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Age-dependent concomitant changes in synaptic function and GABAergic pathway in the APP/PS1 mouse model

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Synaptic dysfunction is a well-documented manifestation in animal models of Alzheimer's disease pathology. In this context, numerous studies have documented reduction in the functionality of synapses in various models. In addition, recent research has shed more light on increased excitability and its link to seizures and seizure-like activities in AD patients as well as in mouse models. These reports of hyperexcitability contradict the observed reduction in synaptic function and have been suggested to be as a result of the interplay between inhibitory and excitatory neuronal mechanism. The present study therefore investigates functional deficiency in the inhibitory system as complementary to the identified alterations in the glutamate excitatory pathway in AD. Since synaptic function deficit in AD is typically linked to progression/pathology of the disease, it is important to determine whether the deficits in the GABAergic system are functional and can be directly linked to the pattern of the disruption documented in the glutamate system. To build on previous research in this field, experiments were designed to determine if previously documented synaptic dysfunction in AD models is concomitantly observed with excitation/inhibition imbalance as suggested by observation of seizure and seizure-like pathology in such models. We report changes in synaptic function in aged APPPS1 mice not observable in the younger cohort. These changes in synaptic function are furthermore accompanied by alteration in the GABAergic neurotransmission. Thus, age-dependent alteration in the inhibitory/excitatory balance might underpin the symptomatic changes observed with the progression of Alzheimer's disease pathology including sleep disturbance and epileptic events.

Key words: GABAergic, glutamatergic, seizures, Alzheimer's disease, APP/PS1, long-term potentiation, expression profile

INTRODUCTION

Dual mutant mice models carrying various known mutations in the amyloid processing protein and the presinilin genes are widely used in the study of the pathology of Alzheimer's disease (AD) (Jankowsky et al. 2001). These models have been shown to mimic various of the AD related clinical pathology such as amyloid beta (Aβ) plaque load accumulation, deficits in synaptic function and structure, as well as deficits in learning and memory (Jankowsky et al. 2001, Trinchese et al. 2004, Radde et al. 2006, Huang et al. 2016).

Deficits in memory and changes in behavior remain the first clinical indications of AD (Alzheimer's-Association 2015). The changes in cognitive functioning are however considered a result of accrued pathology. On the one hand empirical evidence exists showing that increased concentration of Aβ is directly linked to synaptic pathology observed in AD mouse models, even in the absence of plaque pathology (Lue et al. 1999, Zhang et al. 2011). On the other hand, studies have also documented changes in long and short term plasticity to be restricted to a later age in AD models when plaque pathology is clearly established (Trinchese et al. 2004, Gengler et al. 2010, Huang et al. 2016).

In addition to alterations in synaptic functioning, mouse models display increased aberrant network activity which has previously been linked to an imbalance in the excitation/inhibition ratio in such models (Palop and Mucke 2009, 2010, Hazra et al. 2013, 2016). Transgenic mice of the APP/PS1 models overexpress Swedish family mutated forms of the human amyloid precursor protein (APPswe) and various mutation in Presenilin1 result in

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age-dependent and region-specific amyloid deposition (Jankowsky et al. 2001, Fitzjohn et al. 2010, Huang et al. 2016). APP/PS1 mice spontaneously show seizures, which are believed to be associated to an imbalance in the excitatory/inhibitory function (i.e. altered glutamatergic and/or gamma-aminobutyric acid [GABAergic pathways]) in the central nervous system in the pathology of AD (Howell et al. 2000, Ramos et al. 2006, Shankar et al. 2007, Li et al. 2011, Hazra et al. 2013).

In the laboratory, long-term potentiation (LTP) is a well--established electrophysiological phenomenon used in the detection of alterations or impairments in the molecular mechanism of learning and memory. LTP induction and sustenance are physiological synaptic events that are considered to be crucial for the proper functioning of the brain, i.e. memory formation (Shors and Matzel 1997, Malenka and Nicoll 1999, Bliim et al. 2016). Furthermore, the fact that LTP deficit is a common theme observed in AD mouse models strengthens the link between LTP and memory deficits observed in both clinical and/or preclinical research (Gengler et al. 2010, Sheng et al. 2012, Webster et al. 2014, Viana da Silva et al. 2016).

The present paper uses the APP/PS1 mouse model of AD pathology to investigate the relationship between synaptic function, LTP, and GABAergic function and pathway signaling (Radde et al. 2006). To this end, the ability to induce LTP was assessed in an in vitro paradigm that gives insight into the ability of the hippocampus to strengthen synaptic association required for acquiring new memory and processing old memory alike (Lynch 2004, Bliim et al. 2016). Furthermore, the experiments were conducted at two time points in order to determine excitability threshold, short-term plasticity as well as LTP induction in slices obtained from APP/PS1 mice in relation to plaque deposit as a function of age/disease progression. Lastly, alterations in GABA pathway functioning and related gene expression in the prefrontal cortex and hippocampus were assessed using microarray technology (Chebib and Johnston 1999, Johnston 2013).

METHODS

Animals: APP/PS1 mice

This study is based on the well-characterized, previously reported and published APP/PS1 mouse with the amyloid precursor protein APP Swedish mutation (KM670/671NL) and the aggressive mutation in the Presenilin1 (PS1-L166P) on a C57BL/6 background (Radde et al. 2006). Mice were provided with standard laboratory rodent food and tap water ad libitum, while every effort was made to minimize pain in animal use and disturbances in animal well-being. All protocols have been carried out in accordance with guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), and of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by Janssen Pharmaceutica Ethical Committee.

To examine the effects of age, two time points ('ages') were determined for the experimental design. These include a previously reported initial time point of 1.5 month of age, which is before the appearance of plaques in the APP/PS1 mouse model, while the second time point was chosen at 8 months of age when the plaque load is already clearly established in this mouse strain (Radde et al. 2006).

Electrophysiology: Synaptic function and long-term potentiation (LTP)

Mice were euthanized with carbon dioxide, decapitated, and whole brains were quickly removed and placed in ice cold artificial cerebrospinal fluid (aCSF). Slices were made from obtained brain samples using a vibratome (Leica Microsystems, Germany) while submerged in a chamber filled with ice-cold cutting solution containing (in mM) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 2.5 $MgCl_2$, 1.25 KH_2PO_4, 25 $NaHCO_3$, 25 glucose, saturated with 95% O_2/5% CO_2 (pH 7.2~7.3 and 325 mOsm/kg). Hippocampi were horizontally cut at 300 µM trimmed of cortical tissue and allowed to recover in oxygenated ACSF solution at room temperature for at least 1 hour. Hippocampal slices were transferred onto a MEA chip obtained from Qwane Bioscience® on the 200/30 MEA system and continuously perfused at 1.5ml/min with oxygenated aCSF (in mM) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 KH_2PO_4, 25 $NaHCO_3$, 25 glucose, saturated with 95% O_2/5% CO_2 (pH 7.2~7.3 and 325 mOsm/kg) at 31°C. Stimulating electrode was placed in the Shaffer collateral and recording electrode(s) were positioned in the Cornu Ammonus 1 (CA1) area of the hippocampus.

Threshold for maximum response in the slices were determined by measuring the peak to peak response obtained with increases in stimulation strength (from 1 to 150 µA or 200 µA) before conducting the LTP experiment in each slice (Xiong et al. 2013). Responses to IO curve obtained are compared between APP/PS1 and littermate mice.

To determine short-term plasticity paired pulse ratio (PPR) experiment were carried out with changes in the frequency used between pulses. The interval between the paired pulse range from 0.02 s to 20 s (frequencies ranging from 50 to 0.05 Hz) and are reported as the ratio of the peak to peak measurement of the second pulse divided by the first pulse (PPR).

Long-term potentiation (LTP) was induced after 15 min of baseline recording using a high frequency stimulation protocol containing 3 trains each composed

of 100 pulses at 100 Hz separated by 20 s. Continuous recording was maintained 60 min after the induction of LTP. All sweeps were normalized to the mean of the baseline sweeps. Analysis was done by measuring the slope of the recorded fEPSP response at baseline and after induction of LTP. Comparison was made between the baseline slope fEPSP and sweeps recorded immediately after LTP induction (10 min post HFS) or 50–60 min after LTP induction (50–60 min post LTP).

Electrophysiology: GABA inhibitor application

To access the involvement of the GABAergic pathway in the pathology of AD, we conducted a set of experiments recording extracellular synaptic activities, namely field excitatory post synaptic potentials (fEPSP), using the new developed technology of Multi-Electrode Array (MEA) (Howell et al. 2000, Ramos et al. 2006, Yoshiike et al. 2008, Limon et al. 2012, Jo et al. 2014). These recording, in hippocampal brain slices, were used to determine if a differential effect is observed by the disinhibition of GABAergic signaling by comparing the effect of applying a GABA inhibitor on field potentials in APP/PS1 mice (Chebib and Johnston 1999, Johnston 2013). Similarly, the effect of application of benzodiazepine, a γ-aminobutyric acid receptor A (GABAA) agonist, previously shown to result in a decrease in the established paired pulse depression ratio in earlier studies using rats is employed in this study to further characterize the functionality of the GABA pathway (Xie and Tietz 1991, Karnup and Stelzer 1999).

Horizontal brain slices were obtained and processed as above from APP/PS1 mice and littermate. Slices obtained were placed on a multi-electrode array chip obtained from Qwane Biosciences®. Stimulating electrode was placed in the Shaffer collateral and recording electrode(s) were selected in the CA1 area of the hippocampus. Samples were obtained from cohorts of 1.5 months and 8 months old mice for each genotype. After 15 min of baseline recording in normal ACSF a cocktail of low concentrations inhibitors of the GABA receptor A type, Bicucculline (5 μM) and GABA receptor B type, 2-Hydroxysaclofen (20 μM) were co-applied for 15 min. Analysis was performed using 3 sweeps repeats at 5 min and 15 min of inhibitors application. All data points represent the calculated slope of the decay in the population spike. Comparison was made between application and baseline in order to determine the effect of inhibition application on the responses recorded.

In a follow-up experiment, hippocampal slices from 8 months old APP/PS1 and littermate mice were obtained. Using the multi-electrode array technology, as previously described, a stimulating electrode was placed in the Shaffer collateral and recording electrode(s) were selected in the CA1 area of the hippocampus. Baseline recording was established for 15 min before 30 min of application of 1 μM diazepam (a GABA receptor A type subunit agonist). Only slices with a paired pulse ratio greater than 0.5 and less than 1 (i.e. paired pulse depression) were selected and included in the analysis (King, Knox et al. 1985, Xie and Tietz 1991). Comparisons were made between genotype and application of the GABA agonist to determine the effect of agonist application and/or genotype on the GABAA specific function. Drugs used in this study were supplied by Tocris for 2-hydroxysaclofen and bicucculline while diazepam was synthesized within Janssen Pharmaceutica.

Electrophysiological data were collected at a 10 kHz sampling frequency and 1100 amplifier gain. Triggered field Excitatory Postsynaptic Potentials (fEPSP) were recorded and processed with the use of MC_Rack®, Clampfit 10.3® and MC_Data Tool software® (Multi Channel Systems, Germany). Analyzes were performed using GraphPad Prism 6®. Statistical analysis of all electrophysiological data was performed by using standard student t-tests. Two-way ANOVA analysis was used to determine the interaction between observed differences in APP/PS1 mice and age.

Microarray analysis: GABAergic pathways

In order to further strengthen our findings on the deficits in the GABA pathways in APP/PS1 mice, we focus on gene expression in the prefrontal cortex and hippocampus using microarrays. The brain tissue selection was based on the existing evidence on close involvement of these two brain areas in cognitive processes as found disturbed in the course of progressive AD pathology (DeKosky and Scheff 1990, Grady et al. 2001, Mu and Gage 2011). At the pre-specified age of 1.5 and 8 months, brains from transgenic and wild-type mice were removed immediately following decapitation and dissected on dry ice. For this specific microarray study, an additional group of 18 months old animals was added to provide a time point at which we have observed full blown seizure and seizure-like events in this mouse model of AD (Oyelami et al. unpublished data). Tissue samples were obtained from the prefrontal cortex as well as the hippocampus from each mouse. After dissection all samples were stored at –80°C.

Total RNA was prepared starting from tissue obtained from the hippocampus and prefrontal cortex (50–100 mg). Qiazol Lysis Reagent (Qiagen 79306) was added directly to each frozen brain tissue containing a Stainless steel bead with a diameter of 5 mm. Tissues were then disrupted and homogenized using the TissueLyser at maximum

speed for 5 min and chloroform was added. After phase separation by centrifugation and recovery of the aqueous phase, ethanol was added to provide appropriate binding conditions. Each sample was then applied to the wells of the RNeasy 96 RNeasy plate and washed according to the RNeasy 96 Universal Tissue procedure. RNA was eluted with 80 µl RNase free water (Eppendorf) OD260/280 and measured using the Nanodrop 8000 instrument.

All microarray-related steps for target preparation, including the amplification of total RNA and labeling, were carried out as described in the GeneChip® HT 3' IVT PLUS Reagent Kit User Manual (Affymetrix 902417). In this protocol total RNA is reverse transcribed to synthesize first-strand cDNA which is then converted into a double-stranded DNA template for transcription. In vitro, further transcription of synthesized amplified RNA (aRNA) is performed and incorporated into biotin-conjugated nucleotide. The aRNA is then purified to remove unincorporated NTPs, salts and enzymes using Agencourt RNA Clean beads. After fragmentation the biotin-labeled target samples were hybridized to GeneChip HT HG-MG430Array plates. Target hybridization, wash, stain and scan was processed according to the instructions provided in the Affymetrix® GeneTitan™ Instrument User's Guide.

All microarray data were processed using R – the free software environment for statistical computing (R Development Core Team, 2006) as well as Bioconductor tools (Gentleman et al. 2004). The gene expression values were normalized using RMA (Irizarry et al. 2003). Grouping of the individual probes into gene-specific probe set was based on Entrez Gene using the metadata package htmg430ammentrezg (Dai et al. 2005). For this study, we focus on the expression of interneuron specific genes i.e. glutamate decarboxylase 1 (Gad1), Cholecystokinin (CCK), Somatostatin (SST), Parvabulmin, Calretinin, Vasoactive intestine peptide (VIP); gamma-aminobutyric acid solute carriers and receptors as well as association proteins. This resulted in a list of 40 genes of interest: *Cck – Cckar – Cckbr – Gabarap – Gabarapl1 – Gabbr1 – Gabra1 – Gabra2 – Gabra3 – Gabra4 – Gabra5 – Gabra6 – Gabrb1 – Gabrb2 – Gabrb3 – Gabrd – Gabre – Gabrg1 – Gabrg2 – Gabrg3 – Gabrp – Gabrq – Gabrr1 – Gabrr2 – Gad1 – Gad1os – Gad2 – Gadl1 – Slc32a1 – Slc6a1 – Slc6a11 – Slc6a12 – Slc6a13 – Sst – Sstr1 – Sstr2 – Sstr3 – Sstr4 – Sstr5 – Vip*.

For these genes of specific interest, we compared APP/PS1 mouse group with the littermate group at different ages (i.e. 1.5, 8 and 18 months) in the 2 brain regions of interest i.e. frontal cortex and hippocampus. At each age, expression levels are reported as log2 of the fold change between the transgenic and the littermate (logFC), average expression across both conditions and p values (corrected for multiple testing) for the differential expression between transgenic and littermates. The p values were computed using limma (Smyth 2004).

RESULTS

Electrophysiology: synaptic function and long-term potentiation (LTP)

At 1.5 months of age no statistically significant deficit was found in APP/PS1 mouse at the two time points of interest after induction of LTP (Figs 1A, 1C). However, we observed deficits in LTP at 8 months of age in APP/PS1 mouse as compared to littermates (Figs 1B, 1D). With induction of LTP, the slope of the fEPSP immediately after induction is increased to a ratio of 1.43±0.11 in littermates, which is significantly higher than the 1.16±0.04 increase observed in the APP/PS1 mouse (t_{22}=2.49, P<0.05). One hour after induction, magnitude of LTP induced in the littermates remained at 1.66±0.15 of normalized baseline, while in the APP/PS1 mice it remained significantly lower at 1.22±0.08 (t_{22}=2.78, P<0.05) (Fig. 1D). Two-way ANOVA, however, revealed no interaction between the age and genotype and/or recorded responses in the early and later stage of LTP.

While no change was observed at 1.5 months of age, a lower response was recorded at 8 months in the IO curve obtained from APP/PS1 mice when compared to littermates (Fig. 2A, 2B). This reduction was found to be statistically significant at stimulation strengths 50µA (littermate=173±23 µV, APP/PS1=–109±10 µV; t_{83}=2.60, P<0.05), 100 µA (littermate=–343±42 µV, APP/PS1=–202±22 µV; t_{83}=3.04, P<0.01) and 150 µA (littermate=–619±64 µV, APP/PS1=–397±42 µV; t_{73}=2.92, P<0.01). Two-way ANOVA revealed an interaction ($F_{(6, 475)}$=2.33, P<0.05) between the different stimulation strength ($F_{(6, 475)}$=101.4, P<0.0001) and age ($F_{(1, 4754)}$=11.95, P<0.001) in APP/PS1 mice but not in littermates.

Paired Pulse ratio (PPR), calculated as a ratio of the amplitude of the second to the first pulse at various inter-pulse-interval of stimulation, were observed to be significantly decreased in APP/PS1 mice at 8 months when compared to littermates, while no change is observed at 1.5 months of age (Fig. 2C, 2D). The observed changes, at 8 months of age, are recorded at lower stimulation intervals (corresponding to higher frequency) of 0.02 s (littermate=1.04±0.02, APP/PS1=1.16±0.07; t_{48}=2.10, P<0.05), 0.05 s (littermate=1.32±0.03, APP/PS1=1.50±0.10; t_{48}=2.32, P<0.05), 0.1 s (littermate=1.32±0.02, APP/PS1=1.48±0.08; t_{48}=2.56, P<0.05) and 0.2 s (littermate=1.20±0.02, APP/PS1=1.38±0.08; t_{48}=2.97, P<0.01). When applying age as a factor of changes in inter-stimulation interval, we observe an interaction ($F_{(8, 522)}$=10.72, P<0.0001) between age ($F_{(1, 522)}$=116.2, P<0.0001) in the littermates, i.e. higher PPR in the older littermates ($F_{(8, 522)}$=78.99, P<0.0001) while this interaction is not significant in the APP/PS1 cohort.

Electrophysiology: GABA inhibitor application

At 1.5 months of age we observed no difference in the rise time of the population spike with the application of GABA inhibitors either in littermates and transgenic. However, when the same experiment was repeated at 8 months of age we observe statistical difference in the rise slope of the population spike in APP/PS1 mice compared to littermate. This is represented in the inset graph (Fig. 3) at two predetermined point, i.e. at 5 min (littermate=1.06±0.01 μV/ms, APP/PS1=1.45±0.06 μV/ms; t_{41}=2.05, P<0.05) and 15 min (littermate=1.07±0.01 μV/ms, APP/PS1=2.04±0.14 μV/ms; t_{41}=2.09, P<0.05) into the application of the GABA inhibitor cocktail. Two-way ANOVA analysis revealed a significant difference in the interaction ($F_{(3, 128)}$=2.762, P<0.05) with respect to genotype ($F_{(3, 128)}$=2.86, P<0.05) and the application of the GABA inhibitors ($F_{(1, 128)}$=3.915, P<0.05) at 15 min into application. No interaction was observed between the effect of GABA inhibitor application and age or genotype.

Electrophysiology: GABA agonist application

At 8 months of age we investigated the effect of diazepam on the paired pulse ratio in slices. We observed

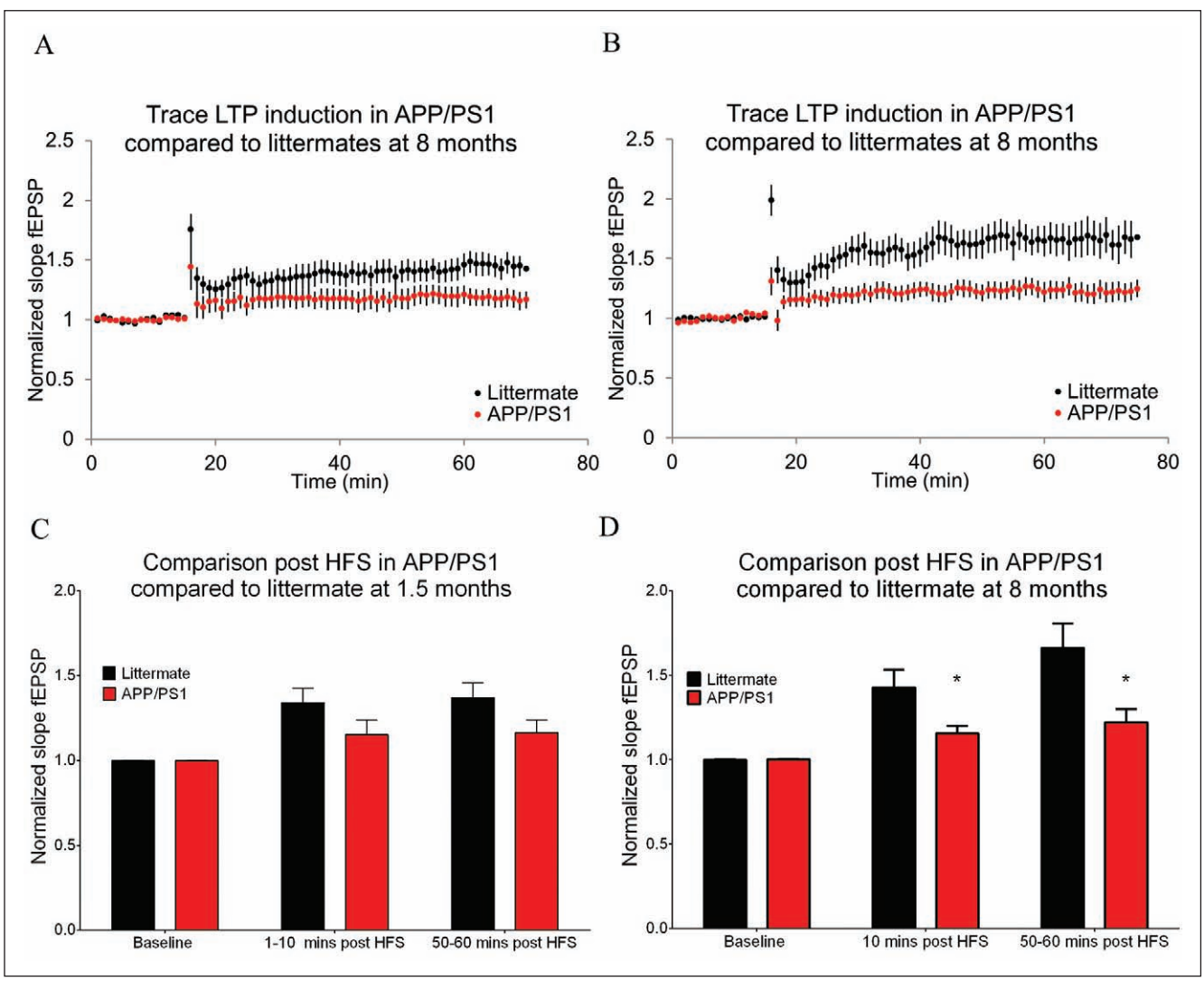

Fig. 1. LTP induction in APP/PS1 mouse model at 1.5 months and at 8 months of age. No significant difference is observed in the Long-term potentiation (LTP) when induced with a high frequency stimulation protocol at 1.5 months in APP/PS1 mice (n=5 animals; 14 slices) in comparison to littermates (n=6 animals; 17 slices) (see panels A and C). Using the same protocol, less LTP is recorded in APP/PS1 mice (n=5 animals; 13 slices) at 8 months of age when compared to age matched littermate (n=3 animals, 11 slices) (see panel B). Furthermore, at 8 months, statistical analysis shows deficits in the amount of LTP induced when normalized to baseline both at 1–10 min (littermates=1.43±0.11, APP/PS1=1.16±0.04; p<0.05) immediately after induction and at 50–60 min after induction (littermates=1.66±0.15, APP/PS1=1.22±0.08; p<0.05) (see panel D).

a significant decrease in the paired pulse ratio in the littermate mouse with application of diazepam (PPR: baseline=0.74±0.0.03; diazepam application=0.64±0.03; percentage change=10.±0.05%; t_{30}=2.38, P<0.05). This change was not significant in the APP/PS1 cohort (PPR: baseline=0.80±0.06; diazepam application=0.72±0.08; percentage change=7.3±0.05%). Using a 2-way ANOVA we observed statistical significance based on genotype ($F_{(1, 56)}$=6.22, P<0.05) and application of diazepam ($F_{(1, 56)}$=8.93, P<0.01) but no interaction between the two.

Microarray analysis of GABAergic pathway

In the hippocampus, at 8 and 18 months of age, we observe decreased expression level of 3 of the 40 selected genes of interest (i.e. *Cck*, *Sst* and *Vip*), none of which is involved in the pathology of the APP/PS1 in the hippocampus at 1.5 months of age (Table I). The expression levels of both *Sst* and *Vip* were decreased by 8 months of age while *Cck* is only affected at a much later time point (18 months). In comparison to the limited transcriptional effects in the hippocampus, we

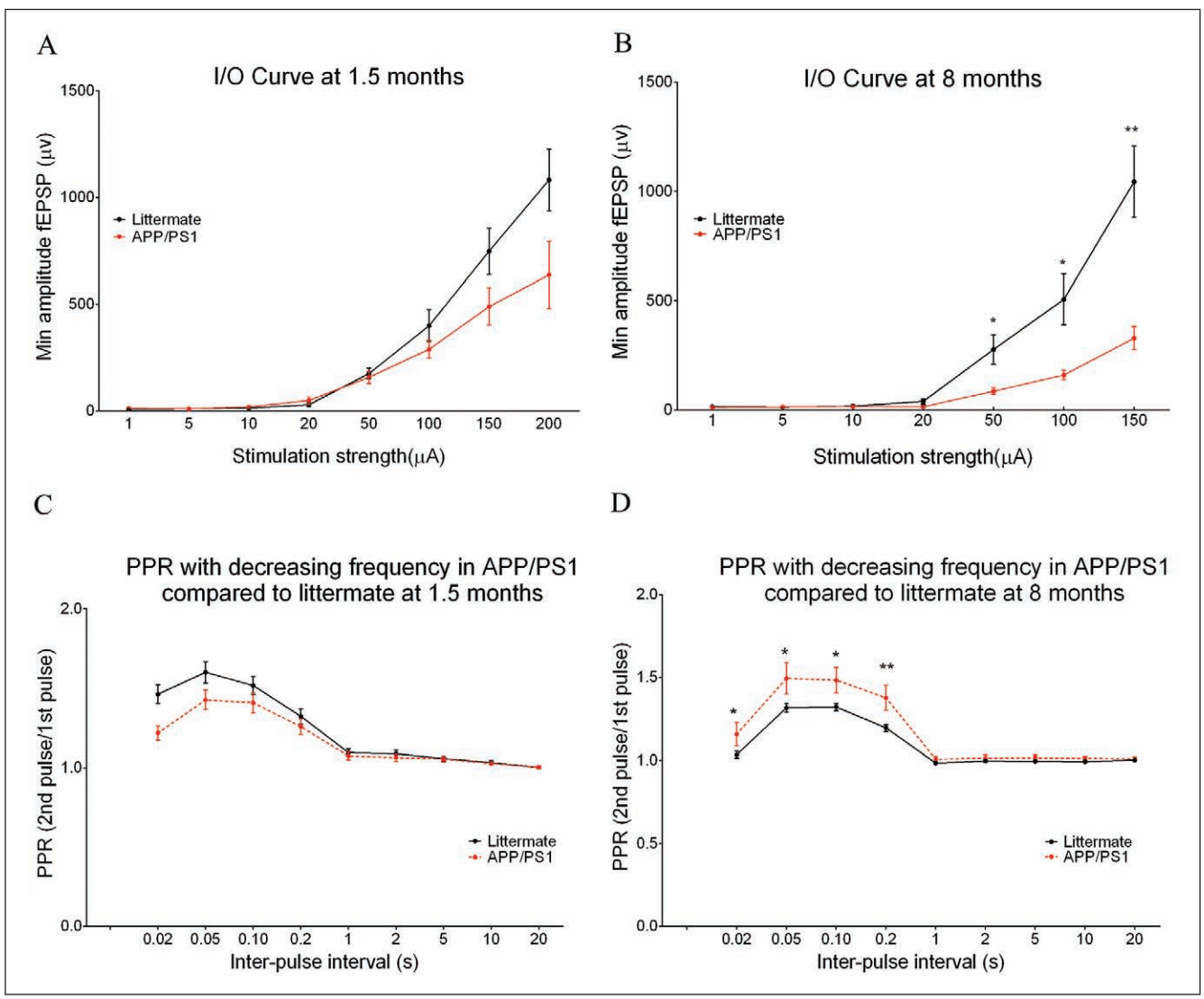

Fig. 2. Synaptic function is significantly altered in APP/PS1 mouse at 8 months but not at 1.5 months of age. The synaptic function is altered in APP/PS1 mice in comparison to littermates. Change in Input/Output (I/O) curve is not statistically significant at 1.5 months of age when comparing APP/PS1 cohort (n=5 animals; 14 slices) with age matched littermates (n=6 animals; 17 slices) (see panel A) similarly, no significant change was observed in the paired pulse ratio (PPR) at different frequencies of stimulation between APP/PS1 cohort and age matched littermates at this age (see panel B). When the same parameters are used, at 8 months of age we observed a lower response in the I/O curve obtained from APP/PS1 mice (n=5 animals; 13 slices) when compared to littermates (n=3 animals; 11 slices) (see panel B). This reduction is statistically significant at stimulation strengths 50 μA (littermate=173±23, APP/PS1=−109±10; p<0.05) 100 μA (littermate=−343±42, APP/PS1=−202±22; p<0.01) and 150 μA (littermate=−619±64, APP/PS1=−397±42; p<0.01), The PPR in the APP/PS1 cohort (n=8 animals; 33 slices) increased in comparison the age-matched littermate cohort (n=5 animals; 17 slices) when delivered at a frequency of 20–50 Hz (see panel D).

observe more transcriptional effects on these genes in the prefrontal cortex. Of the 40 genes of interest presented, 17 were observed to be statistically significant at one or more time points when comparing the APP/PS1 cohort with littermates (Table II). While no changes were observed in the expression levels of GABA receptor types in the hippocampus, we observed decreases in the expression of the genes coding for different GABA receptors in the prefrontal cortex. These include changes in the *Gabra1*, *Gabra3*, *Gabra4*, *Gabra5*, *Gabrb2*, *Gabrb3*, *Gabrg1* and *Gabrg2* gene transcription. Decrease was also observed in the GABA receptor associated protein *Gabarapl1* while the level of *Gabarap* was increased. Finally, we observed exclusively in the prefrontal cortex decreases in the expression of the somatostatin receptors *Sstr2* and *Sstr3*.

Results obtained from the microarray data, confirms an involvement of the GABAergic system in the pathology observed in the APP/PS1 mouse especially in the prefrontal cortex. Additionally, the lack of changes in the GABA receptors expression in the hippocampus further explains

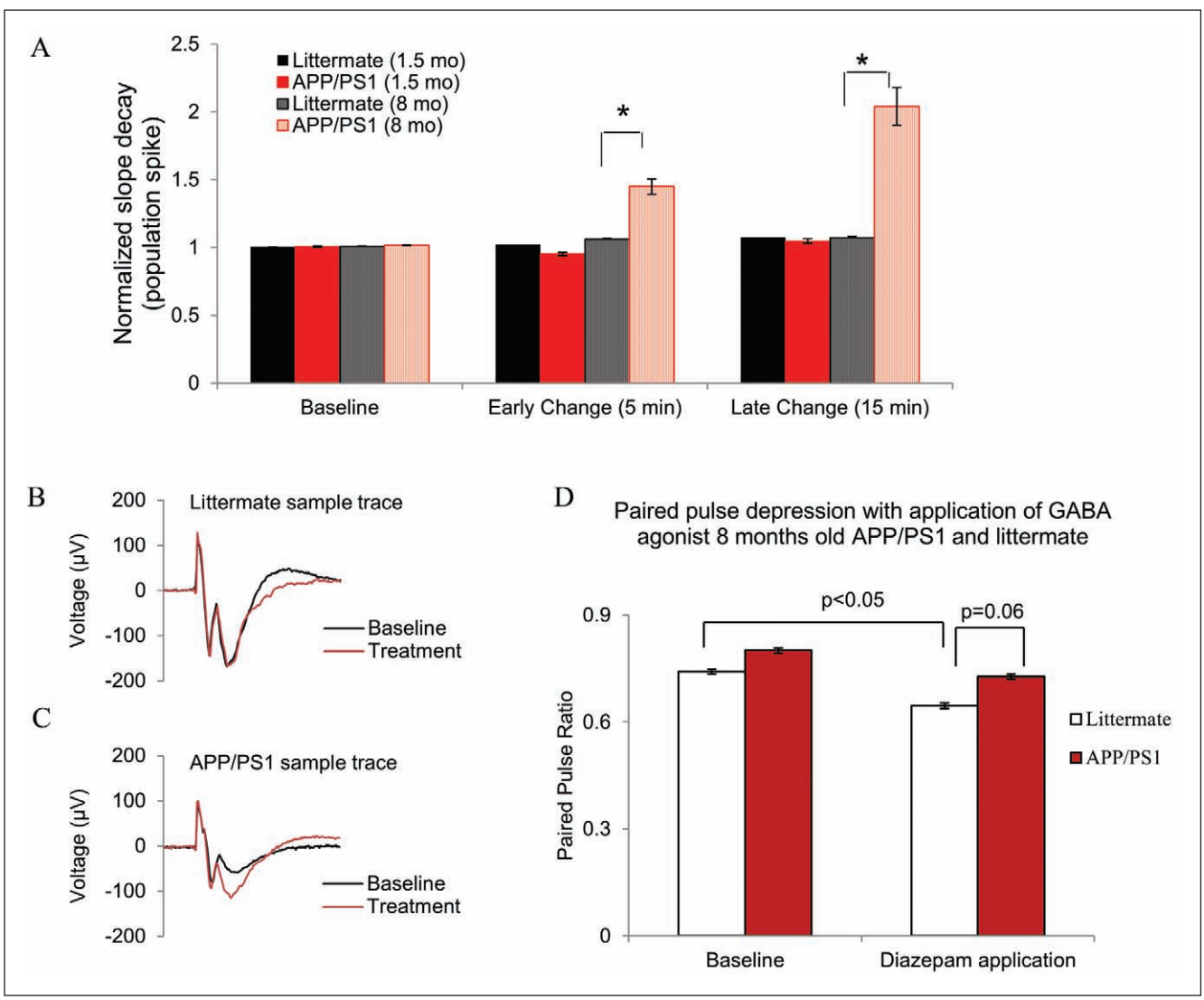

Fig. 3. Alteration in the slope of population spike in APP/PS1 mouse at 8 months with application of GABA inhibitor compared to littermates. By applying a low concentration of bicucculine (5 μM) and 2-hydroxysaclofen (20 μM) in slices obtained from APP/PS1 mouse at 1.5 months (APP/PS1: n=3 animals; 12 slices and littermates: n=3 animals; 13 slices) and 8 months (APP/PS1: n=6 animals; 17 slices and littermates: n=9 animals; 23 slices) we were able to monitor the age-dependent involvement of the GABA pathway in the pathology of APP/PS1 mice compared to littermates. No changes were observed in between the littermate and transgenic group at 1.5 months of age. However, an increase in the decay slope of the population spike is observed at 8 months of age (see panel A). Example traces from baseline and during application of the GABA antagonists in sample slice recorded from a littermate mouse and APP/PS1 mouse are provided (see panels B and C). The analysis of the changes in paired pulse depression in slices with application of benzodiazepine, a γ-aminobutyric acid receptor A (GABAA) agonist, at 8 months in APP/PS1 (n=6 animals; 12 slices) mouse cohort showed no statistical significance when compared to littermates (n=5 animals; 16 slices) (see panel D).

why we were unable to obtain immunohistochemistry difference in the hippocampus of this mouse model at 8 months of age.

DISCUSSION

Although all evidence from electrophysiological observations, such as a decrease in the input/output curve and a reduction in the probability of release, point to a decreased synaptic response in the APP/PS1 mouse, it is generally accepted that the observed synaptic depression is not sufficient in explaining all of the pathology observed in AD (Palop et al. 2007, Palop and Mucke 2009, 2010). Various mutations in the APP protein and over-production of the protein itself have both been shown to lead to an increase in seizure susceptibility in patients and in AD models (Palop et al. 2007, Irizarry et al. 2012). In a similar vein, the PS1 protein mutations and specifically mutations in the Leucine 166 also present in the specific mouse model used in this study have also been shown to result in an increase in the susceptibility to seizures (Rossor et al. 1996, Mann et al. 2001, Moehlmann et al. 2002). In the clinical setting, AD patients have been shown to exhibit seizures or seizure-like activity, indicative of an an increase in excitability that seemingly contradicts the well-documented synaptic depression in the mouse models as well as in AD patients (Amatniek et al. 2006, Minkeviciene et al. 2009, Palop and Mucke 2009). While the glutamatergic pathway is established to be deficit in the disease state, the documented alterations in the glutamatergic pathway alone fails to explain the synaptic depression in AD coupled with hyperexcitability (Hoxha et al. 2012).

Taken together the synaptic depression and hyperexcitability observed in the disease pathology, it has been hypothesized that the changes in the function of the excitatory glutamate pathway may be causally, related to or at least correlated with changes in the counterbalancing GABAergic inhibitory pathway (Amatniek et al. 2006, Minkeviciene et al. 2009, Verret et al. 2012, Hazra et al. 2013). Moreover, the preferential susceptibility of interneurons to Aβ toxicity has been previously documented (Davies et al. 1980, Ramos et al. 2006, Takahashi et al. 2010, Verret et al. 2012).

The present study found that synaptic depression resulting in LTP deficits and decreased response during stimulation in the APP/PS1 mouse was observed at 8 months, but not at 1.5 months. This finding confirms that the neuronal and functional pathology in the APP/PS1 mouse is age/pathology related (Trinchese et al. 2004). Increase in the short-term plasticity which corresponds to a reduced probability of release of neurotransmitters in the APP/PS1 mouse is observed at an older age of 8 months but not at 1.5 months: also in line with the progressive and pathology-related deficits in the functioning of the brain in AD. Previous studies have indeed reported that the altered synaptic plasticity known to occur in AD mouse models are in fact age/pathology dependent (Bergado and Almaguer 2002, Fitzjohn et al. 2010, Gengler et al. 2010). We report here specific changes that are statistically linked to the age/pathology in the APP/PS1 mouse model. Similar to the observed synaptic depression, the susceptibility to seizures in AD patients as well as in mouse models is increased with the progression of the pathology of the disease (Hauser et al. 1986, Amatniek et al. 2006, Cloyd et al. 2006). It is therefore reasonable to assume that changes in the GABAergic pathway are also subjected to the progressive pathology of AD.

In the second set of experiments conducted, we observed changes in the synaptic response with the blockade of the GABA pathway, i.e. an increased excitability of slices that is consistent with an increase in the seizure susceptibility/hyperexcitability observed in previous studies (Davies et al. 1980, Minkeviciene et al. 2009, Hoxha et al. 2012). The concomitant involvement of the GABAergic system and synaptic depression in AD pathology is further strengthened

Table I. Differential expression of GABA related genes in the hippocampus of APP/PS1 mice compared to littermate over 3 time points as observed by microarray based expression profiling

Hippocampus GABA related genes		1.5 months			8 months			18 months		
Symbol	Genename	logFC	Ave Expr	adj. P.Val	logFC	Ave Expr	adj. P.Val	logFC	Ave Expr	adj. P.Val
Sst	somatostatin	−0.23	9.83		−0.38	9.88	*	−0.80	9.71	***
Vip	vasoactive intestinal polypeptide	−0.01	7.80		−0.37	7.66	*	−0.35	7.65	
Cck	cholecystokinin	0.04	11.02		−0.13	10.91		−0.29	10.81	**

The time points studied are 1.5 months (littermate: n=8, APP/PS1: n=8), 8 months (littermate: n=8, APP/PS1: n=8), and 18 months (littermate: n=5, APP/PS1: n=6). The represented genes have been selected from a list of 40 genes of potential interest being of relevance to the γ-aminobutyric acid pathway (GABAergic). The listed genes are significant at one or more of the three time points. These include genes encoding for γ-aminobutyric acid (GABA) receptors, solute carriers and associated proteins. For each gene and at each time point, the log2 fold change (logFC), the mean expression across groups (AveExpr) and the p value for differential expression after correcting for multiple testing (adj.P.Val) are reported. The genes are ordered based on increasing p values at 8 months of age, followed by 18 months and finally at 1.5 months of age. Non-significant genes are excluded from the table. We observe very little change in the hippocampal expression of the selected genes. The observed differences were restricted to the interneuron related/specific genes *Vip*, *Sst* and *Cck*. No significant change was observed at 1.5 months of age.

Table II. Differential expression of GABA related genes in the prefrontal cortex of APP/PS1 mice compared to littermate over 3 time points as observed by microarray based expression profiling

Prefrontal cortex GABA related genes		1.5 months			8 months			18 months		
Symbol	Genename	logFC	Ave Expr	adj. P.Val	logFC	Ave Expr	adj. P.Val	logFC	Ave Expr	adj. P.Val
Cck	cholecystokinin	0.11	11.53		−0.33	11.21	***	−0.12	11.27	
Gabrb2	gamma-aminobutyric acid (GABA) A receptor, subunit beta 2	0.10	9.60		−0.32	9.44	***	−0.27	9.54	***
Sstr2	somatostatin receptor 2	−0.04	6.68		−0.82	6.35	***	−0.44	6.59	
Gabrb3	gamma-aminobutyric acid (GABA) A receptor, subunit beta 3	0.08	8.04		−0.31	7.88	***	−0.25	7.86	*
Gabarapl1	gamma-aminobutyric acid (GABA) A receptor-associated protein-like 1	0.01	10.56		−0.24	10.57	***	−0.07	10.67	
Gabra1	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 1	0.14	9.91		−0.42	9.81	***	−0.28	10.02	
Sst	somatostatin	−0.01	9.95		−0.49	9.55	***	−0.63	9.74	***
Gabrg2	gamma-aminobutyric acid (GABA) A receptor, subunit gamma 2	0.11	8.70		−0.41	8.48	***	−0.27	8.70	
Gabra5	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 5	−0.07	7.49		−0.33	7.37	***	−0.29	7.31	*
Gabra3	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 3	0.12	8.22		−0.40	8.12	***	−0.18	8.23	
Gabra4	gamma-aminobutyric acid (GABA) A receptor, subunit alpha 4	0.18	8.82		−0.25	8.48	**	−0.19	8.61	
Sstr3	somatostatin receptor 3	−0.05	4.10		−0.19	3.98	*	−0.08	3.92	
Gabarap	gamma-aminobutyric acid receptor associated protein	0.03	9.61		0.17	9.77	*	0.21	9.78	
Cckbr	cholecystokinin B receptor	0.12	7.38		−0.32	7.27	*	0.01	7.29	
Slc32a1	solute carrier family 32 (GABA vesicular transporter), member 1	−0.19	7.56		−0.18	7.62		−0.36	7.45	*
Gabrg1	gamma-aminobutyric acid (GABA) A receptor, subunit gamma 1	−0.21	6.12		0.06	6.23		−0.38	5.94	*
Slc6a11	solute carrier family 6 (neurotransmitter transporter, GABA), member 11	−0.55	6.87	*	0.23	7.25		−0.12	6.87	

The time points studied are 1.5 months (littermate: n=8, APP/PS1: n=8), 8 months (littermate: n=8, APP/PS1: n=8), and 18 months (littermate: n=5, APP/PS1: n=6). The represented genes have been selected from a list of 40 genes of potential interest being of relevance to the γ-aminobutyric acid pathway (GABAergic). The listed genes are significant at one or more of the three time points. These include genes encoding for γ-aminobutyric acid (GABA) receptors, solute carriers and associated proteins. For each gene and at each time point, the log2 fold change (logFC), the mean expression across groups (AveExpr) and the p value for differential expression after correcting for multiple testing (adj.P.Val) are reported. The genes are ordered based on increasing p values at 8 months of age, followed by 18 months and finally at 1.5 months of age. Non-significant genes are excluded from the table. We observe changes in several receptor subunits (*Gabra1*, *Gabarap*, *Gabara3*, *Gabara4*, *Gabara5*, *Gabrb2*, *Gabrb3*, *Gabrg1* and *Gabrg2*and *Gabrg2*); interneuron specific/related genes (*Cck*, *Cckbr*, *Sst*, *Sstr2* and *Sstr3*) and in the solute carrier for the GABA amino acid (*Slc6a11*). Almost all of these changes are recorded in the 8 and 18 months cohort while only the *Slc6a11* gene is significantly decreased at 1.5 months of age.

by previous study showing that manipulation of the GABAergic/inhibitory system can rescue LTP and memory deficiency in APP/PS1 mouse model (Meilandt et al. 2008, Yoshiike et al. 2008, Andrews-Zwilling et al. 2010, Tong et al. 2014). Our results confirm this finding, and in addition show that the involvement of the GABAergic system is restricted to a later point in the pathological progression of the disease (i.e. at 8 months and not at 1.5 months).

The present results concur with findings of studies that support an involvement of the GABAergic pathway in the pathology (synaptic depression) of the APP/PS1 mouse model (Yoshiike et al. 2008, Palop and Mucke 2009, Hoxha et al. 2012, Jo et al. 2014). Two possible hypotheses may aid in the interpretation of the results we obtained with the blockade of the GABAergic pathway: the first hypothesis would view the results in terms of a decreased GABAergic pathway innervation and function that is irrespective of possible changes in excitation. A second possible hypothesis and alternative interpretation is that there is an increased GABA pathway innervation/function with no change in the excitation.

The involvement of the glutamatergic pathway in the pathology of AD has previously been documented based on changes in the distribution of the different glutamate receptor types and also evident by the fact that Memantine, a NMDA agonist remains one the most successful symptomatic treatment for AD (Nankai et al. 1995, Blumcke et al. 1996, Winblad and Poritis 1999, Shankar et al. 2007). Thereby justifying the dismissal of the second hypothesis proposed above that the glutamatergic/excitatory pathway is unchanged. In fact, a partial augmentation of the GABAergic system by application of diazepam shows a trend towards a decreased functionality of the GABAA receptor at 8 months of age. In order to further confirm the first hypothesis on a decreased GABA pathway function, we studied the results obtained from the microarray analysis of GABAergic pathway-related genes. Previous research has established the loss of different interneurons or a reduction in the function of such interneurons in AD (Takahashi et al. 2010). Compared to the observed gene changes in the hippocampus, results from the microarray analysis show more differentially expressed genes in the prefrontal cortex of APP/PS1 mice as compared to the littermates. This hints towards a preferential involvement of the prefrontal cortex specifically in the changes observed in the GABAergic pathway in AD (Davies et al. 1980, Howell et al. 2000, Harris et al. 2010). In the hippocampus, GABAergic gene changes were restricted to decreases for 3 different interneuron markers, i.e. *Cck*, important for mood disorder and anxiety, *Vip and Sst* (Davies et al. 1980, Freund and Katona 2007). In the prefrontal cortex, the observed gene changes include decreases in different GABA receptor subunits and associating proteins, some of which have been previously reported (Yoshiike et al. 2008, Limon et al. 2012, Schwab et al. 2013).

Focusing on the differences in expression observed in the prefrontal cortex for the GABAergic genes, it is clear that the greatest effects are observed at the age of 8 months. This finding supports the age-dependent/disease progression as key factors in GABA pathology. At 1.5 months, only 1 of the 40 GABAergic genes is affected, i.e. *Slc6a11*, encoding for *Gat3*, a GABA transporter, expressed in astrocytes (Durkin et al. 1995, Borden 1996). *Gat3* is an important tonic modulator for GABAergic signaling in the hippocampus (Borden 1996, Kersante et al. 2013, Schwab et al. 2013). Thus, while at least this one change can be found at 1.5 months of age, majority of the changes in the pathway seem to occur at a later time point of the disease progression.

At the age of 8 months, the number of significantly affected GABAergic genes is higher than at the 2 other time points studied. Most, but not all, of the affected genes are in fact down regulated in the mutant mice. Overall, the decrease in the expression of GABA related genes indicates a decrease in the efficiency of the GABA pathway. This study further shows that these changes occur concomitantly with previously documented changes in the glutamatergic pathway.

From our findings, we report that APP/PS1 mouse model present deficits in synaptic function, some of which are affected with age. In addition, functional and transcriptional deficits were observed in the GABAergic pathways. These documented synaptic dysfunction in AD models is increased with age and concomitantly observed with excitation/inhibition imbalance therefore suggesting underlying evidence for observation of symptoms such as sleep disturbances, seizures and seizure-like effects observed in AD pathology (Amatniek et al. 2006, Palop et al. 2007, Palop and Mucke 2009, Irizarry et al. 2012).

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