Methods for studying nonhuman primates in neurobehavioral toxicology and teratology

Thomas M. Burbacher*, Kimberly S. Grant

Department of Environmental Health, Box 357234, School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195, USA

Received 23 February 1999; accepted 18 January 2000

Abstract

The behavioral repertoire of nonhuman primates is highly evolved and includes advanced problem-solving capabilities, complex social relationships, and sensory acuity equal or superior to humans. These factors make nonhuman primates valuable animal models for studies of the functional effects of neurotoxicants. This review provides descriptions of tests designed to study learning, memory, schedule-controlled behavior, information processing, social behavior, sensory functioning, and visual-motor coordination and/or visuospatial orientation in macaque monkeys. Whenever possible, the results of studies in primate behavioral toxicology are provided for individual test measures. The primate model is especially useful for studies of developmental exposures because monkeys, like humans, have relatively prolonged periods of gestation, infancy, and adolescence. In recognition of this, a special section is provided for tasks that are specifically designed to study behavioral processes in infant monkeys. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Behavior; Methods; Nonhuman primates; Monkeys; Toxicology; Teratology

1. Introduction

Nonhuman primates are capable of advanced behaviors that share important and fundamental parallels with humans. These parallels include highly developed cognitive abilities and binding social relationships. The behavioral repertoire of these animals makes them valuable models for research on the functional effects of exposure to neurotoxic agents. A voluminous literature exists on the behavior of primates (e.g., see Fobes et al. [21]) but the intent of this review is to provide select information that is useful to toxicologists and teratologists engaged in studies of the effects of chemicals on the central nervous system. Investigators are frequently interested in asking whether a specific domain of behavior has been altered following exposure to a particular toxicant. To this end, this review will focus on describing what tests can successfully be implemented with primates to assess selected functional endpoints. Brief descriptions of tests designed to study learning, memory, schedule-controlled behavior, information processing, social behavior, sensory functioning, and visual-motor coordination and/or spatial orientation are provided. All of the tests described are appropriate for juvenile or adult animals and, in many cases, can be used with infants. Studies of developmental exposure are particularly valuable in these animals because the various stages of growth are prolonged. During these periods of development, windows of sensitivity for neurotoxicant exposure can be studied. A special section will outline tasks that are specifically designed to study behavioral processes in infant monkeys.

Apes, old world monkeys, and new world monkeys have all played important roles in toxicological studies, but most researchers in primate behavioral toxicology have relied upon the macaque monkey. This genus of old-world monkey includes *Macaca fascicularis* (long tailed or crab eating), *Macaca nemestrina* (pig tailed), *Macaca mulatta* (rhesus), and *Macaca arctoides* (stumptailed). Although this review is primarily dedicated to tests that are appropriate for macaque monkeys, it is important to note that the testing procedures described in this manuscript can be used with most nonhuman primate species.

2. Learning

There are two commonly employed methods to test learning in nonhuman primates. The oldest method is the
Wisconsin General Testing Apparatus (WGTA), developed in the 1930s by Harlow [37]. The WGTA can be used with infant and adult monkeys and requires the animal to physically displace objects to indicate choice behavior. Briefly, the WGTA consists of a stimulus presentation board that rests on a table-like structure. The stimulus presentation board contains recessed wells in which food rewards (fruit, peanuts, candy, etc.) can be placed. A superstructure above the stimulus board, equipped with rope pulleys, supports doors that prevent the animal from seeing the test stimuli, typically three-dimensional objects, until the onset of a trial. A human tester sitting behind a one-way viewing window sets up the trial, raises the door, and records responses to the test stimuli. The WGTA is a low-cost testing method that has a rich history with primates and is useful with animals of all ages.

Alternatively, monkeys can be assessed using automated test methodology where animals work in a computer-controlled environment [47,61]. Automated testing frequently takes place in a sound-controlled chamber where animals are presented with two-dimensional stimuli (e.g. slides, colored lights) and responses are recorded on buttons, switches, or levers. The precise electronic control over variables such as presentation of stimuli, timing of trial sequences, and recording of responses lends this methodology to the assessment of sensory functions such as vision and audition. Historically, this type of testing has been referred to as operant. The term operant was adopted to refer to the fact that animals must operate or manipulate a specific part of their environment to receive a reinforcer. A more contemporary consideration of the term operant takes into account that virtually all tests of learning require some operation to be performed on the environment, whether it is pulling a lever or displacing an object. In recognition of this, a more accurate distinction between test methods for primates may be based on whether the task is automated or can be automated.

In general, two distinct types of primary data are derived from studies of learning and memory. For tests designed to measure learning, the most important data are often collected during the original solution of the problem. The rate and quality of learning behavior can be examined with group and individual learning curves. Alternatively, for measures of memory and some schedule-controlled behaviors, the most vital data are generated after the primary task has been learned. When animals have solved or mastered the basic task, subtle changes in the presentation of test trials can easily target other cognitive processes such as memory (e.g., introduction of delay periods).

2.1. Discrimination learning tests

Discrimination tests can vary in complexity so that they can be used with subjects of all ages. A basic procedure to study discrimination learning entails the presentation of two distinct stimuli. One of the stimuli is designated correct and is associated with reinforcement whereas the other is designated incorrect and is not associated with reinforcement. Trials are presented, with the location of the correct stimulus randomly alternated between left and right, until the animal consistently selects the correct stimulus. The simplest type of discrimination tests use stimuli that differ on only one dimension such as color (e.g., black vs. white, Color Discrimination Test), shape (e.g., triangle vs. square, Shape Discrimination Test), or pattern (e.g., vertical vs. horizontal stripes, Pattern Discrimination Test). These tests are typically used with infant monkeys younger than 6 months or older animals with significant cognitive impairment. A more difficult paradigm for assessment of discrimination learning is the Spatial Discrimination Test. For this test, spatial cues are relevant for solution of the task and the animal is trained to learn a specific physical location, typically the left or the right. In the most simplistic form of this task, identical test objects are used as test stimuli [101].

All of the above tests (Color, Shape, Pattern, and Spatial Discrimination) can be made more difficult by using stimuli that vary on more than one physical dimension. These more complex discrimination tests use stimuli that vary in color and shape (e.g., a black square and a white triangle) or color, shape, and pattern (e.g., a black square with horizontal stripes and a white triangle with vertical stripes). The animal must learn to identify which dimension is relevant to problem solution and to disregard the irrelevant object characteristics. A challenging discrimination task, especially for young animals, is the Object Quality Discrimination Test. This test uses three-dimensional objects, such as plastic toys, that differ on multiple dimensions [34]. Object Discrimination Learning Set is a task closely related to Object Quality Discrimination but includes multiple problems instead of a single problem [35]. Animals are given a predetermined number of consecutive trials, frequently six, to solve an object discrimination problem and improvement over trials is assessed as a measure of intraproblem learning. A series of these problems is presented to the monkey during a single test session so that the subject is required to solve a number of discriminations within a short time period. We have used Color Discrimination and Object Quality Learning Sets in our laboratory to examine the effects of in utero exposure to methylmercury on early learning. Results indicate that methylmercury does not disrupt performance on either task.

The Concurrent Discrimination or Serial Discrimination Test uses multiple object pairs (commonly referred to as a list) that are presented consecutively for one trial each before the list of pairs is presented again [49]. For a list length of six, the first stimulus pair is presented, then the second, then the third, and so on until all six object pairs have been presented once. This sequence is shown repeatedly throughout a test session, with the order of stimulus presentations remaining invariant. The monkey must remember the reinforced object for all six pairs presented within a session. Multiple sessions are run over days until the monkey learns
to chose the correct object in each pair consistently. Malamut et al. [55] adapted this procedure so that successive trials of the object pairs were separated by 24-h intervals. The Concurrent Discrimination Test has been used to study the effects of lead exposure during different periods of development [71]. Relative to controls, monkeys exposed continuously from birth to adulthood were the most impaired whereas animals dosed only during infancy were the least impaired. Animals whose dosing began after infancy and continued into adulthood showed an intermediate level of lead-related effects on discrimination learning.

The Conditional Discrimination Test is a relatively challenging task where animals are trained to make an object discrimination on a stimulus board of one color [86]. After a variable number of trials, the color of the stimulus presentation board is changed and the nonreinforced member of the object pair becomes the correct stimulus. The animal must use the change in board color as the relevant cue to guide the selection of test objects. Once the original discrimination has been mastered and the animal can reliably use the board color to direct test object selection, new board colors and/or new object pairs can be introduced to increase the difficulty of the task.

2.2. Reversal or alternation learning tests

Reversal tests are presented following the successful mastery of a discrimination problem and are used to examine the animal’s ability to acquire new strategies and inhibit a previously learned response. For example, after learning a spatial discrimination in which the stimulus on the left was reinforced, a Spatial Reversal Test can be introduced. In this case, the stimulus on the right would now be reinforced. Correct solution requires inhibition of responses to the left and adoption of a new “chose right” strategy. In the closely related Successive Spatial Reversal Test, a series of reversal problems is used and the physical location of the reward is switched after the animal solves each reversal. This progression is continued until a predetermined number of reversals has been completed. Reversal tests can also be implemented following color, shape, pattern, or object discriminations by simply switching the reward to the previously incorrect member of the stimulus pair.

Studies have indicated that monkeys exposed to lead showed treatment-related deficits on spatial reversal tasks, relative to controls, with performance most disrupted in the presence of irrelevant cues [70]. Bushnell and Bowman [14] found that lead exposure during the first year of life resulted in persistent spatial reversal learning deficits up to 3 years of age. Spatial discrimination and successive reversals were also studied in offspring after maternal inhalation exposure to methanol [10]. The performance of the treated infants did not provide evidence for a methanol exposure effect on early spatial discrimination or reversal learning. Tests of nonspatial (e.g., color, shape, pattern, object) discrimination reversal learning have also been successfully used to detect behavioral impairment in adult monkeys developmentally exposed to lead [66,82]. Lead-exposed infant monkeys also displayed impairments in the acquisition of nonspatial discrimination reversal learning [15]. Studies such as these have lead Rice to propose that one of the hallmark features of lead neurotoxicity is the inability to keep pace with changing environmental contingencies. In contrast, infant monkeys exposed to methylmercury both pre- and postnatally did not exhibit impaired performance on nonspatial discrimination reversal tasks and showed marginal evidence of solving the reversals more quickly than controls [72].

In a two-part study, the effects of gestational and lactational exposure to polychlorinated biphenyls (PCBs) on discrimination and reversal learning was investigated by Schantz et al. [93]. In part one, exposure to Aroclor 1248 did not influence the ability of exposed offspring to solve spatial, shape, or color reversal problems. However, in the second study, offspring exposed to high-dose Aroclor 1016 experienced difficulty solving the initial spatial reversal problem but were unimpaired on all other learning measures.

An additional study of the effects of exposure to a PCB mixture found no treatment-related differences in performance accuracy on a series of nonspatial discrimination reversal problems although some exposed monkeys initially had an increased error rate [85]. In a separate study of 2,3,7,8-tetrachlordibeno-p-dioxin (TCDD), exposed animals were impaired in solving shape reversal tasks requiring a win-shift, lose-stay strategy. For example, on a Spatial Alternation Test, the original reward can be located on the left for trial one, the right for trial two, back to the left for trial three, and so on. Color, shape, pattern, or object discriminations can be easily adapted to the alternation procedure by simply switching the reinforced stimulus on each trial [5].

2.3. Concept learning tests

Concept learning tests (e.g., nonmatch to sample, match to sample, oddity) are considered complex tasks for monkeys and require the animal to attend to abstract properties of objects [45]. Many of these tasks are inherently flexible so that innumerable variations can be applied. For example, both the Nonmatch and Match-to-Sample Tests can be administered using visual, tactual, or auditory stimuli. Vision is, however, the primary mode of test presentation for primates and the following descriptions are restricted to this sensory modality. On the Nonmatch-to-Sample Test, subjects are initially shown a sample object. After viewing a sample, the subject is immediately presented with two test objects, one that matches the previously viewed sample and one that does not. The subject is reinforced for selecting the test object that is different from the sample. The Match-to-Sample Test is identical to the nonmatch-to-sample procedure with the exception that the subject is reinforced for selecting the test object that matches the sample [102]. The Conditional Nonmatch and Match-to-Sample Test is a more difficult variation of the above tests. Solving this test is de-
Dependent on the subject learning the conceptual rule "if A, then B." For this test, a cue is presented on each trial that indicates which strategy (match or nonmatch) should be used in order to correctly solve the problem [39]. One method of presenting this cue is to change the background color of the stimulus presentation panel to indicate whether the current trial is a nonmatch or match problem. For example, nonmatch trials could be presented with a blue stimulus background whereas match trials could be presented with a yellow stimulus background. Learning the association between the stimulus background color and the type of trial is critical to successfully mastering the test.

The Oddity Test is very similar to the Nonmatch-to-Sample Test, but it is substantially more difficult to master [36]. For the Oddity Test, three objects are simultaneously displayed on each trial, two identical and one different or "odd." Subjects are reinforced for selecting the odd object. The critical difference between the oddity and nonmatch tasks may lie in the manner in which the trials are presented. Oddity problems typically present all three stimuli simultaneously. In contrast, nonmatch problems are presented in two parts, with the sample stimulus presented for viewing before the test choice portion of the trial is presented. This allows animals to solve nonmatch problems by using a "view sample, then shift" strategy whereas oddity problems can only be solved by determining the relationship between the stimuli.

3. Memory

Many of the above learning tests can easily be modified to study memory by introducing a delay period between stimulus presentation and the response portion of the trial. Dependent on the length of the delay, both short- and long-term memory can be studied. Most memory tasks for primates are derived from the Delayed Response Test, a procedure developed over 80 years ago [40]. This was the first cognitive test for animals that required a response after the stimulus was no longer observable. On the Delayed Response Test, subjects are shown the concealment of a desired reinforcer at one of two or more response locations. Following a delay period, the animal is allowed to displace the object at the response location of its choice. Delayed response performance is typically evaluated as percentage of correct responses relative to the length of the delay periods.

The Delayed Spatial Alternation Test is identical to spatial alternation with the exception that a delay period is introduced between successive trials. The animal must remember the location of the reward from the previous trial, across the delay period, to correctly solve the subsequent trial. Delay periods typically range from 5 to 40 s. Monkeys exposed to lead have been evaluated on this procedure and deficits in performance were documented [81]. Exposed animals show increasing impairment as delay periods are lengthened and exhibit an increase in perseverative (repetitive) responding relative to controls. Using a pulse-chronic exposure model, Levin and Bowman [51] found that monkeys exposed to lead demonstrate significant impairments on the Delayed Spatial Alternation Test. Decrements in performance were most pronounced at short retention intervals suggesting an attentional problem or deficit in strategy selection. These results could not be replicated, however, with chronic lead exposure only [52]. It has been proposed that spatial memory tasks may be the most sensitive measures in characterizing lead-induced changes in cognition [81]. Adult monkeys exposed in utero to methylmercury did not display treatment-related deficits on this task [26]. In utero and lactational exposure to PCBs produced delayed spatial alternation learning deficits more than 3 years after exposure ended [53]. The authors stressed that low-level exposure to PCBs early in life may have long-term consequences for cognitive development. In a separate study of early postnatal exposure to a PCB mixture, exposed monkeys had difficulty mastering a delayed alternation task and were impaired relative to controls at short delays but not at longer delay values [85]. Study results were interpreted as characteristic of a disruption in performance or learning, not in spatial memory processing, and lend support to the belief that exposure to PCBs can produce long-term changes in behavior. In contrast, monkeys exposed to TCDD were not impaired on this task [90].

There are two variants of the nonmatch and match-to-sample concept tests that increase difficulty through the introduction of memory requirements. The Delayed Nonmatch-to-Sample Test has been called the single most widely used test of memory in monkeys [97]. The Delayed Nonmatch-to-Sample Test is identical to the Nonmatch-to-Sample Test with the exception that a delay period is initiated between the viewing of the sample object and the presentation of the test stimuli [57]. The subject must retain the sample object in memory over the delay to correctly select the nonmatching object during the test choice portion of the trial. Delays typically range from 5 s to 60 s, although longer delays such as 120 s can be employed. Infants exposed in utero to methanol were not impaired on the Delayed Nonmatch-to-Sample Test and mastered both the learning and memory components of the task as quickly as controls [10]. Delay periods have also been used to create the Delayed Match-to-Sample Test. Monkeys exposed to low levels of lead from birth showed deficits in delayed match-to-sample performance as adults [65]. Interestingly, treated animals did not have difficulty learning the matching principle. Only when delay periods were introduced did the performance of the lead monkeys deviate from controls. Rice notes that the findings of this study are consistent with reports of short-term memory deficits in children with high body burdens of lead.

An alternative to the simple use of delay periods to study memory is procedures that increase the amount of information to be held in memory. The Nonmatch or Match-to-Sample List Length Tests differ from the standard para-
in that the subject is shown a number of consecutive sample objects (list) before the test choice portions of the trials are presented. In this way, the subject must retain representations of multiple sample items in memory to correctly solve the subsequent test trials. Using a Match-to-Sample List Length procedure, Overman and Doty [60] found that adult rhesus monkeys showed robust memory for lists as long as 20 items. Results from our laboratory showed that in utero exposure to methylmercury did not adversely impact adult performance on either the Delayed Nonmatch-to-Sample Test or the Nonmatch-to-Sample List Length Test [27].

4. Schedule-controlled behavior

Schedule-controlled behavior represents models of learning in which response patterns, usually in the form of bar presses, are studied in relation to experimenter-controlled reinforcement schedules. There are a great number of schedules that can be implemented but, in general, most are variations of a few simple schedules. Each individual schedule is based on the number of responses required for reinforcement or the temporal relationship between reinforcement deliveries [22]. Schedule-controlled learning can be a valuable test measure in behavioral toxicology [68]. Using this approach, both the temporal and serial properties of behavior can be subjected to analysis to reveal the consequences of chemical exposure [103].

The Fixed Interval (FI) Test is one of the most common schedule-controlled behavior tests. In this paradigm, the subject is reinforced for the first response after a specific interval of time has elapsed. Subjects tend to display a characteristic pattern of responding during the interval prior to reinforcement, initially pausing and then slowly accelerating the rate of responses as the end of the interval nears. Patterns of responses allow the examination of a number of performance variables. Overall response rate may be indicative of the activity of the animal whereas changes in response rate across the interval may reflect temporal discrimination (ability to judge time). A study of chronic lead exposure found that treatment resulted in accelerated rates of responding under the FI schedule [69]. This result is particularly interesting in light of the fact that human children exposed to lead frequently exhibit hyperactivity. Developmental methylmercury exposure was not associated with differences in overall FI response rates but did appear to result in a different temporal pattern of responding [72].

A closely related schedule is the Fixed Ratio (FR). On this task, the subject must respond a specified number of times to obtain reinforcement. Fixed Ratio performance is typified by an initial pause and then a high steady rate of responding. Outcome measures on this task include mean pause time (time to first response following cue light), run rate (number of responses per second minus the pause time), and interresponse time (elapsed time between responses). In a study of lead-exposed infant monkeys, treated offspring exhibited increased mean pause times and this difference became more significant with increasing FR values [69]. Both the FI and FR schedules can be adapted so that variable, rather than fixed, intervals and ratios are used. Though employed less frequently, the Variable Ratio (VR) schedule has been used with infants exposed in utero to caffeine [28]. Relative to controls, caffeine-exposed infant monkeys exhibited an altered pattern of responding that included consistently longer pause times and increased interresponse times.

Fixed interval and FR schedules can be combined to create more complex tasks so that within the same test session, the differential sensitivity of both FI and FR schedules to toxicant exposure can be examined (Multiple FI-FR Test). Monkeys exposed to lead during different developmental periods were tested at 3 and 7 years under an FI-FR schedule [73]. Results of the FI assessment at 3 years were generally negative. However, FI testing at 7 years showed lead-related performance effects (increased run rates and decreased average interresponse times) in all treated groups. Effects on the FR component were minimal at both time points.

Monkeys exposed early in postnatal life to a PCB mixture similar to that found in human breast milk exhibited a number of treatment-related performance differences on an FI-FR schedule [76]. The FI component showed heightened sensitivity to the behavioral effects of PCB exposure in that both the mean interresponse time and pause time were altered. Monkeys exposed in utero to methylmercury were tested on a multiple FI-FR schedule as adults and only very limited, sex-specific effects, were observed [29].

Another operant procedure used to study temporal discrimination and response inhibition is the Differential Reinforcement of Low Rate (DRL) schedule. The DRL schedule requires that a minimum time elapse between successive responses before the animal is reinforced. The minimum time can also take place between a reinforcement delivery and the next response. Premature responses on the DRL task reset the time contingency, thus seemingly making it a more difficult task than the FI schedule. Monkeys exposed to lead from birth were impaired in their DRL performance in a delay-dependent fashion [79]. Treated animals made more nonreinforced responses and received fewer rewards than controls.

A relatively new approach to using schedule-controlled behavior with toxicant-exposed primates has been developed by Newland et al. [59]. Squirrel monkeys prenatally exposed to either methylmercury or lead were tested at 5 to 6 years of age using a procedure in which different reinforcement schedules operated independently on two test levers. Reinforcement densities were varied between the levers so that one lever was “richer” than the other, providing the majority of reinforcers. As the experiment progressed, reinforcement densities on the levers were changed and animals had to keep pace with the changing reward contingencies. While control animals gradually and reliably switched...
their responses to the “richer” of the two levers, monkeys exposed to lead or mercury had great difficulty with this task. Exposed animals were significantly impaired in their ability to change their behavior in the correct direction. The authors speculate that these results suggest a potential behavioral mechanism, insensitivity to changing reinforcement contingencies, by which neurotoxicant exposure can impair learning. These results were particularly compelling given that the exposures were relatively low and corresponded to levels found in human occupational environments.

A battery of behavioral tests, the National Center for Toxicological Research (NCTR) Operant Test Battery, has been designed by scientists at the NCTR for use with both monkeys and children [63]. This test series was designed to study specific aspects of cognitive behavior and combines automated technology with standard learning measures as well as schedule-controlled behavior. Performance of rhesus monkeys on this battery is virtually indistinguishable from that of human children [61]. The tests included in this series are:

1. **Incremental Repeated Acquisition (IRA).** The initial task requires the subject to determine which of four retractable levers is correct. After this has been solved, the task difficulty is increased so that the subject must now press two response levers in a specified order for reinforcer delivery. Following mastery of the two-lever response, the difficulty is increased to a three-lever sequence and so on up to six-lever sequences. The IRA is designed to study general learning ability.

2. **Conditioned Position Responding (CPR).** This task uses a centrally located cue light to direct response selection. If the center plate is illuminated with red or yellow, the correct response for the subject is to choose the left panel. If the cue light is green or blue, the correct response is to select the right panel. Performance on the CPR test is thought to reflect color and position discrimination learning.

3. **Progressive Ratio (PR).** Mastery of this test requires the subject to increase the number of lever presses for each subsequent reward. Initially, ten presses are required for a reinforcer. For each subsequent reinforcer, the subject must increase the number of presses by ten. Performance is thought to be a measure of motivation.

4. **Delayed Matching-to-Sample (DMTS).** For this task, the subject is initially shown a geometric symbol that serves as a sample stimulus. Following a delay period, the sample stimulus and two test choices (also geometric patterns) are displayed. One test choice is the same as the sample and the other is different. The subject is reinforced for selecting the test choice that matches the sample stimulus. This test measures short-term memory and attention.

5. **Temporal Response Differentiation (TRD).** On this task, the subject must learn to hold down a lever for a specified number of seconds to obtain a reinforcer. The subject is not reinforced if the lever is released too early (before 10 s) or too late (after 14 s). Performance is thought to reflect time perception.

The NCTR battery of behavioral tests has been used with monkeys exposed to a wide range of drugs. In utero cocaine exposure did not adversely affect acquisition of any measure in the battery [58]. Performance on the Temporal Response Differentiation task (time estimation) was the most sensitive behavioral measure of exposure to chlorpromazine [19], pentobarbital [20], caffeine [7], diazepam [95], physostigmine [25], morphine sulfate [96], and lysergic acid diethylamide (LSD) [24]. The ability to perform the Progressive Ratio task (motivation) was quite sensitive to treatment with morphine sulfate [96], LSD [24], and marijuana [62]. Drugs such as phencyclidine (PCP) produced an overall disruptive effect on all test measures [23].

5. **Information processing**

The speed and accuracy of information processing can be measured using a variety of test measures, but the most commonly employed is the Reaction Time Test. The Reaction Time Test was designed to allow the precise separation of cognitive decision time from movement/response time. Two features make this test an attractive choice for primate behavioral work with toxicants. First, studies with humans have found that performance on certain reaction time tests is related to intelligence or problem-solving abilities [44] and scores may reflect central processing speed and neural integrity. Second, this test is routinely used in human test batteries designed to detect signs of neurotoxicity in the workplace [2, 16]. The use of comparable methods in behavioral toxicology is helpful when extrapolations must be made from animal data for purposes of human risk assessment.

Reaction time testing is divided into two primary parts, simple reaction time and complex reaction time. In the simple reaction time test, the monkey is trained to respond to a single stimulus (e.g., colored light) as quickly as possible. To accomplish this, the animal must learn to hold down a bar until the stimulus is presented and then to release the bar and reach forward to depress a button under the illuminated stimulus. This provides two response measures, cognitive decision time (latency between stimulus onset and release of bar) and movement time (latency between release of bar and depression of button under illuminated stimulus). The complex reaction time task is different in that there are multiple stimuli in the test array. The animal must scan the test array (ranging from two to eight stimuli) to determine which stimulus has been illuminated. In humans, the complex reaction time task is more sensitive to individual differences in cognitive ability and is probably the more useful measure for research in primate behavioral toxicology [44].
In adult primates, simple reaction time performance was not affected by chronic low-level exposure to lead [67]. Developmental methylmercury exposure did not affect performance on a complex reaction time task [77]. Studies with humans, using modified test procedures, have shown that fetal alcohol exposure alters reaction time responses in both infants and children [43,98].

6. Adult social behavior and mother-infant interactions

Assessment of social interactions can provide useful information on how neurotoxicants influence normal patterns of behavior. One of the drawbacks to measuring adult social behavior is simply that, in the context of toxicological studies, monkeys are rarely housed in groups. However, new government guidelines require periods of socialization and these periods can be exploited to study temperament characteristics, such as aggression, as well as patterns of affiliative behavior. Maternal care has been studied by a small number of investigators in relation to chemical exposures. From the standpoint of the infant, chemical exposure may alter the mother’s motivation or ability to properly care for her offspring. From the mother’s perspective, chemical exposure may be responsible for behavioral changes that make the infant more difficult to care for [18]. Deficiencies in the social relationships between mother and infant may permanently disrupt the normal development of species typical behavior.

In laboratory primates, mother-infant interactions are typically studied in the homecage. Observers score the behavior of each member of the mother-infant dyad for some predefined observation time during each test session, recording behaviors in categories such as social contact, social interaction, and nonsocial behaviors. Maternal care was studied in infant monkeys exposed to either lead or TCDD and results indicated that both lead- and TCDD-exposed dyads spent more time in close social contact relative to controls [92]. Increased maternal care has also been reported in mother monkeys caring for blind or physically handicapped infants [87]. In contrast to the lead and TCDD results, adult female monkeys exposed to delta-9-tetrahydrocannabinol demonstrated patterns of decreased social contact and earlier weaning of their offspring [30].

7. Sensory function

Tests of sensory functions are frequently omitted in studies of primate behavioral toxicology but they are valuable tools for evaluating toxicant-related injury to the central nervous system. They also provide a measure of neurotoxicity that is relatively unencumbered by psychological variables such as learning ability. Visual impairment can have serious consequences on cognitive performance and investigators of behavior should strongly consider visual screening measures prior to tests of learning and memory that involve visual stimuli. This ensures that stimuli can be seen by both treated and control animals and allows differences on cognitive tests to be interpreted as ability based, not sensory based.

The most straightforward test of visual function evaluates a parameter known as visual acuity, the ability to see objects. Visual acuity develops in monkeys and humans in much the same way and both species rely on vision as the dominant sensory modality [6]. Testing of acuity in adult monkeys typically takes place in an automated apparatus. Briefly, the monkey sits in a large testing chamber that is painted flat black and faces two oscilloscopes at equal viewing distances. On each trial, one oscilloscope displays a vertical sinusoidal grating of a particular spatial frequency (black and white stripes of a given width). The second oscilloscope displays a blank screen of equivalent luminance. The animal is trained to press the button corresponding to the oscilloscope that is displaying the black and white stripes, not the blank field. If the correct button is selected, the animal is reinforced with fruit juice. Lower spatial frequencies (wider black and white stripes) are typically presented first, followed by the gradual introduction of higher frequency gratings (thinner stripes) until the animal can no longer reliably distinguish the gratings from the blank screen.

Visual acuity can also be assessed in young monkeys using acuity cards such as those developed for human infants by Teller [41,100]. This procedure relies on the native preference of human and nonhuman primate infants for patterned over plain visual surfaces. The infant is held in front of a screen where two stimuli are simultaneously presented: One stimulus is a black and white grating (stripes) and the other is a gray field. The observer, who is unaware of the location of the grating, watches the infant’s eyes to determine the left-right position of the grating. Acuity cards are presented in the order of decreasing stripe widths and the acuity threshold is calculated as the finest stripe width that the infant reliably fixates.

Contrast Sensitivity provides a more fine-grained assessment of visual function and both spatial and temporal sensitivity can be studied. In Spatial Contrast Sensitivity testing, gratings are presented at various contrasts. Contrasts are decreased until the borders between the black and white stripes become indistinct and fade to gray. The assessment of contrast sensitivity can take place under high or low luminance, assessing cone and rod vision respectively. Tests of Temporal Contrast Sensitivity provide a measure of motion perception, including flicker. Stimuli are varied by frequency (rate of flicker) and depth of modulation (equivalent to contrast) until a threshold for motion detection can be ascertained. Like spatial contrast sensitivity, temporal sensitivity can be tested under conditions of high or low luminance.

Monkeys exposed to chronic low-level methylmercury from birth exhibited impaired spatial vision relative to controls under conditions of both high and low luminance [78]. Visual deficits in treated animals were more pronounced under the low-luminance test conditions. Rice and Gilbert
[80] also studied animals exposed to methylmercury in utero plus postnatally for 4 years and found treatment-related changes in high- and low-luminance spatial vision. Temporal visual functioning was less affected by methylmercury exposure. Whereas some changes were documented under high-luminance conditions (restricted to middle and low frequencies), treated monkeys displayed temporal vision superior to controls under conditions of low luminance. Results from our laboratory also indicated that in utero exposure to methylmercury impairs spatial vision in adulthood [8]. This finding indicates that exposure during the prenatal period is sufficient to produce long-term disruption of postnatal visual development.

The signal detection paradigm can also be used in Tests of Pure Tone Auditory Detection, to measure hearing thresholds, and Vibration Sensitivity, to assess somatosensory thresholds. For tests of audition, subjects are trained to respond when a tone is detected. Tones are systematically decremented until the monkey no longer reliably responds to them. Typically, tones that vary in frequency from 125 to 31,500 Hz are used with nonhuman primates. Monkeys exposed to methylmercury for the first 7 years of life showed a selective high-frequency hearing loss relative to control animals when tested at 14 years of age [83]. Results from our laboratory indicate that this effect may be unique to postnatal methylmercury exposure [8]. Monkeys exposed to methylmercury in utero alone demonstrated normal hearing thresholds. Work by Lasky and coworkers has taken a different approach to auditory assessment in primates [46]. Rather than using a signal detection paradigm, evaluation of hearing in lead-exposed monkeys was accomplished with otoacoustic emissions (sounds generated in the cochlea and recorded in the outer ear canal) and auditory-evoked potentials. A small subset of exposed animals exhibited abnormal distortion product otoacoustical emissions and had reduced or absent evoked potentials. There was also a modest but significant group mean difference in the auditory brain stem–evoked responses of lead-exposed animals when compared to controls. The effects of lifetime lead exposure on pure tone detection has also been examined [75]. Consistent with elevated detection thresholds found in children environmentally exposed to lead, a subset of lead-exposed monkeys also exhibited pure tone thresholds outside the range of control values. The effect was, however, somewhat diminished from what would have been expected based on the human results.

Somatosensory function has been evaluated in monkeys exposed to methylmercury or lead [84]. Vibration sensitivity of the fingertip was studied across frequencies ranging from 25 to 250 Hz. The underside of the monkey’s middle finger on one hand was positioned over a probe attached to an accelerometer. The monkeys were trained to release a bar with the free hand to signal that vibration had been detected. Animals exposed to methylmercury demonstrated clearly elevated thresholds on this procedure whereas results from lead-treated monkeys were somewhat equivocal. Monkeys exposed to acrylamide are also impaired in their ability to accurately detect a vibratory stimulus on the fingertip [56]. Results from the acrylamide study suggest that vibration sensitivity testing can parallel the course of intoxication and recovery for chemical agents causally linked to peripheral neuropathies.

8. Visual-motor coordination and visuospatial orientation

A quick and effective task for obtaining quantitative measurements of visual-motor (eye-hand) coordination entails the use of a stimulus presentation board that is composed of Plexiglas wells of varying depths (Pick-up Test) [74]. The varying depths of the wells introduce varying degrees of difficulty for each individual trial. The animal is trained to reach through an opening and retrieve a reinforcer, typically a small piece of fruit or peanut, from the well that has been baited by the tester. The time required to retrieve the reward and the accuracy of the animal’s movements are recorded. This simple test helped to successfully identify impaired motor coordination in monkeys previously exposed to methylmercury [74]. Although years had passed since the animals were treated, exposed animals took longer than controls to retrieve the rewards from the test wells. Follow-up assessment of these animals with a vibration sensitivity test revealed that somatosensory functioning was impaired, thus providing strong evidence for long-term or delayed neurotoxicity. Monkeys exposed to acrylamide also showed treatment related difficulties with eye-hand coordination on this task, relative to controls [56].

Visuospatial orientation can be quantitatively measured with the Lifesaver Test [4]. This test requires the monkey to move a Lifesaver candy along metal rods that have been bent into routes of varying complexity. To conduct the test, a metal rod is inserted into a vise clamp and a Lifesaver is threaded along the rod until the start point is reached. On each trial, the start point is either to the immediate left or right of the centrally located metal rod. The preparation of the test trial is conducted behind an opaque barrier to prevent the monkey from observing. After the Lifesaver has been threaded, the opaque barrier is raised and the monkey is allowed to retrieve the reward by threading it to the free end of the metal rod. The latency to retrieve the Lifesaver is recorded. This task has been shown to be sensitive to the effects of aging in nonhuman primates but has not been widely used in behavioral toxicology [4]. Preliminary results from our laboratory suggest that in utero methylmercury exposure does not affect visuospatial orientation, as measured by the Lifesaver Test, in adult monkeys.

9. Infant behavioral development (birth to 12 months of age)

The study of development requires unique considerations. Foremost among these are the effects of pre- and postnatal variables on behavior and the specialized test procedures that are necessary to observe emerging abilities in young animals [11,89]. Many of the tests described in this
section were adapted from assessments used with human infants. Macaque monkey infants and human infants share certain limitations and abilities, particularly during the first months of life. Both lack language, display poorly developed motor skills, and undergo a prolonged period of infancy. Much of what is described in this section has been developed for monkeys at the Infant Primate Research Laboratory at the University of Washington and is published in a laboratory handbook [88]. Listed in chronological order for infant age at time of assessment, the following test procedures have been successfully used to describe the developmental course of both normal and toxicant-exposed macaque infants.

The Simian Newborn Assessment was developed to determine the integrity of the newborn’s physical state within minutes of delivery and is based on the procedure developed for humans by Apgar [3]. Using numerical ratings from 0 to 2, evaluations of respiration rate, color, muscle tone, heart rate, and arousal are made 5 and 10 min after birth.

The Simian Neonatal Behavioral Assessment Scale was adapted for monkeys from the Brazelton Neonatal Behavioral Assessment Scale used with human infants [1,94]. This test is designed to identify more subtle aspects of central nervous system functioning such as temperament variables, ability to orient and respond to the environment, and capacity to perform integrated motor movements. The examination includes reflexes such as suck, snout, root, clasp, grasp, blink, and righting. Consistent with human test procedures, state variables such as irritability and consolability are recorded as is overall response quality. On this test, infants developmentally exposed to lead exhibited lower muscle tone and increased agitation/arousal when compared to controls [54]. Results from our laboratory have shown that in utero exposure to methylmercury did not affect performance on this test (unpublished data) while in utero exposure to methanol was related to ratings of “low arousal” [10]. The effect of methanol, however, should be interpreted with caution because it may be associated with the high number of cesarean sections in the exposed animals.

Tests of Motor Milestones provide information on motor and social development within the first 6 months of life. Infants are placed in mixed sex groups in a playroom equipped with ramps, chains, and shelves while testers observe and record the date that specific behavioral milestones are first observed. Motor achievements such as first trip away from diaper, onset of bounce play, onset of straight-legged quadrupedal gait, exploration of toys, and ability to climb up (positive geotaxis) and down (negative geotaxis) the playroom ramps are recorded. Social milestones such as facial expressions (e.g. earflips, lipsmacks) and vocalizations (e.g. hoot, screech) are also noted.

The Object Concept Test is used to assess emerging cognition in humans and nonhuman primates [9,64]. The concept of the physical permanence of objects (i.e., objects continue to exist when no longer in view) is one of the primary cognitive milestones characteristic of the sensorimotor period and was considered by Piaget to form the cornerstone of future cognitive operations. During the sensorimotor stage, the infant progresses from abandoning objects that are hidden from view to actively searching and retrieving hidden objects (object permanence). Believed to be an early test of reaching and spatial memory, infants are presented with test trials in which objects are not hidden, partially hidden, or fully hidden from view. The ability to retrieve objects that are not hidden or partially hidden requires coordinated reaching skills whereas the retrieval of a fully hidden object requires spatial memory for that object. Infants exposed in utero to methylmercury were mildly delayed in their ability to reach for and pick up objects but, more importantly, showed retarded development of object permanence [9]. Treated infants required nearly twice as many sessions as controls to retrieve fully hidden test objects. Using a similar procedure, Levin et al. reported that postnatal exposure to lead did not affect the attainment of object permanence [54]. More recent data from our laboratory indicate that prenatal exposure to methanol did not disrupt object permanence development but was associated with a delay in the development of visually directed reaching in male infants [10].

The Visual Exploration Test can be given to infant monkeys as early as 2 months of age using an apparatus that consists of a two-chamber box with peepholes at both ends [50]. The monkeys are placed in the apparatus and allowed to locomote freely between chambers. Slide projectors are used to display images on screens that can only be seen when the monkey is looking through either peephole. Infrared photo beams detect when an animal is looking through a peep hole and data collection is initiated when the photo beam is broken. Visual exploration is measured with variables such as number of looks, length of looks, and total looking time during the test session. This task can also be used with older animals, particularly if attentional deficits are suspected. Significant effects of lead exposure have been documented using this task. Lead-treated infants displayed fewer and shorter looks relative to controls, exhibiting a pattern of looking behavior indicative of decreased visual attentiveness or exploration [54]. Offspring of monkeys exposed to delta-9-tetrahydrocannabinol (THC) also displayed altered patterns of visual exploration and attention on this task [31].

The Visual Recognition Memory Test was developed to assess early cognitive processing in human infants and has been adapted for use with monkeys. Using a test paradigm in which novel visual stimuli are paired with familiar stimuli, looking time to both are recorded [17]. Visual preferences for novel stimuli are considered evidence for recognition memory because some aspects of the familiar stimuli must be retained in memory for the novelty response to occur. Using an adaptation of the test developed by J. Fagan (copyright 1981 by J.F. Fagan III), deficits in visual recognition memory have been documented in a number of studies examining monkeys at high risk for poor developmental
outcome, including those that are teratogen exposed (methylmercury [32], ethanol [33], methanol [10]). Studies with human infants have reported reduced visual recognition scores in infants prenatally exposed to PCBs [42].

Social behavior is an important and defining characteristic of primate development. In infancy, perhaps the most important social behavior is play, contributing to motor coordination and establishing the foundation for communication and placement within the group’s dominance hierarchy. Play behavior can be chronologically characterized by the following stages: (1) presocial, (2) rough and tumble, (3) approach-withdrawal, (4) integrated, and (5) aggressive [38]. These stages of play and other aspects of social behavior can be studied by placing animals in stable play groups and observing the development of species typical social behavior. This is most often accomplished by recording the behavior of a focal animal, using a coding system, in relation to other group members over the first year of life.

The development of primate social behavior appears relatively sensitive to neurotoxicant exposure. Infants exposed in utero to methylmercury exhibited reduced levels of social play and spent longer periods of time engaged in passive, nonsocial behaviors [12]. Infant monkeys exposed daily to lead from birth to 1 year of age showed a pattern of suppressed play, increased clinging, and hypersensitivity to changes in the play environment [13]. It is noteworthy that the introduction of control monkeys to the play groups reduced the magnitude of lead-related changes in social behavior. This effect is reminiscent of the therapeutic role of young, socially competent peers in normalizing the behavior of isolate-reared monkeys [99]. In a more recent study of the differential effects of lead exposure and diet on social development, persistent changes in social play were observed in treated animals [48]. The lead-induced changes were apparent across multiple forms of play behavior and persisted long after exposure was terminated. Among the exposed infants, reductions in social play were accompanied by increases in maladaptive behaviors such as self-stimulation and fearfulness. Infants exposed in utero to TCDD initiated more rough-tumble play, retreated less frequently, and were displaced less often from preferred positions in the playroom [91]. TCDD monkeys also displayed increased levels of self-directed behaviors.

10. Summary

In closing, the tests outlined in this review are intended to provide a framework of primate behavior that will be useful to toxicologists and teratologists. The selection of test measures should be driven by the specific compound under study, the duration, level, and route of exposure, and the age of the animal during exposure and at testing. Monkeys represent a unique resource given the close evolutionary history they share with humans and should be used with discretion and great care. The behavior of these ancestral relatives provides a valuable window in which we, as scientists, can view the important and frequently subtle behavioral consequences of neurotoxicant exposure.

Acknowledgment

The authors thank Noelle Liberato for her dedicated assistance in helping to prepare this manuscript.

References


