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A novel operant testing regimen for multi-construct cognitive characterization of a murine model of Alzheimer's amyloid-related behavioral impairment

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ABSTRACT

A common method for modeling pathological and behavioral aspects of Alzheimer's disease (AD) is the transgenic mouse. While transgenic strains are often well characterized pathologically, behavioral studies of cognitive deficits often employ a limited set of aversively motivated, spatial learning and memory tests, under brief testing periods. Here we illustrate an alternative operant behavioral methodology to provide a comprehensive characterization under repetitive testing conditions, and with appetitive motivation. In this study, we employed the commonly used Tg2576 murine model of Alzheimer's disease amyloid pathology, since it has been the subject of many previous behavioral studies. In these mice, we compared the learning of simple and complex, as well as spatial and non-spatial rules. The mice were assessed on a progressively more complex and interlocking battery of operant tasks, ranging from simple rule learning to delayed recall, as well as tests of motor and sensory ability. In general, as compared to wild type control mice, within-group variability was high in the Tg2576 mice, and deficits were most apparent in more complex discrimination tasks. Furthermore, a consistent decrease in the rate at which Tg2576 mice completed testing trials was observed, pointing to a potential motivation difference or speed–accuracy tradeoffs as a defining characteristic of this strain under these test conditions. Using sensitive adjusting retention interval procedures, it was also possible to isolate a difference in retention interval and separate it from non-mnemonic processes. Overall, these experiments demonstrate the utility of this novel operant approach for characterizing the cognitive deficits of transgenic murine models of dementia.

1. Introduction

Alzheimer's disease (AD) is a progressive, debilitating disease pathologically characterized by amyloid plaques, neurofibrillary tangles and neuronal loss. The disease clinically manifests with memory loss, disorientation, and other behavioral changes (Alzheimer, 1907; Davies & Maloney, 1976; Murrell, Farlow, Ghetti, & Benson, 1991; Price, Walker, Martin, & Sisodie, 1992; Selkoe, 2001, 2004). AD has been studied in spontaneous mammalian animal models, including primates and rodents by using lesions of various specificities by using pharmacological challenges such as anticholinergic drugs, and by using aged animals (Bartus, 2000; Sherman, Kuster, Dean, Bartus, & Freidman, 1981). In the last two decades, transgenic murine lines have become one of the dominant methods for studying the etiology, neuropathology, and treatment strategies for AD (Götz et al., 2007; Iqbal et al., 2005). Most transgenic mouse lines were created from introducing gene mutations related to familial early-onset forms of AD, alone or in combination, into transgenes encoding human amyloid ß-protein precursor (AßPP) and characterized by the appearance over the lifespan of various histopathological and behavioral abnormalities, particularly learning and memory alterations.

One such well-studied transgenic model is the Tg2576 mouse line, introduced in 1996 by Hsiao et al. which contains the Swedish double mutation (K670N/M671L) under the control of a Hamster PrP promoter. As early as 6 months, but virtually, universally by 12–14 months of age, these mice show substantial accumulations of human Aβ species, especially Aβ42, congophilic plaques, and inflammation in cortical and limbic structures. While this is one of the most thoroughly studied human AßPP transgenic mouse lines, one important limitation is in the behavioral characterization of this line. There is reliance on tasks such as passive/active avoidance and object recognition, or on spatially intensive learning and memory tests such as spontaneous alternation, the Morris water

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maze, and the Barnes maze (early studies reviewed in Ashe (2001), Arendash and King (2002), Barnes and Good (2005), Corcoran, Lu, Turner, and Maren (2002), Deacon, Koros,Bornemann, and Rawlins (2009), Good and Hale (2007), Hale and Good (2005), King and Arendash (2002a, 2002b), Middei, Daniele, Caprioli, Ghirardi, and Ammassari-Teule (2006), Ognibene et al. (2005), Westerman et al. (2002) and Zhuo et al. (2007)). These tasks are often aversively motivated (e.g. Morris water maze and passive/active avoidance most notably) or take place in exposed settings, such that they presumably evaluate subjects exclusively in a high state of vigilance and arousal. And while some incorporation of operant tasks exist in this literature (e.g. Adriani et al., 2006; Lagadec et al., 2010), relatively little work has evaluated the Tg2576 or other related lines along a progressive spectrum of tasks, where simple components are initially assessed and then more complex and demanding tests drawing on those simpler skills are introduced. Operant techniques, using appetitive reinforcers, are especially well suited to these progressive, moderate arousal analyses of cognitive functions, and to potentially sensitive operant tests likely to reveal deficits potentially missed in other less complex learning and memory tests.

In the present study, we sought to demonstrate the feasibility of the operant approach for making sophisticated dissociations of advanced cognitive skills in mice well trained in the underlying procedures. Our experimental model was based on an interlocking, progressive operant regimen that has been employed successfully in rats pathology (Kritzer, Brewer, Montalmont, Davenport, & Robinson, 2007), and altered it to be conducted using Tg2576 mice, during their early development of underlying Aβ-related pathology (Westerman et al., 2002). Much work has been done with several of the tasks employed in this study using rats as subjects, including signal detection, Delayed Nonmatch-to-Position, and progressive ratio. These tasks have been used to evaluate ascending cholinergic system deficiencies (e.g. Jensen, Stephens, Sarter, & Petersen, 1987; Sarter, Hasselmo, Bruno, & Givens, 2005), hippocampal damage (Dunnett, Evenden, & Iverson, 1988; Heyser, Hampson, & Deadwyler, 1993; Winters & Dunnett, 2004), and mesolimbic dopamine alterations (Hodos & Kalman, 1963; Zhang, Balmadrid, & Kelley, 2003), respectively. This paper represents a first step in investigating the utility of adding operant regimens to systematically evaluate transgenic models of Alzheimer's disease symptomology.

2. Methods

2.1. Subjects

Tg2576. (B6/129) mice were obtained from Jackson Laboratories. The study employed eight Tg2576 animals and eight wild type controls of the same age and genetic background, both groups were mixed-sex (four and four of each). All mice were singly housed in standard 27.5 cm long × 16.5 cm wide mouse cages throughout the duration of the experiments in a vivarium with controlled temperature and humidity on a 12-h light/dark schedule. Operant training and testing procedures began at 6 months of age, and concluded at approximately 12 months. The subjects were then euthanized and the brain tissue harvested for histological and neurochemical analysis. The Stony Brook University Institutional Animal Care and Use Committee approved all procedures.

2.2. Apparatus and setting procedures

The operant chamber (MED Associates) was located inside a sound-attenuating chamber, with an exhaust fan providing white noise. The chamber was 19 × 22 cm at the floor with two front nose-poke ports that are 4.5 cm directly right and left of the water dipper, and one nose-poke port in the rear, directly opposite of the dipper. Nose poke ports were 1.5 cm in diameter and contained a small light, and a single photo-beam that registered nose-poke responses. The chamber had a metal rung floor, and was lit by a small house light.

During the operant testing, all the mice had no access to water for 23 h, followed by a 30 min test session in which water was available as reinforcers, and then 30 min of free water consumption in their home cage following the test session.

2.3. Behavioral testing

All mice received an identical program of behavioral testing, described as follows and summarized in Table 1. Trial initiation and reinforcement procedures were uniform across all behavior tests. Trials always began with a nose-poke into an illuminated front nose-poke port. Following a correct response, both nose-port well lights were extinguished, and the water reinforcer ladle or "dipper" was made available for 10 s. When the dipper was removed, the nose-port lights were illuminated, beginning the next trial. Following an incorrect response, both nose-port lights were extinguished for the ten-second intertrial interval, and then re-illuminated to indicate the start of the next trial. All sessions were 30 min in duration.

In order to normalize performance on each task, no animal progressed to the next step/task until they had successfully acquired the previous one. In the early tasks, a set number of responses per 30 min trial was required, in the later tasks, the requirement was a percentage of correct responses, out of all trials that were attempted. As a result, there was a short break between tasks for the better performing animals, while the animals who had not acquired the task continued until reaching criteria. The operant programs progressed in a set order, the same for all mice, as follows:

2.3.1. Magazine (dipper) training

In this procedure, both front nose-poke ports were illuminated at the start of all trials. The water dipper was activated non-contingently every 30 s and remained raised for 10 s in the testing chamber. A nose-poke response in either port when lights were illuminated produced an additional reinforcer. The mice were exposed to this procedure for 2 days. This task helped shape the behavior of the mice to nose-poke, in preparation for later tasks.

2.3.2. Fixed ratio

In this task, both front nose-poke ports were lit and mice were required to nose-poke in order to obtain a reward. The mice could poke in either port, at any time when the dipper was not already activated. This program continued until the mice reached a criterion of a total of fifteen or more responses in one session combined to either port, or at least ten more responses on one side than the other.

2.3.3. Alternation

During this task, only one of the front nose poke wells was lit at a time, the lit lamp alternating from right to left on subsequent correct response trials and resetting to the same side following error trials. This program forced mice to use both left and right response equally to maximize reinforcement rate. This alternation program continued until animals completed a criterion of thirty or more responses per session.

2.3.4. Light dark discrimination

During this task, only one front nose-poke well light was lit at a time, and alternated at random, separated by a 10 s intertrial interval. A correct response was made in the illuminated port.