The role of glutamatergic signaling in post-traumatic stress disorder studied in animal models

Ph.D. thesis

Diána Gabriella Balázsfi

Semmelweis University

János Szentágothai School of Neurosciences

Supervisor: József Haller, Ph.D

Official reviewers: Árpád Dobolyi, Ph.D
Vilmos Altbäcker Ph.D.,

Head of the Final Examination Committee:
Zoltán Rihmer, MD, Ph.D

Members of the Final Examination Committee:
Júlia Tímár, MD, Ph.D.
István Gacsályi, Ph.D.

Budapest

2017

1. INTRODUCTION

Different stressors occure during a lifetime, but when somebody is unable to cope with them, pathological consequences may develop leading to psychiatric disorders, like post-traumatic stress disorder (PTSD). PTSD is a mental and/or physical distress that can occure after traumatic event(s), and is based on recalling and re-experiencing traumatic memories (often thought to be lost), and induces the symptoms of fear response („fight-or-flight response”). In most cases one serious traumatic event, battlefield explosion or sexual assault is enough for the development of PTSD. 10-30% of traumatized persons develop this disorder, and it is determined by the genes in approximately 1/3 of cases, while the remaining 2/3 is detremined presumably by external and environmental factors and the individual's coping strategy.

PTSD is often (80-90%) associated with other psychiatric disorders (anxiety, major depression). The recommended treatment of the disease is the combination therapy, which consist of psychotherapy and pharmacotherapy. One of the most common technique of psychotherapy is the prolonged exposure therapy, during which the patient has to confront repeatedly with situations and objects that caused the distress, but are not inherently dangerous. These practices help to improve their ability to discriminate safe and unsafe situations. Unfortunately, in most cases pharmacological treatments are only suitable for the treatment of secondary symptoms. The U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved selective serotonin reuptake inhibitors (SSRI), a type of antidepressants, for the treatment of PTSD, as well as anxiolytics for secondary symptoms. A study showed, that 6 years after the onset of PTSD the treatment helped only the 14 % of patients.

Therefore, it is very important to detect the neurological and neuroendocrinological processes underlying the development of PTSD in order to develop more effective treatments. To investigate the underlying mechanisms of the disease different animal models are available. In our experiment, we used a validated conditioned fear test, which can model the human symptoms defined by DSM IV/V. On the first day of the behavioral test, animals get electric footshock and the animals create an association between the environment and the negative experience (electric footshock). Then the animals are put back to the same box after a shorter (1 day) or a longer (7 or 28days) period of time without any stimulation. In response to the footshock they show a species-specific fear-like behavior, „freezing” (complete immobility, except breathing).

Based on the literature the three most important brain areas encoding fear memories are the amygdala, hippocampus and prefrontal cortex. The background of PTSD was studied through changes in their glutamatergic system. The glutamatergic signaling plays an important role in synaptic plasticity, consequently in the formation of fear memories. Recently, calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (CP-AMPA) receptor indicated as an important player of synaptic plasticity, besides of the well known N-methyl-D-aspartate (NMDA) receptor. Pharmacological modulation of the CP-AMPA receptor provides a more specific modulation of glutamatergic system and indicates new therapeutic options for the treatment of PTSD.

Glutamatergic neurons are characterized by 3 types of glutamate transporters (VGluT1,2,3). We concentrated on VGluT3 - distribution of which follows a restricted pattern in the brain - to get more specific effects. The complete lack of VGluT1 or 2 is lethal, but VGluT3 knockout (KO) mice are viable. VGluT3 mainly colocalizes with other classical neurotransmitters, such as serotonin (5-hydroxytryptamine, 5HT) in the raphe nuclei. Approximately 30% of neurons in median raphe (MR) contains this transporter. The other indication to investigate the role of MR in PTSD was the effectiveness of SSRIs, acting on the serotonergic system, deriving from the raphe nuclei.

In addition, the hypothalamic–pituitary–adrenal axis (HPA) is also important in the stress adaptation. HPA axis regulation has changed in PTSD patients. The glutamatergic signaling has an effect on all levels of the HPA axis. Till now there is no information about the contribution of VGluT3 in stress axis regulation.

2. AIMS

We addressed the following questions:

1. **The acute and remote effects of fear conditioning in rats and mice**
    a. Compare the behavioral effect of fear conditioning in rodents with human PTSD symptoms
    b. Investigate the effects of fear conditioning on the HPA axis (analysing stress hormone levels)
    c. Investigate the involvement of different brain regions in the formation of fear memory (by c-Fos immunhistochemistry)

2. **The role of the glutamatergic system**
    a. The role of CP-AMPA receptor in fear conditioning

i. Changes in mRNA levels of AMPA receptor subunits during fear conditioning (by RT-PCR)
ii. Pharmacological manipulation of CP-AMPA receptor, a potential therapeutic target of PTSD

b. Characterization of the VGluT3 KO mice
i. Basal physiological and behavioral variables
ii. Measurement of anxiety and stress reactivity in pups
iii. The effect of VGluT3 on fear conditioning
iv. The effect of VGluT3 on HPA axis

3. Optogenetic modulation of the VGluT3 containing MR region

a. Behavioral consequencies of the stimulation and inhibition of MR region
b. Examination of brain regions relevant to fear conditioning during activation of the MR region (by c-Fos immunhistochemistry)

3. METHODS

3.1 Animals

Subjects were male Wistar rats (approximately 10 weeks old) and C57BL/6J or C57BL/6N mice (pups were 7-8 days old, adults were 14-18 weeks old) (Charles River Laboratories, Budapest, Hungary). Genetically modified mice (C57BL/6J, homozygous (VGluT3+/+ wild type (WT) és VGluT3-/- knockout (KO))) were obtained from heterozygous mating of local breeding pairs at the Institute of Experimental Medicine, Budapest, Hungary. Tail tissue was collected for verification of VGluT3 genotype by qPCR. Animals were kept under a standard 12h light–dark cycle (lights on at 6 am), with food and water available *ad libitum*, and their body weights were recorded weekly. In the animal facility the temperature was 22 ± 2 °C and humidity was 60 ± 10%. All manipulations of the animals were approved by the local committee for animal health and care and performed according to the European Communities Council Directive recommendations for the care and use of laboratory animals (2010/63/EU).

3.2. Behavioral tests

Behavioral tests were recorded and analyzed by experimenters blind to treatment conditions by a computer-based event recorder (H77, Dr. Haller József, Budapest, Hungary). In some cases (open field test, forced

swim test, for representative pictures) automated video tracking software was used (Noldus EthoVision 10.1, Noldus Information Technology, Wageningen, Netherlands).

3.2.1. Conditioned fear test (CFT)

Shocks of 0.8 mA (mice) or 3mA (rats) were administered via the grid floor of a Plexiglas cage (26×26×31 cm) in a separate room. Two shock trains were administered per min for 5 min (i.e. each subject received 10 shocks). Each shock train was 1s long at 50Hz. Control mice were placed into a similar box for 5 min, but shocks were not delivered. Next day and a 28 days (rats) or one week later (mice) animals were exposed to the same context for 5 min without shocks (contextual fear test). The box was cleaned between sessions with soapy and tap water. The following behaviors were all scored: *freezing* complete immobility, no movements of the snout; *exploration* sniffing movements directed towards the floor and walls of the test cage as well as sniffing in the air; *grooming*washing with forepaws and scratching with hindpaws; *resting* no locomotion, small postural changes allowed. In optogenetic experiments we defined a few other variables: *ambulation* number of virtual line crossings (with all four legs) of a 3x3 grid that divided the cage into nine 10x10 cm squaresand separated from exploration, *shock and stimulation runs* rapid ambulation without engagement in other behaviors that covered at least one cage-length. The result of the 5 min test was further divided into 10 sec *(ON response)* and 20 sec *(OFF response*) time bins in accordance with the optogenetical stimulation.

3.2.2. Young animals

3.2.2.1. Model of bacterial infection

On postnatal day (PND) 14-15 lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, Mo. USA; O55:B5; 100 µg/ml/kg, dissolved in saline) or saline was injected i.p. to the animals (n=3-8/group). One hour later blood samples were taken for hormone measurements (adrenocorticotropic hormone (ACTH), corticosterone) and tail tissue was collected for verification of VGluT3 genotype by PCR. Thus, the animals were blindly assigned into different treatment groups and the genotype was determined only after the experiment.

3.2.2.2. Ultrasonic vocalization (USV)

Pups at PND 7-8 were brought to a soundproof room and placed in a 600 ml glass beaker without bedding and heating. USV was followed for 10 min. Briefly, individual calls were detected using an ultrasonic-sensitive frequency division detector (CIEL electronique, cdb205, Koenigslutter, Germany) fixed on a holder 12 cm above the bottom of the glass beaker coupled to a computer. Vocalizations were recorded using a free Audacity 2.0.5. software. Data were automatically counted in the power spectrum 30-50kHz (typically occuring after maternal separation) using an USV Counter software (developed by S. Zsebők). Total number and total duration of calls per session were measured. In addition, USV frequency was calculated as the total number /10 min.

3.2.3. Adult animals

3.2.3.1. Motor coordination (rotarod)

Tested animals were placed on a commercially available accelerating rod with diameter of 3 cm (IITC Life Science, Woodland Hills, USA). The rotation speed started from 5 rpm and accelerated to 25 rpm during 1 min and the time the animal could stay on the rotarod was measured (latency to fall, cut off: 1 min). Each animal was placed always at the same lane, under similar lighting condition as in the animal facility. This protocol was repeated three times with 30 min intertrial intervals and the latencies to fall were averaged.

3.2.3.2. Pain perception tests (hot plate and flinch-jump test)

The thermal pain threshold was investigated on an electrically heated metal plate. On the day of testing animals were habituated to the testing apparatus (IITC Life Science, Woodland Hills, CA, USA) (10 min; 35 °C). Right after the habituation period the plate was heated with a constant rate of 6°C/min, until the animals showed nocifensive behavior (frequent paw lifting and/or licking). The measurement was repeated three times consecutively with 1 min intertrial intervals. The temperature at which the animal showed the first sign of nocifensive behavior was taken as the paw withdrawal threshold, expressed in °C and the average of three values was taken as the thermal nociceptive threshold.

Investigation of the electric pain threshold started after a 3 min habituation. Shock titrations were continued upwards in a stepwise manner

(from 0.05 mA; 0.05-1.2 mA range), and the cut-off was 1.2 mA to avoid foot damage. The interval between shocks was 30 sec like in our fear conditioning protocol. Pain threshold was defined as the lowest shock intensity, that elicited simultaneous removal of at least three paws from the grid. The flinch and jump threshold were expressed in mA.

3.2.3.3. Forced swim test (FST)

Our test based on the method described by Porsolt et al. Mice were placedfor 6 min in a cylindrical tank (40 cm height and 10 cm diameter) and filled with tap water (24±1°C). We defined floating as passive coping strategy a typical posture of immobility.

3.2.3.4. Startle response

Subjects were placed in a test cage inside a sound attenuated chamber (Animal Acustic Startle System; Coulbourne Instruments, Holliston, USA). Following 5 min habituation subjects were presented a 40 ms long, 120 dB acoustic stimuli (noise) for 5 times in every 20 s to standardize startle. Then the same trial was repeated 5 times with variable intertrial interval and the program automatically recorded the startle response. Response to the 0 dB pulse was the weight of the subject and was subtracted from subsequent startle response data.

3.2.3.5. Elevated plus maze test (EPM)

The apparatus was made of metal and painted black (elevated 70 cm above the floor, arm length: 50 cm; arm width: 10 cm; central platform: 10 × 10 cm; closed arm walls height: 40 cm). Lighting varied between 50-120 lux in different compartments. Each mouse was transferred in the homecage from the housing room to the test room. Immediately after arrival in the test room the animal was placed on the central area of the EPM, with the head facing an open arm. EPM exposure lasted 5 min. The surface of the maze was washed with soapy and tap water before an animal was placed in it. The percentage of time spent in open arms and open/total (open/open plus closed frequency) entries ratio (an entry was defined as having three paws of animal in a defined compartment) were calculated and used as measures of anxiety-like behavior. The number of closed arm entries was used to estimate the general locomotor activity of the animal.

3.2.3.6. Open field test (OF)

Each mouse was placed inside a white Plexiglas chamber (40 cm × 36 cm × 19 cm) for 5 min or 15 min. The central area was defined as occupying 60% of the total area. The travelling distance (cm), velocity (cm/s) and the frequency of entries into the center of the arena were determined.

3.2.3.7. Immunohistochemistry

90 minutes after the behavioral tests mice were anaesthetized (ketamine-xylazine cocktail) and transcardially perfused. The position of the tip of the optical fiber and the size of the virus infected area were determined by immunohistochemistry. The neuronal activation was measured by c-Fos immunohistochemistry. In rats the prefrontal cortex (PFC: cingular (Cg1)) infralimbic (IL), prelimbic (PrL)), the amygdala (central (CeA), medial (MeA), basolateral (BLA))) and the hippocampus (HC: CA1,2,3) were analyzed. In mice after optogenetic modulation the number of positively labelled cells were measured in the raphe (medial (MR) és dorsal (DR)), PFC (Cg1, IL, PrL), paraventricular nucleus (PVN), periaqueductal grey (PAG) bed nucleus of stria terminalis (BNST), amygdala and hippocampus.

3.2.3.8. PCR measurements of gene expression

Brains were collected under RNAase free conditions, were cooled on dry ice. The dissected, frozen tissues were homogenized and total RNA was isolated from the samples. cDNA synthesis was performed with the High Capacity cDNA Reverse Transcription Kit. The gene expression was analyzed using real-time PCR.

3.2.3.9. Hormone concentration

Blood was centrifuged at 4 °C. The plasma was separated and kept at -20 °C till radioimmunoassay (RIA) analysis. The ACTH antibody (no. 8514) directed against the midportion of the h-ACTH1–39 molecule was raised in rabbit and corticosterone antibody was raised in rabbits against corticosterone-carboxymethyloxime bovine serum albumine in the Institute of Experimental Medicine, Hungarian Academy of Sciences (Budapest,

Hungary). All the samples from a particular experiment were measured in one RIA to exclude the interassay variation.

3.2.3.10. Pharmacological treatment

Rats were treated with 0 (vehicle: 0,9% NaCl), 1, or 3 mg/kg IEM-1460 (Tocris Bioscience, Hungary) CP-AMPA receptor antagonist. One and 28 days after fear conditioning, rats were exposed to the stress-associated cage 60 min after the pharmacological treatment.

3.2.3.11. Optogenetic modulation

For the optical control of the MR region, 40 nL adeno-associated virus vector (AAV; Penn Vector Core, PA, USA) encoding ChR2 (AAV2.5.hSyn.hChR2(H134R)eYFP.WPRE.hGH; 1.3e12 GC/ml; Addgene26973) or NpHR (AAV9.hSyn.eNpHR3.0-eYFP.WPRE.hGH; 2.04e12 GC/ml; Addgene26972) were injected into the MR region (coordinates: -4.10 mm from Bregma; 0.0 mm lateral to midline and -4.60 mm ventral to the skull). Two weeks after the injection mice were implanted with multimodal optic fibers (core diameter: 105 μm), implants were secured by screws and acrylic resin (coordinates: angle 10°; -4.80 mm from Bregma; 0.0 mm lateral to midline and -4.1 mm ventral to the skull). Behavioral experiments started after 4-7 days recovery. Net energy output (10-20 mW) was measured by laser power meter before and after the experiments.

The laser stimulation was conducted without electric footshock and the stimulation pattern was 20 Hz and 50 Hz theta burst frequency. In case of optogenetic inhibition a continuous light was administered during electric footshock.

3.2.3.12. Statistics

Data were analyzed using STATISTICA 12.0 software package (StatSoft, Inc., Tulsa, OK, USA). Main effects were investigated by one- or factorial analysis of variance (ANOVA), or with repeated measures ANOVA. Significant main effects where further analyzed by the Newman-Keuls/Duncan post-hoc test. Multiple regression method was used to analyze the correlation. Data were shown as means ± standard error of the mean. $P < 0,05$ was considered significant.

4. RESULTS

4.1. The acute and remote effects of fear conditioning in rats and mice

4.1.1. Behavioral results

During fear conditioning freezing was elevated, exploration and grooming was decreased both in rats and mice. If animals were put back to the fear conditioning box after a shorter or a longer time period, they showed similar or higher freezing behavior than during footshocks and it was independent of the time. Data show the traumatic event induced similar behavioral changes in animals like symptoms in PTSD patients. The psychological distress has appeared in response to environmental cues and maintained for a long time.

Based on the behavioral data we have a reliable animal model of PTSD allowing to investigate the neuronal and hormonal background.

4.1.2. Stress hormones

Interestingly, the detected changes in behavior, was not fully observable in stress hormone levels. In rats the ACTH level was elevated during the fear conditioning and in 1 and 28 days later compared to the non-shocked animals. In contrast, it seems that the 5 min was not enough to induce changes in corticosterone levels. In mice we did not find detectable effect in stress hormone levels.

Patients with PTSD also show elevated and decreased hormone levels too. Glucocorticoids act on HPA axis via negative feedback, but it has receptors in different brain areas too – e.g. it can increase the neuronal activity in BLA -, thus it may use other pathways to influence the behavior.

4.1.3. Measurement of neuronal c-Fos activity

The measured neuronal activity by c-Fos immunhistochemistry was time-dependent - in rats - compared to behavioral data. One day after fear conditioning less c-Fos labelled cells were found in BLA and HC, but other relevant brain areas did not differ significantly from controls. 28 days after fear conditioning neuronal activity was decreased in PFC and increased in amygdala. One explanation of these results is that lower HC

activity was measured in PTSD patients, as a result they cannot learn that the environment is not dangerous anymore. Following this idea, due to the reduced activity of PFC, animals cannot create new associations.

4.2. The role of the glutamatergic system

4.2.1. The role of CP-AMPA receptor

The involvement of AMPA receptors in fear and extinction learning appears established, but findings are not entirely congruent regarding their roles. For instance, both fear expression and extinction were altered in AMPA receptor KO mice while pharmacological interventions affected fear extinction only. The different subunit ratio in AMPA receptors may contribute to these results. Decrease in GluA2 mRNA level indirectly suggests an increase in the formation of CP-AMPA receptors. Our findings on GluA1/GluA2 ratios appear to be reflections of the role played by amygdalar CP-AMPA receptors in fear learning-related neuronal plasticity, but also show that AMPA receptor dynamics is rather different in the PFC. CP-AMPA receptor antagonist IEM-1460 administration decreased fear response one and 28 days after fear conditioning. Based on our findings this compound may have clinical implications.

4.2.2. Investigating the effects of VGluT3

4.2.2.1. Postnatal period

Our data demonstrated that anxious phenotype appears during the early postnatal period, namely there was a tendency for enhanced ultrasonic vocalization induced by maternal separation. Anxiety disorders are stress dependent, so we measured the changes in the stress hormones after stress exposure. VGluT3 KO mice react to an immune stressor with enhanced ACTH and corticosterone secretion compared to WT. Until this point, there was no evidence in the literature about the presence of VGluT3 in HPA axis. Therefore it is more likely, that observed changes are due to the changes in co-transmission of serotonergic, cholinergic and/or GABAergic neurons. Disturbance of the fine balance among all these systems may result in a borderline enhanced anxiety-like behavior in vGluT3 KO pups.

4.2.2.2. Fear conditioning in adult mice

VGluT3 KO mice showed exaggerated fear during conditioned fear test, which was not context dependent (generalised fear) and was accompanied by enhanced anxiety represented by reduced open-arm time in EPM and reduced distance travel as well as decreased number of entrances into the centrum in OF test. We investigated the general characteristics of the VGluT3 KO mice, because these variables (e.g. motorcoordination, pain sensitivity) can influence the interpretation of further tests, but there was no difference between the genotypes.

4.2.2.3. Changes in HPA axis modulation in adult animals

Disruption in stress-regulation may also contribute to the anxious phenotype. Among resting HPA axis variables only the corticotropin-releasing hormone (CRH) mRNA level in the hypothalamus was significantly enhanced in VGluT3 KO mice. Although this elevated CRH did not seem to lead to changes in HPA axis (no genotype difference was detected at lower levels of the axis), it might contribute to the development of innate fear. We found a smaller elevation in corticosterone levels in VGluT3 KO mice in response to different stressors (electric footshock, open field test with or without previous footshock) compared to WT. Indeed, it is widely accepted, that low cortisol response to trauma may be a risk factor for PTSD and PTSD patients react to new stimuli with reduced stress-hormone level elevation. These data suggest that reduced stress-hormone reaction in VGluT3 KO mice might be an important contributor to their exaggerated emotional state.

4.3. Optogenetic modulation of VGluT3 containing MR region

During fear conditioning induced by electric footshock MR region silencing was able to decrease the shock induced behavioral changes in some parameters (e.g. decreased freezing, increased exploration). The optogenetic activation of the MR region - without electric footshock - did not trigger freezing acutely, demonstrating that this brain region detaches from fear-effector systems (like e.g. PAG), which elicit freezing when stimulated. On the contrary, MR region stimulation enhanced the unpleasantness of the context reflected by reduced exploration and enhanced escape behavior (shock-runs). This agitation behavior commonly

observed in mice exposed to aversive stimuli e.g. electric footshocks. Moreover, when optogenetic stimulation was administered rhythmically at 50Hz theta burst frequency it entrained a behavioral rhythm. Noteworthy, the behavioral effects of stimulation were reversible on a very short time-scale and this pattern was not observable in controls and during 20 Hz stimulation. Our data suggest a fast, phasic neurotransmitter release from MR region, which is the opposite of the well-known slow, tonic serotonin release. In MR region there are different type of neurons - smaller percentage of neurons are purely serotonergic, the bigger part is glutamatergic or colocalized with glutamate (VGluT3) -, so we have to assume a more complex function of this region. MR region activation did not evoke, nor did its silencing diminish conditioned fear responses the next day, which indicates that MR region stimulation alone was not sufficient to form an associative memory trace to link an adverse experience to the context. By contrast, MR region activation resulted in an incubation-like enhancement of fear-like behavior, showing more freezing remotely. Thus, it transformed into a long-term fear memory trace after a week.

During optogenetical stimulation, we found c-Fos elevation in MR and DR. Raphe has extensive and complex connections with brain areas important in fear memory formation (prefrontal, entorhinal cortex, hippocampus). The aversiveness of stimulation is further supported by the activation of the PVN, which orchestrates the stress response, the BNST, which maintains arousal, the Cg1, which is involved in the processing of aversive stimuli and the PAG, which coordinates behavioral responses to aversive situations. However, the aversiveness of MR region stimulation was insufficient to result in fear as demonstrated by the absence of freezing, and by the lack of activation of the amygdala and hippocampus, which are strongly involved in the processing of fear.

5. CONCLUSIONS

Based on our findings we can conclude the followings:

1. **The acute and remote effects of fear conditioning**
   a. Fear conditioning is an effective model to induce PTSD-like behavioral changes

   b. Hormonal and c-Fos labeled neuronal activity changes were found and it were time-dependent, indicating a complex, long-term effect on the regulation of HPA-axis and the reorganization of neural network involved in fear learning

2. **The role of the glutamatergic system**

   a. Changes of the GluA1/GluA2 ratio confirms the role of amigdalar CP-AMPA receptor in fear learning via synaptic plasticity, but indicating different processes in PFC

   b. Administration of CP-AMPA receptor antagonist (IEM-1460) decresed fear response, which can be a possible therapeutic target

   c. Lack of VGluT3 has an important role of the development of innate fear and induces a sensitive phenotype for the possible negative life events in the future

   d. The lack of VGluT3 causes decreased stress reactivity which can be a risk factor for the development of PTSD

3. **Optogenetic modulation of VGluT3 containing MR region**
   a. Silencing of the MR region decreased the effect of electric footshock in few parameters. MR region stimulation enhanced the unpleasantness of the context and induced agitation acutely, and incresed neuronal activity was found in raphe, PVN, BNST, Cg1, IL and PAG

b. Optogenetic modulation of MR region was not sufficient to promote fear memories in short-term, but it was capable to induce fear responses in long-term

PUBLICATIONS OF THE AUTHOR

Publications that form the basis of the Ph.D. dissertation

1. **Balázsfi D**, Fodor A, Török B, Ferenczi S, Kovács KJ, Haller J, Zelena D. Enhanced innate fear and altered stress axis regulation in VGluT3 knockout mice. Stress. 2018 Mar;21(2):151-161. doi: 10.1080/10253890.2017.1423053. IF:2,590
2. Horvath HR, Fazekas CL, **Balazsfi D**, Jain SK, Haller J, Zelena D. Contribution of Vesicular Glutamate Transporters to Stress Response and Related Psychopathologies: Studies in VGluT3 Knockout Mice. Cellular And Molecular Neurobiology 2018 Jan;38(1):37-52.doi: 10.1007/s10571-017-0528-7; IF:2,93
3. **Balazsfi DG**, Zelena D, Farkas L, Demeter K, Barna I, Cserep Cs, Takacs VT, Nyiri G, Goloncser F, Sperlagh B, Freund TF, Haller J. Median raphe region stimulation alone generates remote, but not recent fear memory traces. Plos One 12:(7) p. e0181264. (2017) doi: 10.1371/journal.pone.0181264; IF:2,806
4. Gölöncsér F, Baranyi M, **Balázsfi D**, Demeter K, Haller J, Freund TFF, Zelena D, Sperlágh B. Regulation of Hippocampal 5-HT Release by P2X7 Receptors in Response to Optogenetic Stimulation of Median Raphe Terminals of Mice. Front Mol Neurosci. 2017 Oct 12;10:325. doi: 10.3389/fnmol.2017.00325. IF:3,902
5. **Balázsfi D**, Farkas L, Csikota P, Fodor A, Zsebők S, Haller J, Zelena D. Sex-dependent role of vesicular glutamate transporter 3 in stress-regulation and related anxiety phenotype during the early postnatal

period. Stress. 2016 Jul;19(4):434-8. doi: 10.1080/10253890.2016.1203413; IF: 2,590

6. Zelena D, Mikics É, **Balázsfi D**, Varga J, Klausz B, Urbán E, Sipos E, Biró L, Miskolczi C, Kovács K, Ferenczi S, Haller J. Enduring abolishment of remote but not recent expression of conditioned fear by the blockade of calcium-permeable AMPA receptors before extinction training. Psychopharmacology (Berl). 2016 Jun;233(11):2065-76. doi: 10.1007/s00213-016-4255-4; IF:3,308

Other publications of the author

1. Biro L, Sipos E, Bruzsik B, Farkas I, Zelena D, **Balazsfi D**, Toth M, Haller J. Task Division within the Prefrontal Cortex: Distinct Neuron Populations Selectively Control Different Aspects of Aggressive Behavior via the Hypothalamus. J Neurosci. 2018 Apr 25;38(17):4065-4075. doi:10.1523/JNEUROSCI.3234-17.2018; IF:5,970
2. Zelena D, Demeter K, Haller J, **Balázsfi D**. Considerations for the use of virally delivered genetic tools for in-vivo circuit analysis and behavior in mutant mice: a practical guide to optogenetics. Behav Pharmacol. 2017 Dec;28(8):598-609. doi: 10.1097/FBP.0000000000000361; IF:1,854
3. Mikics E, Guirado R, Umemori J, Toth M, Biro L, Miskolczi C, **Balazsfi D**, Zelena D, Castren E, Haller J, Karpova NN. Social Learning Requires Plasticity Enhanced by Fluoxetine Through Prefrontal Bdnf-TrkB Signaling to Limit Aggression Induced by Post-

Weaning Social Isolation. Neuropsychopharmacology x:(x) p. x. Paper in press. (2017) doi: 10.1038/npp.2017.142; IF:6,403

4. Csikota P, Fodor A, **Balázsfi D**, Pintér O, Mizukami H, Weger S, Heilbronn R, Engelmann M, Zelena D. Vasopressinergic control of stress-related behavior: studies in Brattleboro rats. Stress.2016Jul;19(4):349-61. doi:10.1080/10253890.2016.1183117; IF:2,590
5. Fodor A, Kovács KB, **Balázsfi D**, Klausz B, Pintér O, Demeter K, Daviu N, Rabasa C, Rotllant D, Nadal R, Zelena D. Depressive- and anxiety-like behaviors and stress-related neuronal activation in vasopressin-deficient female Brattleboro rats. Physiol Behav. 2016 May 1;158:100-11. doi: 10.1016/j.physbeh.2016.02.041; IF:2,341
6. Zelena D, Pintér O, **Balázsfi DG**, Langnaese K, Richter K, Landgraf R, Makara GB, Engelmann M. Vasopressin signaling at brain level controls stress hormone release: the vasopressin-deficient Brattleboro rat as a model. Amino Acids. 2015 Nov;47(11):2245-53. doi: 10.1007/s00726-015-2026-x; IF:3,173
7. **Balázsfi D**, Pintér O, Klausz B, Kovács KB, Fodor A, Török B, Engelmann M, Zelena D. Restoration of peripheral V2 receptor vasopressin signaling fails to correct behavioral changes in Brattleboro rats. Psychoneuroendocrinology. 2015 Jan;51:11-23. doi: 10.1016/j.psyneuen.2014.09.011; IF:4,788
8. Fodor A, Klausz B, Pintér O, Daviu N, Rabasa C, Rotllant D, **Balazsfi D**, Kovacs KB, Nadal R, Zelena D. Maternal neglect with reduced depressive-like behavior and blunted c-fos activation in Brattleboro mothers, the role of central vasopressin. Horm Behav. 2012 Sep;62(4):539-51. doi: 10.1016/j.yhbeh.2012.09.003; IF:3,378