The role of endocannabinoid signaling in the regulation of responses to environmental challenges and trauma

Doctoral Thesis

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INTRODUCTION

In the central nervous system, the endocannabinoid (eCB) system to our knowledge is composed of eCB receptors (CB_1R, CB_2R), the endogenous receptor ligands anandamide (AEA), 2-arachidonoylglycerol (2-AG) and the enzymes responsible for eCB synthesis and degradation. The eCB system is a so-called retrograde neurotransmitter system, in which the postsynaptically released eCBs exert their biological activity on CB_1R located in the presynaptic membrane. By retrograde signaling, neurons can regulate their own inputs and reduce the amount of neurotransmitter released into the synaptic cleft. This eCB-based signaling has a fundamental role in the regulation of short-, long-term and spike-timing-dependent synaptic depression, thereby regulating synaptic plasticity considered as the molecular basis of learning and memory processes.

From a pharmacological point of view, eCB signaling can be modulated in two main ways: regulation of CB_1R activation (e.g., receptor agonist, antagonist, allosteric modulator) or manipulation of endogenous ligand metabolism (synthesis, degradation, transport). The two main types of pharmacological manipulations have different effects and are therefore suitable for studying different functions. By the induction or inhibition of receptor activation, specific effects of CB_1R signaling can be examined while the manipulation of the eCB metabolism, e.g. by blocking the degradation of AEA and 2-AG, ligand-specific not only CB_1R dependent effects can be detected. The most prominent representatives of the latter approach - used in our own research - are the monoacylglycerol lipase (MAGL) inhibitor JZL184, which is used for the enhancement of 2-AG signaling and the fatty acid amide hydrolase (FAAH) inhibitor URB597 used for the enhancement of AEA signaling.

The eCB system plays a fundamental role in regulating emotions and related behavioral responses: the elements of the eCB system are present in every brain area responsible for the regulation of emotional behavior (e.g., prefrontal cortex, hippocampus, amygdala), but its role is multifaceted. The source of versatility is the high-density expression of CB_1R in many brain regions and its simultaneous presence in excitatory and inhibitory synapses, which have different functions and varying magnitude in the regulation of emotional behavior. In general, CB_1R activation is anxiolytic in aversive conditions in behavioral pharmacological tests, but there are several contradictory results in the literature. To overcome these contradictions, observations suggest that endocannabinoid signaling is not responsible for inducing or inhibiting specific kind of behavior, but rather modulates the nature of the behavioral response to environmental challenges. Thus, eCB signaling context-dependently influences the interpretation of environmental stimuli, so potentially plays a role in regulating coping strategies, aggressive interaction, and fear learning, among others. Most of our knowledge is based on the effects of receptor agonists and antagonist and we have limited knowledge about the specific role and possible interaction of the two endocannabinoids, AEA and 2-AG in the regulation of behavioral responses. Therefore, we wanted to investigate the specific behavioral effects and possible interactions of AEA and 2-AG by examining coping strategies, territorial aggression and acute and long-lasting fear response.

AIMS

During our work, we wanted to answer the following questions:

1. **Effect of enhanced AEA signaling by systemic inhibition of FAAH enzyme on coping strategy and fear response in mice**

1.1. Does enhanced AEA signaling modify the coping strategy in the back test?

1.2. Does enhanced AEA signaling affect acute or long-lasting conditioned fear response?

2. Effect of enhanced 2-AG signaling by systemic inhibition of MAGL enzyme on aggressive interaction in mice

2.1. Does enhanced 2-AG signaling affect territorial aggression and stress axis activation in an aggressive interaction?

2.2. Does enhanced 2-AG signaling affect aggression and stress axis activation of the intruder in an aggressive interaction?

2.3. Does the effect of increased 2-AG signaling on aggression develop in correlation with corticosterone level elevation in intruders?

2.4. Does the effect of increased 2-AG signaling on aggression in residents related to the activation of cannabinoid receptor type 1?

3. Effect of enhanced AEA and 2-AG signaling and their interaction by systemic and local inhibition of FAAH and MAGL respectively on acute fear response and acquisition of traumatic memory in rats

3.1. Do systemically enhanced AEA and 2-AG signaling, or their interaction affect the acute fear response and the acquisition of traumatic memory?

3.2. Do systemically enhanced AEA and 2-AG signaling, or their interaction have an analgesic effect?

3.3. Do locally in the prelimbic cortex enhanced AEA and 2-AG signaling, or their interaction affect the

acute fear response and the acquisition of traumatic memory?

3.4. Do locally in the ventral hippocampus enhanced AEA and 2-AG signaling, or their interaction affect the acute fear response and the acquisition of traumatic memory?

3.5. Do locally in the basolateral amygdala enhanced AEA and 2-AG signaling, or their interaction affect the acute fear response and the acquisition of traumatic memory?

4. Effect of enhanced AEA and 2-AG signaling and their interaction by systemic inhibition of FAAH and MAGL respectively on the extinction of traumatic memory in rats

4.1. Do systemically enhanced AEA and 2-AG signaling, or their interaction affect the extinction of traumatic memory?

METHODS

Animals

Subjects were 2-3-month-old male CD1 mice weighing 30-35 g and 3-month-old Wistar rats weighing approximately 250 g. Laboratory food and water were available *ad libitum*, temperature and relative humidity were kept at 22 ± 2°C and 60 ± 10%. Animals were housed individually in normal light cycle with lights on at 7:00. Experiments were carried out in accordance with European Communities Council Directive (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine.

Drugs, doses and brain site specific administration

The MAGL inhibitor JZL184, the CB1R antagonist AM251 and the FAAH inhibitor were dissolved in 1.5% dimethylsulfoxide (DMSO), which were diluted to a final volume with saline that contained 0.4% methylcellulose. Mice were treated with URB597 at the dose of 0, 0.3 mg/kg, with JZL184 at the dose of 0, 8, 16 mg/kg, with metyrapone at the dose of 0, 30 mg/kg, with AM251 at the dose of 0, 0.5, 1 mg/kg in a volume of 10 ml/kg 40 min intraperitoneally prior to the behavioral testing. Rats were treated systemically with URB597 at a dose of 0,3 mg/kg, with JZL184 at a dose of 16 mg/kg in a volume of 1 ml/kg 40 min intraperitoneally prior to the behavioral testing. For brain site specific enhancement of eCB signaling JZL184 and/or URB597 were infused in a volume of 0.5 µl 30 min prior to testing. JZL184 was injected at the concentration of 1 µg/0.5 µl; URB597 was injected at the concentration of 1 ng/0.5 µl via a Hamilton microsyringe.

Behavioral tests and analysis

Procedures were performed during the first 4 hours of the light phase in a separate room. During the tests, the behavior of the animals was recorded with a camcorder, and the recordings were automatically analyzed using the H77 event recording software and the EthoVision XT behavior tracking software.

Back test on CD1 mice

During the test, the mice were turned to their backs manually and held for one minute. The escape attempts shown in the test were considered as active coping, while inactive behavior was considered as passive coping strategy.

Fear conditioning and contextual reminder on CD1 mice

During conditioning mice were introduced into the Plexiglas box (30×30×30 cm). Shocks were administered via

the grid floor of the box. Two shock trains of 1 s were administered per minute for 5 min (i.e., each mouse received ten shocks). Each shock train (100 V, 3 mA) was 1 s in length and consisted of 0.01-s shocks separated by 0.02-s-long breaks. During the contextual reminder, the individuals returned to the cage once for 5 minutes previously used for conditioning 14 days after the shock.

Resident intruder test on CD1 mice

In the resident-intruder test, an intruder of smaller size was placed into the home cage of residents for 10 min. Aggressive grooming, tail rattling, and wrestling were summed up as offensive behaviors, while defensive upright, avoidance, and flight were summed up as defensive behaviors. Number of bites delivered to and received from opponents were counted.

Fear conditioning and contextual reminder on Wistar rats

Rats were placed in a Plexiglas chamber (30 × 30 × 30 cm), and after 3 min of habituation, three 2.4 mA 2 sec long shocks were administered through the stainless-steel grid floor with 30 s inter-shock intervals. During contextual reminders rats were re-exposed to the conditioning context daily for 5 minutes in the next 7 consecutive days and on the 28th day after conditioning to assess the dynamics of conditioned fear responses.

Hot-plate test on Wistar rats

Changes in nociception were assessed using an increasing-temperature hot plate system (IITC Life Science, Woodland Hills, CA, USA). Rats were placed on the hot-plate apparatus for 3 min of habituation then the plate was heated with a constant rate of 6 °C/min started from 25 °C. Heating was stopped when rats showed nociceptive behavior, hot plate temperature was recorded as pain threshold then the subject was removed from the apparatus.

Blood sampling and corticosterone measurement

Corticosterone levels were measured from trunk blood. Plasma corticosterone was measured by radioimmunoassay method.

Cannula implantation for brain site specific drug administration

Rats were anesthetized and fixed in a stereotaxic frame. Stainless-steel guide cannulae were implanted bilaterally above the targeted brain sites. After surgery, rats were returned to their home cages and subsequently injected intraperitoneally (i.p.) with 0.5 ml of saline and 1 mg/kg Gentamicine in a volume of 1 ml/kg to facilitate clearance of anesthetics and prevent dehydration and sepsis. Rats were allowed to recover for 7 days following surgery.

Perfusion and verification of cannula locations

After the behavioral experiments animals were transcardially perfused. Brains were removed and 30 μm frozen sections were cut in the frontal plane. Sections were examined under a light microscope and locations of infusion needle tips were determined.

Statistical analysis

Data was analyzed by one-way, factorial and repeated measures analysis of variance (ANOVA). ANOVA assumptions were evaluated by Levene's test, where ANOVA assumptions were not fulfilled, data were square root transformed. Fisher’s LSD and Duncan tests were performed for post-hoc analyses when a main effect was significant, and Bonferroni corrections were applied for multiple comparisons. Correlations between test days were estimated by linear regression. P-values lower than 0.05 were considered statistically significant. All statistical analyses were conducted with Dell Statistica software version 13.

RESULTS

1. Effect of enhanced AEA signaling by systemic inhibition of FAAH enzyme on coping strategy and fear response in mice

1.1. Effects of enhanced AEA signaling on coping strategy in the back test

The behavior of mice was determined by an interaction between treatment (factor 1), coping (factor 2), and trial (repeated-measures factor 3) (Wilk's lambda=0.174; $F_{interaction}(4, 66)=22.97$; $p < 0.0001$). In a second analysis we investigated behavioral changes within treatment groups. In subjects treated with vehicle before both trials, behavior was affected by coping styles but not by the trial ($F_{coping}(2, 32)=9.41$, $p = 0.0006$; $F_{trial}(1, 32)=0.75$, $p = 0.39$; $F_{interaction}(2, 32)=0.66$, $p = 0.52$). The duration of escape attempts was similar in the two trials for all three coping styles ($p > 0.5$). In contrast, in mice treated with URB597 before trial 3, behavior was defined by an interaction between coping and trial ($F_{interaction}(2, 32)=8.63$, $p = 0.001$). Particularly, mice that adopted a passive style in the vehicle trial shifted towards a more active style after URB597 ($p = 0.02$). No similar changes were seen in mice adopting mixed or active styles in trial 2.

1.2 Effects of enhanced AEA signaling on acute and long-lasting conditioned fear response

URB597 treatment before fear conditioning increased locomotor activity between shocks ($F_{treatment}(1, 12)=7,07$; $p<0,03$) and decreased freezing ($F_{treatment}(1,12)=5,32$; $p<0,05$). URB597-treated mice increased the exploration of the space between and beneath the metallic grid by which shocks were delivered ($F_{treatment}(1, 12)=4.77$; $p < 0.05$). 14 days after shock

during contextual reminder URB597 treatment had a marginal effect on freezing ($F_{treatment}(1, 12)=3,37$; $0,1>p>0,05$).

2. Effect of enhanced 2-AG signaling by systemic inhibition of MAGL enzyme on aggressive interaction in mice

2.1. The effect of enhanced 2-AG signaling on territorial aggression and stress axis activation of the residents

JZL184 treatment significantly affected bite counts ($F_{treatment*bite\ orientation}(2, 27)=5,94$; $p=0,007$) and to the nature of behavior ($F_{treatment*agonistic\ behavior}(2, 27)=3,41$; $p=0,047$). JZL184 treatment at a dose of 16 mg/kg significantly decreased bites delivered and offensive behavior. JZL184 had no effect on corticosterone levels ($F_{treatment}(2,27)=2,93$; $p>0,07$).

2.2. The effect of enhanced 2-AG signaling on aggression and stress axis activation of the intruders

In intruders JZL184 treatment significantly affected bite counts ($F_{treatment*bite\ orientation}(2,25)=4,29$; $p=0,025$) and the nature of behavior ($F_{treatment*agonistic\ behavior}(2, 27)=3,41$; $p=0,047$). JZL184 treated intruders received significantly more bites and spent more time with defense. JZL184 treatment significantly increased the corticosterone levels of intruders ($F_{treatment}(2, 24)=4,51$; $p=0,02$).

2.3. The effect of MAGL and corticosterone synthesis inhibition on aggression and stress axis activation of the intruders

Metyrapone in a dose of 30 mg/kg was able to reduce corticosterone levels alone and even when co-administered with JZL184 ($F_{interaction}(1, 35)=10,07$; $p=0,003$). Metyrapone

alone and even when co-administered with JZL184 significantly reduced bites delivered ($F_{interaction}(1, 35)=5,09$; $p=0,03$). and time spent with offensive behavior ($F_{interaction}(1, 35)=6,41$; $p=0,015$). Time spent with defense was increased by metyrapone and JZL184 *per se* but not the co-administration of the 2 compound.

2.4. The effect of MAGL and CB_1R inhibition on aggression and stress axis activation of the residents

The CB_1R antagonist AM251 at a dose of 1 mg/kg affected the aggression of residents *per se*: reduced bites delivered ($F_{interaction}(1, 33)=7,79$; $p=0,009$), and time spent with offense ($F_{interaction}(1, 33)=5,92$; $p=0,02$). This kind of effect on aggression was also detected when JZL184 and AM251 were co-administered. At a dose of 0.5 mg/kg AM251 had no effect on aggression or bite counts neither alone nor with interaction with other factors. But there was a significant interaction between JZL184 treatment and bite counts ($F_{JZL184*orientation\ of\ bites}(1,34)=14,32$; $p=0,005$) and the nature of behavior ($F_{JZL184*agonistic\ behavior}(1,34)=15,35$; $p>0,0004$). JZL184 treatment decreased delivered bites and offensive behavior furthermore increased bites received and defensive behavior. AM251 at a dose of 0.5 ($F_{AM251}(1, 34)=4,57$; $p<0,04$) and 1 mg/kg ($F_{AM251}(1, 30)=11,10$; $p<0,003$) also increased corticosterone levels.

3. Effect of enhanced AEA and 2-AG signaling and their interaction by systemic and local inhibition of FAAH and MAGL respectively on acute fear response and acquisition of traumatic memory in Wistar rats

3.1. The effect of systemically enhanced AEA and 2-AG signaling, and their interaction on acute fear response and the acquisition of traumatic memory

Locomotor activity measured during the first 3 min of the conditioning showed no significant treatment-induced changes ($F(4,43) = 1.82$; $p = 0.14$). In contrast, treatment led to significant changes in the duration of freezing behavior during conditioning and contextual reminders ($F_{treatment}(4,38) = 10.55$; $p < 0.01$; $F_{days}(8,302) = 64.7178$; $p < 0.01$; $F_{group*days}(32,304) = 6.91$; $p < 0.01$). Post-hoc comparisons revealed that electric footshocks markedly increased time spent with freezing during conditioning compared to non-shocked controls, which response was dampened by JZL184-treatment. Duration of freezing behavior during the first contextual reminder was significantly increased by electric footshocks compared to non-shocked controls, which response was unaltered by pharmacological treatments. Freezing returned to non-shocked levels on the seventh day following conditioning in all treatment groups. On the 28th day rats receiving pre-conditioning URB597 injections (either alone or concomitantly with JZL184) showed increased freezing levels compared to non-shocked but not to shocked controls.

3.2. The effect of systemically enhanced AEA and 2-AG signaling, and their interaction on pain sensitivity in the hot plate test

Pharmacological treatments did not alter pain threshold ($F_{group}(2,19) = 0.08$; $p = 0.92$; $F_{group*days}(2,19) = 2.1$; $p = 0.15$).

3.3. The effect of enhanced AEA and 2-AG signaling, and their interaction locally in the prelimbic cortex on acute fear response and the acquisition of traumatic memory

Treatment led to significant changes in the duration of freezing behavior during conditioning and contextual reminders ($F_{treatment}(4,26) = 6.44$; $p < 0.01$; $F_{days}(8,208) = 46.29$; $p < 0.01$; $F_{group*days}(32,208) = 5.72$; $p < 0.01$). Post-hoc

comparisons revealed that electric footshocks markedly increased time spent with freezing during conditioning compared to non-shocked controls, which response was unaltered by pharmacological treatments. Freezing behavior during the first contextual reminder was unaltered by pharmacological treatments. By the seventh day following conditioning, freezing returned to non-shocked control levels in all but the URB597-treated group in which it remained significantly higher than non-shocked and shocked control levels. Concomitant administration of JZL184 blocked this effect of URB597 as freezing in this group did not differ from non-shocked or shocked control levels. Freezing level remained elevated on the 28th day after conditioning in rats receiving pre-conditioning URB597 treatment compared to all treatment groups. Simultaneous pre-conditioning JZL184 injection abolished this effect of URB597.

3.4. The effect of enhanced AEA and 2-AG signaling, and their interaction locally in the ventral hippocampus on acute fear response and the acquisition of traumatic memory

Treatment led to significant changes in the duration of freezing behavior during conditioning and contextual reminders ($F_{treatment}(4,20) = 5.33$; $p < 0.01$; $F_{days}(8,160) = 14.34$; $p < 0.01$; $F_{group*days}(32,160) = 4.34$; $p < 0.01$). Electric footshocks markedly increased time spent with freezing during conditioning compared to non-shocked controls. This response was dampened by URB597-treatment compared to shocked control. Simultaneously administered JZL184 blocked this effect of URB597-treatment without affecting freezing *per se*. Freezing behavior during the first contextual reminder was significantly elevated in all treatment groups receiving footshocks compared to non-shocked controls. By the seventh day following conditioning, freezing returned to non-shocked control levels in all but the URB597-treated

group in which it remained significantly higher than non-shocked and shocked control levels throughout the contextual reminders. Concomitant administration of JZL184 blocked this effect of URB597 as freezing in this group did not differ from non-shocked or shocked control levels. Freezing level remained elevated on the 28th day after conditioning in rats receiving pre-conditioning URB597-treatment compared to all treatment groups. Simultaneous pre-conditioning JZL184 injection abolished this effect of URB597.

3.5. The effect of enhanced AEA and 2-AG signaling, and their interaction locally in the basolateral amygdala on acute fear response and the acquisition of traumatic memory

Treatment led to significant changes in the duration of freezing behavior during conditioning and contextual reminders ($F_{treatment}(4,22) = 6.23$; $p < 0.01$; $F_{days}(8,176) = 9.95$; $p < 0.01$; $F_{group*days}(32,176) = 1.73$; $p = 0.01$). Electric footshocks markedly increased time spent with freezing during conditioning compared to non-shocked control levels which response was unaffected by pharmacological treatments. Freezing behavior during the first contextual reminder was significantly elevated in all treatment groups receiving footshocks compared to non-shocked controls and returned to non-shocked control levels by the seventh day following conditioning. Freezing during the contextual reminders were unaffected by pharmacological treatments.

4. Effect of enhanced AEA and 2-AG signaling and their interaction by systemic inhibition of FAAH and MAGL respectively on the extinction of traumatic memory in rats

4.1. The effect of systemically enhanced AEA and 2-AG signaling, and their interaction on the extinction of traumatic memory

Freezing behavior throughout the contextual reminders was significantly affected by treatment ($F_{treatment}(4,42) = 7.57$; $p < 0.01$), test days ($F_{days}(8,336) = 23.35$; $p < 0.01$) and the interaction of these factors as well ($F(32,336) = 4.94$; $p < 0.01$). Pairwise analyses revealed that robust conditioned fear response (increased levels of freezing behavior) was shown by groups previously presented with electric footshocks which response was dampened by JZL184 treatments on the day of the first contextual reminder. Freezing levels in vehicle treated rats returned to non-shocked control levels on the sixth day, while systemic treatments of URB597, JZL184 or both accelerated this process. No conditioned fear responses were detected at 28 days after conditioning.

CONCLUSIONS

In our study we used MAGL and FAAH enzyme inhibitors to investigate the specific behavioral roles and possible interactions of AEA and 2-AG. Based on the results presented in the dissertation, the following conclusions can be drawn.

1. Enhanced AEA signaling promotes the appearance of proactive coping strategy which modulatory effect depends on the intensity of the aversive environmental stimuli.
2. Enhanced 2-AG signaling is responsible specifically for the reduction of territorial aggression. However, the anti-aggressive effect of 2-AG is independent from CB_1R signaling. Enhanced defensiveness appears due to elevated levels of corticosterone in intruder mice.

3. In the development of acute fear response and fear memory formation enhanced AEA and 2-AG signaling plays a different but interacting role

   3.1. 2-AG on a systemic level and AEA in the vHC are responsible specifically for the reduction of acute fear response.

   3.2. The effect of 2-AG and AEA on acute fear can be abolished by AEA on a systemic level and 2-AG in the vHC, respectively.

   3.3. Enhanced AEA signaling in PrL and vHC promotes the formation of robust, long-lasting fear memory.

   3.4. 2-AG can abolish the effect of AEA on fear memory formation in the PrL and vHC but has no effect *per se* on these phenomena at these brain sites.

4. Extinction of traumatic memory is enhanced by 2-AG, AEA and by two eCBs together. The expression of fear response during the first contextual reminder is reduced by 2-AG signaling, which effect can be abolished by AEA.

SUMMARY

Components of the endocannabinoid (eCB) system – the cannabinoid receptors, the two eCBs, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) – are present in significant amounts in brain areas responsible for the regulation of emotional behavior. According to our knowledge, eCB signaling modifies the nature of the behavioral response to environmental stimuli and is not responsible for the induction or inhibition of a specific behavioral response. The aim of my doctoral thesis was to investigate the specific role and possible interaction of AEA and 2-AG in different environmental challenges, studying coping strategies, aggressive interaction, and acquisition and

extinction of traumatic memory. In our studies, the signaling of eCB AEA and 2-AG was enhanced by the inhibition of specific enzymes responsible for their degradation: fatty acid amide hydrolase and monoacylglycerol lipase, respectively. As a result of enhanced AEA signaling, CD1 mice showed a proactive coping strategy in the back test and conditioned fear test. Mice that showed previously reactive coping strategy after the treatment behaved proactively in the back test, but during fear conditioning the proactive strategy was observed comprehensively. In the resident-intruder test of territorial aggression enhanced 2-AG signaling drastically reduced the aggression of resident and intruder CD1 mice. In Wistar rats the acute fear response in the conditioned fear test was reduced by systemically enhanced 2-AG signaling and by locally enhanced AEA signaling in the ventral hippocampus (vHC). Despite the effect of reduced acute fear response, enhanced AEA signaling locally in vHC and in the prelimbic cortex resulted the formation of permanent fear memory. 2-AG alone had no effect on these phenomena but was able to abolish the effects induced by AEA. Extinction of traumatic memory is enhanced by 2-AG, AEA and the two eCBs together. In summary, we provided evidence that the two eCBs have a specific, in some cases interacting role in the modulation of behavioral responses given to tests modeling different environmental challenges. AEA is involved in the formation of a proactive coping strategy, while 2-AG plays a role in reducing aggression and the two eCB jointly regulate the acute fear response, the development and extinction of long-term fear memory.

PUBLICATIONS OF THE AUTHOR

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

1. Balogh, Z., Szente, L., Biro, L., Varga, Z.K., Haller, J., Aliczki, M., (2019). Endocannabinoid interactions in the regulation of acquisition of contextual conditioned fear. Prog Neuropsychopharmacol Biol Psychiatry, 90: 84-91. **IF: 4,185**

2. Aliczki, M., Varga, Z.K., Balogh, Z., Haller, J., (2015). Involvement of 2-arachidonoylglycerol signaling in social challenge responding of male CD1 mice. Psychopharmacology (Berl), 232: 2157-2167. **IF: 3,540**

3. Haller, J., Aliczki, M., Pelczer, K.G., Spitzer, K., Balogh, Z., Kantor, S., (2014). Effects of the fatty acid amide hydrolase inhibitor URB597 on coping behavior under challenging conditions in mice. Psychopharmacology (Berl), 231: 593-601. **IF: 3,875**

Other publications of the author:

1. Aliczki, M., Fodor, A., Balogh, Z., Haller, J., Zelena, D., (2014). The effects of lactation on impulsive behavior in vasopressin-deficient Brattleboro rats. Horm Behav, 66: 545-551. **IF: 4,632**

2. Fodor, A., Barsvari, B., Aliczki, M., Balogh, Z., Zelena, D., Goldberg, S.R., Haller, J., (2014). The effects of vasopressin deficiency on aggression and impulsiveness in male and female rats. Psychoneuroendocrinology, 47: 141-150. **IF: 4,944**

3. Aliczki, M., Balogh, Z., Tulogdi, A., Haller, J., (2012). The temporal dynamics of the effects of monoacylglycerol lipase blockade on locomotion, anxiety, and body temperature. Behav Pharmacol, 23: 348-357. **IF: 2,301**

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