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Research article

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

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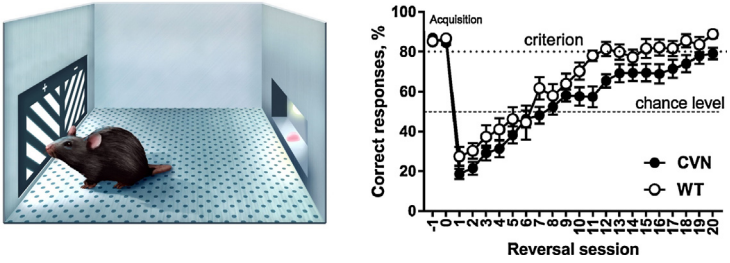
HIGHLIGHTS

- 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

Reversal task, the ratio of CTs to incorrect responses (first presentation only), i.e., "perseveration index" [14], was calculated. Latencies to make a correct and incorrect response as well as reward collection latencies were also evaluated.

To further investigate performance during reversal of visual discrimination, data were split into perseverative sessions, in which performance was below 50% correct, and non-perseverative sessions, which included sessions with performance level from 50% and higher, up to the criterion of at least 80% correct responses on two consecutive days (including the sessions during which the criterion was reached). If the criterion was not reached during Reversal task, non-perseverative sessions included all sessions during which the performance level was 50% or higher.

2.5. Data analysis

Pairwise comparisons were done by the Student's independent samples *t*-test, one-sample *t*-test, or, where the assumption of normality was rejected by the D'Agostino-Pearson test, by the non-parametric Mann-Whitney *U* test. Response and reward collection latencies in individual animals were right-skewed even after log10 or square root transformations. Therefore, for between-genotype comparisons, median rather than mean latency values from individual mice were used as more representative central tendency measures that were robust to the effect of outliers.

Data from repeated measurements across 20 days of Reversal task were analyzed by two-way analysis of variance (ANOVA; within-subject factor – day/session; between-subject factor – genotype). In the beginning of Reversal task, many animals failed to complete usual 30 daily trials (Supplementary Fig. 1). Therefore, because the validity of using RM ANOVA for analysis of the percentage of correct responses in such conditions was questionable, we additionally compared reversal learning curves by fitting the following equation of increasing form of Exponential Decay to the data points:

$$\text{Correct response}\,[\%] = Amax - (Amax - Amin)\,e^{-\lambda t/100}, \qquad (1)$$

where λ is the learning rate, t is time (testing day starting from zero), Amax and Amin are the maximum and initial levels of correct responses, respectively (Supplementary Fig. 2). The following constraints were introduced before fitting: $0 \leq \text{Amin} \leq 100$; $50 \leq \text{Amax} \leq 100$; $\lambda \geq 0$.

To take into account unequal number of trials made on different days, we performed nonlinear regression using number of trials as observation weights, using MATLAB *nlinfit* function based on the Levenberg-Marquardt nonlinear least squares algorithm.

All statistical analyses were conducted with a significance level of 0.05 using GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). Throughout the text, data are presented as the mean ± standard error of the mean. For analysis of covariance (ANCOVA), comparisons of adjusted mean values were deemed valid only if slopes of regression lines for data from CVN and WT mice did not differ significantly ($P > 0.05$), as determined by the tests for homogeneity of regressions.

3. Results

3.1. Visual discrimination pretraining

There was no significant effect of genotype on the number of days required to complete pre-training (Fig. 1A). The relatively long duration of pretraining is likely explained by the fact that initially, a solution of glucose and saccharine was used as liquid reward, and we noted that many animals struggled to achieve the required criteria during the final two pretraining stages ("Must initiate" and "Punish incorrect") in these conditions. However, mice became more motivated when we switched to strawberry flavored liquid milkshake after the initial two weeks of testing. One CVN mouse and one WT mouse were excluded from the analysis because they could not complete pretraining within 40 days. Therefore, all reported data are based on measurements in 17 CVN and 12 WT mice.

3.2. Acquisition of visual discrimination

WT mice had a slight positive bias to the correct stimulus during the first day of visual discrimination testing, while CVN mice did not exhibit any significant bias (WT: 59.1 ± 2.5% correct responses, $P = 0.004$; CVN: 47.6 ± 2.0%, $P = 0.262$; one-sample Student's *t*-test against 50%). The percentage of correct responses in the first trials differed significantly between the genotypes ($P = 0.0014$). Despite this initial bias, there was no effect of genotype on acquisition performance, in terms of the number of days before achieving the criterion, number of trials completed, number of errors and number of received CTs (Fig. 1B–E). Furthermore, both CVN and WT mice reacted with similar latencies to stimuli during correct and incorrect responses (Fig. 1F, G). However, the latency to enter the magazine for reward was slightly but significantly slower in CVN animals (Fig. 1H). The latter phenotype could be caused by higher relative weight of CVN animals. However, during the Visual Discrimination task, the relative weight of CVN mice was actually slightly but significantly lower than that of WT mice, while remaining in the target range (CVN: 87.5 ± 0.69%; WT: 89.6 ± 0.46%; $P = 0.021$, Mann-Whitney *U* test). Thus, longer latency to collect reward in mutant animals was unlikely associated with suboptimal level of food deprivation of mutant animals.

3.3. Reversal of visual discrimination learning

Reversal learning performance of CVN and WT mice is illustrated in Fig. 2 A. By day 20 of reversal testing, many animals (seven CVN mice and one WT mouse) failed to re-learn to a criterion of 80% correct responses for two consecutive days, however the difference in these proportions did not achieve statistical significance ($P = 0.093$, Fisher's exact test). Upon the reversal of the correct stimulus, most animals did not complete all 30 trials during the first several days of reversal testing (Supplementary Fig. 1). There was a clear effect of session ($F_{19,513} = 20.83$, $P < 0.0001$) on the number of daily trials during Reversal task, as mice gradually regained their motivation to complete full set of 30 trials, however the effect of genotype ($F_{1,27} = 1.085$, $P = 0.307$) or interaction between these two factors ($F_{19,513} = 0.699$, $P = 0.821$) were not significant (Supplementary Fig. 1).

RM ANOVA revealed a significant effect of genotype ($F_{1,27} = 5.378$, $P = 0.028$) and session ($F_{19,513} = 66.09$, $P < 0.0001$) on the percentage of correct responses (Fig. 2A), but no significant interaction between these two factors ($F_{19,513} = 0.91$, $P = 0.57$). However, because animals completed variable numbers of trials on different days of Reversal task, analyzing percentages of correct responses by RM ANOVA in such circumstances may not be entirely justified, despite there was no clear effect of genotype on daily trial numbers (Supplementary Fig. 1).

To avoid the limitations associated with day-to-day variation in the number of trials and inability of many mice to re-learn to 80% correct criterion, we additionally compared learning rates by fitting reversal learning data with weighted nonlinear regression, where individual observation weights were set based on the absolute number of trials made on corresponding days, as described in 2.5 Data analysis (Supplementary Fig. 2). Using this approach, we found that reversal learning rates were not significantly different between CVN and WT animals (Fig. 2B). Furthermore, although both A_{max} and A_{min} parameters of Eq. (1) were nominally lower in

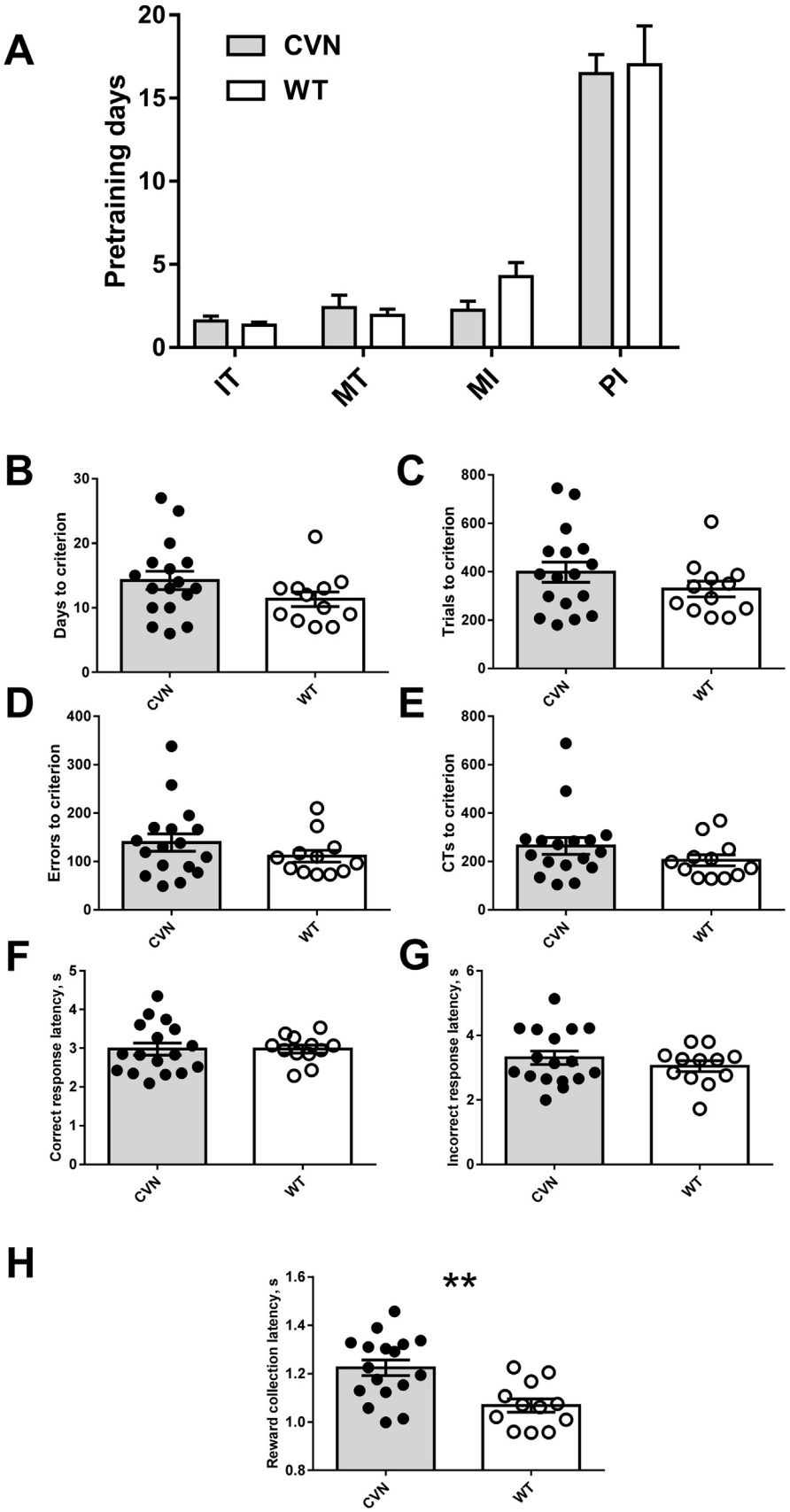

Fig. 1. Touchscreen pretraining and acquisition of visual discrimination in CVN and associated WT mice. (A) Number of days required by CVN and WT mice to complete pretraining stages that introduced mice to touchscreen task requirements. IT, "Initial Touch"; MT, "Must Touch"; MI, "Must Initiate"; PI, "Punish Incorrect". (B–E) Number of days required (B), trials made (C), errors committed (D) and correction trials received (E) before CVN and WT mice achieved the visual discrimination criterion. (F–H) Latencies to respond to correct (F) and incorrect (G) stimuli and to collect the reward (H) by CVN and WT mice. Filled and open circles indicate summed (B–E) or median (F–H) data for individual CVN and WT mice, respectively. Bar charts plot the mean ± standard error of the mean for 17 CVN and 12 WT mice. $^{**}P < 0.01$, two-tailed Student's *t*-test.

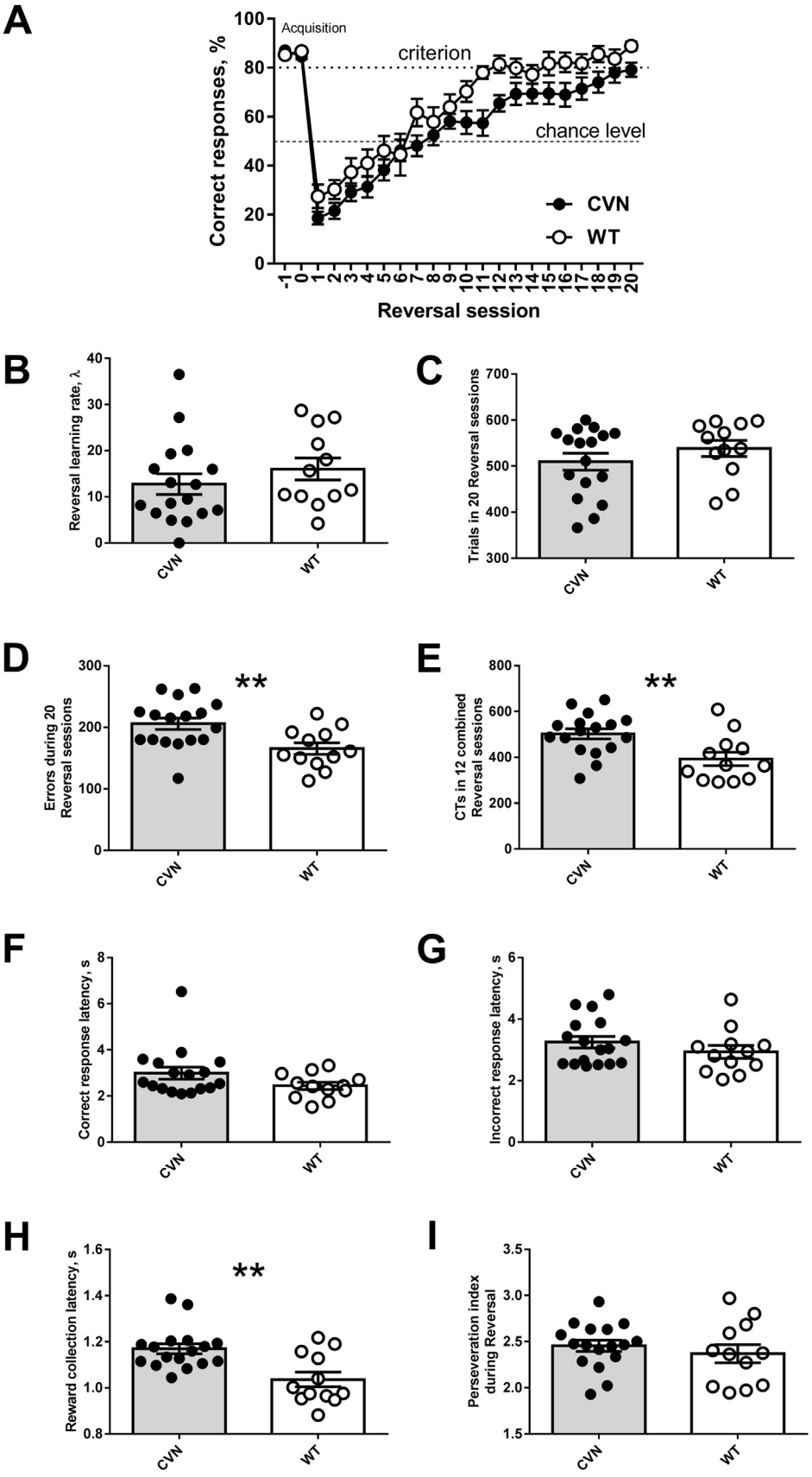

Fig. 2. Reversal of visual discrimination learning in CVN and WT mice. (A) Percentage of correct responses across 20 days of reversal learning sessions. The first two symbols indicate performance during the last two visual discrimination acquisition sessions. Criterion (80%) and chance performance (50%) are indicated by dotted and dashed lines, respectively. (B) Reversal learning rates calculated as described in 2.5 Data analysis and Supplementary Fig. 2. (C–E) Total number of trials made (C), errors committed (D) and correction trials received (E) during 20 days of reversal learning sessions. (F–I) Effect of genotype on latencies to respond to correct (F) and incorrect (G) stimuli, latency

CVN mice, in neither case the effect reached statistical significance ($P > 0.05$). These results contradict the conclusion of repeated-measures ANOVA (Fig. 2A), however it should be noted that in many cases nonlinear fitting did not follow the pattern of the data accurately (Supplementary Fig. 2), which could lead to imprecise calculation of λ values. Furthermore, the summary plot in the lower right corner of Supplementary Fig. 2, drawn using mean learning rates, Amax and Amin values, suggests a difference in the rate of reversal learning between CVN and WT mice. Next, we explored if using learning rate during Visual Discrimination task (Supplementary Fig. 3) as a covariate affected the conclusion about the difference of learning rates during reversal learning. Results of ANCOVA showed that mean reversal learning rates adjusted for the performance during acquisition of visual discrimination were not affected by genotype (CVN: 12.4; WT: 16.5; $F_{1,27} = 2.0$, $P = 0.169$).

Although CVN and WT mice completed a comparable number of trials over 20 days of reversal learning, mutants made significantly more errors and received significantly more CTs in that period (Fig. 2C–E). Significant effect of genotype on these parameters persisted if numbers of errors and CTs during acquisition of visual discrimination were used as covariates (adjusted mean reversal errors: CVN: 202.8; WT: 169.8; $F_{(1,27)} = 6.97$, $P = 0.014$; adjusted mean reversal CTs: CVN: 493.4; WT: 405.4; $F_{(1,27)} = 6.82$, $P = 0.015$). At the same time, RM ANOVA of errors and CTs using genotype and testing stage as between- and within-subject factors showed overall effects of genotype (errors: $F_{(1,27)} = 4.45$, $P = 0.044$; CTs: $F_{(1,27)} = 5.908$, $P = 0.022$) but no significant interaction between genotype and testing stage (errors: $F_{(1,27)} = 0.328$, $P = 0.572$; CTs: $F_{(1,27)} = 1.302$, $P = 0.263$). The latter outcome indicates that AD mutation did not specifically affect reversal learning performance, although error and CT effect size values were larger during the reversal learning (Cohen's $d_{errors} = 1.14$; $d_{CTs} = 1.13$) than during initial acquisition of visual discrimination ($d_{errors} = 0.46$; $d_{CTs} = 0.52$).

Genotype did not affect the average perseveration index (Fig. 2I) and latencies to react to the stimulus during correct (Fig. 2F) and incorrect responses (Fig. 2G). However, as during acquisition of visual discrimination, the speed of entering the magazine to collect the reward during reversal sessions was slightly slower in CVN mice (Fig. 2H). The latter phenotype was not caused by suboptimal food restriction procedure during the Reversal of Visual Discrimination task, as RM ANOVA did not reveal significant effects of genotype or reversal session on relative mouse weight ($P > 0.05$ in both cases).

Separate analyses of examined measures during perseverative and non-perseverative reversal sessions demonstrated that the numbers of errors and CTs as well as reward collection latencies were significantly higher ($P < 0.05$) in CVN mice during both types of reversal sessions, whereas all other parameters were not statistically different between genotypes.

4. Discussion

In this study, we evaluated visual discrimination abilities and reversal learning in the CVN mouse model of AD by using a translational touchscreen-based approach.

The presence of cognitive dysfunction is an important proof of the face validity of AD mouse models. Nonetheless, the vast majority of experiments addressing cognitive behavior in AD mouse models have focused on approaches that are hardly comparable with clinical assessments of individuals with AD. Although tests such as novel object recognition, Morris water maze or fear conditioning are thoroughly validated and relatively quick [15–17], their output may be limited or associated with high levels of anxiety and stress. In contrast, touchscreen-based testing offers a more translational, less stressful, data-rich evaluation of cognition [12]. Moreover, touchscreen tests also permit analysis of executive function, attention, cognitive flexibility and motivation [18–21], i.e., cognitive domains affected in people with AD, but rarely addressed by conventional behavioral tests in rodents. Touchscreen tests have been applied to characterization of several AD mouse models [22–24].

In our experiments, CVN mice acquired visual discrimination with speed and precision comparable to that of age-matched WT animals (Fig. 2B–E). The lack of impairment in the touchscreen Visual Discrimination task was also noted in 4–5-month-old TgCRND8 mice [23]. However, 10-month-old rTg4510 mice, which express P301L-mutated human tau, exhibited lower accuracy during visual discrimination learning [22]. In our study, the only parameter significantly different between genotypes during acquisition of visual discrimination was the speed of entering the food magazine to collect the reward. CVN mice exhibited only slightly slower reward collection than WT counterparts (Fig. 1H), and because latencies to respond to correct and incorrect stimuli were similar in both groups (Fig. 1F, G), it is unlikely that CVN mice had gross motor reaction impairments. We also found that slower reward collection latency was not caused by suboptimal food restriction schedule in CVN mice, because during acquisition of visual discrimination, their relative weight was slightly less than that of WT counterparts, which means that they would be actually somewhat more motivated to work for reward.

Executive function, which comprises planning and exercising behavioral acts as well as attention-guided switching between different behaviors, is impaired in AD patients [25]. Executive function depends on frontal lobe activity [26,27], and structural and metabolic dysfunctions of the frontal lobe frequently accompany AD [28]. Reversal learning, often studied as a measure of executive cognitive flexibility, is disrupted in AD patients [29]. Reversal of left-right discrimination learning in the water T-maze was deficient in 12–14-month-old 3 × Tg-AD mice [30], whereas it was intact in 9-month-old Tg2576 mice [31]. Impaired reversal learning after displacement of the escape platform in a water maze was demonstrated in 4-month-old TgCRND8 mice [32] and in 12- and 18-month old A/T mice overexpressing mutated human APP (APPSweInd) and a constitutively active form of transforming growth factor-β1 [33]. In the appetitively motivated cheeseboard task, deficient reversal learning in 8–9-month old APPSwe/PS1ΔE9 mice was manifested in the lack of preference for the new target zone [34]. Our observations suggest that reversal of visual discrimination in CVN mice may be delayed (Fig. 2A, Supplementary Fig. 2), although this conclusion could be made only following RM-ANOVA, but not on the basis of comparisons of fitted learning rates. However, CVN mice made a higher number of errors during reversal learning (Fig. 2D), which is in agreement with numerous observations of inferior cognitive flexibility revealed in other AD mouse models by using non-touchscreen paradigms. Intriguingly, in the only published report that employed touchscreen-based assessment of the reversal of visual discrimination in a mouse AD model, 4–5-month-old TgCRND8 mice exhibited faster rather than slower reversal learning [23]. There could be various reasons accounting for this discrepancy, e.g., slightly different background or differences in the amount, type and regional specificity of the APP transgene expressed. It is unlikely that background difference played a major role because performance parameters of WT ani-

to collect the reward (H) and on the perseveration index (I) during 20 days of reversal learning sessions. Filled and open circles indicate mean (A), fitted (B), summed (C–E, I) or median (F–H) values for 17 CVN and 12 WT mice, respectively. Bar charts plot the mean ± standard error of the mean for 17 CVN and 12 WT mice. *$P < 0.05$; **$P < 0.01$, two-tailed Student's *t*-test (for D and E) or Mann-Whitney *U* test (H).

mals in our experiments and in the experiments by Romberg et al. were very similar during both Visual Discrimination and Reversal tasks (cf. Figs. 1 B and 2 A of this study with Fig. 2A, B of [23]). Transgenic human APP proteins expressed by CVN and TgCRND8 mice have different sets of mutated residues (CVN – Swedish, Dutch, Iowa; TgCRND8 – Swedish, Indiana). Furthermore, although the levels of the expressed transgene are lower in CVN mice, a concomitant mutation in *Nos2*, which likely precipitates the phenotype by facilitating intracellular aggregation of tau, may be an important factor in causing reversal learning impairment.

Impaired reversal learning in CVN mice could be caused by an overly strong association between the reward and initial stimulus and/or by a weaker ability to inhibit previously rewarded responses. To clarify the roles of these two mechanisms, it would be useful to analyze retention of acquired visual discrimination and extinction of the operant response in CVN mice, as was done in the study of TgCRND8 mutants [23]. In our experiments, RM ANOVA of errors and CTs using genotype and testing stage as between- and within-subject factors reported overall effect of genotype, but no significant genotype × testing stage interaction, which would be expected if reversal learning was specifically affected. Therefore, it is plausible that CVN mice may have a generalized discrimination learning deficit, which perhaps could not be reliably discerned by us due to short duration of the Visual Discrimination task. It will be therefore useful to explore if more taxing paradigms, e.g., using morphed images, will uncover initial visual discrimination learning deficit in CVN animals. Furthermore, it could be of interest to assess the performance of older mice, as 5-month old animals used in this study do not exhibit the maximal extent of AD-related cellular changes [9].

In addition, paradigms like progressive ratio, which have been recently introduced for touchscreen chamber setting [19], could help to determine whether increased latency to collect food reward reflects a motivational deficit in CVN mice. We will employ these and other available touchscreen tests in future studies of CVN mice to explore integrity of a broader set of behavioral modalities frequently dysregulated in AD, such as hippocampus-dependent associative learning, attention and motivation [12,19–21].

5. Conclusions

By using touchscreen technique, we demonstrated that CVN mice, which express mutated human APP and have a homozygous deletion of the *Nos2* gene, acquired visual discrimination at a rate comparable to that in age-matched WT mice. We also 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 elucidation of other cognitive and motivational phenotypes in this AD mouse model.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2017.04.049.

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