ACCEPTED MANUSCRIPT

Running title: Loss of FKBP5 affects neuron synaptic plasticity

Loss of FKBP5 affects neuron synaptic plasticity: an electrophysiology insight

Bin Qiu[1, *], Yuxue Xu[1, *], Jun Wang[1,3], Ming Liu[1], Longyu Dou[1], Ran Deng[1], Chao Wang[1], Kent E. Williams[2], Robert B. Stewart[4], Zhongwen Xie[3], Wei Ren[6], Zhenwen Zhao[6], Weinian Shou[5], Tiebing Liang[2, #], Weidong Yong[1, #]

[1]Comparative Medical Center, Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100021, China

[2]Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA

[3] College of Life Sciences, Anhui Agricultural University, Hefei, Anhui 230036, China

[4]Department of Psychology, Purdue School of Science, Indiana University–Purdue University of Indianapolis, Indianapolis, Indiana 46202, USA

[5]Herman B Wells Center for Pediatric Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana, USA;

[6]Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry Chinese Academy of Sciences, Beijing 100190, China

* These authors contributed equally to this work.

[#] Correspondence should be addressed to:

- Weidong Yong, Ph.D.
  Institute of Laboratory Animal Science
  Chinese Academy of Medical Sciences& Peking Union Medical College Beijing 100021, China
  Tel/fax: +86 10 6776 2060
  Email: wyong@cnilas.org or yongwd@hotmail.com
- Tiebing Liang, Ph.D.
  Indiana Alcohol Research Center
  Department of Medicine
  Indiana University, School of Medicine
  Indianapolis, IN 46202, USA
  Tel: +1 317 274 7813
  Fax: +1 317 274 2695
  Email: tliang@iu.edu

Keywords
Fkbp5 KO, GABAergic, Glutamatergic, LTP, Synaptic Plasticity

This is the author's manuscript of the article published in final edited form as:
Qiu, B., Xu, Y., Wang, J., Liu, M., Dou, L., Deng, R., … Yong, W. (2019). Loss of FKBP5 affects neuron synaptic plasticity: an electrophysiology insight. Neuroscience. https://doi.org/10.1016/j.neuroscience.2019.01.021

ACCEPTED MANUSCRIPT

Abstract

FKBP5 (FKBP51) is a glucocorticoid receptor (GR) binding protein, which acts as a co-chaperone of heat shock protein 90 (HSP90) and negatively regulates GR. Its association with mental disorders has been identified, but its function in disease development is largely unknown. Long-term potentiation (LTP) is a functional measurement of neuronal connection and communication, and is considered one of the major cellular mechanisms that underlies learning and memory, and is disrupted in many mental diseases. In this study, a reduction in LTP in *Fkbp5* knockout (KO) mice was observed when compared to WT mice, which correlated with changes to the glutamatergic and GABAergic signaling pathways. The frequency of mEPSCs was decreased in KO hippocampus, indicating a decrease in excitatory synaptic activity. While no differences were found in levels of glutamate between KO and WT, a reduction was observed in the expression of excitatory glutamate receptors (NMDAR1, NMDAR2B and AMPAR), which initiate and maintain LTP. The expression of the inhibitory neurotransmitter GABA was found to be enhanced in *Fkbp5* KO hippocampus. Further investigation suggested that increased expression of GAD65, but not GAD67, accounted for this increase. Additionally, a functional GABAergic alteration was observed in the form of increased mIPSC frequency in the KO hippocampus, indicating an increase in presynaptic GABA release. Our findings uncover a novel role for *Fkbp5* in neuronal synaptic plasticity and highlight the value of *Fkbp5* KO as a model for studying its role in neurological function and disease development.

ACCEPTED MANUSCRIPT

Introduction

FKBP5 (FK506-binding protein 51, also known as FKBP51) belongs to a subclass of immunophilin proteins and exhibits peptidyl-prolyl *cis–trans* isomerase (PPIase) activity crucial for protein folding (Schiene and Fischer 2000). It functions as a co-chaperone of heat shock protein 90 (HSP90) and forms a glucocorticoid receptor (GR) complex with additional components (Reynolds, Ruan et al. 1999, Westberry, Sadosky et al. 2006, Stechschulte and Sanchez 2011). Previous research revealed that FKBP5 is highly expressed in the hippocampus (Scharf, Liebl et al. 2011) and it appears to be essential for hypothalamic-pituitary-adrenal (HPA) axis function, including the physiological stress response that shapes neuroendocrine reactivity and coping behavior (Binder 2009, Costin, Wolen et al. 2013). In humans, single nucleotide polymorphisms (SNPs) within the *FKBP5* gene are associated with increased recurrence of depressive episodes and increased susceptibility to post-traumatic stress disorder (PTSD), bipolar disorder, major depressive disorder, and suicide attempts (Binder, Salyakina et al. 2004, Binder, Bradley et al. 2008, Lekman, Laje et al. 2008, Tatro, Everall et al. 2009, Willour, Chen et al. 2009, Costin, Wolen et al. 2013, Ellsworth, Moon et al. 2013, Szczepankiewicz, Leszczynska-Rodziewicz et al. 2014). *FKBP5* has also been implicated in the development of addiction and PTSD-alcohol use disorder comorbidity (Xie, Kranzler et al. 2010, McClintick, Xuei et al. 2013, Levran, Peles et al. 2014), as well as alcohol consumption (Qiu, Luczak et al. 2016) and alcohol withdrawal severity (Huang, Schwandt et al. 2014).

ACCEPTED MANUSCRIPT

The associations of FKBP5 with these conditions suggests a critical role in neuroadaptation following stress, alcohol, or other insults. Animal studies have revealed that *Fkbp5* mRNA expression is increased in the hippocampus following the stress of chronic social defeat (Wagner, Marinescu et al. 2012) and increased in the paraventricular nucleus (PVN) and central amygdala (CeA) following restraint stress (Scharf, Liebl et al. 2011). Our studies have found that relative to WT mice, *Fkbp5* KO mice consume more alcohol and suffer more severe alcohol withdrawal as measured by handling-induced convulsions (HICs) following both acute and chronic alcohol exposure (Huang, Schwandt et al. 2014, Qiu, Luczak et al. 2016). The expression level of FKBP5 has been correlated with several mental illnesses (Ising, Depping et al. 2008, Lekman, Laje et al. 2008, Binder 2009, Levran, Peles et al. 2014), and is responsive to stress, alcohol, and morphine (Treadwell and Singh 2004, McClung, Nestler et al. 2005, Balsevich, Uribe et al. 2014). Even though elimination of *Fkbp5* has been found to elicit some behavioral changes (Hartmann, Wagner et al. 2012), electrophysiological examination and molecular analyses are necessary to ascertain differences in neuronal function and neurotransmitter regulation, respectively.

Long-term potentiation (LTP) is critical in learning and memory, and its dysfunction underlies many mental diseases. LTP is defined as an increase in postsynaptic responses lasting hours to days following a high-frequency activation of excitatory synapses (Bliss and Gardner-Medwin 1973, Bliss and Lomo 1973), and is thought to be the functional basis underlying memory formation (Bliss and Collingridge, 1993; Bliss et al., 2014). In addition to variations in LTP, differences in neurotransmitter activity, particularly the glutamatergic (Nakanishi 1994, Swanson, Bures et al. 2005, Gos, Gunther et al. 2009) and GABAergic (Saba, Bennett et al.

ACCEPTED MANUSCRIPT

2011) systems require investigation to understand brain function. A variety of NMDA receptor subunits have been identified: the ubiquitously expressed NR1 subunit; a family of four distinct NR2 subunits (A, B, C, and D); and two NR3 subunits (Moriyoshi, Masu et al. 1991, Sugihara, Moriyoshi et al. 1992, Das, Sasaki et al. 1998). All NMDARs appear to function as heteromeric assemblies composed of multiple NR subunits (Das, Sasaki et al. 1998). NMDAR1 is necessary for plasticity in the CA1 region (McHugh, Blum et al. 1996, Tsien, Huerta et al. 1996). Deletion of NR2B is associated with impairment of LTP in hippocampus (Li, Erzurumlu et al. 1994, Kutsuwada, Sakimura et al. 1996), conversely, overexpression of NR2B enhances LTP and has been shown to enhance learning and memory (Tang, Shimizu et al. 1999). In addition, activation of the GABAergic system, particularly enhanced GABA release and GABA receptor trafficking, contributes to alcohol consumption (Enoch 2008, Saba, Bennett et al. 2011) and other mental illnesses (Sajdyk, Johnson et al. 2008, Luscher, Shen et al. 2011, Abdallah, Jackowski et al. 2015). GABA synthesis may also play a key role in maintaining a high level of GABA activity. However, these signaling systems have not previously been studied in *Fkpb5* KO mice, and little is known about the overall impact of *Fkbp5* on synaptic output, which may be crucial for understanding its role in disease development.

In this study we have examined LTP function in *Fkbp5* KO and WT mice, and measured alterations in the GABAergic and glutamatergic systems. The levels of Glutamate and GABA were measured, and NMDA receptor expression levels and GABA synthesis enzymes were quantified. Behavioral differences were also measured.

Materials and Methods

Animals

ACCEPTED MANUSCRIPT

All experimental protocols were reviewed and approved by the Animal Care and Research Advisory Committee in the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences, and the Indiana University School of Medicine. The animals were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). Development of *Fkbp5* knockout (*Fkbp*5 KO) mice was described in a previous publication (Yong, Yang et al. 2007). *Fkbp*5 KO and WT littermates were bred through heterozygous mating and were back crossed to C57BL/6J inbred mice for at least 5 generations.

Brain slice preparation for electrophysiological measurement

Brain slices for electrophysiology were prepared from WT and KO male mice at 8 weeks of age as described previously (Hou et al., 2006). In brief, mice were deeply anaesthetized and brains were rapidly removed from the skull and transferred into ice-cooled artificial cerebrospinal fluid (ACSF) (in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 10 D-Glucose, 2.5 $CaCl_2$, and 1.5 $MgCl_2$) and saturated with 95 % O_2 and 5 % CO_2 at pH 7.3. Transverse slices of 400 μm thickness containing the hippocampus were cut with a Vibratome (Leica, VT 1000 S, Germany), which was filled with cold ACSF. The prepared slices were incubated in oxygenated ACSF at room temperature for at least 1 hour before being transferred to a recording chamber and bubbled with oxygenated ACSF at 32 ± 1 ºC.

Recording Long term synaptic plasticity response

Field excitatory postsynaptic potential (fEPSP) population responses were evoked by stimulation in the radiatum with a bipolar electrode placed on the CA3 area using acute brain slices (two slices per animal) prepared from WT (N=3) and

ACCEPTED MANUSCRIPT

KO (N=3) male mice at 8 weeks of age; a total of 6 recordings from each genotype. Extracellular recording electrodes were filled with 2 M NaCl and placed in the stratum radiatum of CA1. Data were digitized at 3 kHz using an Axopatch 700B amplifier and analyzed with Clampfit 10.5 software. Single test pulses at a stimulation intensity that elicited 40% of a maximal response of fEPSP slope were delivered as the baseline level. The slices were stimulated with single test pulses every 30 s for at least 30 min followed by Theta-burst stimulation (TBS) and 60 min of test stimulation without changing intensity. All TBS contained 10 bursts at 200 ms inter-stimulation intervals, in which one burst consisted of four pulses of 100 Hz, repeated 3 times with 10 s inter-stimulation intervals. The time course of changes in the fEPSP slope was calculated in relation to the signals obtained during the last 10 min prior to TBS (100%), normalizing all responses to this baseline and then averaging across experiments. The degree of LTP was expressed as a percentage of the original control level. All changes in long-term synaptic plasticity were evaluated by averaging the 10 responses at 51-60 min post-TBS and comparing these data to the 10 control signals during the 10 min prior to TBS. All data are presented as means ± SEM. Student's t-test was performed for statistical evaluation of the data.

Analysis of L-glutamic acid content in hippocampus using LC–MS/MS

Both sides of hippocampi (10 mg) from WT (n=3) and KO (n=3) were homogenized with 800 μL of 80% acetonitrile (containing 0.2% formic acid and 5 mM ammonium formate) and further extracted by ultrasonication for 5 min. After vortex and centrifugation at 13,225 g for 10 min at room temperature, the supernatants were collected for L-glutamic acid measurement. Aliquots of 5 μL were injected onto the LC-MS/MS system. Liquid chromatography was performed with the LC system (I-

ACCEPTED MANUSCRIPT

class Acquity ultra performance liquid chromatography, Waters) including an autosampler and ultra-high performance binary pump. MS/MS detection was performed on an API 4500 QTRAP mass spectrometer (Applied Biosystems/MDS SCIEX) equipped with a heated electrospray ionization (ESI) source operated in the positive ionization mode. Nitrogen was used as the nebulizer and desolvation gases. Typical operating parameters were set as follows: curtain gas (CUR) 10, collision gas (CAD) medium, temperature 300 °C, ion source gas 1 (GS1) 45, ion source gas 2 (GS2) 50, and electrospray voltage 5500 V. The ion transitions were m/z 148.1→84.0 for L-glutamic acid (collision energy = 21 V). The peak areas of different concentrations of L-glutamic acid (0.008 ng/mL-20 ng/mL, Sigma-Aldrich, Saint Louis, MO, USA) analyzed by QTRAP 4500 were collected to establish standard curves and further calculate the concentrations of each analyte in real samples.

Western blotting analysis

Hippocampi were harvested on ice in lysis buffer (Beyotime, Jiangsu, China) with 1:10 volume of protease inhibitor (S8800, Sigma-Aldrich, Saint Louis, MO, USA) and 1:100 volume of phosphatase inhibitor cocktail (P0044, Sigma-Aldrich, Saint Louis, MO, USA). After centrifugation, the supernatants were collected and protein concentrations were determined using a BCA kit (Beyotime, Jiangsu, China). The samples were mixed with loading buffer and denatured. Proteins (40 μg each lane) were separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels (Tanon equipment) and electrically transferred onto a nitrocellulose membrane (0.2 μm) (Pall Corporation, Ann Arbor, MI). Membranes were blocked prior to immunoblotting with primary antibodies at 4^{o}C overnight. Blots were then incubated with HRP-conjugated secondary antibodies (1:5000, Santa Cruz, CA) and proteins were detected using ECL western blotting reagent kits. Signals were monitored by the Tanon 5500

ACCEPTED MANUSCRIPT

Chemiluminescent Imaging System (Tanon, Shanghai, China) and quantified using *TanonGis* software (Tanon, Shanghai, China). Density values were normalized to GAPDH signal and provided as the mean ± SEM. Antibodies used in this study: anti-AMPA (1:500), anti-NMDAR1 (1:500), anti-NMDAR2B (1:1000), anti-GAD65 (1:500), anti-GAD67 (1:500), and anti-GAT1 (1:1000) antibodies were purchased from Abcam (Cambridge, UK). HRP-conjugated anti-GAPDH (1:5000) was purchased from KangChen Bio-tech, Inc. (Shanghai, China).

Immunolabeling of brain slices

Immunohistochemical (IH) labeling of brain slices from male mice at 8 weeks of age of both WT (N =3) and KO (N = 3) were performed as previously described (Bin Qiu, 2016). Anti-GABA (1:200) antibody was purchased from Abcam (Cambridge, UK). For immunofluorescence (IF), male mice at 8 weeks age of both WT (N =3) and KO (N = 3) genotypes were anesthetized with an i.p. injection of tribromoethanol (20 mg/ml, 0.018 ml/g BW), and perfused transcardially with PBS (pH 7.4, 4^{o}C) followed by 4% paraformaldehyde (pH 7.4, 4^{o}C). The brains were isolated, embedded in OCT, and sectioned at 10 µm thickness using a cryostat microtome (Leica CM3050S, Germany). Slices were mounted on 3-aminopropyltriethoxysilane (APES) coated slides, blocked, and incubated with primary antibodies overnight at 4^{o}C and secondary antibodies for 1h at 37^{o}C. Nuclei were stained with DAPI mounting media (Zhongshan Goldenbridge Biotechnology, China). The fluorescence signal was captured using confocal laser scanning microscopy (Leica TCS LSI, Germany). The antibodies used in these experiments include rabbit anti-NMDAR1 (1:25), rabbit anti-NMDAR2B (1:25), rabbit anti-AMPAR (1:25), rabbit anti-GABA, and Alexa Flour® 488-conjugated goat anti-Rabbit IgG (1:500) from Abcam (Cambridge, UK). The optical density (AOD) of GABA was quantified by ImageJ.

ACCEPTED MANUSCRIPT

Recording of miniature excitatory postsynaptic currents

Miniature excitatory postsynaptic currents (mEPSCs) of CA1 pyramidal cells were recorded in whole cell mode using acute brain slices (two slices per animal) prepared from WT (N=4) and KO (N=4) male mice at 8 weeks of age as described above; a total of 8 recordings per genotype. The electrophysiological recordings were obtained under visual control by use of an Olympus microscope (Olympus BX50-WI, Olympus, Japan) and a 40x long-working distance objective (NA 0.8). Patch pipettes with 4-6 MΩ resistance were pulled from 110 mm long borosilicate glass capillaries (GB 150F-86-10, Sutter instrument, USA). The ion currents were recorded by an Axopatch 700B amplifier and pClamp10.6. Only cells that showed a high seal resistance (>1 GΩ) and a series resistance <25 MΩ were included. The series- and input-resistances were checked before and after the recordings in each experimental sequence. Cells were excluded if the input resistance or series resistance changed more than 15 % throughout the experiment. Signals were obtained at a holding potential of -70 mV. For pharmacological isolation of AMPA receptor-mediated mEPSCs, 1μM Tetradotoxin (TTX, the voltage-gated sodium channel blocker) and 100 μM picrotoxin (PTX, GABAA receptor antagonist) were added to the ACSF to abolish action potentials and inhibitory postsynaptic current events, respectively. The intracellular solution consisted of the following: (in mM) 140 K-gluconate, 2 $MgCl_2$, 8 KCl, 10 HEPES, 0.2 NaGTP, and 2 Na_2ATP. The pH was adjusted to 7.3 with KOH. For each cell, at least 5 minutes of recording was obtained. For the detection of spontaneous events, the “threshold research” option was used and each event was checked. Data were analyzed off-line by using pClamp10.6 for event Frequency and Amplitude. Unpaired t-test was used for

ACCEPTED MANUSCRIPT

statistical analysis. Results are presented as mean ± SEM, and significance was defined as $p<0.05$.

Recording of miniature inhibitory postsynaptic currents

Miniature inhibitory postsynaptic currents (mIPSCs) of CA1 pyramidal cells were recorded in whole cell mode using acute brain slices (two slices per animal) prepared from WT (N=4) and KO (N=4) male mice at 8 weeks of age as described above. The signal was obtained at a holding potential of -70 mV. For pharmacological isolation of $GABA_A$ receptor-mediated spontaneous inhibitory postsynaptic currents, 10nM glycine, 20 μM DNQX (AMPA receptor antagonist), 25 μM D-AP-5 (NMDA receptor antagonist), and 0.5μM TTX were added to the ACSF. The intracellular solution consisted of the following: (in mM) 135 CsCl, 10 HEPES, 2 $MgCl_2$, 20 TEACl, and 10 EGTA. The pH was adjusted to 7.3 with CsOH with a pipette resistance of 4-5MΩ. The non-parametric Mann-Whitney U test was performed for mIPSC statistical analysis. Results are presented as mean ± SEM and $p<0.05$ was considered significant. Cumulative distribution plots of mIPSC amplitudes and inter-event intervals were compared using the Kolmogorov-Smirnov Goodness of Fit Test.

Saccharin and quinine consumption test and the forced swimming test (FST)

Saccharin and quinine intake was tested in adult male KO (N= 14) and WT (N= 21) mice. Animals were individually caged with free choice of water and 1.03% (W/V) saccharin. Fluid intake was recorded twice during the 1 week test (Pelz, Whitney et al. 1973). Forced swimming test was performed using 3-month-old male KO (N=12) and WT (N=12) mice. Animals were individually placed in a 2-Liter glass beaker filled with water (22 ± 1 °C) to a height of 15 cm, so that the mouse could neither touch the

ACCEPTED MANUSCRIPT

bottom nor escape. The test lasted for 5 min and the time spent floating versus struggling was recorded. The 'floating behavior' (where the animal remains almost immobile and with its head above water) was used as a parameter to analyze behavioral differences.

Statistical analysis

Unless otherwise noted, all values are presented as mean ± standard error of the mean (SEM). Differences between two groups were compared by Student's t-test with GraphPad Prism (GraphPad Software Inc., San Diego, CA). *P* values less than 0.05 were considered to be significant.

Results

LTP is decreased in *Fkbp5* KO hippocampus

Responses to drugs and stress often present with aberrations in LTP, which may indicate the activation of a common substrate, resulting in alterations of synaptic strength (Nestler 2001, Wolf 2003, Niehaus, Murali et al. 2010). To investigate whether elimination of the *Fkbp5* gene produces dysfunctions in LTP, electrophysiological testing was carried out on brain slices of WT and *Fkbp5* KO mice. Stimulation electrodes were placed in the CA3 region of the hippocampus, and recording electrodes were placed in CA1 (Figure 1A), producing a typical change in evoked responses following LTP. Field excitatory postsynaptic potentials (fEPSPs) were recorded in the hippocampi of both WT and KO mice (Figure 1B and 1C). Relative to WT, the fEPSP slope was significantly lower in KO following TBS stimulation (Figure 1D). The bar graphs summarize the differences in percentage of fEPSP slope before and after TBS (Figure 1E). The data indicate that KO mice display significant reductions in LTP.

ACCEPTED MANUSCRIPT

Decreased expression of glutamate receptors in *Fkbp5* KO hippocampus

Classical synaptic LTP requires glutamate and NMDA receptor (NMDAR) activation, which drives increased AMPA receptor (AMPAR) expression in the postsynaptic membrane (Bashir, Bortolotto et al. 1993). NMDA receptor activation occurs when it binds with glutamate and glycine (or D-serine), allowing positively charged ions to flow through the cell membrane. To determine the source of the reduced LTP observed in KO, we first measured the glutamate level in the hippocampi of KO and WT mice. However, no differences of glutamate content, measured by L-glutamic acid, were observed (Figure 2A). Further analyses were conducted to determine whether the observed reductions in LTP are associated with alterations in excitatory glutamate receptors in *Fkbp5* KO mice. Indeed, significant reductions in NMDAR1, NMDAR2B, and AMPAR protein expression in the hippocampus were identified in *Fkbp5* KO mice via Western blot (Figure 2B-D). Concurrent results were evident in immunofluorescence (IF) labeling. The majority of neurons in the CA1 and DG sub-regions displayed an abundance of NMDAR1 (Figure 2E), NMDAR2B (Figure 2F), and AMPAR (Figure 2G) expression in WT mice, while the expression of these proteins was considerably reduced in *Fkbp5* KO mice (Figure 2E-G). These results suggest that the decreased LTP observed in *Fkbp5* KO mice may be partially due to lower expression levels of excitatory glutamate receptors

Decreased frequency of mEPSCs in *Fkbp5* KO mice

Given the lack of change in glutamate abundance coupled with decreased expression of glutamate receptors in *Fkbp5* KO hippocampus, we next investigated the functional glutamatergic synapse alterations in KO hippocampus, particularly

ACCEPTED MANUSCRIPT

those that might account for the observed attenuation of LTP. At a holding potential of -70 mV, AMPA receptor-mediated mEPSCs were monitored with the addition of pharmacological agents (1μM TTX, 100 μM PTX) (Figure 3A). Compared with WT mice, the mEPSC frequency was significantly lower in KO mice (WT: 2.31 ± 0.18 Hz; KO: 1.33 ± 0.15 Hz, $p < 0.001$, Unpaired t-test) (Figure 3B and 3C), but no differences were observed in mEPSC amplitude (WT: 18.89 ± 1.15 Hz; KO: 17.23 ± 1.44 Hz, $p = 0.3809$, Unpaired t-test) (Figure 3D and 3E). A reduced mEPSC frequency is usually interpreted as a decrease in the presynaptic release probability, however this is not consistent with the similar glutamate content observed between the WT and KO. An alternative explanation for the lower mEPSC frequency observed in KO is a reduction in the number of functional synaptic sites, which is consistent with the decreased expression of glutamate receptors in KO. Moreover, the lack of difference in the amplitude of the mEPSCs between WT and KO indicates a lack of change in the activation of the postsynaptic glutamate receptors that are present. Taken together, the reduced LTP in KO is likely due to a decreased number of functional excitatory synaptic sites, but is not associated with a change in the activation of postsynaptic glutamate receptors.

Increased GABA level in *Fkbp5* KO hippocampus

Normal central nervous system function requires maintaining a balance between neuronal excitation and inhibition. Because GABA is the major inhibitory transmitter in the CNS, we examined GABA in *Fkbp5* KO mice via IHC labeling. An increase in GABA was detected in the KO hippocampus relative to WT (Figure 4A and 4B). The magnified CA1, CA2, CA3, and DG sub-regions also displayed these differences, which were quantified (Figures 4C). In line with the observed increase in KO hippocampal GABA level, KO mice exhibited an increase in the expression of

ACCEPTED MANUSCRIPT

GAD65 (also known as glutamate decarboxylase 2), an enzyme that catalyzes the decarboxylation of glutamate to GABA (Figure 5A). In addition to GAD65, KO mice also appeared to possess a slightly higher expression of another enzyme, GAD67 (also known as glutamate decarboxylase 1), although this difference did not reach statistical significance (Figure 5B). Likewise, no significant difference in GABA transporter GAT1 expression could be detected between KO and WT (Figure 5C). These results provide evidence in support of enhanced GABA production in the presynaptic terminal partially via increased GAD65 enzyme in *Fkbp5* KO mice.

Increased frequency of mIPSCs in *Fkbp5* KO mice

Based on the observed increase in GABA level, we next investigated the functional GABAergic alterations present in KO hippocampus, to determine whether such alterations could account for the previously observed attenuation of LTP. Hippocampal slices obtained from animals at 8 weeks of age were assessed using whole cell recording techniques. At a holding potential of -70 mV, miniature IPSCs (mIPSCs) were detected as fast inward currents, which could be blocked by application of 10 μM bicuculline, a competitive $GABA_A$ receptor antagonist (Figure 6A). This result indicated that the mIPSCs were mediated via $GABA_A$ receptor activation. Cumulative probability analysis revealed a significant reduction in mIPSC inter-event intervals, indicating an increased frequency of these signals in KO mice (11.5 ± 1.2 Hz) when compared to WT (8.2 ± 0.5 Hz, $p<0.05$, Kolmogorov-Smirnov (KS)) (Figure 6B and 6C). However, the amplitudes of the mIPSCs demonstrated no significant differences between the two groups (KO: 21.17 ± 1.35 pA; WT: 21.2 ±1.13 pA, $p= 0.99$) (Figure 6D and 6E). The increased frequency of mIPSCs in *Fkbp5* KO mice is consistent with increased GABAergic synaptic activity and could indicate a change in the probability of transmitter release. This is in agreement with the

ACCEPTED MANUSCRIPT

observed increase in KO hippocampal GABA. The unchanged amplitude of mIPSCs suggests that postsynaptic function is apparently unaffected.

Fkbp5 KO male mice display behavior differences compared to WT mice

SNPs in the *FKBP5* gene have been associated with PTSD, depression, anxiety, and bipolar disorder (Binder, Bradley et al. 2008, Willour, Chen et al. 2009, Tatro, Nguyen et al. 2010). Given these associations and the role of GABAergic signaling in these illnesses, it was important to examine behavioral differences between male WT and *Fkbp5* KO mice. Depression-like behavior was assessed using an anhedonia test and the forced swim test (Porsolt, Le Pichon et al. 1977, Lucki 1997). When given free access to water and saccharin, KO male mice exhibited reduced total saccharin consumption via t-test ($p<0.01$) (Figure 7A); and this difference was not due to differences in taste sense as measured by quinine intake (Figure 7B). These results indicate that *Fkbp5* KO male mice display decreased anhedonic behavior. FST has been used to assess the effects of genetic modification on depressive behavior in animals (Porsolt et al., 1977) or the learned immobility to adapt successfully to the inescapable situation (Cryan and Mombereau, 2004). Male KO mice spent significantly more time floating than WT by t-test (p = 0.01) (Figure 7C).

Discussion

FKBP5 plays an important role in various mental illnesses, including PTSD, anxiety, depression, and addiction. It also has important effects on signaling pathways and neuron development. Its role in the homeostatic plasticity of the glutamatergic and GABAergic systems has not previously been explored. In the present study, we discovered that mice lacking *Fkbp5* exhibit reduced LTP,

ACCEPTED MANUSCRIPT

associated with decreased mEPSCs and increased GABAergic synaptic function in the hippocampus. Saccharin consumption and FST behaviors were found to be impacted in the KO male mice. Our findings uncover a role for *Fkbp5* in neuronal synaptic plasticity and highlight the value of *Fkbp5* KO as a model for studying neurological disease.

It has been established that the induction of LTP in the CA1 area of the hippocampus requires glutamate and the activation of NMDA receptors located in the cell membrane of the postsynaptic neuron. NMDA influx triggers an increase in calcium entry, leading to activation of postsynaptic molecular pathways, and increased postsynaptic AMPAR density, which is responsible for a persistent increase in the postsynaptic response (Bliss & Collingridge, 1993). In this study, a reduction of LTP was observed in *Fkbp5* KO mice, suggesting altered neural function in the *Fkbp5* KO hippocampus. As no differences in glutamate abundance were observed between WT and KO, and a reduction in the expressions of excitatory receptors (NMDAR1, NMDAR2B, and AMPAR) were found in KO, we conclude that the synthesis of glutamate is not altered in the ablation of *Fkbp5* , and that the reduced LTP may be partially due to reductions of NMDAR1 and NMDAR2B levels in the postsynaptic membrane. Western blotting and immunofluorescence data indicated that *Fkbp5* KO affects the molecular expression of these receptors in hippocampus (Fig. 2), resulting in altered neuronal activity. In addition to NMDA receptors, AMPA receptors on the postsynaptic membrane are required to drive LTP (Isaac, Nicoll et al. 1995, Liao, Hessler et al. 1995). Consistent with our observations, the reduction of AMPAR in *Fkbp5* KO may also contribute to reduced LTP. Moreover, a significant decrease in mEPSC frequency was observed in KO

ACCEPTED MANUSCRIPT

mice. Reduced mEPSC frequency indicates either a decrease in the presynaptic release probability or a decreased number of functional synaptic sites.

GABAergic activity also plays a fundamental role in the induction of LTP (Wigstrom and Gustafsson 1983). Previous research has demonstrated that increasing doses of the GABA-enhancing diazepam (Riss, Cloyd et al. 2008) inhibits LTP, indicating that GABAergic activity exerts a powerful influence over LTP (Levkovitz, Avignone et al. 1999). In the current study, increased GABA was observed in *Fkbp5* KO hippocampus, potentially due to increased GABA synthesis. GABA is primarily synthesized from glutamate by GAD67 and GAD65, which are expressed in different amounts in cell bodies and axon terminals (Erlander, Tillakaratne et al. 1991). GAD67 immunoreactivity is expressed throughout the cell body and in synaptic terminals (Kaufman, Houser et al. 1991), and is a rapidly synthesized and utilized form of GAD, allowing on-site synthesis in response to cellular stimulation (Esclapez, Tillakaratne et al. 1994). In contrast, GAD65 is localized exclusively in the terminals and is reversibly bound to the membrane of synaptic vesicles, which may represent a depot of GAD that can be recruited upon intense stimulation (Kaufman, Houser et al. 1991, Soghomonian and Martin 1998), and plays a specific role in the control of synaptic GABA release (Pinal and Tobin 1998). Indeed, we observed a significant increase in GAD65 expression and a slightly elevated level of GAD67 expression in KO hippocampus, which may account for the higher level of GABA observed in KO mice. The lack of a significant difference in GABA transporter (GAT1) expression suggests that reuptake of GABA is unaffected. The expression of GAD in brain regions of patients suffering from major depressive disorder (MDD) has been investigated previously, with mixed findings in the pre-frontal cortex (PFC), temporal cortex, and thalamus (Bielau,

ACCEPTED MANUSCRIPT

Steiner et al. 2007, Gos, Steiner et al. 2012), but some consistent observations of increased density of GAD-IR cells in the entorhinal cortex and the hippocampus (Cheetham, Crompton et al. 1988, Gos, Gunther et al. 2009, Gos, Steiner et al. 2012). In this regard, our results are consistent with human studies in the hippocampus. There are considerable data linking altered GABAergic activity with HPA axis function, in which *Fkbp5* plays an important role as a GR regulator. *Fkbp5* is highly expressed in brain regions associated with stress response, and responds to stress itself (Scharf, Liebl et al. 2011, Qiu, Luczak et al. 2016). Similarly GAD mRNA expression is enhanced in several hypothalamic regions, such as the dorsomedial hypothalamus, medial preoptic area, and BST (Bowers, Cullinan et al. 1998), following both acute and chronic stress exposure. GR is expressed in the GABAergic neurons of the anterior hypothalamic area and mediates corticosteroid-induced plasticity (Shin, Han et al. 2011). Therefore it is presently unclear whether *Fkbp5* affects GAD and GABA directly or indirectly (*e.g.*, via GR). In GAD65 KO mice, reductions in synaptic GABA release are attributable to fewer vesicles being released (Tian et al. 1999). Thus, increased GABA may result from enhanced expression of GAD65 in *Fkbp5* KO. This would be consistent with the high frequency of mIPSCs, indicating an increase in presynaptic GABA release. The lack of an effect on mIPSC amplitude in *Fkbp5* KO suggests that postsynaptic function is essentially unaffected. Reductions in LTP can be mediated by an increase in presynaptic GABAergic interneurons, due to the increased frequency of mIPSCs (Levkovitz, Avignone et al. 1999). Thus, decreased glutamate receptors and increased GABA may account for the decreased LTP observed in *Fkbp5* KO.

GABAergic transmission in the brain has been implicated in the pathophysiology of depressive disorder (Abdallah, Jackowski et al. 2015). GABA

ACCEPTED MANUSCRIPT

exerts its major function through the GABA type A receptors ($GABA_ARs$), which inhibit the hyperarousal state and anxiety. Reductions in LTP might be the causal link between *FKBP5* SNPs and many mental illnesses (Szymanska, Budziszewska et al. 2009, Tatro, Nguyen et al. 2010, Schmidt, Buell et al. 2015). Previous studies found decreased GABA concentrations in brain regions, such as dorsolateral PFC and occipital cortex (OCC) of patients with MDD (Rajkowska, O'Dwyer et al. 2007, Sanacora and Saricicek 2007, Maciag, Hughes et al. 2010). Recent findings demand more brain region-specific and a more complex models are needed to study this issue (Pehrson and Sanchez 2015). The limitations of human studies make it difficult to evaluate the brain region-specific expression of neurotransmitters and related enzymes, and argue for more animal model research to delineate the regional molecular mechanisms.

In the current study, both anhedonic behavior and FST were found to be impacted in the male KO mice at baseline. Previous research determined that although young *Fkbp5* KO (10-16 week old) mice do not display general behavioral changes at baseline, they spend significantly less time immobile following restraint stress (Touma, Gassen et al. 2011), a behavior also observed in aged *Fkbp5* KO (17-20 month old) mice with no stress (O'Leary, Dharia et al. 2011). However, we observed a genotype effect, with unstressed 3-month-old male KO mice spending an increased amount of time floating. This difference could be due to the stress treatment utilized. Although the predominant interpretation of FST is that immobility reflects hopelessness and negative mood (Porsolt et al., 1977), other interpretations are that this may only reflect the acute effect of antidepressants (Mann 2005), or the learned immobility to adapt successfully to the inescapable situation (Cryan and Mombereau, 2004). Our interpretation is that the FST difference may indicate a

ACCEPTED MANUSCRIPT

difference in cognitive function (Molendijk and de Kloet 2015). More research is needed to understand *Fkbp5* gene function and its effect on learning and memory.

Previous research has demonstrated that GABA and GABAR agonism enhances immobility, indicating that GABAergic functions play some role in the mechanism of this immobility (Nagatani, Sugihara et al. 1984, Nagatani, Yamamoto et al. 1987, Aley and Kulkarni 1989, Ferre, Fernandez Teruel et al. 1994). More recent research found increasing central GABAergic activity using various drugs results in a depressant-like activity, measured as an increase in the duration of immobility in the FST model of depression (El Zahaf and Salem Elhwuegi 2014). These observations are in line with those of *Fkbp5* KO, with higher GABA and enhanced immobility. One limitation of the present study is not having directly tested the manipulation of GABAergic or glutamatergic systems to identify their association with behavior changes. The use of only male mice in the present study represents another limitation, as comparisons between the sexes may have enriched our understanding.

We conclude that FKBP5 plays a critical role in neuronal synaptic plasticity on both excitatory and inhibitory synapses in the hippocampus. Further research into how the elimination of *Fkbp5* alters neuron development, gene expression, and behavior will provide insights into future treatment strategies for mental illness.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Acknowledgements

This research was supported by grants from CAMS Innovation Fund for Medical Sciences (CIFMS) WY (2017-I2M-3-015), the National Science Foundation of China BQ (No.81700751), WY (No. 81272273), and ZX (No.31571207). NIAAA P60AA007611 and R01AA10707 of National Institutes of Health (NIH). We would also like to express our appreciation to Judy E. Powers for technical support, Dr. Brady Atwood for paper revision, and Dr. Lucinda Carr and Dr. Lawrence Lumeng of the Indiana Alcohol Research Center, for their support in providing useful discussions of this research and their wisdom in long-term research using animal models.

Conflict of Interest

All authors have no conflict of interest to declare.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Figure legends

Figure 1. ***Fkbp5* KO mice exhibit decreased LTP.**

(A) The anatomical placement of electrodes in the mouse hippocampus for LTP measurement. (B) The LTP (blue) responses of WT mice. (C) The LTP (red) responses of KO mice. (D) The time course of changes in fEPSP slope during LTP measurement. (E) The calculated changes and statistical analysis in fEPSP slope during LTP measurement. All changes in long-term synaptic plasticity were evaluated by averaging 10 responses at 51-60 minutes post- theta-burst stimulation (TBS) and normalizing these data to 10 control signals at 11-20 minutes prior to TBS. Comparisons were made using WT (N=3) and KO (N=3) male mice at 8 weeks of age ; a total of 6 recordings from each genotype. Student's *t*-test was applied statistical significant analysis: **represents $p < 0.01$.

Figure 2. *Fkbp5* KO mice possess reduced glutamate receptor expression in the hippocampus.

(A) The content of L-glutamic acid in hippocampus detected using LC–MS/MS by comparing KO (N=3) and WT (N=3) male mice. (B, C, and D) The expression of NMDAR1, NMDAR2B, and AMPAR in WT and *Fkbp5* KO mouse hippocampus were determined by Western blotting. Data are provided as the mean ± SEM. Comparisons were made by Student's t-test: *represents $p < 0.05$; **represents $p < 0.01$; NS represents no statistical significance. (E, F, and G) The localization and expression of NMDAR1, NMDAR2B, and AMPAR in CA1 and DG sub-regions from WT and KO hippocampus were detected by immunofluorescence. The images within the white rectangles show an enlarged view of the boxed regions. Bar = 100 μm.

ACCEPTED MANUSCRIPT

Figure 3. Decreased frequency of mEPSCs in *Fkbp5* KO mice.

(A) Representative signals of AMPA receptor mediated mEPSC recorded at a holding potential of -70 mV. (B) Cumulative probability plots of the mEPSC intervals reveal a shift to the right in the KO curve. (C) The frequency of mEPSCs significantly decreased in KO (N=4) compared to WT (N=4). (D) The cumulative plots of the mEPSC amplitude reveal no differences between the two groups. (E) No significant differences could be detected between the mEPSC amplitudes of the two groups. Comparisons were made by Student's t-test: *** represents $p < 0.001$; NS represents no statistical significance.

Figure 4. *Fkbp5* KO mice possess increased GABA level in hippocampus.

(A) The protein expression of GABA in hippocampus of WT (N=3) and (B) KO (N=3) mice demonstrated by IHC. (C) Comparison of GABA at representative sub-regions of the hippocampus demonstrate a higher level of GABA in the CA1, CA2, CA3, DG1, DG2, and DG3 sub-regions in KO relative to WT. The statistic analysis of the average optical density (AOD) of GABA level that was quantified by ImageJ, and the results were showed as fold change relative to WT. Data are provided as the mean ± SEM. Comparisons were made by Student's t-test: *, **, and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Figure 5. GABA transporter expression in the brain

(A) The expression of GAD65, (B) GAD67, and (C) GAT1 in the hippocampus from WT (N=3) and KO (N=3) mice determined by Western blotting. Comparisons were made by Student's t-test: * represents $p<0.05$,

ACCEPTED MANUSCRIPT

Figure 6. Increased frequency of mIPSCs in *Fkbp5* KO mice.

(A) Representative mIPSC signals recorded at a holding potential of -70 mV in the presence of 0.5 μM TTX, 20μM CNQX, and 25μM D-AP5 (two top traces). Application of 10 μM bicuculline blocks all mIPSCs (two bottom traces) indicating that mIPSCs are mediated by activity from $GABA_A$ receptors. (B) Cumulative probability plots of the mIPSC intervals reveal a shift to the left in the KO curve. (C) The frequency of mIPSCs was significantly higher in KO (N=4) compared to WT (N=4). (D) The cumulative plots of the mIPSC amplitude reveal no differences between the two groups. (E) No significant differences could be detected between the mIPSC amplitudes of the two groups. Comparisons were made by Student's t-test: *represents $p < 0.05$; NS represents no statistical significance.

Figure 7. *Fkbp5* KO mice display anhedonic behavior.

(A) Compared to WT male mice (N=21), *Fkbp5* KO mice (N=14) exhibited a significant reduction in saccharin intake. (B) No significant differences in quinine intake were observed between *Fkbp5* KO and WT male mice. (C). Male *Fkbp5* KO mice (N=12) exhibited increased floating time in FST relative to WT (N=12). Comparisons were made by Student's t-test: * and ** represent $p < 0.05$ and $p < 0.01$, respectively.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Reference:

Abdallah, C. G., A. Jackowski, J. R. Sato, X. Mao, G. Kang, R. Cheema, J. D. Coplan, S. J. Mathew and D. C. Shungu (2015). "Prefrontal cortical GABA abnormalities are associated with reduced hippocampal volume in major depressive disorder." Eur Neuropsychopharmacol **25**(8): 1082-1090.

Aley, K. O. and S. K. Kulkarni (1989). "GABA-mediated modification of despair behavior in mice." Naunyn Schmiedebergs Arch Pharmacol **339**(3): 306-311.

Balsevich, G., A. Uribe, K. V. Wagner, J. Hartmann, S. Santarelli, C. Labermaier and M. V. Schmidt (2014). "Interplay between diet-induced obesity and chronic stress in mice: potential role of FKBP51." J Endocrinol **222**(1): 15-26.

Bashir, Z. I., Z. A. Bortolotto, C. H. Davies, N. Berretta, A. J. Irving, A. J. Seal, J. M. Henley, D. E. Jane, J. C. Watkins and G. L. Collingridge (1993). "Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors." Nature **363**(6427): 347-350.

Bielau, H., J. Steiner, C. Mawrin, K. Trubner, R. Brisch, G. Meyer-Lotz, M. Brodhun, H. Dobrowolny, B. Baumann, T. Gos, H. G. Bernstein and B. Bogerts (2007). "Dysregulation of GABAergic neurotransmission in mood disorders: a postmortem study." Ann N Y Acad Sci **1096**: 157-169.

Binder, E. B. (2009). "The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders." Psychoneuroendocrinology **34 Suppl 1**: S186-195.

Binder, E. B., R. G. Bradley, W. Liu, M. P. Epstein, T. C. Deveau, K. B. Mercer, Y. Tang, C. F. Gillespie, C. M. Heim, C. B. Nemeroff, A. C. Schwartz, J. F. Cubells and K. J. Ressler (2008). "Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults." JAMA **299**(11): 1291-1305.

Binder, E. B., D. Salyakina, P. Lichtner, G. M. Wochnik, M. Ising, B. Putz, S. Papiol, S. Seaman, S. Lucae, M. A. Kohli, T. Nickel, H. E. Kunzel, B. Fuchs, M. Majer, A. Pfennig, N. Kern, J. Brunner, S. Modell, T. Baghai, T. Deiml, P. Zill, B. Bondy, R. Rupprecht, T. Messer, O. Kohnlein, H. Dabitz, T. Bruckl, N. Muller, H. Pfister, R. Lieb, J. C. Mueller, E. Lohmussaar, T. M. Strom, T. Bettecken, T. Meitinger, M. Uhr, T. Rein, F. Holsboer and B. Muller-Myhsok (2004). "Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment." Nat Genet **36**(12): 1319-1325.

ACCEPTED MANUSCRIPT

116 Bliss, T. V. and A. R. Gardner-Medwin (1973). "Long-lasting potentiation of synaptic
117 transmission in the dentate area of the unanaestetized rabbit following stimulation of the
118 perforant path." J Physiol **232**(2): 357-374.
119 Bliss, T. V. and T. Lomo (1973). "Long-lasting potentiation of synaptic transmission in the
120 dentate area of the anaesthetized rabbit following stimulation of the perforant path." J
121 Physiol **232**(2): 331-356.
122 Bowers, G., W. E. Cullinan and J. P. Herman (1998). "Region-specific regulation of glutamic
123 acid decarboxylase (GAD) mRNA expression in central stress circuits." J Neurosci **18**(15):
124 5938-5947.
125 Cheetham, S. C., M. R. Crompton, C. L. Katona, S. J. Parker and R. W. Horton (1988).
126 "Brain GABAA/benzodiazepine binding sites and glutamic acid decarboxylase activity in
127 depressed suicide victims." Brain Res **460**(1): 114-123.
128 Costin, B. N., A. R. Wolen, S. Fitting, K. L. Shelton and M. F. Miles (2013). "Role of adrenal
129 glucocorticoid signaling in prefrontal cortex gene expression and acute behavioral responses
130 to ethanol." Alcohol Clin Exp Res **37**(1): 57-66.
131 Das, S., Y. F. Sasaki, T. Rothe, L. S. Premkumar, M. Takasu, J. E. Crandall, P. Dikkes, D.
132 A. Conner, P. V. Rayudu, W. Cheung, H. S. Chen, S. A. Lipton and N. Nakanishi (1998).
133 "Increased NMDA current and spine density in mice lacking the NMDA receptor subunit
134 NR3A." Nature **393**(6683): 377-381.
135 El Zahaf, N. A. and A. Salem Elhwuegi (2014). "The effect of GABAmimetics on the duration
136 of immobility in the forced swim test in albino mice." Libyan J Med **9**: 23480.
137 Ellsworth, K. A., I. Moon, B. W. Eckloff, B. L. Fridley, G. D. Jenkins, A. Batzler, J. M.
138 Biernacka, R. Abo, A. Brisbin, Y. Ji, S. Hebbring, E. D. Wieben, D. A. Mrazek, R. M.
139 Weinshilboum and L. Wang (2013). "FKBP5 genetic variation: association with selective
140 serotonin reuptake inhibitor treatment outcomes in major depressive disorder."
141 Pharmacogenet Genomics **23**(3): 156-166.
142 Enoch, M. A. (2008). "The role of GABA(A) receptors in the development of alcoholism."
143 Pharmacol Biochem Behav **90**(1): 95-104.
144 Erlander, M. G., N. J. Tillakaratne, S. Feldblum, N. Patel and A. J. Tobin (1991). "Two genes
145 encode distinct glutamate decarboxylases." Neuron **7**(1): 91-100.
146 Esclapez, M., N. J. Tillakaratne, D. L. Kaufman, A. J. Tobin and C. R. Houser (1994).
147 "Comparative localization of two forms of glutamic acid decarboxylase and their mRNAs in
148 rat brain supports the concept of functional differences between the forms." J Neurosci **14**(3
149 Pt 2): 1834-1855.

ACCEPTED MANUSCRIPT

Ferre, P., A. Fernandez Teruel, R. M. Escorihuela, E. Garcia, A. Zapata and A. Tobena (1994). "Struggling and flumazenil effects in the swimming test are related to the level of anxiety in mice." Neuropsychobiology **29**(1): 23-27.

Gos, T., K. Gunther, H. Bielau, H. Dobrowolny, C. Mawrin, K. Trubner, R. Brisch, J. Steiner, H. G. Bernstein, Z. Jankowski and B. Bogerts (2009). "Suicide and depression in the quantitative analysis of glutamic acid decarboxylase-Immunoreactive neuropil." J Affect Disord **113**(1-2): 45-55.

Gos, T., J. Steiner, H. Bielau, H. Dobrowolny, K. Gunther, C. Mawrin, M. Krzyzanowski, R. Hauser, R. Brisch, H. G. Bernstein, Z. Jankowski, K. Braun and B. Bogerts (2012). "Differences between unipolar and bipolar I depression in the quantitative analysis of glutamic acid decarboxylase-immunoreactive neuropil." Eur Arch Psychiatry Clin Neurosci **262**(8): 647-655.

Hartmann, J., K. V. Wagner, C. Liebl, S. H. Scharf, X. D. Wang, M. Wolf, F. Hausch, T. Rein, U. Schmidt, C. Touma, J. Cheung-Flynn, M. B. Cox, D. F. Smith, F. Holsboer, M. B. Muller and M. V. Schmidt (2012). "The involvement of FK506-binding protein 51 (FKBP5) in the behavioral and neuroendocrine effects of chronic social defeat stress." Neuropharmacology **62**(1): 332-339.

Huang, M. C., M. L. Schwandt, J. A. Chester, A. M. Kirchhoff, C. F. Kao, T. Liang, J. D. Tapocik, V. A. Ramchandani, D. T. George, C. A. Hodgkinson, D. Goldman and M. Heilig (2014). "FKBP5 moderates alcohol withdrawal severity: human genetic association and functional validation in knockout mice." Neuropsychopharmacology **39**(8): 2029-2038.

Isaac, J. T., R. A. Nicoll and R. C. Malenka (1995). "Evidence for silent synapses: implications for the expression of LTP." Neuron **15**(2): 427-434.

Ising, M., A. M. Depping, A. Siebertz, S. Lucae, P. G. Unschuld, S. Kloiber, S. Horstmann, M. Uhr, B. Muller-Myhsok and F. Holsboer (2008). "Polymorphisms in the FKBP5 gene region modulate recovery from psychosocial stress in healthy controls." Eur J Neurosci **28**(2): 389-398.

Kaufman, D. L., C. R. Houser and A. J. Tobin (1991). "Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions." J Neurochem **56**(2): 720-723.

Kutsuwada, T., K. Sakimura, T. Manabe, C. Takayama, N. Katakura, E. Kushiya, R. Natsume, M. Watanabe, Y. Inoue, T. Yagi, S. Aizawa, M. Arakawa, T. Takahashi, Y. Nakamura, H. Mori and M. Mishina (1996). "Impairment of suckling response, trigeminal neuronal pattern formation, and hippocampal LTD in NMDA receptor epsilon 2 subunit mutant mice." Neuron **16**(2): 333-344.

ACCEPTED MANUSCRIPT

Lekman, M., G. Laje, D. Charney, A. J. Rush, A. F. Wilson, A. J. Sorant, R. Lipsky, S. R. Wisniewski, H. Manji, F. J. McMahon and S. Paddock (2008). "The FKBP5-gene in depression and treatment response--an association study in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Cohort." Biol Psychiatry **63**(12): 1103-1110.

Levkovitz, Y., E. Avignone, Y. Groner and M. Segal (1999). "Upregulation of GABA neurotransmission suppresses hippocampal excitability and prevents long-term potentiation in transgenic superoxide dismutase-overexpressing mice." J Neurosci **19**(24): 10977-10984.

Levran, O., E. Peles, M. Randesi, Y. Li, J. Rotrosen, J. Ott, M. Adelson and M. J. Kreek (2014). "Stress-related genes and heroin addiction: a role for a functional FKBP5 haplotype." Psychoneuroendocrinology **45**: 67-76.

Li, Y., R. S. Erzurumlu, C. Chen, S. Jhaveri and S. Tonegawa (1994). "Whisker-related neuronal patterns fail to develop in the trigeminal brainstem nuclei of NMDAR1 knockout mice." Cell **76**(3): 427-437.

Liao, D., N. A. Hessler and R. Malinow (1995). "Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice." Nature **375**(6530): 400-404.

Lucki, I. (1997). "The forced swimming test as a model for core and component behavioral effects of antidepressant drugs." Behav Pharmacol **8**(6-7): 523-532.

Luscher, B., Q. Shen and N. Sahir (2011). "The GABAergic deficit hypothesis of major depressive disorder." Mol Psychiatry **16**(4): 383-406.

Maciag, D., J. Hughes, G. O'Dwyer, Y. Pride, C. A. Stockmeier, G. Sanacora and G. Rajkowska (2010). "Reduced density of calbindin immunoreactive GABAergic neurons in the occipital cortex in major depression: relevance to neuroimaging studies." Biol Psychiatry **67**(5): 465-470.

Mann, J. J. (2005). "The medical management of depression." N Engl J Med **353**(17): 1819-1834.

McClintick, J. N., X. Xuei, J. A. Tischfield, A. Goate, T. Foroud, L. Wetherill, M. A. Ehringer and H. J. Edenberg (2013). "Stress-response pathways are altered in the hippocampus of chronic alcoholics." Alcohol **47**(7): 505-515.

McClung, C. A., E. J. Nestler and V. Zachariou (2005). "Regulation of gene expression by chronic morphine and morphine withdrawal in the locus ceruleus and ventral tegmental area." J Neurosci **25**(25): 6005-6015.

McHugh, T. J., K. I. Blum, J. Z. Tsien, S. Tonegawa and M. A. Wilson (1996). "Impaired hippocampal representation of space in CA1-specific NMDAR1 knockout mice." Cell **87**(7): 1339-1349.

ACCEPTED MANUSCRIPT

Molendijk, M. L. and E. R. de Kloet (2015). "Immobility in the forced swim test is adaptive and does not reflect depression." Psychoneuroendocrinology **62**: 389-391.

Moriyoshi, K., M. Masu, T. Ishii, R. Shigemoto, N. Mizuno and S. Nakanishi (1991). "Molecular cloning and characterization of the rat NMDA receptor." Nature **354**(6348): 31-37.

Nagatani, T., T. Sugihara and R. Kodaira (1984). "The effect of diazepam and of agents which change GABAergic functions in immobility in mice." Eur J Pharmacol **97**(3-4): 271-275.

Nagatani, T., T. Yamamoto, T. Sugihara and S. Ueki (1987). "The effect of agonists at the GABA-benzodiazepine receptor complex on the duration of immobility of mice in the forced swimming test." Eur J Pharmacol **142**(1): 17-22.

Nakanishi, S. (1994). "Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity." Neuron **13**(5): 1031-1037.

Nestler, E. J. (2001). "Molecular basis of long-term plasticity underlying addiction." Nat Rev Neurosci **2**(2): 119-128.

Niehaus, J. L., M. Murali and J. A. Kauer (2010). "Drugs of abuse and stress impair LTP at inhibitory synapses in the ventral tegmental area." Eur J Neurosci **32**(1): 108-117.

O'Leary, J. C., 3rd, S. Dharia, L. J. Blair, S. Brady, A. G. Johnson, M. Peters, J. Cheung-Flynn, M. B. Cox, G. de Erausquin, E. J. Weeber, U. K. Jinwal and C. A. Dickey (2011). "A new anti-depressive strategy for the elderly: ablation of FKBP5/FKBP51." PLoS One **6**(9): e24840.

Pehrson, A. L. and C. Sanchez (2015). "Altered gamma-aminobutyric acid neurotransmission in major depressive disorder: a critical review of the supporting evidence and the influence of serotonergic antidepressants." Drug Des Devel Ther **9**: 603-624.

Pelz, W. E., G. Whitney and J. C. Smith (1973). "Genetic influences on saccharin preference of mice." Physiol Behav **10**(2): 263-265.

Pinal, C. S. and A. J. Tobin (1998). "Uniqueness and redundancy in GABA production." Perspect Dev Neurobiol **5**(2-3): 109-118.

Porsolt, R. D., M. Le Pichon and M. Jalfre (1977). "Depression: a new animal model sensitive to antidepressant treatments." Nature **266**(5604): 730-732.

Qiu, B., S. E. Luczak, T. L. Wall, A. M. Kirchhoff, Y. Xu, M. Y. Eng, R. B. Stewart, W. Shou, S. L. Boehm, J. A. Chester, W. Yong and T. Liang (2016). "The FKBP5 Gene Affects Alcohol Drinking in Knockout Mice and Is Implicated in Alcohol Drinking in Humans." Int J Mol Sci **17**(8).

Rajkowska, G., G. O'Dwyer, Z. Teleki, C. A. Stockmeier and J. J. Miguel-Hidalgo (2007). "GABAergic neurons immunoreactive for calcium binding proteins are reduced in the prefrontal cortex in major depression." Neuropsychopharmacology **32**(2): 471-482.

ACCEPTED MANUSCRIPT

Reynolds, P. D., Y. Ruan, D. F. Smith and J. G. Scammell (1999). "Glucocorticoid resistance in the squirrel monkey is associated with overexpression of the immunophilin FKBP51." J Clin Endocrinol Metab **84**(2): 663-669.

Riss, J., J. Cloyd, J. Gates and S. Collins (2008). "Benzodiazepines in epilepsy: pharmacology and pharmacokinetics." Acta Neurol Scand **118**(2): 69-86.

Saba, L. M., B. Bennett, P. L. Hoffman, K. Barcomb, T. Ishii, K. Kechris and B. Tabakoff (2011). "A systems genetic analysis of alcohol drinking by mice, rats and men: influence of brain GABAergic transmission." Neuropharmacology **60**(7-8): 1269-1280.

Sajdyk, T., P. Johnson, S. Fitz and A. Shekhar (2008). "Chronic inhibition of GABA synthesis in the bed nucleus of the stria terminalis elicits anxiety-like behavior." J Psychopharmacol **22**(6): 633-641.

Sanacora, G. and A. Saricicek (2007). "GABAergic contributions to the pathophysiology of depression and the mechanism of antidepressant action." CNS Neurol Disord Drug Targets **6**(2): 127-140.

Scharf, S. H., C. Liebl, E. B. Binder, M. V. Schmidt and M. B. Muller (2011). "Expression and regulation of the Fkbp5 gene in the adult mouse brain." PLoS One **6**(2): e16883.

Schiene, C. and G. Fischer (2000). "Enzymes that catalyse the restructuring of proteins." Curr Opin Struct Biol **10**(1): 40-45.

Schmidt, U., D. R. Buell, I. A. Ionescu, N. C. Gassen, F. Holsboer, M. B. Cox, B. Novak, C. Huber, J. Hartmann, M. V. Schmidt, C. Touma, T. Rein and L. Herrmann (2015). "A role for synapsin in FKBP51 modulation of stress responsiveness: Convergent evidence from animal and human studies." Psychoneuroendocrinology **52**: 43-58.

Shin, S. Y., T. H. Han, S. Y. Lee, S. K. Han, J. B. Park, F. Erdelyi, G. Szabo and P. D. Ryu (2011). "Direct Corticosteroid Modulation of GABAergic Neurons in the Anterior Hypothalamic Area of GAD65-eGFP Mice." Korean J Physiol Pharmacol **15**(3): 163-169.

Soghomonian, J. J. and D. L. Martin (1998). "Two isoforms of glutamate decarboxylase: why?" Trends Pharmacol Sci **19**(12): 500-505.

Stechschulte, L. A. and E. R. Sanchez (2011). "FKBP51-a selective modulator of glucocorticoid and androgen sensitivity." Curr Opin Pharmacol **11**(4): 332-337.

Sugihara, H., K. Moriyoshi, T. Ishii, M. Masu and S. Nakanishi (1992). "Structures and properties of seven isoforms of the NMDA receptor generated by alternative splicing." Biochem Biophys Res Commun **185**(3): 826-832.

Swanson, C. J., M. Bures, M. P. Johnson, A. M. Linden, J. A. Monn and D. D. Schoepp (2005). "Metabotropic glutamate receptors as novel targets for anxiety and stress disorders." Nat Rev Drug Discov **4**(2): 131-144.

ACCEPTED MANUSCRIPT

Szczepankiewicz, A., A. Leszczynska-Rodziewicz, J. Pawlak, B. Narozna, A. Rajewska-Rager, M. Wilkosc, D. Zaremba, M. Maciukiewicz and J. Twarowska-Hauser (2014). "FKBP5 polymorphism is associated with major depression but not with bipolar disorder." J Affect Disord **164**: 33-37.

Szymanska, M., B. Budziszewska, L. Jaworska-Feil, A. Basta-Kaim, M. Kubera, M. Leskiewicz, M. Regulska and W. Lason (2009). "The effect of antidepressant drugs on the HPA axis activity, glucocorticoid receptor level and FKBP51 concentration in prenatally stressed rats." Psychoneuroendocrinology **34**(6): 822-832.

Tang, Y. P., E. Shimizu, G. R. Dube, C. Rampon, G. A. Kerchner, M. Zhuo, G. Liu and J. Z. Tsien (1999). "Genetic enhancement of learning and memory in mice." Nature **401**(6748): 63-69.

Tatro, E. T., I. P. Everall, E. Masliah, B. J. Hult, G. Lucero, G. Chana, V. Soontornniyomkij, C. L. Achim and H. I. V. N. R. Center (2009). "Differential expression of immunophilins FKBP51 and FKBP52 in the frontal cortex of HIV-infected patients with major depressive disorder." J Neuroimmune Pharmacol **4**(2): 218-226.

Tatro, E. T., T. B. Nguyen, C. A. Bousman, E. Masliah, I. Grant, J. H. Atkinson and I. P. Everall (2010). "Correlation of major depressive disorder symptoms with FKBP5 but not FKBP4 expression in human immunodeficiency virus-infected individuals." J Neurovirol **16**(5): 399-404.

Touma, C., N. C. Gassen, L. Herrmann, J. Cheung-Flynn, D. R. Bull, I. A. Ionescu, J. M. Heinzmann, A. Knapman, A. Siebertz, A. M. Depping, J. Hartmann, F. Hausch, M. V. Schmidt, F. Holsboer, M. Ising, M. B. Cox, U. Schmidt and T. Rein (2011). "FK506 binding protein 5 shapes stress responsiveness: modulation of neuroendocrine reactivity and coping behavior." Biol Psychiatry **70**(10): 928-936.

Treadwell, J. A. and S. M. Singh (2004). "Microarray analysis of mouse brain gene expression following acute ethanol treatment." Neurochem Res **29**(2): 357-369.

Tsien, J. Z., P. T. Huerta and S. Tonegawa (1996). "The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory." Cell **87**(7): 1327-1338.

Wagner, K. V., D. Marinescu, J. Hartmann, X. D. Wang, C. Labermaier, S. H. Scharf, C. Liebl, M. Uhr, F. Holsboer, M. B. Muller and M. V. Schmidt (2012). "Differences in FKBP51 regulation following chronic social defeat stress correlate with individual stress sensitivity: influence of paroxetine treatment." Neuropsychopharmacology **37**(13): 2797-2808.

Westberry, J. M., P. W. Sadosky, T. R. Hubler, K. L. Gross and J. G. Scammell (2006). "Glucocorticoid resistance in squirrel monkeys results from a combination of a transcriptionally incompetent glucocorticoid receptor and overexpression of the glucocorticoid receptor co-chaperone FKBP51." J Steroid Biochem Mol Biol **100**(1-3): 34-41.

ACCEPTED MANUSCRIPT

Wigstrom, H. and B. Gustafsson (1983). "Facilitated induction of hippocampal long-lasting potentiation during blockade of inhibition." Nature **301**(5901): 603-604.

Willour, V. L., H. Chen, J. Toolan, P. Belmonte, D. J. Cutler, F. S. Goes, P. P. Zandi, R. S. Lee, D. F. MacKinnon, F. M. Mondimore, B. Schweizer, J. R. DePaulo, Jr., E. S. Gershon, F. J. McMahon and J. B. Potash (2009). "Family-based association of FKBP5 in bipolar disorder." Mol Psychiatry **14**(3): 261-268.

Wolf, M. E. (2003). "LTP may trigger addiction." Mol Interv **3**(5): 248-252.

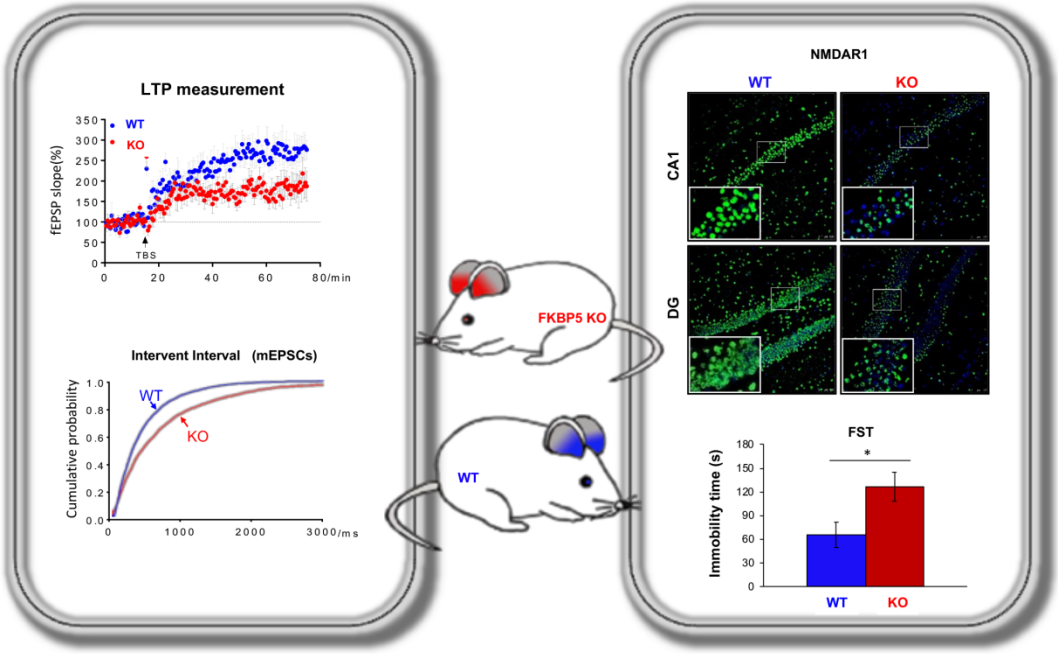
Xie, P., H. R. Kranzler, J. Poling, M. B. Stein, R. F. Anton, L. A. Farrer and J. Gelernter (2010). "Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder." Neuropsychopharmacology **35**(8): 1684-1692.

Yong, W., Z. Yang, S. Periyasamy, H. Chen, S. Yucel, W. Li, L. Y. Lin, I. M. Wolf, M. J. Cohn, L. S. Baskin, E. R. Sanchez and W. Shou (2007). "Essential role for Co-chaperone Fkbp52 but not Fkbp51 in androgen receptor-mediated signaling and physiology." J Biol Chem **282**(7): 5026-5036.

Highlights of Fkbp5 KO mice electrophysiology research

- LTP reduced in *Fkbp5* KO male mice relative to WT, indicating altered neuron function.
- Expression of excitatory glutamate receptors (NMDAR1, NMDAR2B, and AMPAR) and mEPSC frequency reduced in KO.
- Increased GABA expression and mIPSC frequency in KO hippocampus.
- Male Fkbp5 KO mice display low saccharin intake and higher immobility

ACCEPTED MANUSCRIPT


LTP measurement
WT
KO
fEPSP slope(%)
TBS
80/min
Intervent Interval (mEPSCs)
Cumulative probability
WT
KO
3000/ms
FKBP5 KO
WT
NMDAR1
WT
KO
CA1
DG
FST
Immobility time (s)
WT
KO

350

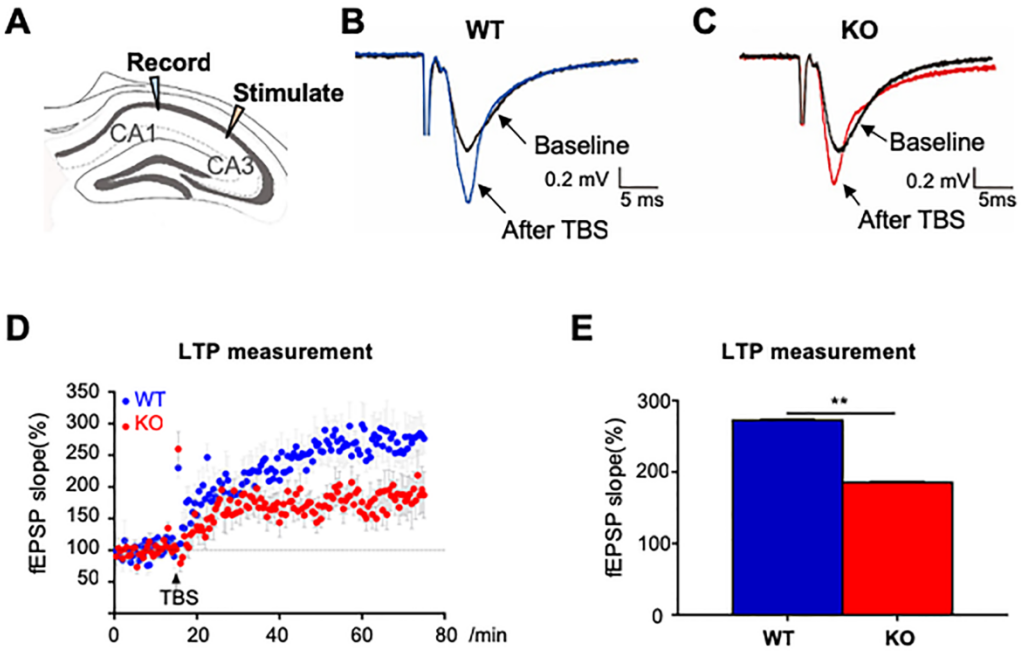

A
Record
Stimulate
CA1
CA3
B
WT
Baseline
0.2 mV
5 ms
After TBS
C
KO
Baseline
0.2 mV
5ms
After TBS
D
LTP measurement
WT
KO
fEPSP slope(%)
TBS
80 /min
E
LTP measurement
fEPSP slope(%)
WT
KO

Fig. 1

351

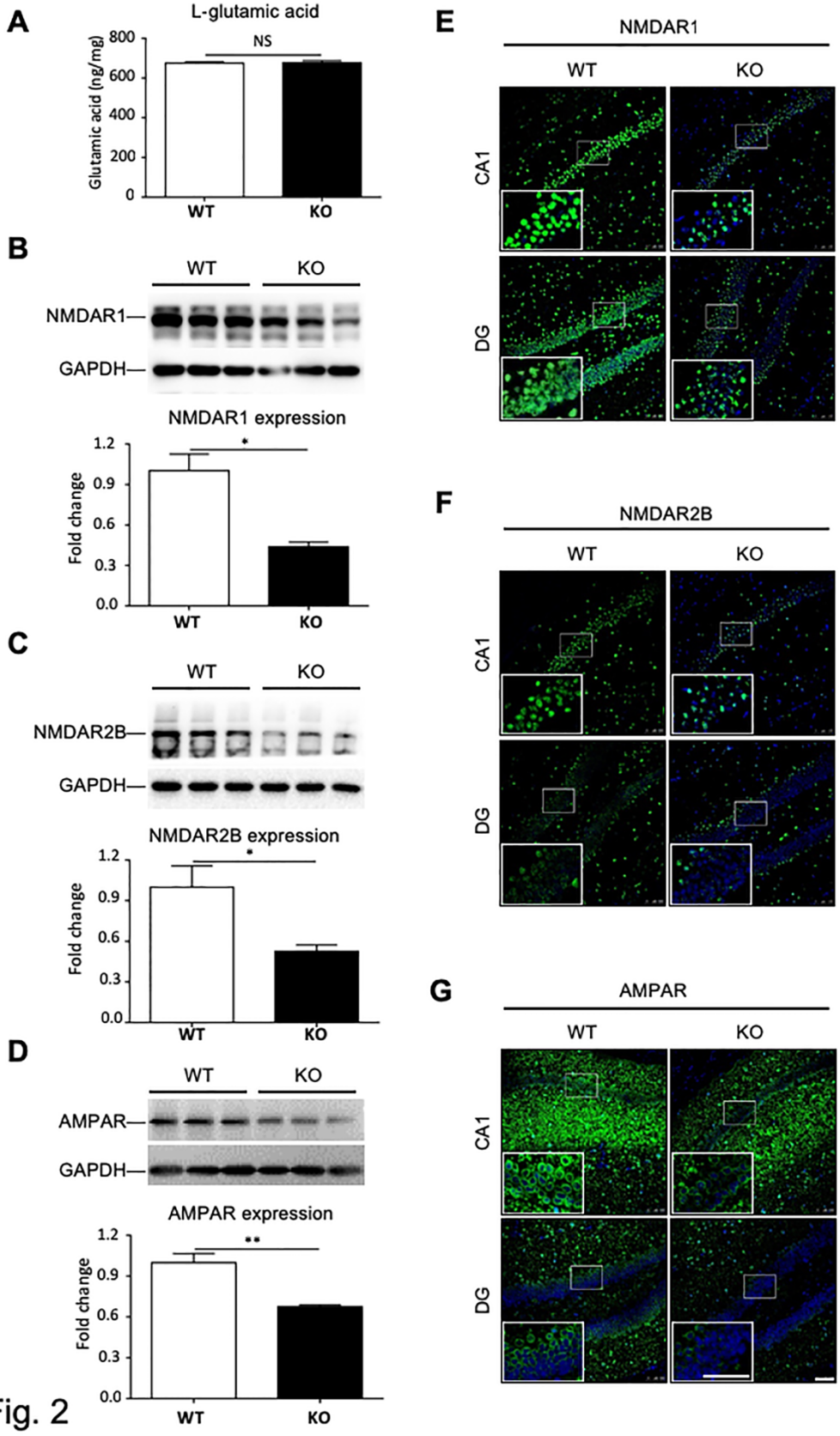

A
L-glutamic acid
NS
Glutamic acid (ng/mg)
800
600
400
200
0
WT
KO
B
WT
KO
NMDAR1
GAPDH
NMDAR1 expression
*
Fold change
1.2
0.9
0.6
0.3
0.0
WT
KO
C
WT
KO
NMDAR2B
GAPDH
NMDAR2B expression
*
Fold change
1.2
0.9
0.6
0.3
0.0
WT
KO
D
WT
KO
AMPAR
GAPDH
AMPAR expression
**
Fold change
1.2
0.9
0.6
0.3
0.0
WT
KO
E
NMDAR1
WT
KO
CA1
DG
F
NMDAR2B
WT
KO
CA1
DG
G
AMPAR
WT
KO
CA1
DG

Fig. 2

352

ACCEPTED MANUSCRIPT

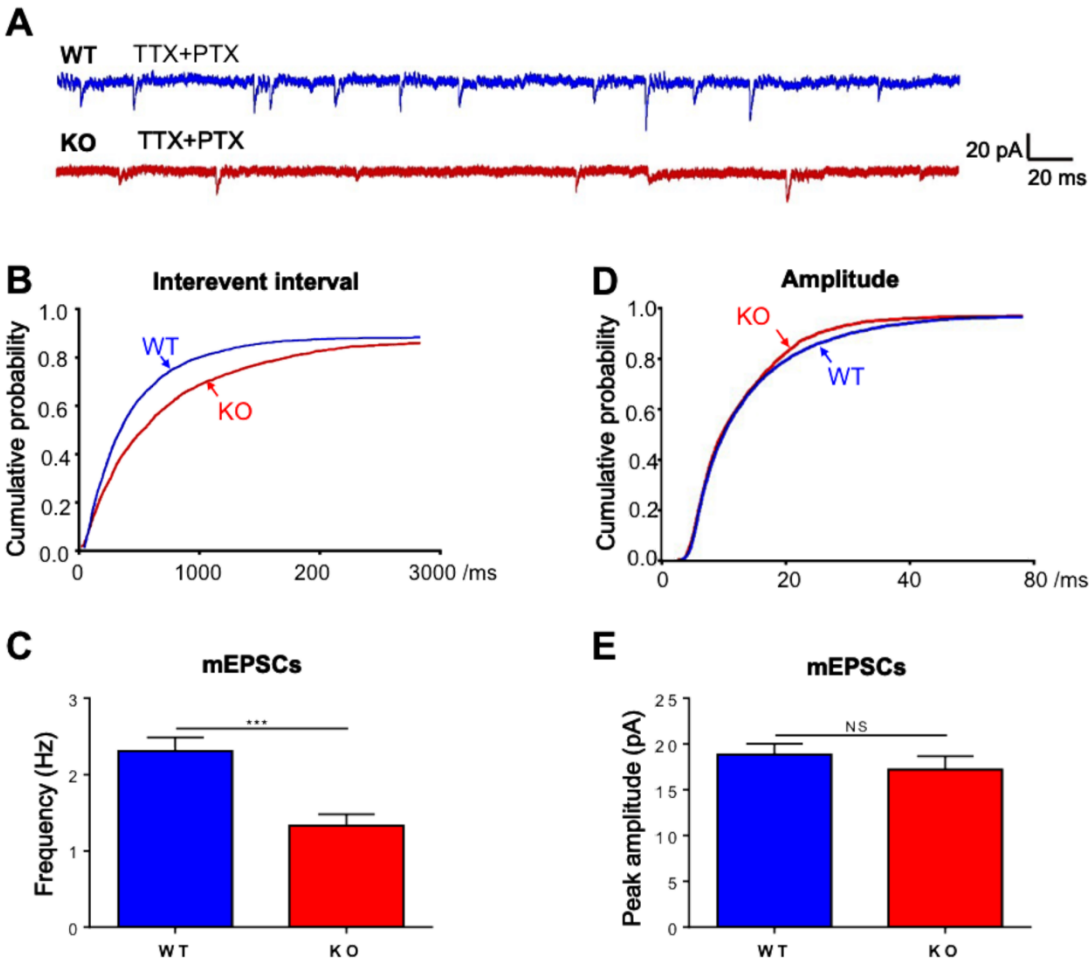


Fig. 3

353

ACCE

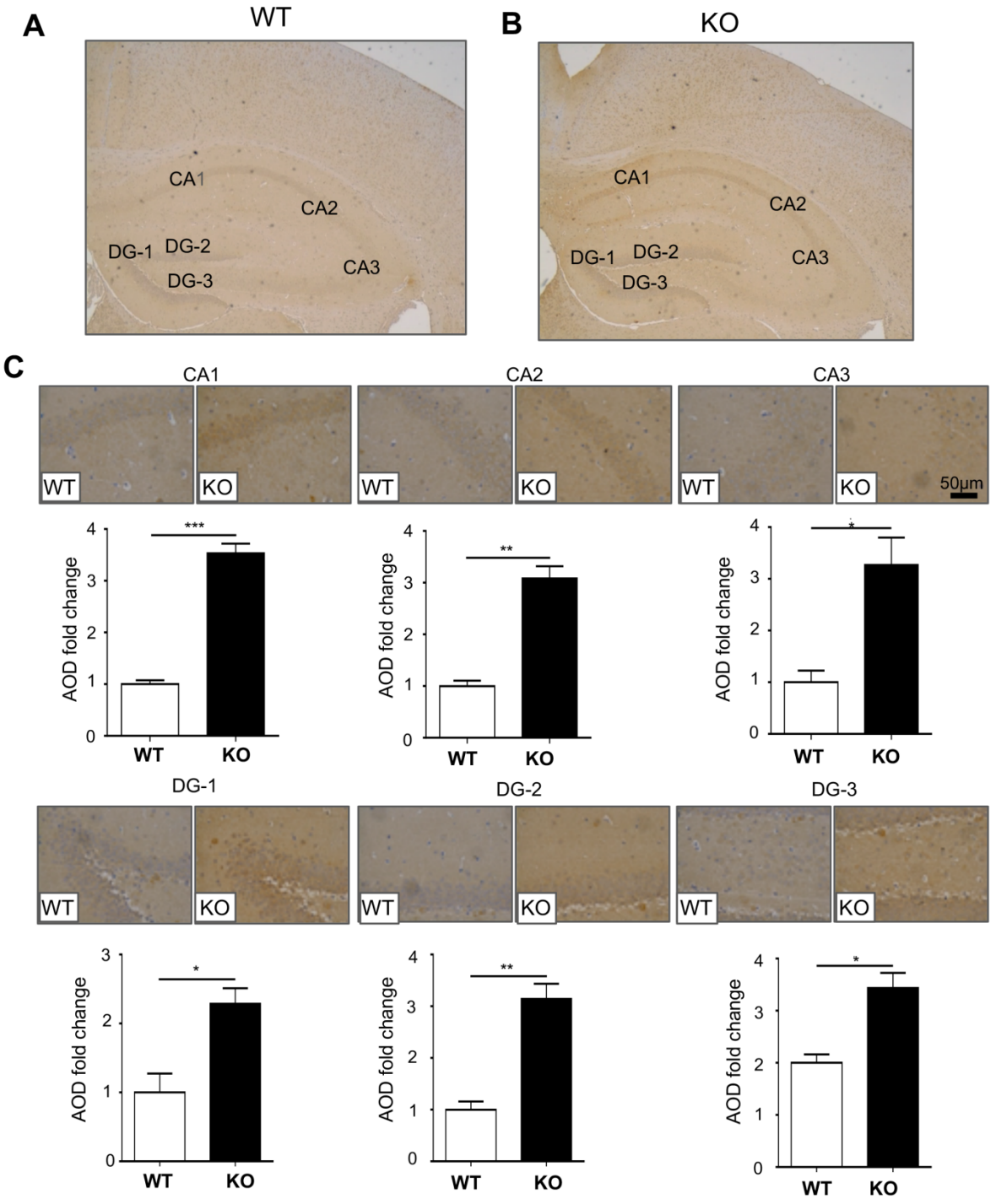

A
WT
CA1
CA2
DG-1
DG-2
DG-3
CA3
B
KO
CA1
CA2
DG-1
DG-2
DG-3
CA3
C
CA1
CA2
CA3
WT
KO
50μm
AOD fold change
***
**
*
DG-1
DG-2
DG-3

Fig. 4

354

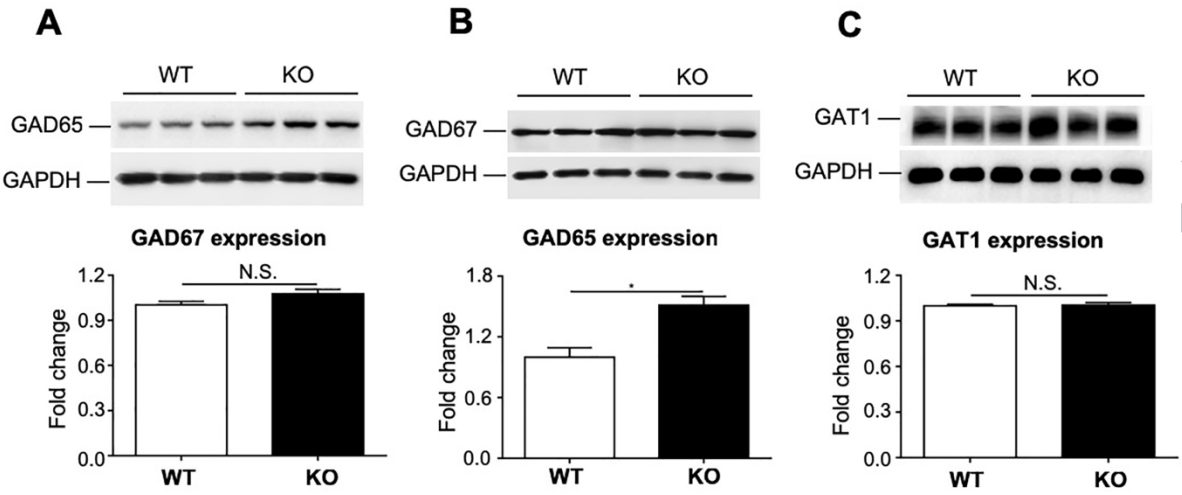


Fig.5

355

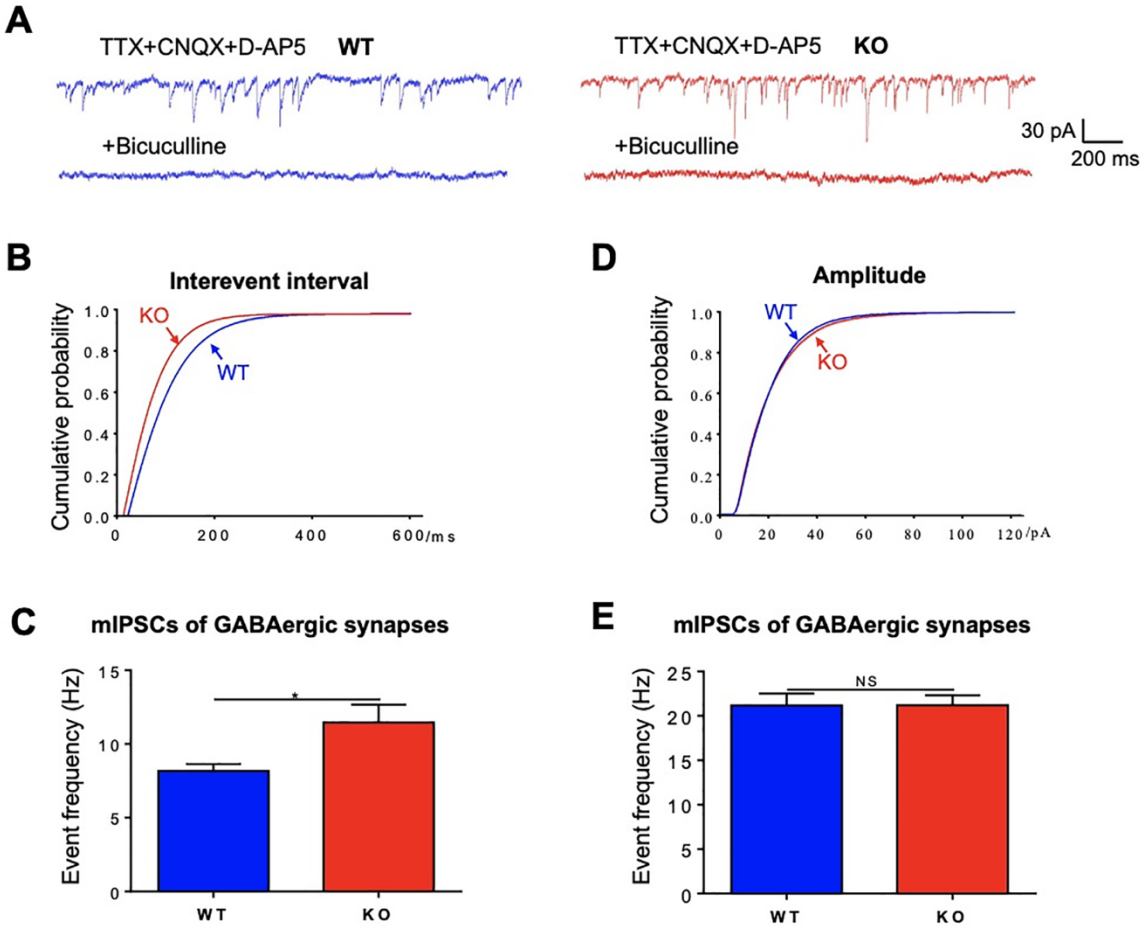


Fig. 6

356

ACCEPTED MANUSCRIPT

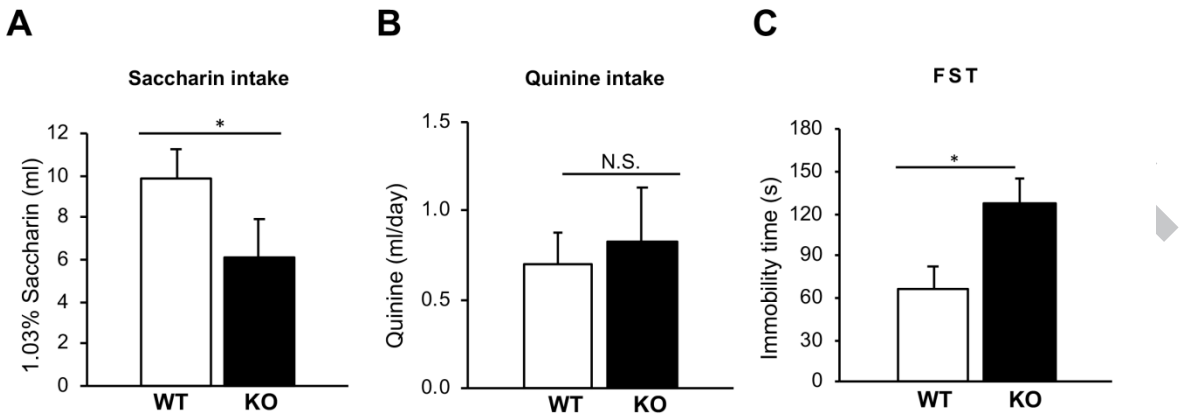


Fig. 7

357

ACCEPTED MANU