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Research article

# Acquisition and reversal of visual discrimination learning in APPSwDI/*Nos2*$^{-/-}$ (CVN) mice

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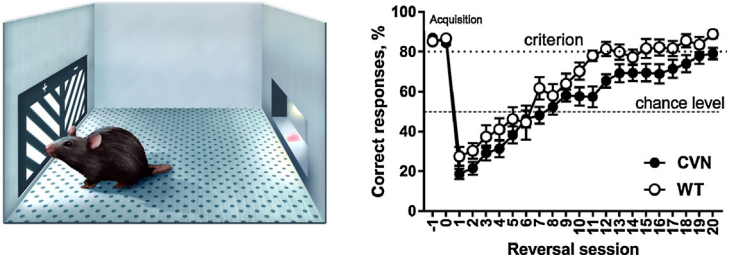
## HIGHLIGHTS

- CVN mice acquired Visual Discrimination at a rate comparable to that of WT mice.
- Reversal of Visual Discrimination learning in CVN mice was slower than in WT mice.
- CVN mice made more errors during the Reversal of Visual Discrimination learning.
- CVN mice were slower to collect liquid food reward.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Studies of cognitive behavior in rodent models of Alzheimer's disease (AD) are the mainstay of academic and industrial efforts to find effective treatments for this disorder. However, in the majority of such studies, the nature of rodent behavioral tests is considerably different from the setting associated with cognitive assessments of individuals with AD. The recently developed touchscreen technique provides a more translational way of rodent cognitive testing because the stimulus (images in different locations on the screen) and reaction (touch) are similar to those employed in human test routines, such as the Cambridge Neuropsychological Test Automated Battery. Here, we used Visual Discrimination and Reversal of Visual Discrimination touchscreen tasks to assess cognitive performance of *APPSwDI/Nos2*$^{-/-}$ (CVN) mice, which express mutated human APP and have a homozygous deletion of the *Nos2* gene. We revealed that CVN mice made more first-time errors and received more correction trials than WT mice across both discrimination and reversal phases, although mutation effect size was larger during the latter phase. These results indicate sensitivity of touchscreen-based measurements to AD-relevant mutations in CVN mice and warrant future touchscreen experiments aimed at evaluating other cognitive and motivational phenotypes in this AD mouse model.



*Abbreviations:* AD, Alzheimer's disease; APP, amyloid precursor protein; CT, correction trial; CVN mice, *APPSwDI/Nos2*$^{-/-}$ mice; ITI, inter-trial interval; PI, perseveration index; RM ANOVA, repeated measures analysis of variance; WT, wild type.

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## 1. Introduction

Transgenic mouse models are indispensable for efforts to counteract the dramatic burden of Alzheimer's disease (AD) [1–3]. In the majority of AD mouse models, at least one of the three characteristic features of human AD pathology is recapitulated, namely extracellular deposits of Aβ, intracellular neurofibrillary tangles formed by hyperphosphorylated tau protein and/or neuronal loss. Because amyloid plaques have been traditionally regarded as hallmarks of AD [4], and autosomal dominant mutations in the amyloid precursor protein gene (*APP*) have been described in people with AD [5], numerous genetic mouse models of AD expressing mutated human *APP* have been created [1–3]. However, many such amyloid-centric models did not phenocopy accumulation of hyperphosphorylated tau, whereas it is tau dysregulation that correlates most closely with AD severity in humans [6].

CVN mice express human APP isoform 770 that contains Swedish (K670N/M671L), Dutch (E693Q), and Iowa (D694N) mutations under the control of the mouse Thy1 promoter, and a targeted loss-of-function mutation in the mouse *Nos2* gene, which encodes nitric oxide synthase 2 [7]. NO production by inducible NOS may have anti-apoptotic and pro-survival effects [8], and its ablation in CVN mice dramatically potentiated the effects of the human transgene expressed at comparatively low levels [7]. Specifically, in addition to expected amyloid pathology, manifested as microvascular Aβ accumulation, CVN mice developed aggregations of hyperphosphorylated tau, demonstrated pronounced metabolic brain disturbances and exhibited significant neuronal loss in the hippocampus and subiculum [7,9]. These impairments were likely responsible for inferior performance of CVN mice in several learning and memory tests, such as the radial-arm water maze, Barnes maze and fear conditioning [7,9].

To investigate performance of CVN mice in a more translatable setting, we have initiated studies of this AD mouse model in touchscreen operant chambers. The touchscreen approach to evaluating cognition in humans, exemplified by the Cambridge Neuropsychological Test Automated Battery (CANTAB), has been gaining increasing popularity in the clinical setting [10]. High translatability of mouse touchscreen testing is ensured by the fact that the stimulus (images in different locations on the screen) and reaction (touch) are similar to those employed in the human battery. Therefore, analogous cognitive tests can be administered in both species [11]. Here, we present data on the performance of CVN mice in the Visual Discrimination and Reversal of Visual Discrimination touchscreen tasks [12].

## 2. Material and methods

### 2.1. Animals

Eighteen male CVN mice, produced by crossing mice that express Swedish K670N/M671L, Dutch E693Q, and Iowa D694N human APP mutations under control of the Thy-1 promoter (10) with *Nos2*$^{-/-}$ (B6 129P2Nos2 tau1Lau/J) animals [7], and 13 age-matched C57Bl/6J wild-type (WT) counterparts were bred at the Charles River breeding facility in Sulzfeld (Germany). For touchscreen experiments, 4–5-month-old animals were transferred to the animal facility at Charles River Discovery Research Services (Kuopio, Finland). Animals were acclimatized for one week before testing. The mean ages of mice at the start of testing were similar: CVN, 135.5 ± 1.95 days; WT, 134.8 ± 2.41 days. Mice were housed singly in a temperature- and humidity-controlled environment under a 13:11 h light/dark cycle (lights on at 07:00 am and off at 8:00 pm). Cages (IVC type II, Allentown, Inc., Allentown, NJ, USA) were kept at negative pressure and furnished with corn cob-derived bedding (Scanbur, Karlslunde, Denmark), nesting material (aspen wool, Tapvei Oy, Kortteinen, Finland), and a tinted polycarbonate tunnel (Datesand, Manchester, UK). Mice were fed Teklad Global 16%-protein rodent diet (Envigo, Huntington, UK) and kept on a restricted food regimen, at 85–95% of their free-feeding weight in order to maintain motivation for the task, with water *ad libitum*. Mice received one training session per day between 1 and 4 pm, 5–7 days per week.

All experiments were carried out according to the protocols reviewed by the internal animal welfare body and approved by the National Ethics Committee of Finland.

### 2.2. Apparatus

Experiments were conducted in 16 Campden Instruments touchscreen chambers (Campden Instruments, Loughborough, UK) located in a dedicated room. A house light was fitted in all chambers and was on as standard. Two-window mouse Campden VD masks were used for the pretraining, Visual Discrimination and Reversal tasks (see Table 1 in [12]). During the first two weeks of pretraining tests, a 1.5% glucose/0.4% saccharin solution was used as liquid reward [13]. However, it was discontinued because of apparently suboptimal performance of mice and Valio Profeel strawberry-flavored milk drink (Valio, Helsinki, Finland) was used for the remainder of the experiments.

### 2.3. Touchscreen pretraining

Before visual discrimination testing, mice were trained on basic touchscreen task requirements, which were introduced in several consecutive stages ("Initial touch", "Must touch", "Must Initiate" and "Punish Incorrect"), as described previously [12]. Once the mice completed all pretraining criteria [12], they were moved on to Visual Discrimination task training.

### 2.4. Visual discrimination and reversal tasks

The Visual Discrimination task was similar to that previously described [12]. After initiating each trial, the mouse was presented with a choice between two (spatially pseudorandom) "Lines Grid-Right" and "Lines Grid-Left" stimuli, one in each response window. During acquisition of visual discrimination, the stimuli were counterbalanced such that for approximately half of the animals the "Lines Grid-Right" stimulus was correct (S+; rewarded) and the "Lines Grid-Left" stimulus was incorrect (S−), whereas for another half of the mice, the contingency was opposite. Mice were assigned to either the "Lines Grid-Right" or the "Lines Grid-Left" group sequentially, based on their performance during pretraining. Responses to the S− stimulus were "punished" with a 5-s "time out" followed by a correction trial (CT). The ITI was 20 s, and correction ITI was 5 s. The mice were considered to have acquired discrimination, when they reached a performance criterion of at least 80% of trials correct (not including CTs) in two consecutive 30-trial sessions. Mice were moved on to the reversal phase of the task individually, immediately after they attained the acquisition criterion. The Reversal task was identical to the initial acquisition task, except that S+ and S- were reversed. All mice received at least 20 days of reversal sessions.

Several parameters were calculated to assess performance during Visual Discrimination and Reversal tasks. Initial stimulus bias was analyzed during the first 30 trials of visual discrimination acquisition by comparing the observed percentage of correct responses to the chance level (50%). For both tasks, the numbers of trials (first presentation only, i.e., excluding CTs), errors (incorrect choices on first presentation trials) and CTs were analyzed. For quantitative assessment of the perseverative behavior during