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Learning about cognition risk with the radial-arm maze in the developmental neurotoxicology battery

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ABSTRACT

Cognitive dysfunction has been found in epidemiological studies to be among the most sensitive impairments associated with developmental exposure to a variety of environmental contaminants from heavy metals to polyhalogenated hydrocarbons and pesticides. These chemicals have been also shown to impair cognitive function after developmental exposure in experimental animal models. The radial-arm maze (RAM) has proven to be a sensitive and reliable way to assess both learning and memory in a variety of species, most often in rats and mice. The RAM is a very adaptable test method that takes advantage of rodents' instinct to explore new places in the environment to forage. That is, rodents do not need to be trained to run through the maze; they will normally do this from the initial session of testing. Training with differential reinforcement for arm choices provides a more rigorous test of learning and memory. The RAM is quite adaptable for assessing various aspects of cognition. Although the RAM has been mostly used to assess spatial learning and memory, it can be configured to assess non-spatial memory as well. Both working and reference memory can be easily distinguished. The RAM can be run with both appetitive (food reinforced) and aversive (water escape) motivators. The RAM has been found to be sensitive to a wide variety of developmental toxicants including heavy metals such as mercury and pesticides such as chlorpyrifos. There is an extremely rich literature especially with rats showing the effects of many types of brain lesions and drug effects so that the participation of a wide variety of neural systems in RAM performance is known. These systems, notably the hippocampus and frontal cortex, and acetylcholine and glutamate neurotransmitter systems, are the same neural systems that have been shown in humans to be critical for learning and memory. This considerably aids the interpretation of neurobehavioral toxicity studies.

1. Introduction

Cognitive dysfunction is one of the most common findings of developmental environmental neurotoxicity in epidemiological studies, most notably seen in studies of lead, polyhalogenated hydrocarbons, and pesticides (Eskenazi et al., 2007; Lanphear et al., 2005; Rauh et al., 2006). Cognitive impairments from developmental exposure to these chemicals have also been documented in experimental animal studies, with species ranging from zebrafish to rhesus monkeys, of course also including the widely use rodents as well (Levin et al., 2001, 2002; Schantz et al., 1991). Experimental animal testing demonstrates the cause-and-effect relationship in a rigorous way not possible with epidemiological studies. Experimental animal studies also provide important brain-based complex mechanistic information about cognitive effects of developmental neurotoxicity unavailable with *in vitro* studies. Inclusion of cognitive tests in the developmental neurotoxicology screening battery provides information critical about an important adverse outcome of developmental neurotoxic exposure in humans. The radial-arm maze (RAM) provides a sensitive and readily adaptable technique with which to determine developmental neurotoxic effects on cognition.

2. Protecting against developmental neurotoxic risks of cognitive impairment

Cognitive testing is important for risk assessment. However, the way in which cognitive tests are currently used in screening is not as useful as it should be. Given that cognitive impairment is a sensitive indicator of developmental neurotoxicity in human studies, the fact that cognitive tests as currently performed in experimental animal developmental neurotoxicology screening test batteries are not very sensitive, is an indictment against how those tests are currently used, not against the importance of conducting cognitive tests in the screening battery. (See the overview article in this special issue). Screening for cognitive impairments with animal models using insensitive tests will not be very informative and will not provide sufficient protection against toxicant induced cognitive impairments occurring in people. The answer is not

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to abolish cognitive testing in the developmental neurotoxicity test battery, but to use efficient cognitive tests that are more predictive of cognitive impairment in people. Improving the sensitivity of cognitive testing will not only help in the prediction of cognitive effects of particular compounds in people, it will provide important information about the functional mechanisms of toxicity of a wide variety of chemicals. It is important to use cognitive tests that are not only efficient and sensitive, but that are informative about the neural processes disrupted by toxicant exposure.

Cognitive tests range from very simple quick tests to much more complex tests that take considerable amounts of time to perform. Tests also range in sensitivity. All cognitive tests also involve other neurobehavioral functions, from sensory and motor processes, to motivational and emotional function. For example, active or passive shock avoidance tests of learning and memory are also sensitive to simple changes in locomotor activity and to changes in sensitivity to shock as a motivating influence. Many spatial navigation learning and memory tests rely on visual cues; visual disturbances would affect performance on these tests.

Sensitivity can be lessened in several ways. If the motivating influence is substantial, such as in shock-motivated tests, sensitivity can be diminished. The source of this lessened sensitivity may occur because maximum motivation may bring into use the maximum cognitive resources to solve the task. This would measure peak cognitive performance, but the generalizability of these findings to the more common expression of cognitive function under more modest motivation would be limited. Also, shock induces emotional responses which rather than serving to motivate the subject to learn or remember more accurately could disrupt these processes, again diminishing from the tests' interpretability as well as sensitivity. There is a window of sensitivity to the effects of exposure dependent on the overall sensitivity of the test. If a test is too easy, then it would take a substantial neurobehavioral impairment to influence the outcome of the test, decreasing the sensitivity of the test. If the test is too difficult then the controls would fail, decreasing the sensitivity of the test.

Integrity of controls is essential to a sensitive test. Tests can become quite insensitive even to exposures causing substantial impairment if the performance of the control group is disrupted or made quite variable due to co-exposures, problems with husbandry or variability in test conditions. Like biochemical tests, there need to be regular positive controls to confirm test integrity, that is, a test of the test. These can include positive controls of known amnestic drug treatments such as scopolamine or dizocilpine, internal dynamics of the test such as acquisition curves or forgetting curves or the effects of brain lesions impairing accuracy on the test.

Spatial discrimination is an important cognitive function shared by great variety of species from honeybees to humans. Simple tests of spatial discrimination such as the RAM are sensitive to toxicant exposures such as developmental exposure to lead and chlorpyrifos as well as antagonists of transmitter receptors critical for cognitive function such as scopolamine (muscarinic acetylcholine antagonist) and dizocilpine (NMDA glutamate antagonist). Rats and mice normally run in the RAM in an efficient food foraging pattern and chose different arms above chance rates even with minimal training. With training they learn in a reproducible fashion improving accuracy over a small number of sessions to an asymptote of performance to index memory. Working and reference memory can be differentiated by selectively baiting some arms, but not others. Response speed can be measured in a manner orthogonal to choice accuracy since the same effort is required to make correct or incorrect choices. Of course the set of paradigms in the RAM is only one of many different ways in which cognition can be tested in an efficient and sensitive manner. These tests include T-maze alternation, novel object recognition, operant conditioning and the Morris and other water maze tasks. The other articles in this series provide excellent discussions of those methods.

3. Using the radial-arm maze to assess learning and memory

The RAM is a widely used apparatus to assess spatial working and reference memory. David Olton pioneered its modern use and provided much of the early literature concerning the neural and behavioral systems necessary for accurate performance in the RAM (Olton and Samuelson, 1976). Olton and co-workers adapted previous tests with simultaneous multi-choice configurations that were developed by Hamilton, Tolman and others in the early (Hamilton, 1911, 1916) and mid-(Tolman et al., 1946) 20th century. Earlier reviews have covered the historical use of the radial-arm maze for addressing the memory effects of drugs (Levin, 1988) and neurotoxicants (Olton, 1983; Walsh and Chroback, 1987), particularly the persistent cognitive effects of developmental neurotoxic exposure. The radial-arm maze has become a very widely used method for the examination of spatial learning and memory in rats, mice and other animals including monkeys and humans. RAM methods have been developed for human testing as well (Braun et al., 2012).

The RAM is quite versatile with a variety of different procedures providing assessment of learning and memory. The win-shift task is the most common way of using the RAM. With this procedure all of the arms are baited at the beginning of the test session and then allow the subject to freely choose arms and retrieve the baits until all the different arms had been chosen. The optimal strategy for this task is to shift response choice after a reinforced entry (win-shift). Working memory is tested by counting the errors, which are re-entries into previously baited arms. The difficulty of the task increases as the session progresses. If all the arms are baited then the first choice is always reinforced. Then as each new arm is chosen the subsequent choice is more difficult. Working and reference memory can be distinguished in the RAM. This test can be run with some of the arms baited but others never baited, such that the first entry into the baited arms is reinforced but not subsequent entries and the never-baited arms are not reinforced at all. The never baited arms stay constant throughout testing. Re-entries of the subject into formerly baited arms are the test of working memory while any entry into a never-baited arm is the test of reference memory. Typically, we have found that 18 sessions of training are sufficient to reach asymptotically good performance on the win-shift radial arm maze task. Delayed matching to sample can be run with the maze initially configured to force the subject to enter one particular arm. Then the subject is allowed access to all of the arms. Errors are counted with the number of arm entries until the subject returns to the initially sampled arm. Learning can be assessed with the repeated acquisition procedure developed by Peele and Baron (1988). In any given session three different arms are rewarded. The subject is given five trials to solve the new problem. The number of errors per trial is counted and the decrease in errors per trial is the index of learning. Non-spatial memory can be assessed by pairing reinforcement with visual or textural cues.

Many great studies have been conducted investigating the effects of various brain lesions, drugs and natural phenomena such as aging on RAM performance. Mazes containing from three to 24 arms have been used in these studies. Because every alternative arm choice is possible every time a choice is made, the radial-arm maze is particularly amenable to computer modeling. Spetch and Wilkie (1980) and Eckerman (1980) have previously designed computer programs to simulate choice behavior in radial arm mazes. The author has written a Monte Carlo computer randomization program which produces random chance accuracy scores for several measures for mazes of different sizes as well as the effect of different levels of memory or response bias (see supplemental file tables).

Lesion studies have provided information concerning brain areas important for memory function as measured by the RAM. As has been seen with other numerous tests, the hippocampus and related structures are of critical importance for memory function in the RAM (Becker et al., 1980). In addition, other limbic structures such as the

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