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Electrophysiological characterization of networks and single cells in the hippocampal region of a transgenic rat model of Alzheimer's disease

Running Title: Electrophysiology of the rat AD hippocampus

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Abstract

The hippocampus and entorhinal cortex (EC) are areas affected early and severely in Alzheimer's disease (AD), and this is associated with deficits in episodic memory. Amyloid beta (Aβ), the main protein found in amyloid plaques, can affect neuronal physiology and excitability, and several AD mouse models with memory impairments display aberrant network activity, including hyperexcitability and seizures. In this study, we investigated single cell physiology in EC and network activity in EC and dentate gyrus in the McGill-R-Thy1-APP transgenic rat model, using whole-cell patch clamp recordings and voltage-sensitive dye imaging in acute slices. In slices from transgenic animals up to 4 months of age, the majority of the principal neurons in layer II of EC, fan cells and stellate cells, expressed intracellular Aβ. Whereas the electrophysiological properties of fan cells were unaltered, stellate cells were more excitable in transgenic than in control rats. Stimulation in the dentate gyrus resulted in comparable patterns in both groups at 3 and 9 months, but at 12 months, the elicited responses in the transgenic group showed a significant preference for the enclosed blade, without any change in overall excitability. Only transient changes in the local network activity were seen in the medial entorhinal cortex. Although the observed changes in the McGill rat model are subtle, they are specific, pointing to a differential and selective involvement of specific parts of the hippocampal circuitry in Aβ pathology.

Significance statement

The hippocampal region, essential for episodic memory, is affected in the early stages of Alzheimer's disease. Here, we use the McGill-R-Thy1-APP transgenic rat model to study the effects of Aβ pathology on networks and single cells in the hippocampal region. In young animals, we observed widespread intracellular Aβ accumulation, which later progressed to extracellular plaques. However, the in vitro physiology was largely unaltered, with only changes in single cell excitability of stellate cells in layer II of MEC and network activation patterns in dentate gyrus. Thus, these two components of the entorhinal-hippocampal network emerge as potentially more vulnerable in the context of Aβ pathology.

Introduction

Alzheimer's disease (AD), the most common cause of dementia, is a progressive neurodegenerative disorder. The neuropathological hallmarks include extracellular amyloid plaques and intracellular neurofibrillary tangles consisting of hyperphosphorylated tau, as well as cortical atrophy and cell loss. Areas affected by plaques and tangles in early stages of AD include the entorhinal cortex (EC) and the hippocampus (Braak & Braak, 1991; Thal et al., 2002). Neuron loss has been reported in subregions of the hippocampus (West et al., 1994; Simic et al., 1997; Price et al., 2001), and in particular layer II of EC exhibits a substantial cell loss in patients in the early stages of AD as well as with mild cognitive impairment (Gomez-Isla et al., 1996; Kordower et al., 2001). The two main groups of principal neurons in layer II, stellate cells in medial EC (MEC) and fan cells in lateral EC (LEC) (Canto & Witter, 2012a; b), provide input to the hippocampus via the perforant path (Cappaert et al., 2015). In transgenic mice, it has been shown that both tau and amyloid-β (Aβ) pathology can spread

104 through transsynaptic transmission, starting in EC (Harris et al., 2010; de Calignon et al.,
105 2012), further implicating the entorhinal-hippocampal region in early stages of AD.
106
107 The original “amyloid cascade hypothesis” was formulated 25 years ago (Hardy & Higgins,
108 1992). Although the exact role of Aβ in the initiation and progression of AD is still highly
109 debated, it is clear that Aβ is an important contributor to the pathological processes (Herrup,
110 2015; Musiek & Holtzman, 2015). The research focus has shifted to include effects of soluble
111 forms of Aβ (Haass & Selkoe, 2007) and Aβ peptide levels have been shown to have a higher
112 correlation with cognitive decline than amyloid plaque load does (McLean et al., 1999;
113 Naslund et al., 2000). Studies have shown toxic effects of Aβ oligomers on synaptic function
114 and structure (Selkoe, 2008; Marchetti & Marie, 2011), which could lead to disruption of the
115 normal neuronal function and subsequent aberrant network activity (Palop & Mucke,
116 2010a). Recent studies report changes in single neuron excitability in mouse models,
117 including pyramidal cells of CA1 (Brown et al., 2011; Kerrigan et al., 2014), and frontal cortex
118 (Kellner et al., 2014), as well as EC (Marcantoni et al., 2013; Xu et al., 2015) and dentate
119 gyrus (DG) (Hazra et al., 2013). Additionally, intracellular delivery of Aβ has been shown to
120 increase neuronal excitability (Scala et al., 2015), and intracellular Aβ (iAβ) is found in EC and
121 hippocampus of AD patients (Gouras et al., 2000; D'Andrea et al., 2002). As intracellular
122 accumulation and cognitive deficits have been observed in animal models prior to formation
123 of plaques (Billings et al., 2005; Leon et al., 2010), it is hypothesized that iAβ may play an
124 important role in neuronal dysfunction in AD (Bayer & Wirths, 2010).
125
126 In this study we use the McGill-R-Thy1-APP transgenic rat model which harbors human
127 amyloid β precursor protein (AβPP) with the Indiana and Swedish double mutations (Leon et

al., 2010), and is one of the few rat models with a progressive plaque pathology. The first plaques appear in the subiculum at 9 months of age and then spread to other parts of the hippocampus as well as EC (Heggland et al., 2015). A subtle cell loss (~20%) has also been reported in the subiculum at 18 months (Heggland et al., 2015). By one week after birth, iAβ is observed (Leon et al., 2010), and layer II of EC is one of the areas with initial high expression (Kobro-Flatmoen et al., 2016). From 3 months, the rats display cognitive impairments (Iulita et al., 2014) and metabolic alterations (Nilsen et al., 2014), and pre-plaque inflammation and changes in long-term potentiation have been described at later ages (Hanzel et al., 2014; Qi et al., 2014). In the present study, we investigated changes in excitability and activity patterns of the networks of the hippocampus and EC in acute slices. We used young pre-plaque animals, when only iAβ accumulation is present, as well as older animals, when plaques have started to appear. With the use of whole-cell patch clamp recording in acute slices from young animals, we investigated possible changes in the excitability of stellate and fan cells in layer II of EC. We also assessed whether changes in electrophysiological properties at the network level were related to the developing pathology over time, with the use of voltage sensitive dye imaging (VSDI).

Materials and methods

Animals

All the experimental procedures were approved by the Local Animal Research Authority and followed the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. The animals were kept on a 12 hour light/dark cycle under standard laboratory conditions (19-22°C, 50-60% humidity) and had free access to food and water. A colony of transgenic McGill-R-Thy1-APP rats, based on two breeding pairs obtained from McGill University (Leon et al., 2010), was maintained at our university. The McGill-R-Thy1-APP rats carries the human $A\beta PP_{751}$ including the Swedish double mutation and the Indiana mutation under the control of the *Thy*1.2 promoter. Quantitative PCR (qPCR) was used to decide the genotype of the transgenic rats (negative, homozygous or hemizygous for the transgene). Genomic DNA was isolated from samples of ear tissue with a High Pure PCR Template Preparation Kit (11796828001, Roche Diagnostics, Basel, Switzerland). The transgene (human AβPP) and a normalization gene (GAPDH or beta-actin) were detected using RT^2 qPCR Primer Assays from Qiagen (PPH05947A, PPR06557 and PPR06570C, Venlo, Netherlands) with FastStart Universal SYBR Green Master (04913850001, Roche Diagnostics) on an Applied Biosystems StepOnePlus real-time PCR system (Life Technologies Ltd, Thermo Fisher Scientific, Waltham, MA, USA). The $\Delta\Delta C_T$ values were calculated from the qPCR with a known homozygous sample as reference (Livak & Schmittgen, 2001).

Slice preparation

For the VSDI, homozygous (+/+) transgenic rats and wild type control animals (wt; WistarHan, Taconic, Hudson, NY, USA) of both sexes at the ages of 3, 9 and 12 months were

used. For whole-cell patch clamp recordings, homozygous (+/+) transgenic rats and negative (-/-) littermates of both sexes at the ages of 1 and 3-4 months were included. The animals were anesthetized with isoflurane (IsoFlo vet., Abbott Laboratories, Chicago, IL, USA), decapitated and the brain quickly removed from the skull and placed in ice-cold (0-4°C), oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) solution containing (in mM): 100 D-Mannitol, 119 choline chloride, 2.5 KCl, 7 $MgCl_2$, 0.5 $CaCl_2$, 25 Glucose, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 11.5 sodium ascorbate and 3 sodium pyruvate. For rats of 3 months and older, a transcardial perfusion with ice-cold ACSF was done before decapitation, to remove blood and cool down the brain as quickly as possible.

For VSDI, the brain was cut in 400 µm thick horizontal entorhinal-hippocampal slices on a vibratome (Vibratome 300 sectioning system, Vibratome, IL, USA). Slices ranged from approximate interaural levels 2.4 to 4.68 mm (Paxinos & Watson, 2007), containing mid to ventral levels of the hippocampus. The slices were placed on a membrane filter (JHWP01300, Omnipore membrane filter, PTFE, Merck Millipore, Darmstadt, Germany) glued to a thin Plexiglas ring (11 mm inner diameter, 15 mm outer diameter) and held in a oxygenated moist interface chamber at 32°C for at least one hour before transfer to the recording chamber. For holding and recording, the following ACSF was used (in mM): 126 NaCl, 3 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 10 glucose, 1.2 NaH_2PO_4, 26 $NaHCO_3$.

For whole-cell patch clamp recordings, entorhinal slices of 400 µm were cut on a vibratome (Leica VT1000S, Leica Biosystems, Nussloch, Germany), either in the horizontal or semicoronal plane (20° angle with the vertical plane). Horizontal slices from middle dorsoventral levels, approximately interaural 2.9 to 4.4 mm (Paxinos & Watson, 2007) were

193 used for recording MEC II cells, with the majority of the recorded cells found in the center of
194 the mediolateral axis within each slice. Semicoronal slices were used for recording LEC II cells
195 close to the rhinal fissure, at approximate rostrocaudal levels of 4.3 to 6 mm posterior to
196 bregma (Paxinos & Watson, 2007). The slices were held in a submersion chamber with ACSF
197 containing (in mM): 126 NaCl, 3 KCl, 3 $MgCl_2$, 0.5 $CaCl_2$, 10 glucose, 1.2 NaH_2PO_4, 26 $NaHCO_3$
198 at 37°C for one hour, and then at room temperature until recording.
199
200 ***Voltage-sensitive dye imaging***
201 The slice was perfused with oxygenated ACSF at 34°C in a recording chamber mounted on a
202 fluorescent microscope (Axio Examiner.D1, Carl Zeiss, Oberkochen, Germany), and stained
203 with the voltage-sensitive dye RH 795 (0.5mg/ml ACSF; R-649, Molecular Probes, Invitrogen,
204 Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) for 3 minutes and the excess
205 dye was washed out by perfusion of ASCF for 15 minutes before recording. The slice was
206 illuminated from a halogen lamp (MHAB-150W, Moritex, Saimata, Japan) through a
207 bandpass excitation filter (535 ± 25 nm) and a dichroic mirror (half reflectance wavelength of
208 580 nm) and the dye emission was passed through a longpass filter (50% transmittance at
209 590) and detected with a CMOS-camera (100 x 100 pixel array; MiCAM Ultima, Brainvision,
210 Tokyo, Japan). For the 3 month age group a non-immersion Zeiss Fluar objective was used
211 (NA = 0.25). For the 9 and 12 month groups a water-immersion objective from Brainvision
212 (NA = 0.35) was used, as we obtained this after the 3 month group was recorded. A shutter
213 (HL-151, Brainvision) controlled by the Brainvision acquisition software built into the light
214 source was opened 500ms before the start of the recording to reduce mechanical noise. The
215 images were acquired at 1.0 ms/frame for 512 frames and the first 50 frames were used to
216 measure the optical baseline. An extracellular stimulation was applied after 50 ms with a

tungsten bipolar electrode (tip separation of 150 µm) using either a single pulse with an amplitude of 0.2 or 0.6 mA of 300 µs duration, or 4 pulses at a frequency of 40 Hz with an amplitude of 0.2 mA. Eight recordings separated by 3 seconds were averaged to reduce noise. The stimulation electrode was placed in different areas of the hippocampal region: the border of the molecular and granule layer of the DG and layers II/III of entorhinal. In the 9 and 12 month age group the majority of the slices (38 of 44 slices) were also recorded with the $GABA_A$ antagonist bicuculline added to the ACSF (5 µm; Bicuculline methiodide; 14343, Sigma-Aldrich, St Louis, MO, USA), to block the inhibition in the slice.

Whole-cell patch clamp

All single cell recordings were performed at 34°C with perfusion of oxygenated ACSF containing (in mM): 126 NaCl, 3 KCl, 1.5 $MgCl_2$, 1.6 $CaCl_2$, 10 glucose, 1.2 NaH_2PO_4, 26 $NaHCO_3$. Principal cells in layer II of MEC and LEC were identified using infrared differential interference contrast (IR-DIC) on an Axio Examiner.D1 microscope (Carl Zeiss, Oberkochen, Germany) with a Zeiss Plan-Apochromat water dipping objective (20x; NA = 1.0), or an Olympus BX51WI microscope (Olympus, Tokyo, Japan) with an Olympus LC Plan FL objective (40x; NA = 0.8). Recording pipettes pulled from standard-walled borosilicate capillaries (3-8 MΩ pipette resistance; GC120F-10, Harvard Apparatus, Harvard Bioscience, Holliston, MA, USA) were filled with intracellular solution containing (in mM): 120 K-gluconate, 10 KCl, 10 HEPES, 4 MgATP, 10 Na_2-phosphocreatine, 0.3 GTP. Biocytin (3-4%; B4261, Sigma-Aldrich, St Louis, MO, USA) was added to the recording solution for later anatomical analysis of cell location and morphology. For a few of the cells (n = 34 cells) an Alexa Fluor hydrazide dye (405, 488, 468 or 633; Molecular Probes, Invitrogen, Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) was added to the intracellular solution instead of biocytin.

Whole-cell recordings in current clamp mode were performed on two different setups. Recordings on the first setup was done with MultiClamp 700A and 700B amplifiers (Axon Instruments, Molecular Devices, Sunnyvale, CA, USA) in bridge mode and digitized with an InstruTECH ITC-1600 A/D interface (HEKA Elektronik, Lambrecht, Germany) in combination with the acquisition software Chartmaster (HEKA). The second setup was equipped with a Multiclamp 700B amplifier and data was acquired with an InstruTECH ITC-18 board (HEKA) and the acquisition software Patchmaster (HEKA; RRID: SCR_000034). Recordings were made at sampling rates of 10, 25 or 50 kHz, depending on the length of the recording. Capacitance compensation was maximal and series resistance was compensated, and the seal resistance was above 1 GΩ. We did not correct for the liquid junction potential, which was calculated to be 15.8 mV.

To study general electrophysiological properties and firing frequencies of neurons, voltage responses to a series of 1 s long current steps of 50 or 30 pA starting from – 300pA were recorded. A protocol of 10 pA steps starting from 0 pA was used to measure the rheobase. In addition, we injected a sinusoidal current with a linearly increasing frequency (from 0 to 20 Hz) with a duration of 15 seconds, a so-called ZAP-protocol (Erchova et al., 2004), while recording the membrane voltage, and estimated the resonance frequency (frequency with largest amplitude response). In 11 cells, the resonance frequency could not be estimated, due to traces with noise or action potentials.

Histology

After recordings, the slices were fixed for minimum 24 hours in 4% freshly depolymerized paraformaldehyde (w/v in 125 mM phosphate buffer (PB), pH 7.4) and then transferred to 20 % glycerol and 2% dimethyl sulfoxide (DMSO) in 125 mM PB.

The slices from VSDI were subsequently cut at 50 µm on a freezing microtome (Microm HM430, Thermo Fischer Scientific, Waltham, MA, USA). Half of the sections were mounted directly on Histobond^{+} slides and stained with Cresyl violet to verify the regions of activity seen with the VSDI. After drying overnight on a heating plate (37 °C) the sections were dehydrated in ethanol, cleared in xylene and rehydrated before staining with Cresyl violet (1 g/L) for 10-15 minutes. The sections were then alternately dipped in ethanol-acetic acid (5 mL acetic acid in 1 L 70% ethanol) and rinsed with cold water until the desired differentiation was obtained, then dehydrated, cleared in xylene and coverslipped with Entellan (Merck KGaA, Darmstad, Germany).

The other half of the sections were stained with free-floating immunohistochemistry using the monoclonal anti-human Aβ antibody McSA1 (MM-0015-P; MédiMabs, Montreal, QC, Canada; RRID: AB_1807985;), which is specific for human Aβ and stains both plaques and intracellular deposits (Grant et al., 2000; Leon et al., 2010). First, heat-induced epitope retrieval was done at 60 °C for 2 h in PB. After washing with PB (2x10 minutes) the tissue was permeabilized with 0.5% Triton-X-100 in Tris-buffered saline (TBS-Tx; 50 mM Tris, 150 mM NaCl, pH 8.0) for 10 minutes and blocked with 10% goat serum in TBS-Tx for 30 minutes, before overnight incubation at 4 °C with the primary antibody, McSA1 (1:4000). The following day, the sections were washed with TBS-Tx (3x10 minutes) and incubated with a

289 biotinylated goat anti-mouse secondary antibody (1:200, Sigma-Aldrich) for 90 minutes.
290 After washing (TBS-TX; 3x10 minutes), incubation in ABC (PK-4000, Vectastain ABC kit,
291 Vector Laboratories, Burlingame, CA, USA) for 90 minutes, washing with TBS-Tx (3x10
292 minutes) and Tris-HCl (50 mM Tris adjusted to pH 7.6 with HCl; 2x5 minutes) and the
293 sections were incubated in 0.67% diaminobenzidine (DAB) with 0.024% H_2O_2 in Tris-HCl for
294 30 minutes. After a final wash with Tris-HCl (2x5 minutes) the sections were mounted on
295 Superfrost slides, dried overnight on heating plates, cleared with xylene and coverslipped
296 with Entellan. A Zeiss Axio Imager.M1 microscope (Carl Zeiss) with a CX9000 camera (MBF
297 Bioscience, Williston, VT, USA) was used to take brightfield photomicrographs of the
298 sections, which were further processed with Adobe Photoshop CS6 (Adobe Systems, San
299 Jose, CA, USA; RRID:SCR_014199).
300
301 The slices from single-cell recordings were processed to visualize the morphology of the cells
302 and to determine the intracellular expression of Aβ. Heat-induced epitope retrieval (HIER)
303 was applied at 60 °C for 2 h in PB and the slices were then washed 2x15 min in PB at room
304 temperature followed by 5x15 min wash in 0.5% Triton-X-100 in TBS-Tx and incubation with
305 the primary antibody, McSA1 (1:1000), at 4 °C for 4 days. After rinsing 5x15 in TBS-Tx the
306 slices were incubated overnight in room temperature with Alexa Fluor 488 conjugated to
307 streptavidin (1:300; S11223) and a goat anti-mouse secondary antibody conjugated with
308 Alexa Fluor 546 (1:200; A11003, Molecular Probes). A subset of the slices was stained with
309 the opposite combination of fluorophores (Alexa Fluor 546 streptavidin, S11225 and Alexa
310 Fluor 488 goat anti-mouse, A11001). Subsequently, the sections were washed 3 x 15 min
311 with TBS-Tx, mounted and coverslipped. The slices were scanned using a laser scanning
312 confocal microscope (LSCM; LSM 510, Carl Zeiss) to determine the cell morphology and

intracellular expression of Aβ. Alexa 488 was excited by an Argon/2 laser and the emission was registered through a 505-550 bandpass filter, whereas Alexa 546 was excited by a DPSS 461-10 laser and the emission was bandpass filtered at 575-615.

Analysis of VSDI data

The Brainvision analysis software (BV_Ana) was used to analyze the optical signals. Changes in membrane potential cause proportional changes in the emission of the voltage sensitive dye (Grinvald et al., 1988), and these were evaluated as fractional changes in the fluorescent signal (ΔF/F). All the optical signals were processed using spatial and cubic filters in BV_Ana. The first 50 frames were used as the average baseline, and the fractional optical signals were color-coded and superimposed on a brightfield image to represent the spread of neural activity in the slice (Fig. 1A). In the recordings from DG stimulation we quantified the neural activation by calculating the integral (area under the curve) from optical traces in voxels in the molecular layer of DG and CA3, as this measure would represent the magnitude of the total membrane potential changes (Koganezawa et al., 2008). In addition, the total activated area in the whole slice after DG stimulation was quantified as the total number of pixels above threshold, with the threshold set to be 0.05% ΔF/F. The paired-pulse ratio (PPR) was calculated by dividing the maximal amplitude of second pulse by the first pulse, with a pulse interval of 25 ms. This was done for the voxels in each of the two blades of DG and an overall average PPR for DG was calculated for each slice. In recordings with stimulation in superficial MEC, stripes of voxels were analyzed. This was done both across layers and within the superficial layers in MEC, to evaluate the spread of the signal in the local network (Fig. 1B).

Analysis of whole-cell patch clamp data

Analysis of the whole-cell current-clamp recordings was performed using the software Fitmaster (HEKA). The input resistance was calculated from the steady-state voltage response to injected current steps that did not elicit action potentials (APs) by fitting the quadratic equation:

$$\Delta V = R_{N,0}\Delta I + c_{AR}\Delta I^2$$

Where $R_{N,0}$ is the voltage-independent input resistance and c_{AR} is the coefficient of anomalous rectification (Waters & Helmchen, 2006). The membrane time constant, τ, was estimated by fitting a double exponential to the voltage response of a -300 pA current injection and using the higher value. The rebound potential was measured from a -300 pA current step (if the trace did not include a rebound AP), as the difference between the maximal value after the end of the stimulus (V_{max}) and the baseline measured before the start of the stimulus ($V_{baseline}$). The sag ratio was defined as:

$$Sag = (V_{baseline} - V_{ss})/(V_{baseline} - V_{min})$$

where V_{min} is the minimal value reached after the onset of the stimulus and V_{ss} is the steady-state value of the voltage response to a -300 pA current step. The resting membrane potential (V_m) was estimated by averaging a 10 s spontaneous recording. The following action potential parameters were estimated from the first action potential of the rheobase trace (the first trace in the rheobase protocol to elicit an AP): AP threshold (defined as maximum of the double derivative of the voltage response, found using a fit), AP amplitude (difference between maximum amplitude and AP threshold), AP half width (width at 50% of max AP amplitude), fast afterhyperpolarization potential (fAHP; minimum value directly after AP) and the depolarizing afterpotential (DAP; difference between the maximum value after the AP and the fAHP, in five cells with doublet spikes this could not be measured). The

parameters fAHP and DAP were only measured in cells from MEC LII. We calculated AP amplitude, AP width at 0 mV and interspike interval (ISI) as a function of AP number from a positive current step (+200 or 210 pA), as well as the ratio between the first and the second ISI and the adaptation ratio (ISI_{first}/ISI_{last}). To look at the relationship between firing frequency and current we measured the average firing frequency from current steps ranging from 200 to 500 pA. We also measured the instantaneous frequency between the two first APs (f_0) and the two last APs (steady state, f_{ss}). The afterhyperpolarization potential (AHP) after the end of the current injection was also measured, for current steps ranging from 50 to 500 pA. The measures of firing frequencies and AHP as a function of current were only done ona subset of the cells, and for MEC this dataset only included cells from the 1 month old animals. Cells that had a V_m > -57 mV, AP amplitude < 75 mV or a bridge balance > 22 MΩ were excluded from the analysis, as well as putative interneurons.

The images from the LSCM were used to classify the neurons based on morphology. Cells in LEC LII that had a clear pyramidal (n = 9) or multiform (n = 17) morphology and cells in MEC LII with a clear pyramidal (n = 7) morphology, but not the intermediate cells types, were excluded from the analysis. Some cells were not filled well enough with biocytin to visualize the morphology (n = 9 for LEC and n = 5 for MEC). As the vast majority of the cells that were filled sufficiently were classified as fan cells (101 of 127 cells in LECII; 80%) or stellate cells (73 of 80 cells in MECII; 91%), we assumed that most of the non-filled cells would be of these types, and these were therefore included in the analysis. All the included cells from MEC displayed the known typical electrophysiological properties of stellate cells, including prominent sag and rebound.

Statistical analysis

The quantitative VSDI data obtained in DG and MEC, was analyzed with respect to effect of genotype within each age group using a linear mixed model. Fixed factors were sex, genotype (+/+ or wild type) and where relevant, area (exposed and enclosed blade of DG) or distance from electrode in MEC, as well as the interaction between genotype and area/distance from electrode. A repeated effects variable with a diagonal or compound symmetry covariance structure (chosen based on convergence and information criteria) was included to account for several voxels (the regions of interest) being measured in each slice (intraslice variance).

A linear mixed model was used to estimate the effect of genotype on the measured electrophysiological parameters from the single cell recordings. Rat ID was added as a random effect to account for several cell recordings within one animal, and thus the values from each cell will not be independent. Genotype and age were included as fixed effects with two levels each (+/+ and -/-; 1 month and 3 months). In addition sex and experimental setup was included as a fixed effect to correct for possible differences that might bias the results. An extended model was also run to test for the possible interactions between genotype and age, and genotype and sex. On parameters with several measurements within the same cell (e.g. for several APs or current steps) the AP number or injected current was included as factors and as repeated measures with cell ID as the subject variable. The covariance structure for the repeated measures was compound symmetry or unstructured, based on which one had lower information criteria. The possible interaction between genotype and AP number or genotype and injected current was also included in the statistical model.

No corrections were done for multiple testing and results were considered statistically significant when $p < 0.05$. IBM SPSS Statistics, version 22 (IBM Corporation, New York, USA; RRID: SCR_002865) was used for the statistical analysis.

Results

No changes in fan cell physiology in lateral entorhinal cortex and subtle changes in stellate cell physiology in the medial entorhinal cortex in homozygous McGill-R-Thy1-APP rats

To investigate whether basic electrophysiological properties or firing behavior were altered in the McGill-R-Thy1-APP transgenic rat, we performed whole-cell patch-clamp recordings in the current clamp mode of principal cells in layer II of LEC and MEC in rats aged 1 and 3-4 months of age.

We included 111 fan cells from LEC in the electrophysiological analysis (n = 47 cells from transgenic animals and n = 64 cells from control animals; aged 1 and 3-4 months). Since iAβ reportedly aggregates preferentially in LEC close to the rhinal fissure (Kobro-Flatmoen et al., 2016), we selectively recorded fan cells in LEC superficially in layer II and just ventral to the rhinal fissure, in semicoronal slices (Fig. 2A). Most of them displayed the typical morphology, with apical dendrites fanning out towards the pial surface and only a few or no basal dendrites (Tahvildari & Alonso, 2005; Canto & Witter, 2012a; Fig. 2B). Fan cells showed a low sag and rebound potential, no spike doublets/triplets or DAP, but had a relatively high input resistance and time constant (Fig. 2C; Canto & Witter, 2012a; b). The majority of the fan cells

recorded in homozygous (+/+) transgenic rats (89%; 31 of 35 neurons) stained positive for intracellular Aβ (Fig. 2G). A few neurons in the +/+ animals were not Aβ immunoreactive (4 of 35 fan cells; example in fig. 2H), whereas in negative littermates (-/-) none of the neurons showed immunoreactivity to human Aβ.

None of the measured basic electrophysiological or action potential parameters of fan cells differed between the transgenic and control animals (Table 1 and Extended Data Table 1.1). Similarly, there was no significant effect of genotype on the measured waveform parameters and firing properties (Table 2 and Extended Data Table 2.1).

In total, 78 stellate cells in layer II of MEC were included in the analysis (n = 38 cells from homozygous transgenic rats and n = 40 cells from negative littermates, aged 1 and 3-4 months). The stellate cells, recorded in horizontal slices, were mainly located superficially in layer II (Fig. 2D), and not at extremes of the mediolateral axis (i.e. not close to the border to parasubiculum or LEC). The majority of the stellate cells displayed the typical morphology, with dendrites radiating from the soma (Fig. 2E), though some cells had intermediate stellate to pyramidal morphologies (Canto & Witter, 2012b; Fuchs et al., 2016). All included cells showed a prominent sag (low sag ratio) and rebound potential, and rebound spikes after a hyperpolarizing pulse were not uncommon (Fig. 2F). In addition spike doublets or triplets could be seen in the start of spiking trains, and a fAHP and DAP was clearly seen after single APs (Canto & Witter, 2012b). When staining for intracellular Aβ, 96% of the recorded stellate cells in slices from transgenic were Aβ-immunoreactive (25 of 26 cells; example in fig. 2I). No Aβ-immunoreactive neurons were observed in the control slices (Fig. 2J).

455

456 Of all the electrophysiological properties measured in stellate cells, two parameters were
457 altered in transgenic compared to control rats (Tables 3 and 4, Extended Tables. 3.1 and 4.1).
458 The fAHP displayed a slight but significantly increased hyperpolarization in the homozygous
459 +/+ rats compared to the controls (Table 3, row k), and f_0 was also significantly increased in
460 the +/+ transgenic rats (Table 4, row g; estimated effect 36.2 Hz).

461 In the statistical model, age and sex were included as fixed effects, and on several of the
462 electrophysiological parameters these had significant effects (Tables 1-3). In this study, the
463 aim was to investigate the effects of genotype, but these results for age and sex underline
464 the importance of including these as factors in the statistical analyses.

465

466 ***Voltage-sensitive dye imaging and Aβ immunoreactivity of the hippocampal region***

467 In view of the minor increase in excitability observed in layer II stellate cells in MEC,
468 combined with the fact that these neurons provide major inputs to the DG (Cappaert et al.,
469 2015), we decided to record the propagation of neural activity in the hippocampal region
470 using VSDI in acute brain slices of McGill-R-Thy1-APP and wild type rats (Fig. 1). Bipolar
471 electrical stimulation was applied to the dentate gyrus and medial entorhinal cortex (areas
472 shown with red asterisks in Fig. 3A). The slices used for VSDI were also immuno-stained for
473 Aβ-42 and showed that in wild type animals staining was absent (Fig. 3B, D and E), whereas
474 every transgenic animal in all age groups (3, 9, and 12 months) had strong intracellular Aβ
475 immunoreactivity in several areas of the hippocampal region (Fig. 3C, F and G). Expression
476 was particularly strong in the pyramidal cell layer of subiculum (Fig. 3F), CA1, CA3 (Fig. 3C) as
477 well as in layer II of the entorhinal cortex (Fig. 3G). No extracellular plaques were seen in any

of the slices from animals aged 3 or 9 months, whereas at 12 months the plaque levels were highly variable, from no plaques to very high plaque loads (Heggland et al., 2015).

The neural network responses in the two blades of DG show subtle alterations in transgenic rats

Stimulation in the molecular layer in the crest of DG, the area bridging the two blades, with a single pulse (0.2 mA for 300 µs), resulted in activation in both of the blades of DG as well as in the hilus, and in several cases a small change in the optical signal could also be seen in CA3 (Fig. 4). In the wild type animals, the exposed blade (also called the outer, free or infrapyramidal blade) had a higher level of activity than the enclosed blade (also called the inner or suprapyramidal blade), at all ages (Fig. 4, left panels). In the homozygous transgenic animals, this pattern of activation was also seen in the majority of the slices at 3 and 9 months (Fig. 4, right panels). However, at 12 months of age, we observed that some transgenic rats had larger responses in the enclosed than the exposed blade or very similar responses in the two blades after stimulation in the crest (Fig. 4, lower right panel).

Quantification of the membrane potential changes were in line with this altered pattern of DG activation, following single pulse stimulation (Fig. 5; Extended Data Fig 5.1). At 3 and 9 months, there was a larger membrane potential change in the exposed blade than the enclosed blade in both wild type and control animals (Fig. 5A, left and middle panel). The statistical analysis, using the mixed linear model, showed a significant effect of area (blade) at 3 and 9 months, but no significant effect of genotype or interaction between area and genotype (Extended Data Figure 5.1, row a and b). At 12 months, the effect of area was no

longer significant, nor was there a main effect of genotype (Extended Data Figure 5.1, row c). However, at 12 months there was a significant interaction between genotype and area (Extended Data Figure 5.1, row c) and the membrane potential changes in the enclosed blade were significantly higher in the homozygous transgenic animals than in the wild type animals (Fig. 5A, right panel). The same pattern of activation in DG was also found when stimulating with 4 pulses at 40 Hz, and with the addition of the GABAa antagonist bicuculline (Fig. 5B, middle and right panels). The statistical test showed comparable results, with a significant interaction between genotype and area at 12, but not 9 months (Extended Data Figure 5.1, rows d-g).

In all age groups the total activated area (number of pixels) was unaltered in the +/+ transgenic rats compared to wild type (Table 5). In addition, there was no significant difference between the PPR in wild type and +/+ transgenic animals at either 9 or 12 months (Table 5). The addition of bicuculline did not change this pattern of activation. This indicates that although there was a change in the activation pattern of DG in the homozygous transgenic animals at 12 months, the overall excitability of the DG circuitry was not altered.

Transient changes in neural network responses in MEC in transgenic rats

Layer II neurons in MEC that project to the hippocampus, also give rise to local axon collaterals in layer I and II, reaching for around 300 µm with an occasional spread of up to 400 µm along the transverse axis (Tamamaki and Nojyo, 1993; Klink and Alonso, 1997; Schmidt et al., 2017). These local collaterals may innervate neurons in layers II, III and V,

since all have apical dendrites in layers I/II. We therefore aimed to look at differences in local network responses between wild type and transgenic animals upon stimulation in layer II in MEC using VSDI.

Stimulation in superficial MEC (single 0.6 mA pulse) led to changes in the VSDI signal that spread in the superficial layers as well as to the deep layers of MEC (Example image in Fig. 1A). In addition, small changes were observed in the pre- and parasubiculum and DG in many slices. We analyzed changes in fluorescent signal in individual voxels taken at the position of the stimulation electrode and gradually moving away with a maximum distance of approximately 700 µm away from the electrode. As expected, a significant effect of distance from electrode was seen, with a decreasing signal with distance both across and within layers (Table 6; Extended Table 6.1). No main effect of genotype was observed for any age group (Table 6). However, at 3 months there was a significant interaction between genotype and distance from electrode across layers, but this was not seen at 9 and 12 months (Table 6). Similarly, a small but significant interaction between genotype and distance from electrode within layers was seen at 9 months, but not 3 and 12 months (Table 6). These significant interactions indicate alterations in the network responses in the MEC of the transgenic rats. However, the effects are small and transient, as they are only seen in single age groups.

Discussion

Accumulating evidence suggests that the cognitive symptoms of memory loss and learning impairments in the early stages of AD are not mainly due to neuronal loss or atrophy, but can be linked to neuronal and synaptic dysfunction and subsequent abnormal patterns of activation in local neuronal circuits and larger-scale networks (D'Amelio & Rossini, 2012). Aβ peptides likely play an important role in these deleterious processes by affecting synapses and synaptic function (Palop & Mucke, 2010b). Here, we studied changes in the entorhinal-hippocampal network and single cells in acute slices taken from McGill-R-Thy1-APP transgenic rats expressing human mutated APP. We first analyzed the electrophysiological properties and excitability of the main principal cell populations in layer II of EC, fan cells and stellate cells, with the use of *in vitro* whole-cell patch clamp. When comparing transgenic rats and controls, at 1 month and 3-4 months of age, we found no alterations in any of the passive membrane properties, and only subtle differences in the excitability of stellate cells. Further, with the use of VSDI, we observed alterations in the activation patterns of the two blades of DG in 12 months old homozygous transgenic animals, as well as transient changes in the local network activity in MEC.

Other studies report network hyperexcitability, including seizures, in different brain areas of several mouse models of AD (Palop et al., 2007; Minkeviciene et al., 2009; Harris et al., 2010; Verret et al., 2012), including EC (Duffy et al., 2015; Xu et al., 2015) and DG (Hazra et al., 2013). In the current study, we did not find clear evidence for generalized hyperexcitability of EC or DG in the McGill rat using VSDI in slices. This apparent discrepancy likely is caused by the different experimental methods used. The optical signals we recorded represent the averaged membrane voltage changes in the total population of cells, including glial cells. An

571 increased number of hyperactive as well as hypoactive neurons has previously been
572 reported in AD mice using Ca^{2+}-imaging (Busche et al., 2008; Busche et al., 2012). Such
573 changes in single cells or ensembles would sum together and would therefore be difficult, if
574 not impossible, to detect with VSDI. In addition, many of the studies reporting aberrant
575 network activity in transgenic AD models have been done in vivo, with techniques including
576 EEG (Minkeviciene et al., 2009; Verret et al., 2012), single neuron (Kellner et al., 2014) or
577 local field potential recordings (Xu et al., 2015). Thus, we cannot exclude the possibility that
578 in vivo recordings or utilizing a different method to assess network function might reveal
579 other changes in the McGill-R-Thy1-APP rat not seen in the present study.
580
581 We found no alterations of subthreshold intrinsic properties in either fan cell or stellate cells
582 in the homozygous transgenic McGill rats aged 1 and 3-4 months. In Tg2576 mice, fan and
583 stellate cells in EC showed no changes in the input resistance and resting membrane
584 potential (Marcantoni et al., 2013), in agreement with our findings. Similar results with no
585 changes in subthreshold properties have been shown in pyramidal cells in CA1 of the McGill
586 rat (Qi et al., 2014), PSAPP (Brown et al., 2011), PDAPP (Kerrigan et al., 2014), 3xTg-AD (Scala
587 et al., 2015) and CRND8 mice (Wykes et al., 2012) as well as in the frontal cortex of
588 APPswe/PS1dE9 (APdE9) mice (Kellner et al., 2014). In contrast, a depolarization of the
589 resting membrane potential has been found in interneurons in DG and pyramidal cells in
590 neocortex in APdE9 mice (Minkeviciene et al., 2009; Hazra et al., 2013) in addition to
591 parvalbumin-positive interneurons, but not pyramidal cells in parietal cortex of hAPPJ20
592 mice (Verret et al., 2012), suggesting that cell populations might be differentially affected.
593

We identified two suprathreshold properties that showed alterations in stellate cells in the homozygous rats. Stellate cells display a clear fAHP followed by a DAP (Alonso & Klink, 1993) and this fAHP is due to a Ca^{2+}-dependent K^+ conductance (Storm, 1987; Klink & Alonso, 1993). This conductance is thought to be mediated by BK (big potassium) channels, and is also important for spike repolarization (Sah, 1996). The BK channels can facilitate high-frequency firing, likely through limiting the activation of other potassium channels and decreasing the inactivation of sodium channels (Gu et al., 2007). Notably, the BK current is transient, inactivating rapidly, and thus will be most influential in the initial part of a spike train (Shao et al., 1999). Correspondingly, the other alteration we observed in stellate cells in the transgenic rats was increased excitability early in the spike train, a slightly higher f_0 at 1 month. It is thus possible that the hyperexcitability we here describe in the MEC stellate cells in the McGill rat actually results from early changes in ion conductances, in particular the BK potassium current, which might worsen over time. Whether the BK channel, or other channels, are affected specifically in this model, will be of interest for further studies. Several studies report various physiological alterations of single cells in other transgenic mouse models, including changes in excitability, potassium currents and AP waveform (Brown et al., 2011; Wykes et al., 2012; Kerrigan et al., 2014; Scala et al., 2015), highlighting several possible channels as targets for Aβ toxicity.

The VSDI data indicates an alteration of the response pattern in DG, seen in the 12 month homozygous transgenic group, but not at 3 and 9 months. The McGill-R-Thy1-APP rat initially displays extracellular plaques at around 9 months, and although the pattern of plaque deposition is similar across animals, the age of onset and temporal progression of the plaque pathology varied considerably between animals (Heggland et al., 2015). This corresponds to

618 the findings in the current study, with highly variable levels of plaque in the recorded slices
619 from the different homozygous transgenic animals. It is of interest to mention that one
620 animal with the highest plaque load also had the largest change in DG activation pattern. In
621 AD mice models, hyperactive neurons have been found to be associated with plaques
622 (Busche et al., 2008; Busche et al., 2012) and deficits in place cell firing were related to
623 hippocampal plaque burden in the Tg2576 model (Cacucci et al., 2008). Consistent with this
624 are findings that synaptic density is reduced in proximity to plaques (Dong et al., 2007) and
625 neurons in contact with plaques have a loss of perisomatic GABAergic synapses (Garcia-
626 Marin et al., 2009), providing a possible mechanism for some of the observed network
627 changes in AD models.
628
629 The observed responses after stimulation in the molecular layer of DG, which is a major area
630 of termination of the perforant path input from layer II of EC, revealed an asymmetry in the
631 activation of the two blades in wild type animals, with larger amplitudes in the exposed
632 (infrapyramidal) blade than in the enclosed (suprapyramidal) blade. A similar asymmetry has
633 been reported previously with VSDI in rats (Scharfman et al., 2002; Wright & Jackson, 2014)
634 and Ca^{2+} imaging in mice (Yu et al., 2013). The inhibitory circuitry differs in the two blades
635 (Seress & Pokorny, 1981), which could be a possible explanation for these observations.
636 However, the asymmetry, as well as the alterations seen in the 12 month transgenic group,
637 were seen using both normal ACSF and after addition of the $GABA_A$ receptor antagonist
638 bicuculline. This indicates that differences or alterations in inhibition do not play a major role
639 in this case, although effects of $GABA_B$ cannot be completely ruled out. Other known blade
640 differences include the perforant path input from the entorhinal cortex, with the two blades
641 receiving preferential input from different parts of EC (Wyss, 1981; Witter et al., 1989).

642 Although the precise distribution of the perforant path to the two blades is somewhat
643 disputed (Witter et al., 1989), these anatomical differences might play a role in the
644 asymmetric activation of DG.
645
646 Although possible sex differences were not the focus of this study, the observed effects do
647 support the inclusion of sex as a factor in future studies. In the statistical analysis of the
648 electrophysiological parameters, we included age and sex as factors, both to be able to
649 account for possible bias on the estimated effect of genotype, as well as the possibility that
650 genotype had a differential effect on either sex or with increasing age. Women are at higher
651 risk of developing Alzheimer's disease (Li & Singh, 2014) and sex differences have been
652 described in other animal models of AD (Wang et al., 2003). An effect of sex on changes in
653 metabolism has been described in the McGill-R-Thy1-APP rat (Nilsen et al., 2014) although
654 no clear differences were reported between males and females regarding plaque pathology
655 (Heggland et al., 2015) or memory impairments (Leon et al., 2010). Some of the possible
656 effects we find here might be due to different, and fluctuating, hormone levels, but also
657 differences in genes and gene expression between sexes could play a part (Shah et al., 2014)
658 The effects seen with age were also in general minor. Since we recorded in rats aged 1
659 month (juvenile) and 3-4 months (adult), the changes could reflect the transition to
660 adulthood. Previously, stellate cells in MEC of young adult (postnatal day 46) rats have been
661 shown to be less excitable, and have slight alterations in intrinsic electrophysiological
662 properties, compared to juvenile (postnatal day 21) rats (Boehlen et al., 2010).
663
664

In summary, we found that in young animals, there were only minor alterations in the intrinsic electrophysiological parameters of single cells, with a slight hyperexcitability seen in stellate cells and no changes in the fan cells in the homozygous rats, although the majority of these cells displayed accumulation of iAβ. Following up on this, we found that the networks of DG and MEC were largely unaltered in the McGill-R-Thy1-APP transgenic rat. However, at 12 months there was a statistically significant change in the typically asymmetric activation of the DG seen in wild type rats. Additionally, there were transient changes in the local network of MEC. Whether the hyperexcitability of stellate cells plays a major role in the cognitive deficits seen in pre-plaque homozygous McGill rats still remains an open question. Additionally, the results from the VSDI point to the possible involvement of the medial perforant path to the DG in AD dysfunction. Even small alterations in the EC-DG or intrinsic DG circuitry could therefore perturb the normal hippocampal processing and thus affect learning and memory.

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881 ## Figure legends

882 **Figure 1.** A) Example of VSDI imaging with stimulation in MECII in a horizontal slice (top) and
883 the corresponding Nissl stain after histology (bottom). B) Illustration of regions of interest
884 (stripes) chosen to analyzed the VSDI signals in MEC, across layer (top left) and within the
885 superficial layers (bottom left). To the right the corresponding traces for the voxels along the
886 stripe is shown with the traces taken from the three color-coded voxels represented with
887 the corresponding color.
888
889 **Figure 2.** The characteristic morphology and electrophysiology of a fan cell and a stellate cell
890 from entorhinal cortex layer II and examples of intracellular expression of human Aβ in
891 recorded cell. A) Representative example of a semicoronal slice used for recording cells in
892 LEC. The asterisk ventral to the rhinal fissure marks the location of the patched cell shown in
893 (B). B) Confocal image showing the morphology of a typical fan cell in layer II of LEC, close to
894 the rhinal fissure (location marked in A). C) Trace from the fan cell in (B) showing voltage
895 responses to hyperpolarizing and depolarizing current steps of ± 300 pA. D) Representative
896 example of a horizontal slice used for recording cells in the MEC. The asterisk marks the
897 location of the cell shown in (E). E). Confocal image showing the morphology of a typical
898 stellate cell in layer II if MEC (location marked in D). F) Trace from the stellate cell in (E)
899 showing voltage responses to hyperpolarizing and depolarizing current steps of ± 300 pA.
900 LEC, lateral entorhinal cortex; MEC, medial entorhinal cortex. G) Confocal scan from a 3
901 months old homozygous McGill-R-Thy1-APP transgenic rat showing a fan cell in LEC LII with
902 intracellular Aβ. H) Confocal scan from a 1 month old homozygous transgenic rat showing a
903 stellate cell in MEC LII with intracellular Aβ. I) Confocal scan from a 1 month old homozygous
904 transgenic rat showing a fan cell in LEC LII without expression of intracellular Aβ. J) Confocal

scan from a 1 month old negative control animal showing no expression of human intracellular Aβ. The cells were filled with biocytin during recording and visualized with streptavidin labeled with Alexa Fluor 488. The presence of the anti-human Aβ antibody McSA1 was visualized with Alexa Fluor 546. LEC, lateral entorhinal cortex; MEC, medial entorhinal cortex.

Figure 3. Horizontal slices from control animals and homozygous McGill-R-Thy1-APP rats were used for VSDI. A) Example of a horizontal slice stained with cresyl violet showing the different areas targeted for stimulation (red asterisks). B) Example slice from a 9 months old wild type rat showing no immunoreactivity against Aβ, using the human-specific anti-Aβ antibody McSA1. C) Example slice from a 12 months old homozygous transgenic rat with intracellular Aβ immunoreactivity. Insets show higher magnification of the subiculum (D, F) and the medial entorhinal cortex (E, G). Scale bars: 500 µm (bar in A represents all overview images, bar in D all insets).

Figure 4. Voltage sensitive dye imaging of DG showed differences in activation patterns in control and transgenic animals. Evoked activity from a bipolar stimulation electrode (single 0.2 mA pulse) centered in DG in control (left) and transgenic (right) animals. The activity spread to both blades, and in some cases activity could also be seen in CA3. At 3 and 9 months, the membrane potential changes were in general larger in the exposed blade than the enclosed blade. In contrast, at 12 months several of the transgenic animals showed an increased activity in the inner blade. Images are from representative horizontal slices from wild type and +/+ transgenic animals at 3, 9 and 12 months of age. Scale bars: 500 µm

Figure 5. The evoked activity in the two blades of DG was significantly altered in 12 months old transgenic animal, with increased activity in the enclosed blade, with a constant overall activated area. A) Quantification of the relative membrane potential change measured with VSDI in the two blades of DG (ROI are the voxels shown in figure 3) is shown for 3, 9 and 12 months. In control animals the majority of the slices had larger membrane potential changes in the exposed blade than the enclosed blade, but at 12 months this pattern was altered in a portion of the transgenic animals, while the total activated area was similar in all age groups. Asterisk indicates estimated marginal means that have non-overlapping 95% confidence intervals. B) Quantification of the relative membrane potential change measured with VSDI in the two blades of DG and CA3 in transgenic and control animals after a single or 4 pulse stimulation and in the presence of 5 µM bicuculline at 9 and 12 months. At 9 months the pattern of activation was similar in transgenic and control animals (top panels), but at 12 months this pattern was altered in the +/+ transgenic group (bottom panels). For each age group the effects of genotype and area and the interaction was tested with a linear mixed model. Results from the statistical analysis are shown in Extended Data Figure 5.1

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948 ## Tables

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952 **Table 1.** Results from the mixed linear model analysis for electrophysiological parameters of LEC II
953 fan cells in homozygous transgenic animals (+/+) and controls (-/-). Basic electrophysiological and
954 action potential properties from all individual cells are represented in Extended Data Table 1.1.

		Estimated marginal means		p-values for test of fixed effects		
		-/-	+/+	Genotype	Age	Sex
a	Input resistance (MΩ)	138.0	129.8	0.299	0.085	**0.013**[1]
b	Time constant, τ (ms)	28.9	30.7	0.230	0.746	**0.001**[2]
c	Sag ratio	0.803	0.799	0.776	0.284	0.890
d	Rebound (mV)	5.45	5.51	0.926	0.534	0.666
e	V_m (mV)	-69.3	-70.7	0.126	**0.022**[3]	0.204
f	Rheobase (pA)	71	72	0.934	**0.034**[4]	**0.006**[5]
g	AP threshold (mV)	-45.0	-46.8	0.054	0.313	0.119
h	AP amplitude (mV)	91.8	91.7	0.898	0.563	0.098
i	AP half width (ms)	1.071	1.128	0.152	0.665	0.659

955 n = 111 cells from 40 animals. V_m, resting membrane potential; AP, action potential.

956 [1]Effect size: 21.9 MΩ (males higher) [2]Effect size: 5.6 ms (males higher) [3]Effect size: -2.2 mV [4]Effect size: 18.9 pA
957 [5]Effect size: 24.5 pA (males lower)

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961 **Table 2.** Estimated p-values for action potential parameters as a function of AP number and firing
962 properties of LEC LII fan cells. Action potential and firing properties of LEC LII fan cells in homozygous
963 transgenic rats (+/+) and control animals (-/-) are shown in Extended Data Table 2.1.

964

		Genotype	Age	Sex	AP no	AP*Genotype
a	AP amplitude	0.855	**0.002**[1]	**0.024**[2]	**0.000**	0.059
b	AP width	0.388	0.195	0.620	**0.000**	0.123
c	ISI	0.166	0.124	**0.039**[3]	**0.000**	0.317
d	ISI1/ISI2	0.698	**0.007**[4]	**0.006**[5]	-	-
e	Adaptation ratio	0.957	0.237	**0.014**[6]	-	-
		Genotype	**Age**	**Sex**	**Current**	**Current*Genotype**
f	Average frequency, *f*	0.554	0.634	0.577	**0.000**	0.425
g	f_0	0.936	0.334	0.122	**0.000**	0.157
h	f_{ss}	0.666	0.650	0.578	**0.000**	0.977
i	AHP	0.774	0.659	0.434	**0.000**	0.656

965 Values are estimated p-values from the mixed linear model, n = 111 cells from 40 animals; for firing frequencies
966 and AHP n = 67 cells from 26 animals. AP, action potential; ISI, interspike interval; adaptation ratio, first ISI/last
967 ISI; f_0, instantaneous firing frequency between two first APs; f_{ss}, instantaneous firing frequency between two
968 last APs; AHP, afterhyperpolarization potential.
969 [1]Effect size: 2.5 mV [2]Effect size: 1.7 mV (males lower) [3]Effect size: 9.8 ms (males higher) [4]Effect size: -0.07
970 [5]Effect size: 0.07 (male higher) [6]Effect size: 0.08 (male higher)

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Table 3. Estimated marginal means and p-values for electrophysiological parameters of MEC II stellate cells. Basic electrophysiological properties of MEC LII stellate cells in homozygous transgenic rats (+/+) and negative control animals (-/-) for all cells are provided in Extended Data Table 3.1.

		Estimated marginal means		p-values for test of fixed effects		
		-/-	+/+	Genotype	Age	Sex
a	Input resistance (MΩ)	50.3	55.5	0.327	0.762	0.133
b	Time constant, τ (ms)	13.3	13.3	0.945	0.121	**0.004**[1]
c	Sag ratio	0.580	0.607	0.094	0.230	**0.034**[2]
d	Rebound (mV)	7.6	7.3	0.711	0.135	0.849
e	V_m (mV)	-64.7	-64.7	0.937	0.217	0.229
f	Resonance frequency (Hz)	4.5	4.5	0.982	**0.025**[3]	0.069
g	Rheobase (pA)	123	111	0.375	0.690	0.225
h	AP threshold (mV)	-47.9	-48.9	0.229	0.148	0.246
i	AP amplitude (mV)	89.1	86.4	0.105	0.231	0.974
j	AP half width (ms)	0.991	0.980	0.820	0.155	0.856
k	fAHP (mV)	-51.6	-53.1	**0.034**	**0.000**[4]	0.161
l	DAP (mV)	1.9	2.3	0.479	0.125	0.752

Values are estimated marginal means and p-values from the mixed linear model, n= 78 cells from 30 animals. V_m, resting membrane potential; AP, action potential; fAHP, fast afterhyperpolarization potential; DAP, depolarizing afterpotential.

[1]Effect size: 2.2 ms (males lower) [2]Effect size: 0.026 (males higher) [3]Effect size: -0.65 Hz [4]Effect size: -4.0 mV

Table 4. Estimated p-values for action potential parameters as a function of AP number and firing properties of MEC LII stellate cells.

		Genotype	Age	Sex	AP no	AP*Genotype
a	AP amplitude	0.318	0.463	0.812	**0.000**	0.258
b	AP width	0.264	0.670	0.246	**0.000**	0.589
c	ISI	0.576	0.593	0.977	**0.000**	0.685
d	ISI1/ISI2	0.449	0.657	0.416	-	-
e	Adaptation ratio	0.818	0.737	0.862	-	-
		Genotype	**Age**	**Sex**	**Current**	**Current*Genotype**
f	Average frequency, *f*	0.611	-	0.829	**0.000**	0.884
g	f_0	**0.042**	-	0.332	**0.000**	0.884
h	f_{ss}	0.839	-	0.870	**0.000**	0.984
i	AHP	0.561	-	0.629	**0.000**	0.922

Values are estimated p-values from the mixed linear model, n = 78 cells from 30 animals; for firing frequencies and AHP n = 38 cells from 16 animals. AP, action potential; ISI, interspike interval; adaptation ratio, first ISI/last ISI; f_0, instantaneous firing frequency between two first APs; f_{ss}, instantaneous firing frequency between two last APs; AHP, afterhyperpolarization potential.

991 **Table 5.** Results from the mixed linear model for total number of activated pixels and PPR using VSDI

992 in the DG of homozygous transgenic animals (+/+) and controls (-/-).

	Mean (SD)		p-values for test of fixed effects		
	-/-	+/+	Genotype	Sex	N (slices/animals)
Activated pixels (1 pulse)					
3 months	807 (284)	83 (297)	0.549	0.463	13/7
9 months	1698 (579)	1800 (279)	0.641	0.638	21/10
12 months	1687 (485)	1581 (427)	0.564	0.733	23/9
Paired-pulse ratio: Normal ACSF					
9 months	0.860 (0.098)	0.811 (0.032)	0.157	0.198	21/10
12 months	0.920 (0.119)	0.854 (0.098)	0.190	0.718	23/9
Paired-pulse ratio: Bicuculline					
9 months	1.320 (0.076)	1.321 (0.082)	0.970	0.897	15/8
12 months	1.373 (0.088)	1.363 (0.070)	0.859	0.782	15/9

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996 **Table 6.** Results from the mixed linear model for quantified membrane potential change using VSDI
997 in MEC of homozygous transgenic animals (+/+) and controls (-/-). Spread of activity from electrode
998 placed in superficial layers MEC recorded with VSDI for all rats is shown in Extended Data Table 6.1.

999

	p-values for test of fixed effects				
	Genotype	**Sex**	**Distance from electrode**	**Distance from electrode * Genotype**	**N (slices/animals)**
Superficial layers					
3 months	0.095	0.116	**0.000**	0.652	16/6
9 months	0.315	0.431	**0.000**	**0.015**	21/10
12 months	0.445	0.297	**0.000**	0.696	13/7
Across layers					
3 months	0.237	0.266	**0.000**	**0.000**	16/6
9 months	0.639	0.104	**0.000**	0.902	21/10
12 months	0.673	0.750	0.000	1.000	13/7

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Extended Data

Table 1.1. Basic electrophysiological and action potential properties of fan cells in LEC LII in homozygous transgenic rats (+/+) and negative control animals (-/-) at 1 and 3 months of age. A) Input resistance measured from a series of current steps. B) The membrane time constant, τ; C) sag ratio; D) rebound potential; all measured from a current step of -300 pA. E) Resting membrane potential, V_m. F) Rheobase, measured by current steps increasing by 10 pA/step. G) Action potential threshold; H) action potential amplitude; I) action potential half width; all measured from the current step at rheobase. Values from all individual cells are shown (n = 111 cells in 40 animals).

Table 2.1. Action potential and firing properties of LEC LII fan cells in homozygous transgenic rats (+/+) and control animals (-/-), in both age groups. A) Action potential amplitude as a function of action potential number. B) Action potential width at 0mV as function of action potential number. C) ISI, interspike interval as a function of spike interval number. D) Ratio of the two first interspike intervals (ISI1/ISI2) and adaptation ratio (first ISI/ last ISI). Values in A-D are measured from a +200 or 210 pA current (n = 111 cells in 40 animals). E) Average firing frequency, *f*; F) instantaneous firing frequency between two first spikes, f_0; G) instantaneous firing frequency between two last spikes, f_{ss}; H) afterhyperpolarizing potential after end of current step; E-H are all plotted as a function of current (n = 67 cells in 26 animals). All values are shown as estimated marginal means and standard errors from the mixed linear model.

Table 3.1. Basic electrophysiological properties of MEC LII stellate cells in homozygous transgenic rats (+/+) and negative control animals (-/-) at 1 and 3 months of age. A) Input resistance measured from a series of current steps. B) The membrane time constant, τ; C) sag ratio; D) rebound potential; all

measured from a current step of -300 pA. E) Resting membrane potential, V_m. F) Membrane resonance frequency in response to a ZAP current. G) Rheobase, measured by current steps increasing by 10 pA/step. H) Action potential threshold; I) action potential amplitude; J) action potential half width; K) fAHP, fast afterhyperpolarization potential; L) DAP, depolarizing afterpotential; all measured from the first action potential of the current step at rheobase. Values from all individual cells are shown (n = 78 cells in 30 animals).

Table 4.1. Action potential and firing properties of MEC LII stellate cells in homozygous transgenic rats (+/+) and control animals (-/-), for both age groups in A-D, 1 month group in E-H. A) Action potential amplitude as a function of action potential number. B) Action potential width at 0 mV as function of action potential number. C) ISI, interspike interval as a function of spike interval number. D) Ratio of the two first interspike intervals (ISI1/ISI2) and adaptation ratio (first ISI/ last ISI). Values in A-D are measured from a +200 pA current step (n = 78 cells in 30 animals). E) Average firing frequency, f; F) instantaneous firing frequency between two first spikes, f_0; G) instantaneous firing frequency between two last spikes, f_{ss}; H) Afterhyperpolarizing potential after end of current step; E-H are all plotted as a function of current (n = 38 cells in 16 animals). All values are shown as estimated marginal means and standard errors from the mixed linear model.

Figure 5.1. Results from the mixed linear model for quantified membrane potential change using VSDI in the DG of homozygous transgenic animals (+/+) and controls (-/-).

	p-values for test of fixed effects				
	Genotype	**Area**	**Genotype x Area**	**Sex**	**N (slices/animals)**
1 pulse					

a	3 months	0.177	**0.008**	0.316	0.753	13/7
b	9 months	0.757	**0.014**	0.583	**0.022**	**21/10**
c	12 months	0.191	0.243	**0.019**	0.192	23/9
	4 pulses					
d	9 months	0.28	**0.017**	0.68	**0.005**	**21/10**
e	12 months	0.394	0.208	**0.007**	0.169	23/9
	Bicuculline					
f	9 months	0.155	0.069	0.197	0.496	15/8
g	12 months	0.596	0.066	**0.016**	0.596	15/9

Table 6.1. Spread of activity from electrode placed in superficial layers MEC recorded with VSDI in wild type (wt) and transgenic (+/+) rats. The relative membrane potential change at increasing distance from the electrode tip is shown within the superficial layers (left) and across the layers of MEC (right), for 3, 9 and 12 months old rats.