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Research report

Evaluation of study design variables and their impact on food-maintained operant responding in mice

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ABSTRACT

Operant conditioning paradigms are useful for studying factors involved in reward, particularly when combined with the tools of genetic manipulation in mice. Published operant studies involving mice vary widely with respect to design, and insight into the consequences of design choices on performance in mice is limited. Here, we evaluated the impact of five design variables on the performance of inbred male mice in operant tasks involving solid food pellets as reinforcing agents. We found that the use of lever-press or nose-poke during FR1 sessions did not impact the performance of C57BL/6 mice, but that the lever-press approach correlated with enhanced performance during PR testing. While FR1 session duration had a notable impact on the rate of acquisition of food-maintained responding, performance during FR1 and PR sessions was largely unaffected. Higher order schedules of reinforcement (FR3 and FR5) led to elevated responding during both FR and PR sessions, and improved the correspondence between rewards earned and consumed. Single and group-housed mice performed indistinguishably during FR1 and PR sessions, while environmental enrichment combined with group housing accelerated the rate of acquisition of food-maintained responding while decreasing responding during PR testing. Finally, while C57BL/6 and 129/Sv mice exhibited comparable behavior during FR1 sessions, C57BL/6 mice tended to acquire food-maintained responding faster than 129/Sv counterparts, and exhibited elevated responding during PR testing. Altogether, our findings indicate that while operant performance for food in mice is relatively insensitive to many study parameters, experimental outcomes can be shaped predictably with proper design decisions.

1. Introduction

Operant conditioning refers to the use of positive or negative reinforcement to modify the frequency of a particular voluntary behavior. In the 1950s, Skinner and co-workers pioneered automated operant conditioning tasks involving the pigeon as subject, and early studies from this group shed important light on the impact of behavioral variables such as the schedule of reinforcement and their influence on operant behavior [1–3]. Automated operant tasks have also been adapted for rodents, which exhibit several reproductive and biological characteristics advantageous for high-throughput experimentation. Rats have been particularly common subjects in operant studies, and have helped to provide critical insights into the neurochemical and anatomic basis of reward (e.g., Refs. [4–10]).

The use of mice as subjects in operant conditioning studies accelerated in the 1970s when groups began to evaluate the impact of pharmacologic interventions on operant responding in mice. Several groups, for example, studied the effect of drugs of abuse and related agents on food-maintained operant responding in mice [11–22]. Operant-based approaches have more recently been adapted for drug self-administration studies in mice. Mice, like rats and primates, will self-administer nicotine [23], ethanol [24], cocaine [25,26], opioids [27], and cannabinoids [28].

Differences between inbred and outbred mouse strains with respect to operant responding were noted in early studies, highlighting genetic influences on instrumental learning [29,30]. In the last two decades, investigators have generated and utilized mice harboring targeted genetic mutations in operant studies in an attempt to better understand the genetic and molecular underpinnings of drug reward. Approaches involving knockout mice in particular have highlighted the significance of specific receptor types, such as the serotonin 5-HT1B and D2 dopamine receptors, to the reinforcing effects of cocaine [31,32], ethanol [33,34], and morphine [35]. Similarly, the D1 dopamine receptor and melanocortin receptor types 3 and 4, among other targets, have been implicated in the reinforcing effects of food and sweet rewards [36–38]. As

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the spatial and temporal resolution of gene ablation or suppression strategies in mice improves, operant-based studies will be particularly useful for delineating the cellular basis of reward.

A careful comparison of protocols employed in mouse-based operant conditioning studies reveals striking variability with respect to many experimental design variables. For example, published studies differ with respect to the use of lever-press or nose-poke to earn rewards, session numbers and duration, schedule(s) of reinforcement, housing conditions, genetic background, and nature and composition of the reinforcing agent. To date, a systematic evaluation of the influence of most such design variables on operant performance in mice is lacking. As such, the goal of this study was to quantify the influence of manipulanda, session length (30–90 min), schedule of reinforcement (FR1, FR3, and FR5), housing status (isolation, group housing, and environmental enrichment), and mouse strain (C57BL/6 and 129/Sv) on the performance of mice in an operant task involving food as the reinforcing agent.

2. Materials and methods

2.1. Subjects

All studies were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23; revised 1996), and were granted formal approval by the Institutional Animal Care and Use Committee of the University of Minnesota. Efforts were made to minimize the pain and discomfort of the animals throughout the study, and when possible, to reduce the number of animals used in each test. Male C57BL/6 and 129/Sv mice were purchased from Jackson Laboratories (Bar Harbor, ME), group-housed for 2–4 weeks on a 12-h light/dark cycle prior to testing and were given *ad libitum* access to standard rodent chow (#2018, Harlan Teklad Global Diets, Madison, WI). Unless otherwise noted, subjects were single-housed at 7–8 weeks of age. Single-housed mice were given *ad libitum* water access and 1.8 g of standard rodent chow per day until each subject reached 85–95% of their free-feeding weight (~3 days). Subsequently, subjects were given 2.0–2.3 g of rodent chow per day to maintain stable bodyweights throughout testing.

2.2. General experimental design

Five separate studies were conducted, each involving a distinct cohort of mice. Experiments were conducted in six mouse operant chambers as described previously [39]. Subjects were handled individually in the testing room for 3 days prior to the first test session. One day prior to testing, 20 reward pellets (20 mg PJA/100020 dust-free Noyes Precision pellets, Bio Serv, Frenchtown, NJ) were placed into the home cage to blunt the potential effect of food neophobia on operant performance [40]. All subjects were tested in 10 sessions occurring over an 11-day period using a fixed ratio 1 (FR1) schedule of reinforcement unless stated otherwise. Testing was conducted between 1100 and 1600 h. The house light was illuminated throughout the session. The active lever/nose-poke hole was counterbalanced between-subjects for each study. A response on the active manipulandum resulted in stimulus light illumination (3 s) above the lever or inside the nose-poke hole; a single pellet was immediately delivered to the food cup. A response on the inactive manipulandum was recorded but did not lead to pellet delivery or stimulus light illumination. Prior to the start of the session on Day 1, reward pellets were crushed to create powder that was sprinkled on the active lever or in the active nose-poke hole to facilitate instrumental responding on the first training day. Upon session completion, the house light was turned off and the mice were returned to their home cage. After completing the 10 FR1 sessions, mice that had acquired food-maintained operant responding behavior were evaluated in a single 1-h PR test wherein delivery of a food pellet was contingent upon a progressive ratio of active responding (e.g., 1, 2, 4, 6, 9, 12, 15, 20, etc.), as described previously [39,41].

2.3. Analysis

Data are presented throughout as the mean ± SEM and were analyzed using Prism 5 software (GraphPad, La Jolla, CA). Acquisition rate was defined as the first day of the first block of 3 consecutive days during which all acquisition criteria were met (see Section 3 for the criteria). Responding (active and inactive) and the number of pellets (earned, consumed, and uneaten) were determined for each subject by averaging values measured over the 3-day stability period. Active and inactive responding, rewards earned and breakpoint values were recorded from the PR data. The breakpoint is defined as the value associated with the last completed set of active responses that resulted in a reward in a 60-min session. Data were compared between groups using Student's *t*-test or one-way ANOVA followed by Tukey's multiple comparison tests, as appropriate. The threshold for statistical significance in all instances was $P < 0.05$.

3. Results

3.1. Study 1: manipulanda

To determine whether food-reinforced operant responding in mice is influenced by the manipulanda employed during testing, we randomly assigned male C57BL/6 mice to either lever-press (LP) or nose-poke (NP) groups ($n = 10$ per group). The number of active and inactive responses, as well as the number of pellets earned, consumed, and uneaten under a fixed ratio 1 (FR1) schedule of reinforcement were tabulated for 10 days during 90-min test sessions. As shown in Fig. 1, both LP and NP groups engaged in robust active responding and reward consumption over the 10 FR1 sessions.

Criteria used to determine whether and when a particular subject acquires food-maintained operant responding include a minimum number of active responses or rewards earned, a measure of discrimination between active and inactive levers or nose-poke holes, and a measure of performance stability (e.g., Refs. [42–44]). Prior to establishing the acquisition criteria for our studies, we carefully examined the performance of all subjects over the 10 FR1 sessions. Within the first three sessions, all subjects in both the LP and NP groups routinely engaged in active responding >30 times per session. Moreover, all subjects in both groups exhibited reasonable discrimination (>3:1) between active and inactive levers or nose-poke holes within three sessions. Active responding and pellets earned, however, varied dramatically between sessions for many subjects (see Supplemental Fig. S1). Indeed, the coefficient of variation for active responding ranged from 16 to 94% and 17 to 59% for the LP and NP groups, respectively, over the 10 test sessions. In contrast, reward consumption was a more stable within- and between-subjects parameter for the subjects, particularly for the LP group (%CV = 14–38) (Fig. 1; Supplemental Fig. S1).

Given these observations, we used the following criteria to assess whether and when subjects successfully acquired

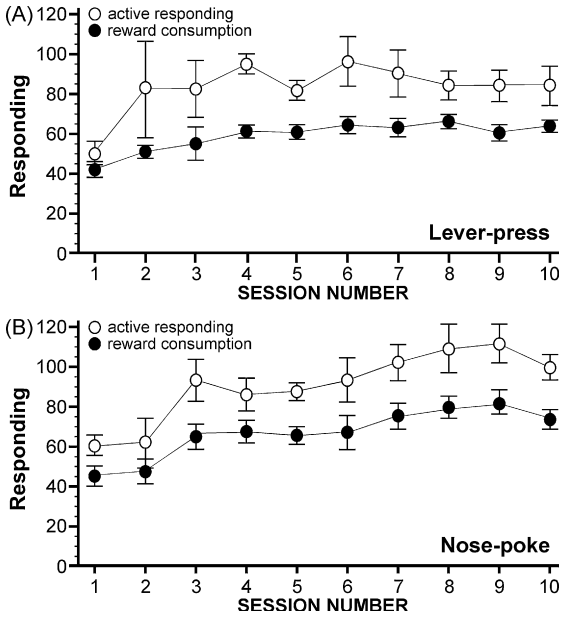

Fig. 1. Active responding and reward consumption for lever-press and nose-poke groups. Active responding and reward consumption for male C57BL/6 mice assigned to lever-press (A, $n = 10$) and nose-poke (B, $n = 9$) groups over 10 days of FR1 training. While no obvious differences between NP and LP groups were noted with respect to these measures, the relative stability of consumption over active responding within groups is evident (note the smaller error bars), particularly for the LP group.