Impaired Synaptic Transmission in the CA1 Area of Hippocampal Slices 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 Previously reported(Colton *et al.*, 2014) and unpublished internal validation studies at Charles River Discovery Finland uncovered a range of biochemical, physiological and behavioral disturbances in CVN mice.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, inferior performance in the Barnes maze and Morris water maze, decreased contextual fear memory and impaired Reversal learning in the touchscreen Visual Discrimination task (*see poster 601.08 Tue Nov 15 1:00 PM*).

Cognitive dysfunction in Alzheimer's disease (AD) is likely to be at least partly mediated by the disruption of synaptic transmission in the brain areas affected by amyloid plaques and/or neurofibrillary tangles. The hippocampus is profoundly affected by AD-like pathologies in human patients as well as in many mouse models of AD, including CVN mice. Therefore, mouse hippocampal slices have become a popular object of experimental studies aimed at elucidation of electrophysiological consequences of AD-relevant mutations and their sensitivity to various therapeutic treatments. However, electrophysiological evidence of potential repercussions of excessive amyloid deposition and expression of hyperphosphorylated tau for synaptic transmission in the hippocampus of CVN has been lacking so far. To this end, we prepared hippocampal slices from 17–18-month-old CVN and aged matched control C57Bl/6J mice and, by using stimulation of Schäffer collaterals via multi-electrode arrays (MEAs), recorded field excitatory postsynaptic potentials (fEPSPs) and explored their short-term and long-term plasticity in the hippocampal CA1 area.

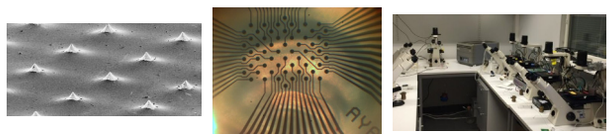

Figure 1. Multi-electrode array-based technique utilized at Charles River Discovery Finland to record field potentials in acute brain slices

2 METHODS

CVN mice and their wild-type (WT) littermates (17–18 months of age, either sex, C57Bl/6J background) were used for the experiments. Whole brain slices were cut at 350-μm thickness by a Vibroslice MA752 (Campden Instruments, Loughborough, UK) in such a way, so that the blade cut through hemispheres at an angle of 20-30^{o} to their horizontal planes. Up to eight slices containing medial segments of the hippocampus with overlaying cortical areas were trimmed of the remaining tissue, placed into a well of a slice chamber (Fine Science Tools, Foster City, CA, USA) and kept interfaced between moist air and subfused fresh artificial cerebrospinal fluid (ACSF) that contained 124 mM NaCl, 25 mM $NaHCO_3$, 1 mM NaH_2PO_4, 4.4 mM KCl, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, 10 mM glucose, and 0.0015 mM phenol red. Temperature in the chamber was slowly increased to 30 °C for the rest of the incubation time. Slices rested in these conditions for at least 2–3 h before experiments commenced.

Field excitatory postsynaptic potentials were recorded by the Multi Channel Systems electrophysiological suite (Multi Channel Systems, Reutlingen, Germany) as described previously (Kopanitsa et al., 2006; Fig. 1). To evoke orthodromic fEPSPs, stimulation electrodes were activated at a frequency of 0.02 Hz. Peak amplitude of the negative part of fEPSPs was used as a measure of the synaptic response. Following at least 10-15 min of equilibration inside an MEA well, I/O relationships were obtained and baseline stimulation strength was set to evoke a response that corresponded to ~40% of the maximal attainable fEPSP at the principal recording electrode. Paired-pulse facilitation (PPF) was observed after stimulating Schäffer collateral/commissural fibres with a pair of pulses at baseline stimulation strength and an interpulse interval of 50 ms. PPF value was calculated as $fEPSP_2/fEPSP_1 \times 100\%$. Average data from five paired-pulse stimulations were used for each slice. LTP was induced after a 60-min period of stable baseline responses by a theta-burst stimulation (TBS) train consisting of 10 bursts given at 5 Hz with 4 pulses given at 100 Hz per burst. LTP plots were scaled to the average of the first five baseline points. To account for a possible drift of baseline conditions, peak values in the test pathway were normalised by peak amplitudes in the control pathway prior to statistical comparison. LTP magnitude was assessed by averaging normalised fEPSPs in the test pathway 60–65 min after LTP induction.

Since several slices were routinely recorded from every mouse, values of the fEPSP peak amplitudes, paired-pulse facilitation (PPF) and long-term potentiation (LTP) were compared using two-way nested ANOVA design with genotype (group) and mice (sub-group) as fixed and random factors correspondingly, applying the Satterthwaite's correction.

Statistical effects were considered significant if $P < 0.05$. Data are presented as the mean ± standard error of the mean with *n* and *N* indicating number of slices and mice, respectively.

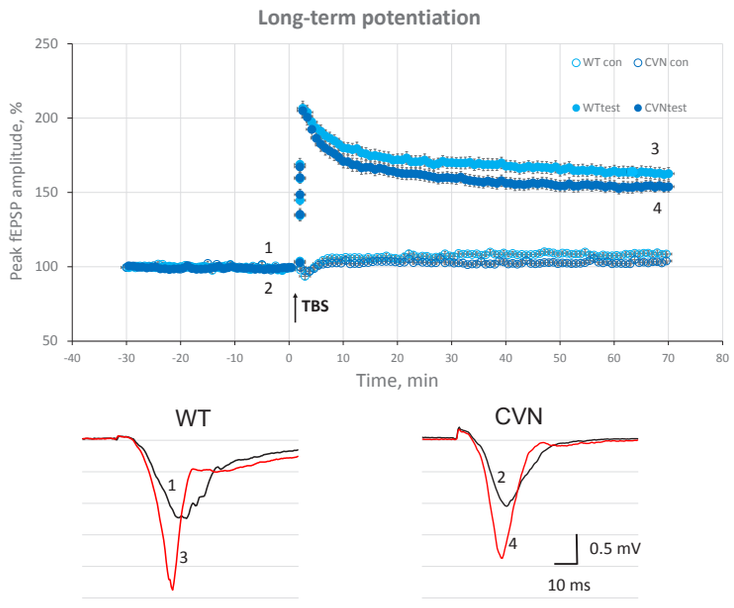

Figure 2. Theta-burst-induced LTP of excitatory synaptic transmission in the synapses between Schäffer collaterals and apical dendrites of CA1 pyramidal neurons. *con* – control pathway; *test* – test pathway. WT: n_{slices} = 34, N_{mice} = 11; CVN: n_{slices} = 35, N_{mice} = 10.

3 RESULTS

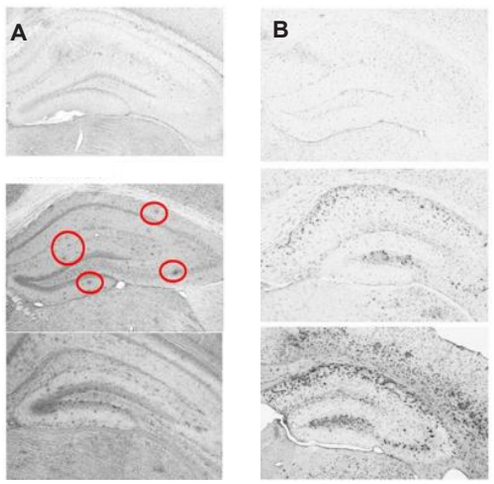

Figure 3. Abundant amyloid plaques (A) and upregulated inflammatory markers (B) can be revealed in the hippocampus of 9-month old CVN mice by staining with antibodies against $Aβ_{1-40}$ and AIF1/IBA-1. More robust staining is evident in the hippocampus of 12-month-old mice.

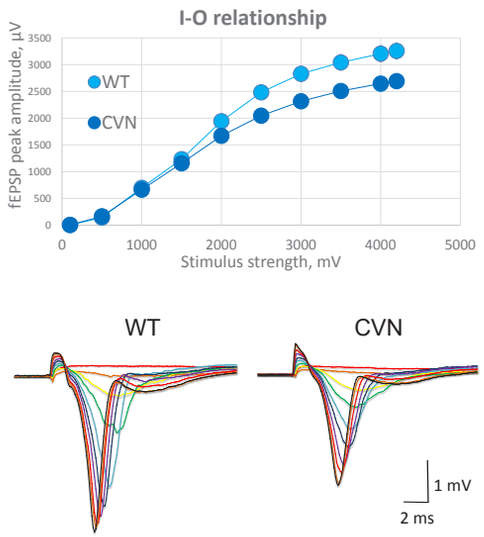

Figure 4. Basal evoked excitatory synaptic transmission in the synapses between Schäffer collaterals and apical dendrites of CA1 pyramidal neurons. WT: n_{slices} = 34, N_{mice} = 11; CVN: n_{slices} = 35, N_{mice} = 10.

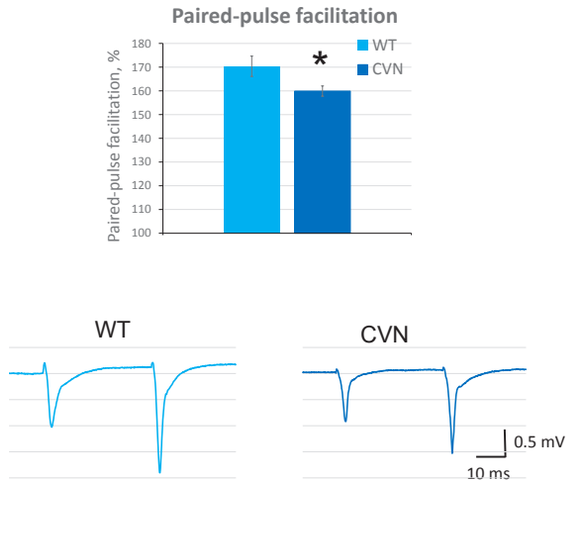

Figure 5. Paired-pulse facilitation of evoked excitatory synaptic transmission in the synapses between Schäffer collaterals and apical dendrites of CA 1 pyramidal neurons. WT: n_{slices} = 34, N_{mice} = 11; CVN: n_{slices} = 35, N_{mice} = 10.

4 CONCLUSIONS

Basal synaptic transmission was moderately reduced in hippocampal slices from CVN mice (Fig. 3): peak amplitudes of fEPSPs evoked during input-output relation recordings were smaller in slices from mutant animals, particularly at higher values of the stimulus strength. The peak amplitude of maximum fEPSPs was 3.3 ± 0.8 mV in slices from control animals, whereas in CVN mice responses were slightly but significantly lower (2.7 ± 0.7 mV; $P = 0.015$; 2-way nested ANOVA, main genotype effect).

PPF of synaptic responses observed at a 50-ms interpulse interval was lower in hippocampal slices from CVN mice (159.9 ± 2.2%) than that in control mice (170.4 ± 4.3%, $P = 0.044$; Fig. 4).

Short theta-burst stimulation elicited LTP of fEPSP amplitudes in slices from CVN and control animals. Sixty-five min after induction, LTP levels were similar in both genotypes (CVN: 151.0 ± 4.2%; WT: 152.5 ± 3.3%; $P > 0.05$; Fig. 5).

We conclude that a moderate impairment of basal synaptic transmission parameters in hippocampal slices from CVN mice is probably a reflection of a neuronal loss in the hippocampus. Disturbances in PPF may be also partly linked to selective degradation of some populations of hippocampal interneurons previously observed in this model (Wilcock et al., 2008).

5 REFERENCES

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