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LABORATORY MANUAL
**PRINCIPLES OF PSYCHOLOGY:
EXPERIMENTAL FOUNDATIONS**

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Experiment 4

Psychopharmacology

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Psychopharmacology of Spatial Learning

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Introduction

Learning fixed locations in our daily environment is deceptively simple. Recall for a moment your first few days on campus. After walking around just a few times, you were probably able to find your way to and from the cafeteria, gym, bookstore, and library. Since you probably spent little, if any, time consciously memorizing the routes you took to those places, you were probably unaware that you learned much in those walks. But you learned a great deal about the relative location of places in your new world. You did so using a process called *spatial learning*, which is distinctly different from the kind of learning you depend upon to pass exams.

Unlike rote learning or other means of learning new voluntary behaviors (e.g., *operant conditioning*), spatial learning occurs almost automatically in all mammals studied. It develops very rapidly, requires no reinforcement, and rarely entails conscious thought (see Barnes, 1988, and Sherry et al., 1992). That may reflect our evolutionary history, in which survival depended on a special ability to form *cognitive maps* indicating the locations of food, water, and escape routes from predators. Even today, in a more civilized world, we suffer greatly when our spatial learning ability begins to fail. That occurs to some extent with advanced aging (see Gage et al., 1988 and Ordy et al., 1988). It occurs more dramatically in Alzheimer's disease (see DSM IV). Indeed, an early and characteristic sign of that disorder is disorientation in space causing the afflicted person trouble finding his or her way to and from work (Henderson et al., 1989; Binetti et al., 1998).

You can appreciate now why an understanding of spatial learning is important for both academic and medical reasons. The medical reasons alone explain the urgency of learning the biological basis of spatial learning. Such knowledge is necessary to find ways of reducing or even stopping the spatial disorientation experienced with advanced aging and especially with the onset of Alzheimer's disease. Research to date indicates a number of changes in brain processes that may account for spatial disorientation, one of the most prominent of which is a reduction in content and release of a neurotransmitter called *acetylcholine* (ACh) in a brain structure called the *hippocampal formation* (see Barnes, 1988 and Sherry et al., 1992; Stancampiano et al., 1999; Fadda et al., 2000). ACh is released in that structure by axon terminals of neurons in another brain structure called the septal area. When the number of those septal neurons decline in the course of aging or Alzheimer's disease, less ACh is

released in the hippocampal formation. When the decline becomes marked, impairments develop in hippocampal formation functions such as formation of long-term spatial memories. That view is consistent with many studies in experimental animals (Gage et al., 1988, Ordy et al., 1988, Muir, 1997; Stancampiano et al., 1999; Fadda et al., 2000).

ACh released in the hippocampal formation influence its functions because neurons there have binding sites (i.e., *receptors*) for ACh embedded in their cell membranes. There are two different types of cholinergic (i.e., ACh-binding) receptors, namely nicotinic and *muscarinic receptors*. Both types are found in the hippocampal formation. In this laboratory, you will perform an experiment on tame laboratory animals to determine if muscarinic receptors contribute to spatial learning and memory processes. You will use *behavioral testing* and *psychopharmacology* to test whether or not spatial learning and memory are affected by administration of *scopolamine*, a drug selectively blocking muscarinic receptors for a short period without causing any harm to the experimental animals. (Definitions of all italicized terms are given at the end of this lab.)

Objectives

- To explore the behavioral features and biological basis of spatial learning abilities in a model mammalian species (i.e., the laboratory rat).
- To gain experience performing an experiment to answer a basic question in psychology, in this case how we learn the location of places in our daily environment.
- To learn the value of behavioral testing methods in determining an animal's learning capacity and the value of psychopharmacology in determining which brain processes are critical to that capacity.
- To discover the importance of acetylcholine in learning and remembering locations in our environment and how the loss of that substance in Alzheimer's disease could explain a key symptom of that disorder.
- To become aware that behavior is the product of brain processes, especially those involved in chemical communication among neurons.

Hypothesis and Identification of Variables

Every experiment tests a hypothesis. Our experiment will test the hypothesis that a subject's ability to learn a new location in its environment is impaired by a drug blocking muscarinic receptors. To test a hypothesis, we must choose a research strategy, which requires a choice of (1) a research design, (2) a research setting, and

(3) a data collection method. For this experiment the following choices have been made:

Our research design is experimental, comparing two groups of subjects (one given a drug blocking muscarinic receptors and a control group given no drug).

Our research setting is a laboratory, allowing us to test subjects under controlled conditions.

Our data collection method is behavioral observation of performance speed in a water maze.

DT

What is an independent variable? What is the independent variable for this experiment?

What is a dependent variable? What is the dependent variable in this experiment?

A research strategy cannot be used, however, without making further choices about how the experiment will be run. What subjects will we use? How will we measure their ability to learn a new location? How will we selectively block muscarinic receptors? Those questions are answered in our methods section, which allow others to repeat the experiment and thus check our results.

Methods

Subjects

Since our study requires manipulating brain processes, we must use laboratory animals for ethical reasons. Our choice is one of the most common of all non-human subjects in experimental psychology, namely albino rats. They are a good choice, because they are easy and inexpensive to house, have brains that serve as a good model of the mammalian brain, and have well-developed spatial learning abilities. Albino rats are also very tame and thus safe to handle freely without gloves so long as you don't grab them suddenly or squeeze them around the neck.

We will use young female rats of the Sprague-Dawley strain (200-225 grams in weight). We choose young animals because they have a greater tendency to explore their environment actively and hence tend to learn its spatial features more rapidly. We use females because they are more likely to display the drug effect we will explore (e.g., Berger-Sweeney et al., 1995).

Materials

Since we obviously lack the option of communicating with the rats by means of language, we must rely upon behavioral testing to determine how they learn the location of a new place in their environment. For testing spatial learning abilities,

psychologists often use mazes. They are essentially puzzle boxes in which many different paths may be followed, only one of which leads all the way from the start box to the goal box without dead-end detours. Our maze is a modified version of the water maze introduced by Richard Morris (1984) for testing spatial learning and memory in rats, which are good swimmers. Our water maze is a galvanized pool 1.5 meters in diameter and 0.6 meters deep. In a predetermined quadrant of the pool stands a platform 13 centimeters wide. Only by climbing onto the platform can a rat escape from the water and rest safely. The platform is thus the goal of the water maze for a rat swimming in it. The animal cannot locate the platform visually during test trials because the platform lies below the surface of the water and because both the pool and the platform are painted flat black.

Try to understand why such a maze is so well suited to studying spatial learning. Only when the rat has truly learned the location of the platform with respect to fixed, visible landmarks around the pool can it quickly and reliably escape the water no matter where it is placed along the wall of the pool at the start of each trial. It cannot do that merely by learning to look for the platform (since that is invisible) or by learning to repeat a standard set of movements (since a different set is required from different starting points around the pool). Quick, reliable navigation through the water maze is thus a measure of learning a location in space, not of some other strategy for finding the platform.

We will use a *latency measurement* as an indicator of learning in the water maze. It is defined as the mean time required for a rat to travel from the edge of the pool to the platform across seven trials.

Testing Procedure

Before the lab session, the student preceptor will set up the Morris water maze and fill it with water at room temperature. The preceptor will also and color-code the bottles with the two substances (drug or non-drug) to be injected, so that only he or she knows which one contains the drug being used to test our hypothesis.

Scopolamine Injection. At the beginning of the lab session, the instructor will randomly assign each of six rats calmed by petting to one of two groups. Those in one group will be injected with the muscarinic receptor blocker scopolamine (1 mg/kg body weight) dissolved in a 0.9% salt solution (i.e., saline). Those in the other group will be injected with an equal volume of the saline solution alone. Both types of injection will be made into the intraperitoneal (i.e., abdominal) cavity. The injections will be given "blind" in the sense that neither you nor the instructor will know which animal receives the drug or just saline. (Neither injection should harm the animals. Saline is similar to normal body fluids. Scopolamine in the dose used is metabolized and cleared from the body in less than a day without damaging any of its tissues or processes.)

The 20 minutes required for the drug to have its effect on the brain will be used to provide an introduction to the hypothesis under study. Toward the end of that time, those taking the lab will break up into six teams identified by number. Each team

will be given lab coats and then assigned one of the rats and shown how to handle it properly. The animal should be held comfortably on one arm and gently petted for a few minutes to become comfortable with its new handlers.

Behavioral Testing. Once each team has comforted its animal, testing will begin. As with the injections, the testing will be done "blind" so that none of the teams know whether its animal received the drug or the saline solution. With only one water maze, the teams must take turns testing their animals. Team 1 will start. Using a random number table, Team 1 will decide in which of the four quadrants of the pool to place the platform. That placement will not be changed for all trials run by that team. Using a random number table again, the starting position for the first trial will be chosen among the four quadrants of the pool. All team members will take positions around the pool as visual cues for their rat and maintain those positions on all trials of their animal. One member of the team will then place the rat at the edge of the water maze facing the side of the pool. Another member of the team will be prepared to record the time the animal was placed in the water and the time when it climbed onto the platform. The rat should be allowed to remain on the platform for 20 seconds to help it learn its location with respect to other landmarks in the room.

If the rat does not reach the platform in 90 seconds after being placed in the water, it should be lifted out of the water, placed on the platform, and kept there for the standard 20 seconds. At the end of a trial, a team member will pick up the wet rat and dry it in a towel, holding it for much of the time (a rest period of 90 seconds) before the next trial. Each team will run their animal in the water maze seven times in a row, each trial ending with 20 seconds on the platform and a subsequent 90-second period for further rest and drying.

During the rest period, the remainder of the team should record the location of the platform, the starting location of the rat, and the time it took the rat to swim to the platform. If the rat did not reach that goal by the 90-second limit, team members should simply record 90+ seconds. In addition to running times, descriptive observations should be made on any changes in the swimming pattern of each rat over its seven trials, especially noting differences between aimless versus platform-directed swimming.

Team 2 can begin its first trial once Team 1 finishes its seventh trial. As with the first team, Team 2 must start by deciding where to place the platform and where to put its rat into the pool. All the instructions given above for Team 1 should be followed. This procedure should be followed until all six teams have run their animals in the maze, each for seven trials.

Data Analysis. For each rat, there should be 7 data points for the time it took to swim from the side of the water maze to the platform. Three of the rats received injections from the same bottle, whereas the other three received injections from a different bottle. Group the data on rats injected from the same color-coded bottle. Calculate the mean and standard deviation of the latency measurements for animals in same group. Run a t-test to determine whether the difference in means between the groups is significant. The preceptor will then remove the color bands from the

injection bottles to reveal which group actually received scopolamine. If our hypothesis is correct, you should find that (1) there is a significant difference in mean latency between the groups and that (2) the group with the higher mean (i.e., the one in which animals took longer on average to find the platform) was the one given scopolamine.

DT

In what way(s) is spatial learning different from operant conditioning?

Why is it important to run a drug study in a "double-blind" manner? Why do we not use double-blind techniques in other situations?

Discussion Questions

1. If the two animal groups you tested differed significantly in time to swim the water maze, what does that imply about the relationship between brain function and behavior?
2. If your results were consistent with the hypothesis tested, what implications does that have for possible treatments of Alzheimer's disease?
3. If you found no significant difference between the animal groups in this experiment, does that necessarily mean the hypothesis was wrong? Did you observe anything during the experiment that might provide an alternative explanation for such a negative result?
4. How do you feel about using animals in research? Are there any conditions you feel must be met before such research should be conducted?

Definition of Terms

Acetylcholine: a neurotransmitter important in many brain functions, including learning and memory processes.

Behavioral testing: observation of overt behaviors under controlled conditions used to infer mental processes.

Cognitive maps: mental imagery used to remember the relative location of fixed places in our environment.

Hippocampal formation: an extension of the cerebral cortex in the temporal lobe critical in forming long-term explicit (= declarative) memories.

Latency measurement: a delay interval between starting and finishing a task (e.g., the time taken to swim from the start point to the submerged platform in the Morris water maze).

Muscarinic receptor: one of two types of acetylcholine receptor. It preferentially binds a drug called muscarine. The other type of acetylcholine receptor is called *nicotinic*, which preferentially binds a drug called nicotine.

Morris water maze: a behavioral testing apparatus consisting of a water pool in which the location of the goal (a submerged platform on which to rest) can only be remembered by learning the platform's relative location to fixed visual cues around the pool.

Neurotransmitter: a molecule released by a neuron to transmit its signals to other neurons across synaptic gaps.

Psychopharmacology: the study of drug effects on overt behavior and mental processes.

Receptors: proteins (usually membrane-bound) specialized to bind with only one type or family of molecules (e.g., muscarinic receptors).

Scopolamine: a drug binding to muscarinic (but not nicotinic) receptors. It thus prevents acetylcholine released in the brain from binding and activating muscarinic receptors.

Spatial learning: the process of learning the relative location of fixed places in our environment.

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