an artificial model that could adequately simulate a complete animal, the model would be essentially the animal that it was designed to mimic. Nonhuman animals are more similar to humans than they are dissimilar and as such they can effectively serve as reasonable surrogates. The proximity of nonhuman primates to humans on the phylogenetic tree generally qualifies them as the model that most closely approximates the human condition. The ethical use of nonhuman primates for research purposes has been eloquently addressed in detail (8). This greater similarity to humans can be very important especially if one is trying to model human attributes that are not readily observable in other animals. For example, the tissues associated with placentation and the cycles of reproductive hormones observed in nonhuman primates are very similar to those of humans as are their complex social structures, prolonged nursing periods and life-span. This is not so with most non-primates. The focus of this review will be on complex brain function after long-term drug exposures during development, areas of research for which the nonhuman primate is likely the most appropriate animal surrogate for humans. The tasks currently being used to assess complex brain function in nonhuman primates have been shown to have direct relevance for, if not direct equivalence in, humans (9). In addition, nonhuman primate metabolism in general, as well as drug metabolism and disposition in particular, are often very similar to that seen in humans. And importantly, the developmental trajectory for nonhuman primates, particularly the rhesus monkey that will be highlighted here, is very similar to that of humans, not only in terms of developmental milestones but also in terms of time-course.

3. METHODS OF ASSESSING COMPLEX BRAIN FUNCTION IN NONHUMAN PRIMATES: OBJECTIVITY, AUTOMATION AND REPEATED MEASURES

About two decades ago, during preparations for a large scale monkey study in which some of the primary endpoints of interest were aspects of complex brain function, a battery of operant behavioral tasks was developed (see (9, 10-13). This instrument has since become known as the National Center for Toxicological Research (NCTR) Operant Test Battery or OTB. The term operant simply means that subjects have to operate something in their environment (in this case response levers or press-plates) in order to obtain reinforcers (in our case, banana-flavored food pellets). Operant tasks require some degree of training and therefore differ from untrained, spontaneous or ethological behaviors that can also serve as metrics of brain function. The beauty and strength of operant behaviors stems from the fact that they can be made extremely specific, allowing for the isolation of brain functions deemed especially important or noteworthy (i.e., visual discriminations, learning, time perception) and they can be generated at the will of the experimenter because their elicitation comes under strong stimulus control after appropriate training (i.e., the presence or absence of cue lights or tones can be used to indicate the availability of reinforcement and prompt subject participation). In order to maximize the value of the food reinforcers often used in such studies, and therefore maximize motivation and the likelihood that subjects will work toward obtaining them, it is imperative that access to food be strictly controlled. It is often necessary to individualize daily food allotments for each subject since animals tend to vary substantially in the amount of food needed to maintain appropriate body weight and food-directed motivation. For young/developing rhesus monkeys we target a monthly weight gain of about 0.1 kg and provide daily rations to reach this target. If animals gain weight faster than desired, daily rations are decreased; if they don't gain weight fast enough, daily rations are increased. The quality of operant procedures to allow for precise scheduling of experimental events provides maximal efficiency of data collection. Operant tasks also lend themselves very nicely to automation: the NCTR OTB, for example, is completely computer-driven. This aspect of task administration not only frees up study personnel for other tasks, but also eliminates the potential confounding interactions that can occur between experimenters and subjects under circumstances where direct observations and/or other kinds

of human-animal interactions are part of the assessment. In addition, the automated nature of task administration and data collection eliminates subjective interpretation of responses and easily provides for repeated testing under identical conditions.

3.1. Functional Domains

As mentioned previously, operant behaviors can be highly specified. That is, very specific responses can be required for reinforcer delivery. Response rates can be reliably and predictably controlled by schedules of reinforcement (14). For example, very rapid response rates can be elicited by scheduling reinforcer delivery after some set number of responses (e.g., every 20 lever presses). Under such schedules, subjects tend to respond at high frequency in order to rapidly attain criteria for reinforcer delivery. This schedule is similar to that used when persons are paid by the job, rather than by the hour. Under fixed time schedules, responses are reinforced at only specific intervals (e.g., every 5 minutes). Under these schedules, subjects tend not to respond much, if at all, during the beginning of the interval, but as the interval progresses, response rates increase such that near the end of the interval, responding occurs at a very high rate. This schedule is similar to those operating in typical public transportation settings, for example: as the scheduled time of bus arrival nears, patrons spend an increasing amount of time focused on the direction from which the bus will come. In addition to precisely controlling response rates, reinforcement contingencies can also be used to train specific types of responses requiring subjects to solve specific problems. Several types of problem solving behaviors are modeled using the NCTR OTB.

Studies begun in the mid-1980s at the Food and Drug Administration's NCTR incorporated the OTB for use with laboratory rhesus monkeys. Initial OTB descriptions and drug effects can be found elsewhere (10-12).It has also been demonstrated that, in general, performance in each of the OTB tasks is not predictive of performance in any of the other OTB tasks: there is little correlation between performance in one task and performance of any of the others (9). This is important because it demonstrates that different brain functions subserve performance in each of the OTB tasks and verifies that each task is measuring something different than that which is measured in the other tasks. This is further demonstrated by the ability of a variety of psychotropic agents to rather selectively alter performance of one task and not others (9). In addition to being used in the studies to be reviewed here, the NCTR OTB has been used fairly extensively by us to develop a library of the acute effects of a variety of prototypic psychoactive compounds (see (9)). The specific brain functions thought to be modeled by performance of the NCTR OTB and the tasks utilized to assess are described here briefly.

3.1.1. Motivation

The NCTR OTB has incorporated a task specifically for the purpose of obtaining an assessment of behavior thought to be indicative of a subject's motivation to 'work' for the reinforcers being used (here they are banana-flavored food pellets). Referred to as a Progressive Ratio task (initially described in (15-16)), responses are made on a single response lever and the number of lever presses required for reinforcer delivery (the response/reinforcer ratio) increases for each reinforcer. Thus, some small number of lever presses (e.g., 2) is required for the first food pellet while the next reinforcer 'costs' 4 lever presses, the next 6 and so on. In this manner, the work required for reinforcement can be ramped up in short order, providing metrics (response rates, number of reinforcers earned, etc.) of motivation.

3.1.2. Visual and Position Discrimination

To determine how well subjects can discriminate between different objects and make decisions based on those differences a Conditioned Position Responding Task using colored stimuli is employed. Here, three press-plates arranged in a horizontal line serve as manipulanda and, initially, a red, yellow, blue or green color is presented on the middle press-plate. Subjects indicate that they have seen the stimulus by pressing the illuminated plate after which it is immediately extinguished and the two side plates are illuminated white. If the middle press-plate had been red or yellow, then a left choice response is reinforced; if it had been blue or green, then a right choice response is reinforced. Task solution is relatively simple for most subjects, as evidenced by rapid responding and average choice accuracies of greater than 90%. Processes underlying these types of abilities are thought to reside in frontal-cortical brain areas (17-18).

3.1.3. Learning

The learning task used in the NCTR OTB is an Incremental Repeated Acquisition Task that is a modification of more traditional repeated acquisition procedures (19). Here subjects must repeatedly acquire knowledge in an incremental fashion. Four response levers (arranged in a horizontal plane) serve as the manipulanda and each test session starts with presentation of a one-lever response 'sequence': a response to the correct one of the four levers results in reinforcer delivery. After this sequence has been mastered, the sequence length (and presumably task difficulty) is incremented to a two-lever response sequence where the subject must learn which of the four levers is the 'new' lever for the 'incremented' sequence and remember to follow a response to it with a response to the correct lever from the previously learned one-lever 'sequence.' Once this two-lever sequence has been mastered, the response requirement is again incremented to a three-lever sequence and so on up to a six-lever sequence. Thus, in each test session, several learning curves are obtainable, one for each lever sequence length mastered and both accuracy and speed of task solution are captured. For those familiar with the children's game "Simon®", this task can be thought of as its monkey version. A key aspect of this task is that the correct sequence of lever presses changes every test session and, thus, the correct response sequence cannot be predicted from session to session. In addition, while there clearly is a short-term memory component to this task (subjects must recall which sequence they learned for the prior, shorter sequence in that same test session), the bulk of the errors

made during typical performance of this task are acquisition-type errors, i.e., those made during determination of which response represents the required incremented response.

3.1.4 Short-term Memory

For assessing processes associated with short-term memory a Delayed Matching-to-Sample (DMTS) procedure is used (see (20) for a mini-review of experimental approaches to the study of short-term memory). Each trial in the task begins with the illumination of the middle one of three press-plates (same manipulanda used for the color discrimination task described above). Stimuli are white-on-black geometric shapes and subjects acknowledge an initial 'sample' stimulus by pressing the illuminated plate after which it is immediately extinguished. Following a recall delay (e.g., from 1-32 seconds) that varies randomly, all three plates are illuminated, each with a different shape, only one of which 'matches' the 'sample' stimulus. A response to the 'matching' shape results in reinforcer delivery. Accuracy of matching after no or very short delays, i.e., with no or little opportunity to forget, is thought to represent a measure of an organism's ability to attend to the task and encode the information to be remembered and response accuracies at very short delays is typically quite high. As recall delay increases, response accuracy decreases, with the slope of the decrease in accuracy over time representing a memory decay or forgetting function. Unlike with the IRA task, here subjects are presented with the solution to the task (i.e., the sample stimulus), having only to recall (match) that solution when presented with choices after a recall delay.

3.1.5. Time perception

For the assessment of timing behavior, a Temporal Response Differentiation task is used (see (21) for a review of issues concerning experimental aspects of timing ability). This task requires subjects to press and hold a response lever in the depressed position for at least 10 but not more than 14 seconds. Thus, subjects must target a 4-second window of opportunity in order to obtain a reinforcer. The data obtained from this task include a variety of measures associated with the distribution of lever-hold durations (time production) which are typically characterized by Gaussian distributions. It is thought that the distribution means of 'timed' responses represent timing accuracy and that the spread or standard deviation of the distribution represents timing precision. Alterations in the characteristics of the response duration distribution are thought to provide insights into the mechanisms of timing such as the speed of an 'internal clock' (22).

4. RELEVANCE TO THE HUMAN EXPERIENCE

Data obtained from children performing these same NCTR OTB tasks (for nickel reinforcers instead of banana-flavored food pellets) have provided important comparative information. First, the OTB performance of children is, in most cases, indistinguishable from that of well-trained monkeys (23) and second, task performance is, in most cases, significantly correlated with IQ (24). A notable exception to this generalization is performance of the motivation task: children with IQ scores of 70 to 130 all respond for nickels with equal vigor (24). Thus, the exact same testing instrument, the NCTR OTB, can be used in both humans and monkeys, allowing one to presumably study the same cognitive processes in both species. In addition, performance of some OTB tasks can serve as surrogates for typical paper and pencil IQ testing.

5. SPECIFIC EXAMPLES OF DERANGED BRAIN FUNCTION AFTER DEVELOPMENTAL DRUG EXPOSURES

5.1. Marijuana and Amotivation

To model human teenagers, rhesus monkeys 2 to 3 years of age were used as subjects to determine the effects of chronic marijuana (MJ) smoke exposure on complex brain function and structure (see (13) for study details). All subjects were trained to perform the NCTR OTB for one year prior to the onset of MJ smoke exposure. For most subjects, performance of each OTB task was stable enough during the last 3 months of the training year to be relatively predictable (i.e., 95% confidence intervals were reasonable). Exposures were effected with the use of a metered smoke generation system that delivered smoke via nose-mouth-only masks (modified infant anesthesia masks) and one-way inhalation valves to subjects (13). To minimize the acute effects of MJ smoke, daily exposures occurred right after behavioral assessments were complete; thus, subjects were behaviorally tested approximately 22-23 hours after their last exposure. Earlier studies on the acute effects of MJ smoke on OTB performance by rhesus monkeys (11) demonstrated that the short-term memory (DMTS) and timing (TRD) tasks were the most sensitive, followed by the learning (IRA) and color and position discrimination (CPR) tasks and then the motivation (PR) task. For the chronic studies, the four exposure groups (n = 15-16/group) included daily MJ smokers, weekend only MJ smokers, daily placebo MJ (marijuana extracted such that it did not contain any psychoactive active cannabinoids) smokers and daily sham exposures (fitted with exposure masks but exposed to room air only). One half of subjects were behaviorally assessed throughout the entire exposure phase (one year) of the study and for seven months following the last exposure. The other half was not behaviorally assessed during the year of exposure and for two months following cessation of exposure. For animals tested throughout the exposure phase, behavioral test sessions occurred just prior to daily exposures such that assessments occurred long after their last exposure. Again, this served to minimize the acute effects of MJ on behavior which can be substantial (11). The smoke from one MJ cigarette or one extracted MJ cigarette was administered during each exposure session.

Behavioral data for the twelve weeks immediately preceding the start of exposures served as 'Baseline' data allowing each animal to also serve as its own control. There were no differences in behavioral performance between any of the treatment groups prior to the start of treatment. For each task the effects of exposure and the effects of abrupt withdrawal from exposure were

determined. For the latter determination, data for the last 12 weeks of exposure served as the pre-withdrawal 'Baseline' data. Individual animal data were also examined to determine whether group effects were due to effects on specific individuals.

Over the entire year of exposure, subjects in both the sham and extracted MJ smoke (placebo) groups (the controls) exhibited steady increases in motivation task responding (increases in response rates, number of food pellets earned, etc.). At the end of the exposure year, these control subjects were putting forth about 3 times as much effort in this task as they were at the start of exposure. They were also putting forth significantly more effort than subjects in either the weekend or daily MJ smoke groups where the amount of effort remained virtually unchanged over the entire year. All measures of motivation behavior increased for both MJ groups during withdrawal beginning about one month after cessation of chronic smoke exposure and these increases stabilized about two to three months after the last exposure at levels that were no different from those for either of the control groups. Thus, during periods of active marijuana 'use' by 'teenage' monkeys either every day or just on weekends, an 'amotivational' syndrome was demonstrated and it took several months of abstinence for this condition to completely abate. In animals that were not behaviorally assessed during the year of exposure or for two months afterward, there was no evidence of a residual amotivational effect of treatment.

There were no systematic effects of chronic MJ smoke exposure on response rates or accuracies in the other OTB tasks. The percent of a given task that was completed during the timed behavioral sessions tended to be lower for the daily MJ smoke group but significant differences were rarely noted. Examination of individual subject data for the daily MJ group did reveal that one of the subjects suffered a substantial decrease in percent task completed in the color and position discrimination task starting about 3 months after daily exposure began. This subject alone accounted for a downward trend in performance of this task exhibited by the daily MJ group as a whole. Here, adverse performance was associated with decreased response speed but not in accuracy. Upon cessation of exposure, the behavior of this animal recovered substantially but never to pre-exposure levels. This observation demonstrates that certain individuals exhibit very different sensitivities to the effects of MJ.

The findings from the MJ study support the contention that chronic MJ use in humans can be associated with a syndrome of decreased motivation in humans. In humans, there is little experimental evidence to document an 'amotivational' effect of marijuana in adults (25-28); in fact the primary data supporting the occurrence of the syndrome in humans comes from studies where the subjects were teenagers or young adults (29-30). Thus, the use of a 'teenage' animal model in the present study may have maximized the likelihood of observing the syndrome. The increase in motivation behavior noted here began at about 3-4 years of age and appeared to be a developmental phenomenon. It is likely that this increase coincided with the subjects' transition into or through puberty, which occurs at around 3.5 years of age in rhesus monkeys. The effect of MJ was to suppress this very slowly increasing expression of motivation. For these reasons, the phenomenon is likely to be very difficult to document in humans.

The finding that weekend only marijuana smoke exposure was sufficient to affect motivation was surprising and demonstrates that this effect occurs even with relatively infrequent exposures. Additionally, the effect was as large as that noted in animals exposed to MJ smoke every day. These observations suggest that there may be a slow accumulation of some compound (or metabolites) in MJ smoke that affects performance for an extended period of time. This latter suggestion is consistent with recent reports of a long half-life for THC in blood (31-32).

5.2. Cocaine

To model human pregnancies, young adult female rhesus monkeys were time-mated. Once pregnancy was confirmed (usually at about gestational day 35, see (33-35) for study details) chronic exposure to cocaine was begun and continued until birth. Initial treatment groups of 3 subjects each included 0.0, 1.0, and 3.0 mg cocaine hydrochloride (HCl) /kg/day, Monday through Friday, given in three divided intramuscular injections throughout the day. These doses represent human equivalent exposures of about one-tenth to a quarter of a gram of cocaine HCl per day and the Monday through Friday dosing regimen served to model binge-use typical in humans. The intramuscular route provides plasma kinetics in the monkey that nicely model those seen after intranasal administration (snorting) in humans (33). Once it was clear that these initial doses did not adversely affect pregnancy outcome, an additional treatment group given escalating doses of cocaine was added to provide for even greater gestational exposures. For this group, the dose started at 1.0 mg/kg/injection given three times per day (3.0 mg/kg/day, same as the high dose group mentioned previously), Monday through Friday. The dose was then increased every two weeks throughout pregnancy by 0.5 mg cocaine/kg/injection. The escalating procedure was necessary to allow subjects to develop tolerance to the appetite-suppressing effects of cocaine and thus insure adequate nutrition throughout gestation. By the end of the pregnancy some subjects were receiving doses up to 8.5 mg/kg/injection or 25.5 mg/kg/day which are about the equivalent of 1.5 to 1.75 grams of cocaine HCl per day for a 70 kg human. While such amounts are relatively high for casual users, they are certainly not out of the ordinary for heavy users. Once it was demonstrated that this treatment regimen was also well tolerated, a larger group of 10 subjects began exposures for several weeks to months prior to mating to provide for total gestation exposure (35); doses were escalated as described above only after pregnancies had been detected. Since the focus of this study was to determine the effects of *in utero* cocaine exposure on the integrity of offspring brain function, no exposures occurred after birth. No significant effects of exposure were found on body weights of either infants or mothers in the smaller groups of three (although there was a dose-related trend

toward decreased body weights in offspring). However, in the total gestational exposure group, the treated animals weighed significantly less and were of smaller stature (decreased overall length and crown circumference) at birth than controls (35): similar findings have been reported in the literature for children exposed to cocaine during gestation. Infants remained with their natural mothers until weaning at six months of age at which time they were singly housed and began OTB training. Metrics of acquisition of OTB responding were monitored for over a year and no exposure-related effects on response acquisition were observed (34).

5.2.1. Pharmacological Challenges

Over the next six years, a number of pharmacological challenges were carried out in these offspring to determine whether alterations in specific neurotransmitter systems might have been caused by gestational cocaine exposure (see (36) for timeline of drug studies). In particular, the focus was on the dopamine system, with which cocaine is known to interact to cause its typical effects. Dose-response curves for OTB performance were obtained for cocaine and amphetamine (another dopaminergic agent), the somewhat selective dopamine (D2) receptor antagonist haloperidol, the dopamine1 (D1) receptor antagonist SCH-23390, the D2/D3 agonist, quinpirole, and the D2 antagonist, spiperone. In addition, the N-methyl-D-aspartate (NMDA) glutamate receptor channel blocker, MK-801 (see Table 1 in (36) for details) was also tested since the excitatory glutamate system is thought to be important in the development of long-term potentiation (LTP) and this process is thought to be critical for the expression of learning. In these acute drug challenge studies, no differences in drug sensitivities were noted among the smaller treatment groups. However, data are now becoming available for the larger, total gestational exposure group where a preliminary report describes that the cocaine-exposed animals are significantly less sensitive to the behaviorally-disruptive effects of cocaine at around 6 years of age (37). In these studies, intravenous cocaine administration significantly disrupted performance of a time estimation task in control subjects at a dose (1.0 mg/kg) that had no effect on responding in animals exposed to cocaine throughout the whole of gestation. These observations suggest that long-term or permanent alterations in brain sensitivity may be effected by developmental drug exposures. Since others have shown that very similar *in utero* cocaine exposure regimens in monkeys alter the normal migration pattern of dopaminergic neurons in brain (38-40), it is perhaps not surprising that such changes can manifest as functional differences.

5.2.2. Age-related sensitivity to dopaminergic compounds

One very interesting finding obtained during the acute drug challenge experiments was the discovery that, irrespective of treatment group, monkeys become increasingly sensitive with maturity to the behavioral effects of cocaine (41-42). For example, young animals (~1.5 years old) are 10 to 30 times less sensitive to an intravenous injection of cocaine than are adults (~10-11 years old) and juveniles (~3 years old) are intermediate in sensitivity. These observations of drug effects on performance of the motivation task clearly demonstrate that the young primate brain is very much different from the mature primate brain in its susceptibility to the actions of cocaine. Subsequent studies showing similar age-related sensitivities to both amphetamine and methylphenidate suggest that this phenomenon likely relates to the maturation of the dopaminergic system (43). This maturation and blossoming of the dopaminergic system and related physiology may have important implications for the abuse of these drugs since children do not tend to abuse these substances, certainly not to the degree seen in older subjects. In addition, it is possible that any altered function of the dopamine system that may be caused by *in utero* exposure to cocaine may not manifest until that system attempts to transition to adult functional status later in life.

5.2.3. Behavioral Rigidity

The lack of findings of any effects of *in utero* exposure to cocaine on the ability of subjects to learn to perform the OTB tasks seems remarkable in light of the recent findings that prenatal cocaine exposure alters the development of the rhesus monkey frontal cortex (39-40) and affects development of dopamine neurons (44-46), as well as expression of dynorphin and enkephalin mRNA in fetal rhesus monkey brain (47). The highly practiced operant tasks for food reinforcement were either insensitive to the effects of *in utero* cocaine exposure or these assessments occurred too early in the life of the animals to show effects. Reversal procedures have been extensively used to examine the extinction of a previously learned behavior and the acquisition of a new behavior (48-51) and are thought to provide a greater behavioral challenge than initial acquisition because the new behavior to be learned in such cases also involves the extinction of a previously learned response. Therefore, a behavioral challenge was employed in our studies wherein the rules of the OTB's color and position discrimination task were 'reversed' and subjects' adaptation to the new situation was monitored (see (36) for study details). Here, instead of a left position response being correct after presentation of a red or yellow color and a right position response being correct after presentation of a blue or green color, the correct positions were reversed: a right position response was now reinforced (correct) after presentation of red or yellow and a left position response was now correct after presentation of blue or green. This behavioral challenge, or reversal, occurred when the animals were seven years of age. As expected, all animals initially showed impaired reversal performance and all were relatively slow in adapting their behavior to match the new rules of reinforcement (as part of the OTB, this task is presented for only five minutes every other test day; thus, in any given two week period the animals have a maximum of only 25 minutes to practice). All cocaine exposed groups performed more poorly (took more sessions to attain or never attained pre-reversal type responding) than did the saline control animals. Animals from the escalating dose group continued to show this impairment for over 285 sessions (about 2 and one half years). In fact, these subjects improved only up to the point of obtaining reinforcers by chance (ca. 50% choice

accuracy), suggesting that the escalating dosing regimen produced very long lasting, if not permanent, impairments in the behavioral ability of these subjects. The number of sessions required by subjects in the other exposure groups to master the reversal indicated that *in utero* cocaine exposure caused dose-related difficulties in behavioral adaptation (36). Motoric abilities were not affected since rates of responding were not systematically affected by gestational cocaine exposure. Thus, the noted effects seem to reflect an inability of subjects to extinguish a heavily over practiced behavior and/or adapt to new task rules. Although there were very few subjects in this study, the results were striking. The effects occurred long after exposure ceased (7 years) and were evident in some cases for another 2 ½ years when the observations were terminated. These findings are similar to those described for rats exposed to cocaine prenatally which have difficulty in performing serial reversal and extradimensional set shifting tasks (52).

5.3. Remacemide

Remacemide is a compound that exhibits neuroprotective and anticonvulsant properties and has proven effective as an adjunct to other anti-epileptic drugs in reducing the frequency of seizures in adult humans (53). The mechanisms thought to underlie its neuroactive properties involve its non-competitive antagonism of N-methyl-D-aspartate (NMDA) receptors and blocking of fast sodium channels. The NMDA receptor is an important target for excitatory amino acid binding and participates in the neural phenomenon of long term potentiation or LTP (54) which is characterized as an increase in synaptic efficiency (55-56). LTP is generally believed to be intimately involved with learning and memory processes (57). In addition to their roles in learning and memory processes, excitatory amino acids play important roles during development by regulating neuronal survival, structure, synaptogenesis and plasticity (58). Thus, if remacemide was to be used chronically in children, it was important to explore the consequences of such exposure on the development and expression of important brain functions such as learning and memory. Observations in humans have indicated marked differences in excitatory amino acid receptor sites from newborns to adults in the 10th decade of life (59-62)) and have led to the speculation that young brains may be more responsive to agents that affect NMDA receptors than are older brains (60).

Our studies of remacemide (detailed in (63-65)) examined in the rhesus monkey model, the developmental effects of chronic administration of remacemide or the classical, non-competitive NMDA receptor antagonist MK-801, on the acquisition of NCTR OTB performance. The emphasis here was on the ability of subjects to learn to perform OTB tasks. In these studies, treatment occurred throughout the training or acquisition of task performance rather than starting and stopping long before training began. This design was used in order to model potential human pediatric consumption, e.g., for the treatment of seizure disorders, in order to determine if such treatment might be associated with risks to normal development. Low and high doses of both drugs (20 and 50 mg/kg/day, remacemide; 0.1 and 1.0 mg/kg/day, MK-801) were administered daily via oral gavage beginning at about 9 months of age and the ability of subjects to acquire task performance was monitored. Drugs were administered within one hour after behavioral test sessions ended; administration of drugs after daily behavioral assessment (rather than before) again served to minimize the impact of acute drug effects and allow analyses to focus on the long-term developmental effects of treatment. The OTB behaviors that were monitored included: Incremental Repeated Acquisition (IRA) for learning; Delayed Matching-to-Sample (DMTS) for short-term memory; Conditioned Position Responding (CPR) for color and position discrimination; and Progressive Ratio (PR) for appetitive motivation.

5.3.1. Chemically-induced mental retardation

The most striking finding of these studies was that chronic treatment with the high dose of remacemide virtually prevented the ability of subjects' to learn how to perform the learning (IRA) task: virtually no increase in response accuracy could be demonstrated after 18 months of training during treatment. Chronic treatment with the low dose of remacemide or with either dose of MK-801 was without effect in this regard. Neither compound significantly altered response rates in this task, suggesting that the effects of high dose remacemide resulted from a specific cognitive impairment (impaired acquisition of task problem solution) rather than from or in addition to an inability of subjects to fulfill the motoric requirements of the task. This effect on learning task performance was further dramatized by a number of other observations that included the failure of the behavior of affected subjects to ever resemble that of control (or any other group) subjects; even 6 months after cessation of treatment: learning task behavior of the high dose remacemide animals was virtually no different from that observed at the beginning of training. While there was a significant effect of high dose remacemide to considerably retard the acquisition of color and position discrimination task performance, animals in this group were able to perform this task as well as all other groups by the end of treatment (64). There was no notable effect of high dose remacemide on the acquisition of the two other OTB tasks (motivation and short-term memory). And perhaps most importantly there was no effect of any drug treatment (remacemide or MK-801) on metrics typically employed in routine toxicity tests (clinical chemistry and hematological measures; general comportment; ophthalmology; spontaneous activity; pharmacokinetics, etc., (65). Thus, had only routine toxicity testing occurred, there would have been no indication whatsoever that remacemide could, under certain circumstances, be problematic.

Given the differential pharmacology of these drugs, it is likely that the effects of remacemide on learning task performance resulted either from its ancillary activity at fast sodium channels or from its effects to block NMDA receptors and sodium channels concurrently. While a role for sodium channel blockade in the effects of remacemide seems likely, it is also possible that, relative to MK-801, its effects resulted in part from its metabolism to an active

desglycinated moiety that has an even greater affinity for the NMDA receptor than does remacemide. Thus, although the time courses for the parent compounds MK-801 and remacemide are similar, the presence of an active remacemide metabolite should result in longer inactivation of NMDA receptors than that which occurs after MK-801 treatment. This difference in functional NMDA receptor blockade may account for the noted differences in toxicity. Irrespective of mechanism(s), the ability of chronic remacemide exposure during development to selectively and apparently permanently ablate the ability of nonhuman primate subjects to acquire performance of a learning task is remarkable. Given that, in children, performance of this same task is significantly correlated with IQ (24), it seems reasonable to conclude that remacemide produced mental retardation in this group of primates. Whether similar findings would have been observed if exposures had occurred at a different period of development or in adult subjects is currently unknown, but the answer to such questions could prove to be very enlightening.

6. PERSPECTIVES

Three different examples of studies in nonhuman primates have been presented in which chronic drug exposures during development resulted in altered functional states of the central nervous system. In some cases, the effects were reversible (marijuana smoke exposure during adolescence) and in others, they were not (*in utero* cocaine exposure; juvenile exposure to remacemide). Nonhuman primates are, in most cases, likely to be the best animal surrogates for humans and should be employed when answers to important public health issues are needed. Utilizing assessment tools such as the automated Operant Test Battery that was featured in the studies described here provides the opportunity to repeatedly assess the functional status of the nervous system within the same subject. Collection of data using behavioral instruments that can also be used with human subjects provides opportunity for cross-species comparisons and for determining the human relevance of such measures.

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