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Research report

Experimenter effects on behavioral test scores of eight inbred mouse strains under the influence of ethanol

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HIGHLIGHTS

- A study of ethanol effects on 8 strains of mice was balanced for experimenters.
- Strain differences and ethanol effects were clearly significant ($P < .00001$).
- Open field activity differed for experimenters ($d = 0.8$) after ethanol injection.
- Experimenters rated head dips on the elevated plus maze differently ($d = 1.2$).
- Fall latency on the accelerating rotarod differed between experimenters ($P = .005$).

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ABSTRACT

Eight standard inbred mouse strains were evaluated for ethanol effects on a refined battery of behavioral tests in a study that was originally designed to assess the influence of rat odors in the colony on mouse behaviors. As part of the design of the study, two experimenters conducted the tests, and the study was carefully balanced so that equal numbers of mice in all groups and times of day were tested by each experimenter. A defect in airflow in the facility compromised the odor manipulation, and in fact the different odor exposure groups did not differ in their behaviors. The two experimenters, however, obtained markedly different results for three of the tests. Certain of the experimenter effects arose from the way they judged behaviors that were not automated and had to be rated by the experimenter, such as slips on the balance beam. Others were not evident prior to ethanol injection but had a major influence after the injection. For several measures, the experimenter effects were notably different for different inbred strains. Methods to evaluate and reduce the impact of experimenter effects in future research are discussed.

1. Introduction

The laboratory mouse now plays a central role in research on animal models of human behavioral disorders [1], and numerous laboratories worldwide work with the same genetically defined mouse strains and mutations to answer complex questions about behavior. Within a laboratory, multiple experimenters often work together in order to increase the amount and rate of data collection, while different labs almost always utilize the services of different experimenters. In terms of research design for genetic studies, the experimenter is part of the laboratory environment and constitutes a control variable rather than a systematically manipulated independent variable in many studies. It is recognized that the laboratory environment can have a noteworthy impact on the results of mouse behavioral tests and can interact with genotype of the research animals [2,3]. Within a lab, the experimenter who

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administers a test can also be an important influence [4–7]. The specific experimenter who conducts a test is a difference between labs that cannot be eliminated. Within a lab, however, the study design can be carefully balanced and randomized so that the experimenter does not bias treatment effects. The principles behind this kind of balancing are well understood [8], but the interpretation of even a perfectly balanced study can be difficult if any experimenter effects interact with the treatment effects of principal interest. It is therefore important for behavioral neuroscience research that we gain a better appreciation of the prevalence and magnitude of experimenter effects.

The problem of experimenter effects [9] and experimenter bias [10] in studies of rodent behavior has been acknowledged, and a few reports of experimenter effects on mouse and rat behavioral tests have appeared. Results for elevated plus maze behaviors of rats differed markedly between two experimenters [11], while rats in an elevated plus maze showed greater variation between six experimenters in anxiety scores when the experimenters were unfamiliar to them [12]. Although many studies of genetic variation in mouse behavior employ more than one experimenter, it is rare to see this factor included in the report and data analysis.

In the present study where the treatment effect of central interest turned out to be very small, the experimenter effect was the largest effect in the entire study, even though experimenter was included in the design as a control variable. The study was originally conceived after a surprising result obtained at the University of Alberta [13]. In 1998, eight genotypes were tested for several behaviors in three labs [2]. Then in 2002, 20 inbred strains of mice were tested on several behaviors following ethanol or cocaine injection as part of the Mouse Phenome Project, and the experiment was replicated with identical apparatus and protocols at the same time at Oregon Health & Science University [3,14,15]. Between 1998 and 2002, the Alberta lab had to be moved out of the Department of Psychology space into the central animal quarters in a different wing of the Biological Sciences building. The same test apparatus was used in the new quarters, and results were quite different for certain of the tests, especially the elevated plus maze. It was noticed at the time that odors of many other species of rodents were present in the central facility. Mice were exposed to those odors when being transported down a hallway to the test room, and some of the odors were circulated through the test room as well. The experimenters, however, also differed between the 1998 and 2002 studies done in Alberta. Either the odors, the experimenters, or other unknown factors could have altered results.

Mice are highly sensitive to different kinds of odors and engage in scent marking for social communication [16]. There is clear evidence that rodents exposed to predator odor (fox and cat odor) show anxiety-like behaviors to the potential threat [17–19]. Additionally, it is clear from nearly 65 years of research (see O'Boyle [20] for a historical discussion) that rats are muricidal, a stereotypic behavior defined by the tendency for rats to express predatory behaviors when a mouse is present and accessible [20]. These behaviors include hunting, killing and consuming the mouse [21]. The predatory behaviors and their influence on mouse behavior have been further characterized by the Blanchards and coworkers at the University of Hawaii, who have developed a mouse defence battery to characterize responses of mice confronted with a rat [22,23]. Mice presented with a recently euthanized or anesthetized rat tend to keep a large distance from the rat and will flee if an awake, restrained rat can follow. If escape is not available, the mouse will perform defensive (defensive upright posture, vocalizations) and attack (biting, jump attack) behaviors [24,25]. More recently, mice presented with a restrained rat were shown to have altered facial expressive patterns with increased nose and cheek swells, and the behaviors were very similar to those manifested to cat odor presentation [26]. For mice exposed to rat odor, stress-related hormone levels were altered [27–29]. Rat odor also suppressed appetite and markedly increased latency to approach and consume food rewards [30], decreased sucrose intake and time spent in the open arms of an elevated plus maze [31], increased time spent freezing [32], and amplified startle response and time spent in the dark of a light–dark test [33,34]. Some effects were so robust that Calvo-Torrent et al. [31] suggested rats and mice should not be housed near one-another.

When D.W. moved his mouse lab to UNCG in 2008, the animal research facility was empty and there were many unused testing and colony rooms. This provided an ideal situation to test the influence of rat odors on mouse behavior. The facility manager stated that all air in the facility was fresh to each room and was not recirculated. During preparation for the study, the smell of rats was never detected by the researchers in any of the testing rooms. A study was then conducted using three groups: (a) mice housed and tested in rooms that only contained mice; (b) mice housed and tested in rooms that contained both mice and rats; (c) mice housed only with mice but tested in a room containing rats. It was expected that mice exposed to rat odors for the first time would express greater anxiety-like behaviors and show greater impairment following an ethanol injections. The study used two experimenters to test the animals during each day. The study was carefully randomized and balanced for experimenter and treatment effects over strain, sex, time (morning versus afternoon), and housing room.

While the study was in progress, it was noticed on several occasions that a distinctive odor of coffee brewing was coming into the mouse testing rooms. Neither mice nor rats were ever fed coffee in this study, and our experimenters never brewed coffee anywhere in the animal facility. It was then determined that the animal care personnel employed by the university were making coffee in their office that was inside the controlled access animal facility. Evidently there was recirculation of air among the various rooms, especially during hot weather when air conditioning was used. This negated the design of our experiment. We decided to complete the study and look at the data. No or very small rat housing effects were found, but there were several substantial experimenter effects.

2. Methods

2.1. Mice

Equal numbers of males and females of eight strains from the Mouse Phenome Database (MPD) Priority list 1 were studied (129S1/SvImJ, A/J, BALB/cByJ, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ, SJL/J). All animals were obtained at 6 weeks of age from the Jackson Laboratory, Bar Harbor, Maine, USA. The rats were six week old Harlan Sprague-Dawley imported directly into the lab and never used in any previous study. At the start of the study they were about 10 weeks old.

2.2. Husbandry

On arrival, mice were randomly assigned, two male and two female mice per strain, to one of three rooms: (a) no rats in colony room or behavioral testing room, (b) no rats in colony room but rats in test room, and (c) rats in both colony room and test room. Mice were habituated in their assigned housing condition for two weeks before behavioral experiments commenced. Animals were housed two of the same sex per cage in standard shoebox cages with open wire frame tops and had free access to Purina 5001 mouse chow and Greensboro tap water.