Reduced Cognitive Flexibility and Altered Metabolic Profile in the Prefrontal Cortex of *APPSwDI - Nos2*$^{-/-}$ (CVN) Mice

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1 BACKGROUND

The CVN mice are bigenic mutants that combine expression of the human APP isoform 770 containing the 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 *NOS2* gene encoding nitric oxide synthase 2. Previously published (Colton *et al.*, 2014) and additional internal validation studies at Charles River Discovery Finland uncovered a range of biochemical, physiological and behavioral disturbances in CVN mice. In particular, CVN mice exhibit gradual, age-dependent accumulation of insoluble Aβ isoforms (Fig. 1), impaired performance in the radial arm Morris water maze (Fig. 2) and Barnes maze, as well as deficient contextual fear memory.

To investigate performance of CVN mice in more translatable mouse behavioral tests, we have initiated a program of cognitive evaluation of this AD mouse model in the touchscreen operant chambers. Touchscreen approach to studying cognition in humans, exemplified by the Cambridge Neuropsychological Test Automated Battery (CANTAB), has been gaining increasing popularity in clinical setting (www.cambridgecognition.com). 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 the ones employed in the human battery. Therefore, analogous cognitive tests can be administered in both species (Nithianantharajah *et al.*, 2015).

Here, we present data on the performance of CVN mice in the Visual Discrimination and Reversal touchscreen tasks and corresponding pathological features of the CVN mice by other behavioral (cognitive) assays, amyloid pathology and metabolic profiling.

2 METHODS

Experiments were performed in 5–6-month-old CVN and age-matched control wild-type (WT) mice. Throughout the test, animals were kept at 85–90% of their free feeding weight to increase their motivation to obtain food reward (strawberry-flavored milkshake). Prior to Visual Discrimination testing, the mice were trained on basic touchscreen task requirements in Bussey-Saksida touchscreen operant chambers. This pre-training phase comprised 5 stages and included habituation to the chamber, learning association between stimulus presentation and reward availability and "punishing" procedure for touching the empty (plain black) location on the screen instead of a stimulus (Horner et al., 2013). During acquisition of Visual Discrimination, a pair of spatially pseudorandom stimuli appeared on the screen, one in each of the two response windows (Fig. 3). One stimulus was correct (S+) and another one was incorrect (S-). Responses to the S+ were rewarded (sound, drop of milkshake, magazine light on, no "time out"); response to the S- was "punished" (house light off for 5 s "time out", absence of milkshake delivery, repeated trial with the same stimuli in the same location until the mouse makes the correct response). The mice were considered to have acquired the discrimination when they reached a performance criterion of at least 80% of trials correct (not including correction trials) 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, so that the original S+ became S- and vice versa.

MR experiments were performed using a horizontal 11.7 T magnet interfaced to a Bruker Avance III console (Bruker Biospin GmbH, Ettlingen, Germany). A volume coil (Bruker Biospin GmbH, Ettlingen, Germany) was used for transmission, and surface two-element array coil was used for receiving (Rapid Biomedical GmbH, Rimpar, Germany). Isoflurane-anesthetized mice were fixed to a head holder and positioned in the magnet bore in a standard orientation relative to gradient coils.

Structural MRI was performed with a standard Turbo-RARE for thirty-one 0.45-mm slices and 78 microns in-plane resolution. Proton (^{1}H) MR spectroscopy data were collected from the voxel area placed in the prefrontal cortex (2.2×1.6×1.8 mm^3, 6.3 µL localized volume). A PRESS sequence (TE = 10 ms) combined with outer volume suppression (OVS) was used for the pre-localization. Data were collected by averaging 512 excitations (frequency corrected for each FID) with 2048 point, TR of 2 s and spectral width of 5 kHz. Excitation frequency was shifted by -2 ppm to minimize the chemical shift phenomenon within the selected voxel area. In addition, a reference spectrum without water suppression (NT = 8) was collected from the identical voxel area using the same acquisition parameters. Peak areas for resolved metabolites were analyzed using LCModel (Stephen Provencher Inc., Oakville, Canada) with an exclusion criterion of CRLB >20% for individual metabolites within the analyzed spectrum.

3 BIOCHEMICAL AND BEHAVIORAL CHANGES IN CVN MICE

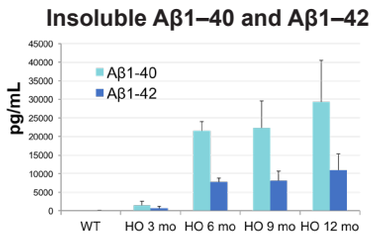

Figure 1. High levels of insoluble Aβ subtypes between 3 and 6 months of age, when mice are, respectively, presymptomatic and showing first signs of memory impairment.

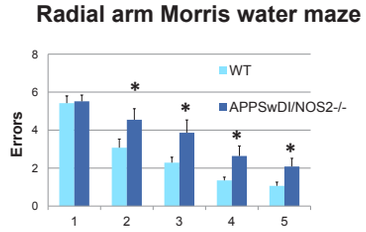

Figure 2. CVN mice are impaired in the radial arm Morris water maze at 12 months of age as indicated by the higher number of errors they make in comparison to WT animals. *P < 0.05 CVN vs. WT

4 RESULTS

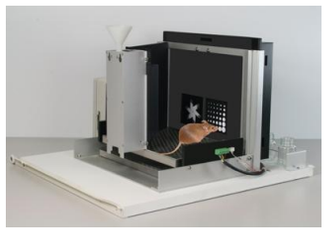

Figure 3. Illustration of a typical Visual Discrimination trial (view from inside the touchscreen chamber)

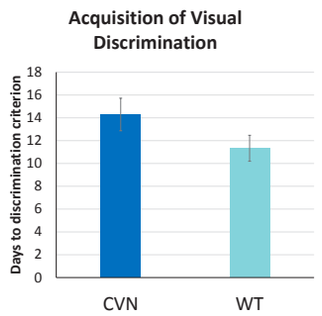

Figure 4. CVN an WT mice required similar number of sessions to achieve the Visual Discrimination criterion.

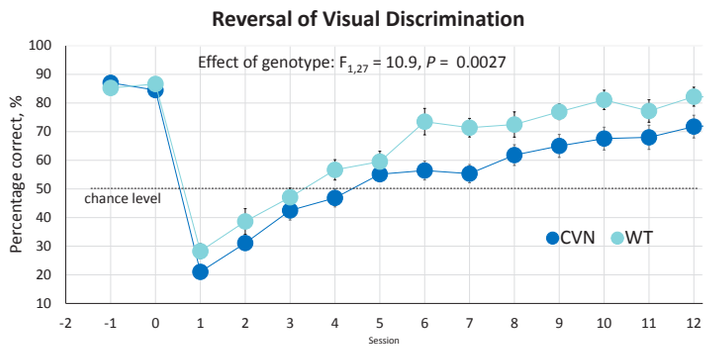

Figure 5. Reversal learning performance was significantly affected by genotype and session.

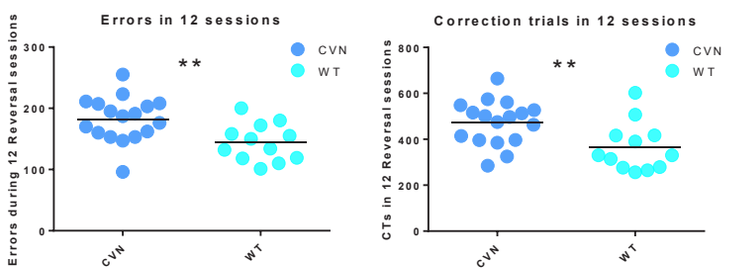

Figure 6. CVN mice committed more errors and made more correction trials than WT mice during the Reversal of Visual Discrimination.

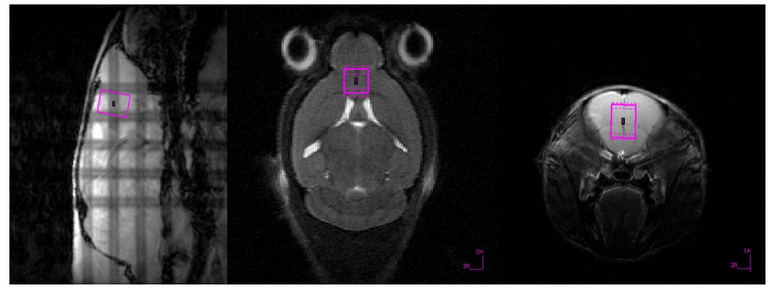

Figure 7. Selection of the voxel of interest for imaging metabolite concentrations in the part of the mouse brain encompassing the prefrontal cortex

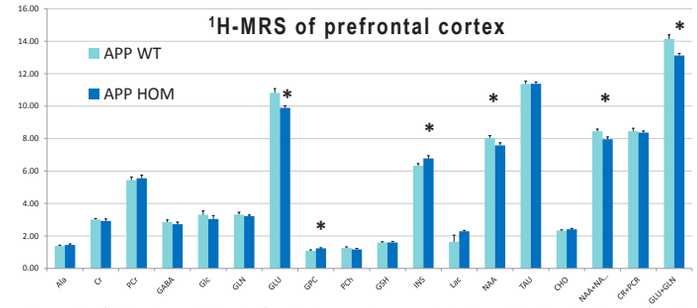

Figure 8. CVN mice exhibit significant changes in several brain metabolites in the prefrontal cortex as determined by ^{1}H-MRS. *P < 0.05 WT vs. CVN.

5 DISCUSSION

There was no significant effect of genotype on the number of sessions required to complete pre-training and to acquire Visual Discrimination (Fig. 4). CVN and WT animals made errors at a similar frequency during Visual Discrimination testing and received comparable numbers of correction trials. No statistically significant differences were revealed in the latencies to react to correct and incorrect stimuli and latency to attend the food magazine for reward (P < 0.05 in all comparisons, Mann-Whitney test). These observations suggest that at the age of 5-6 months CVN mice do not display pronounced deficits in initial instrumental learning, speed of reaction and motivation for reward.

However, after the contingency of reward dispensation was switched from the previously correct to previously incorrect stimulus response, CVN mice displayed impaired reversal learning (Fig. 5). Analysis of response accuracy across 12 sessions revealed a significant effect of genotype ($F_{1,27}$ = 10.9, P = 0.0027) and session ($F_{11,297}$ = 62.83, P < 0.0001), but no significant interaction between these two factors ($F_{11,297}$ = 0.92, P = 0.519). Furthermore, CVN mice made significantly more errors (Fig. 6A; WT: 144.1 ± 8.8; CVN: 182.2 ± 8.8; t_{27} = 2.965, P =0.0063) and received a significantly larger number of correction trials (Fig. 6B; WT: 365.2 ± 30.7; CVN: 473.0 ± 23.3; t_{27} = 2.847, P =0.0083) during the 12 Reversal sessions. There was no significant difference in the latency to react to the stimulus during correct and incorrect responses, however, the speed of attending magazine to collect the reward during the 12 Reversal sessions was faster in WT mice (WT: 1.42 ± 0.16; CVN: 1.61 ± 0.08; U = 49.0, P = 0.02).

As reversal learning depends on the integrity of prefrontal cortex, we analyzed the metabolic profile in this brain region by ^{1}H magnetic resonance spectroscopy using a 11.7 T small animal MRI system (Fig. 7). We observed lower levels of the neuronal marker *N*-acetyl aspartate and excitatory neurotransmitter glutamate, and higher concentration of myo-inositol in the prefrontal cortex and surrounding areas in CVN mice (Fig. 8). We have previously detected similar changes in the hippocampus of 12-month CVN animals. Furthermore, changes in these metabolites are qualitatively similar to alterations seen in the brain of AD patients.