# Schulich MEDICINE & DENTISTRY ADDICTION RESEARCH GROUP

# INHIBITION OF FATTY ACID BINDING PROTEIN-5 ALLEVIATES STRESS-INDUCED ANXIETY AND DEPRESSION-LIKE BEHAVIOURS THROUGH KEY SIGNALING PATHWAYS AND PROLIFERATION MARKERS OF ADULT NEUROGENESIS

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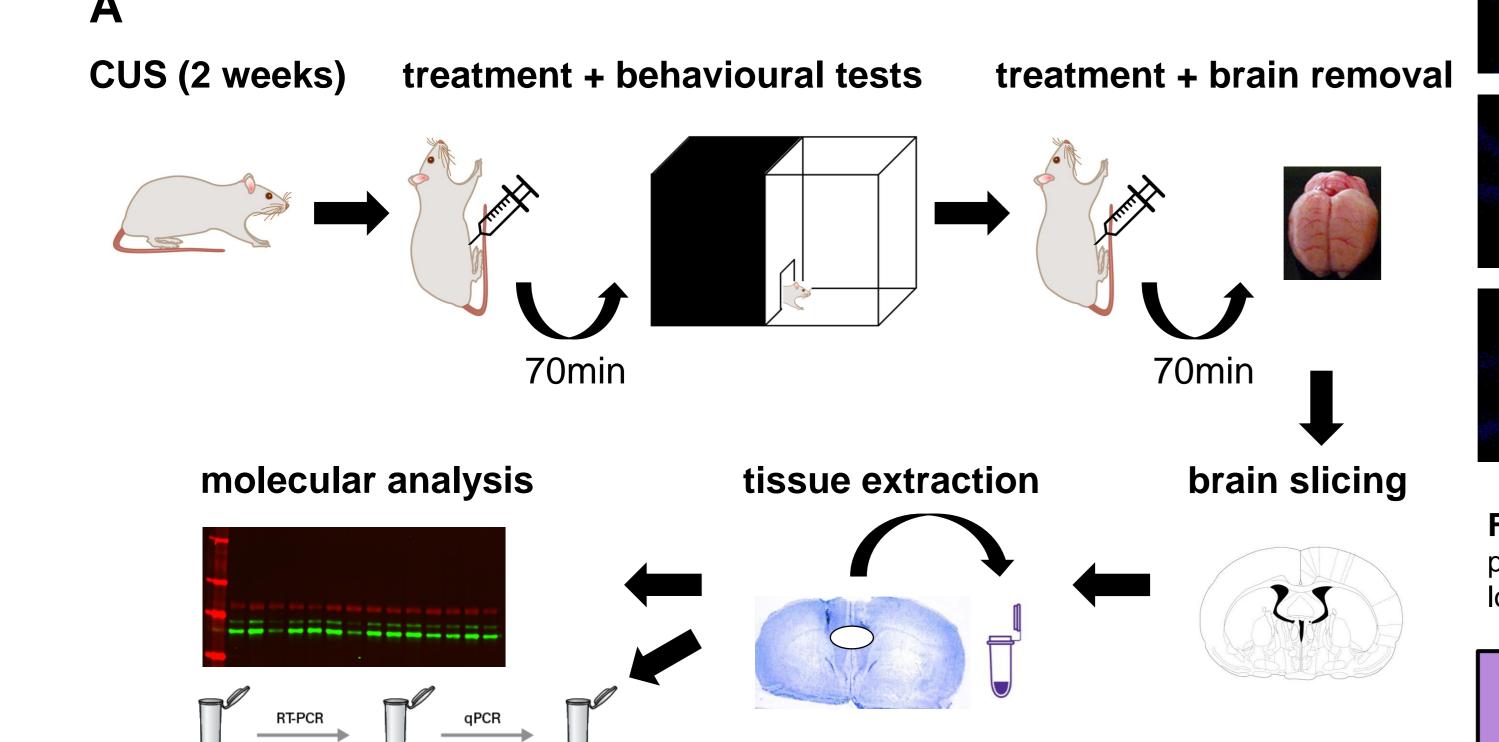
### **BACKGROUND**

Anxiety disorders and depression are the most frequently diagnosed neuropsychiatric disorders worldwide. A promising neurobiological system in which to target the development of novel pharmacotherapies for these neuropsychiatric disorders is the endocannabinoid system (eCB), which has been shown to modulate emotional behaviour and neuronal transmission patterns in both humans and rodents (1,2). Water-insoluble eCB lipids anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) require chaperone proteins for their intracellular transport. Fatty acid binding protein-5 (FABP-5) is a chaperone protein in the eCB system, responsible for the intracellular transport of AEA for degradation by fatty acid amide hydrolase (FAAH). Thus, similar to the inactivation of FAAH or AEA uptake, inactivation of FABP-5 results in elevated AEA-mediated neurotransmission without impacting 2-AG levels (3,4). Importantly, previous findings associate FABP signaling abnormalities with various psychiatric conditions, including anxiety disorders, depression, alcoholism and schizophrenia in both rodents and humans (5,6).

Adult neurogenesis is the process in which newly formed nerve cells are continuously added to the already existing neural network during adulthood. As pro-neurogenic effects were observed following treatment with different classes of antidepressants (7), and as chronic stress was shown to heavily disrupt generation of newborn neurons (8), pharmacotherapeutic interventions to improve adult neurogenesis can be a promising approach against neuropsychiatric disorders.

Previously, we have shown that acute pharmacological inhibition of FABP-5 within the prelimbic cortex of rats altered neuronal activity in key regions of anxiety-related neural circuitry, resulting in an anxiolytic behavioral phenotype in a cannabinoid CB2 receptor dependent fashion (9). We also detected an AEA synthesis-dependent anxiolytic phenotype by pharmacological inhibition of FABP-5 within the basolateral amygdala (BLA, 10). Here, we aimed to investigate the molecular effects of FABP-5 inhibition using **SBFI-103**, a selective inhibitor of FABP-5 (11). Following a 2/3-week long chronic unpredictable stress paradigm, we administered SBFI-103 intraperitoneally for 4 weeks (3 injections per week, 20mg/kg) to adult Sprague-Dawley rats (Figure 1A, B) and investigated anxiety, depression-like behaviour and cognition after each injection. Thereafter, we investigated mRNA expression levels and protein phosphorylation levels in the eCB system within the limbic regions of the rat brain using RT-qPCR and Western blotting, respectively. Furthermore, we examined relevant neurogenesis markers to explore the proliferation and differentiation of newborn neural cells in the subgranular zone of the dentate gyrus using immunohistochemical staining. Our findings provide critical information on understanding mechanistically how inhibition of FABP-5 ameliorates emotional disturbances.

# METHODS



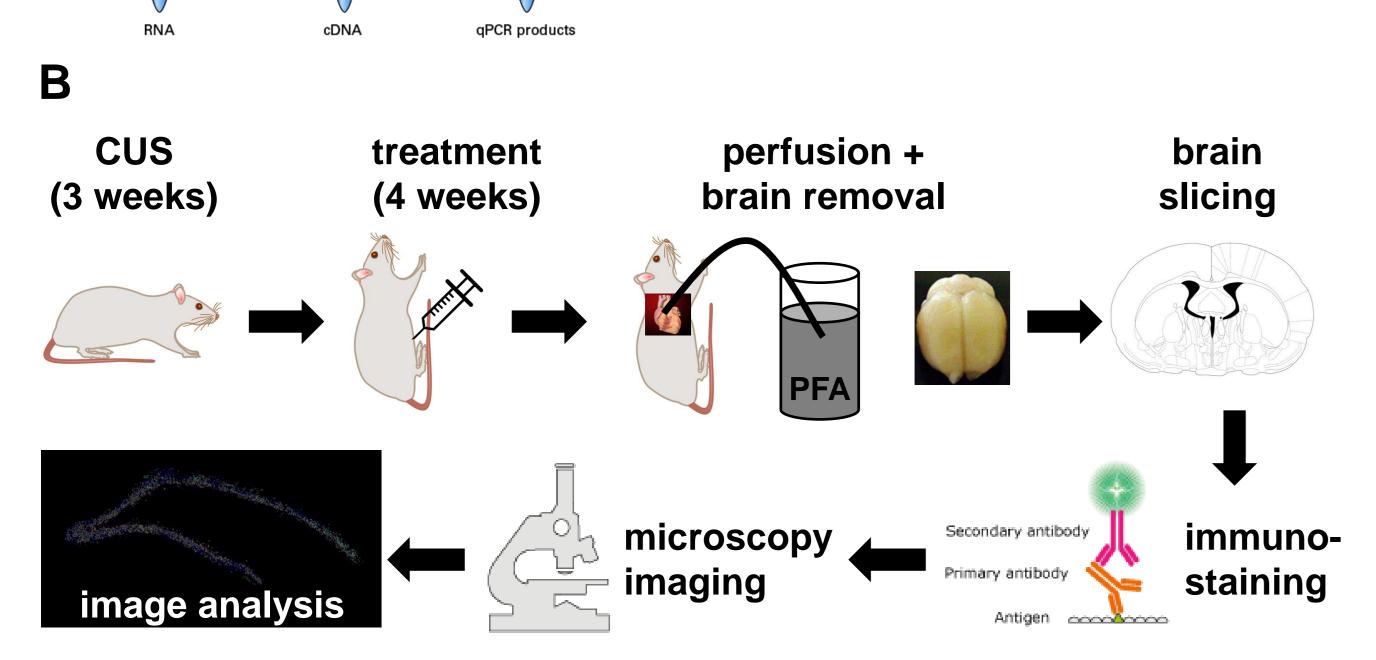


Figure 1: (A) Male Sprague Dawley rats were exposed to a mild chronic unpredictable stress (CUS) protocol. Behavioural tests started a week after the offset of CUS. All behavioural tests were run 70min after an acute injection of VEH or SBFI-103 (20mg/kg). Following the completion of behavioural experiments, rats were sacrificed using sodium pentobarbital. A final injection was done 70min prior to euthanasia in order to investigate the molecular effects of SBFI-103. Brains were removed and flash frozen at -80°C. Coronal sections (100µm) of prefrontal cortex (PFC), nucleus accumbens (NAc), basolateral amygdala (BLA) and ventral hippocampus (VHip) were sliced using cryostat. Microdissection of PFC, NAc, BLA and VHip was performed bilaterally by micropunch method. Collected tissue was processed to be used for protein or RNA extraction in order to perform Western Blotting and q-RT PCR, respectively. (B) A different cohort of male Sprague Dawley rats were exposed to a slightly longer chronic unpredictable stress (CUS) protocol. SBFI-103 treatment started a week after the offset of CUS and was applied for 4 weeks (3 injections per week). BRDU was administered a day before perfusion (3 injections, 250 mg/kg total). Then, animals were transcardially perfused with saline followed by PFA, and their brains were removed. Coronal sections (35µm) of dentate gyrus of dorsal hippocampus were sliced using cryostat. Slices were stained with targets of interest. Images were acquired with Leica SP8 confocal microscope, processed and analyzed using ImageJ.

**Data analysis:** Data are presented as mean + SEM, including individual values. 2-way ANOVA followed by Tukey's post hoc test was conducted to compare groups. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

# **RESULTS - IMMUNOSTAINING**

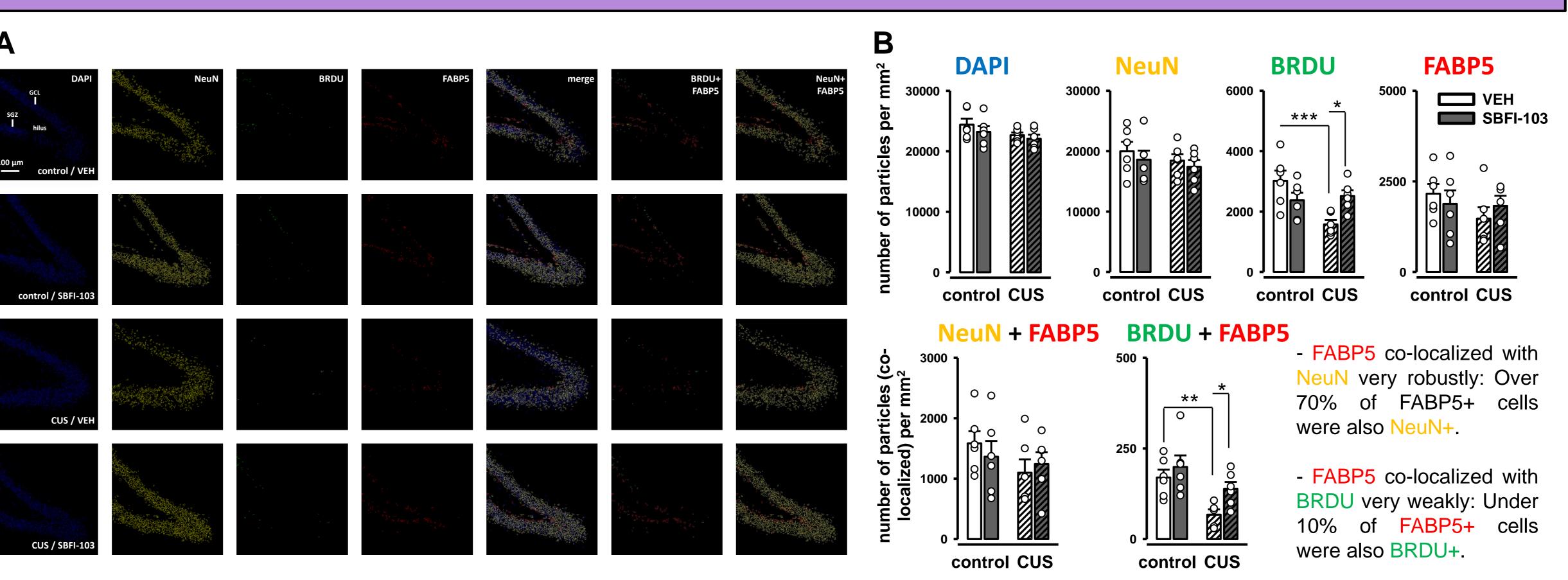


Figure 2: (A) Representative immunofluorescent images from dentate gyrus of dorsal hippocampus, including hilus, subgranular zone (SGZ) and granular cell layer (GCL). (B) Analyzed images were quantified for each target protein (DAPI, NeuN, BRDU and FABP5, top). Number of BRDU+ cells were significantly reduced by stress and reversed by SBFI-103. Colocalization of FABP5 with NeuN and BRDU were assessed (bottom).

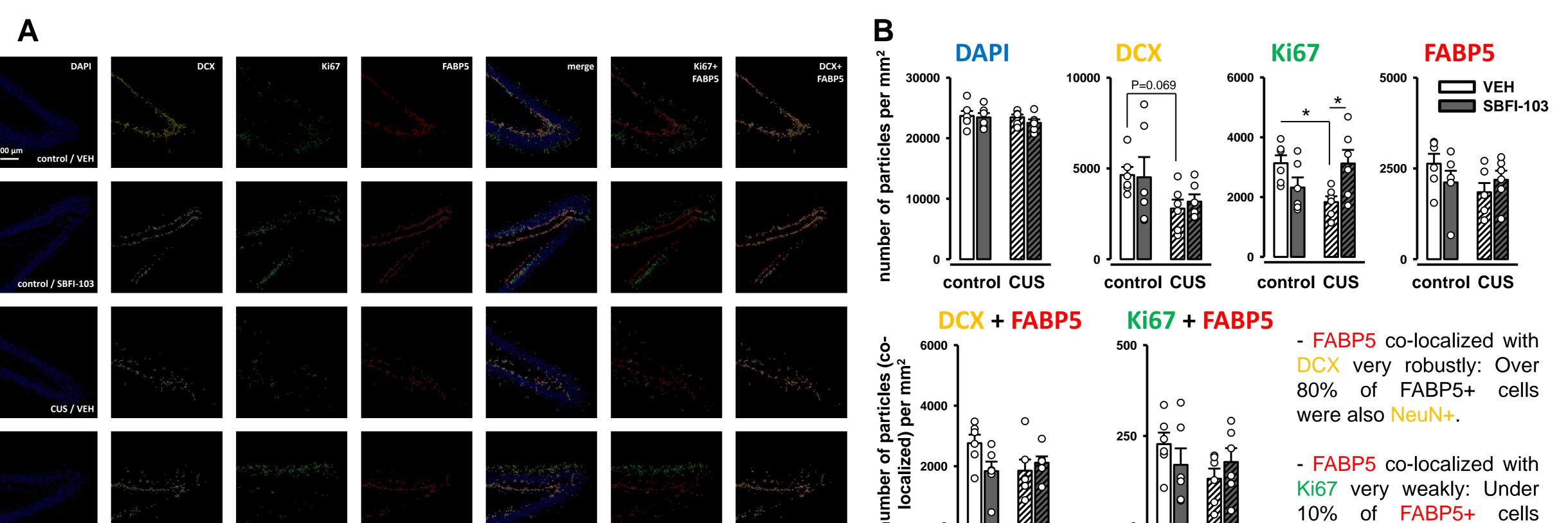
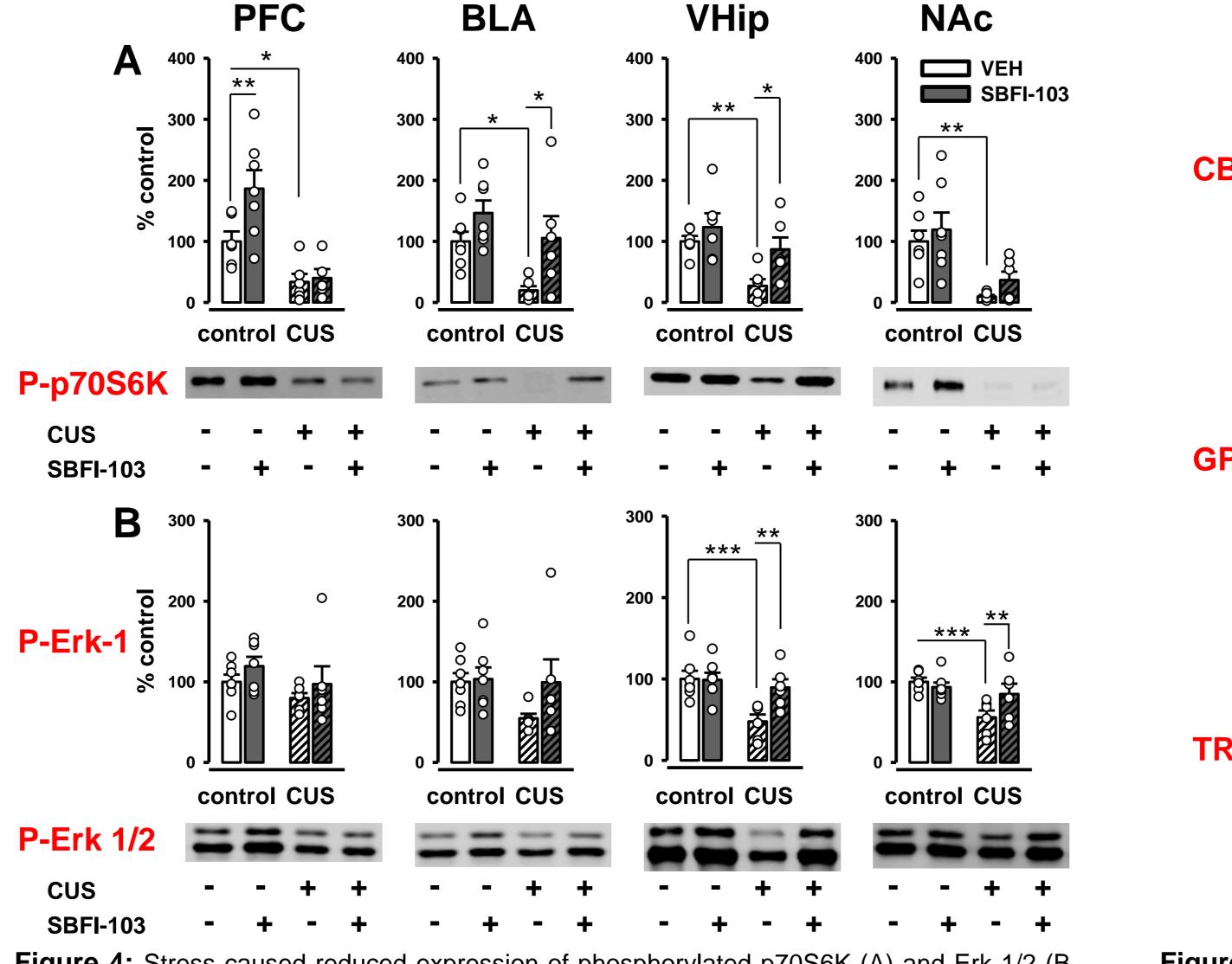


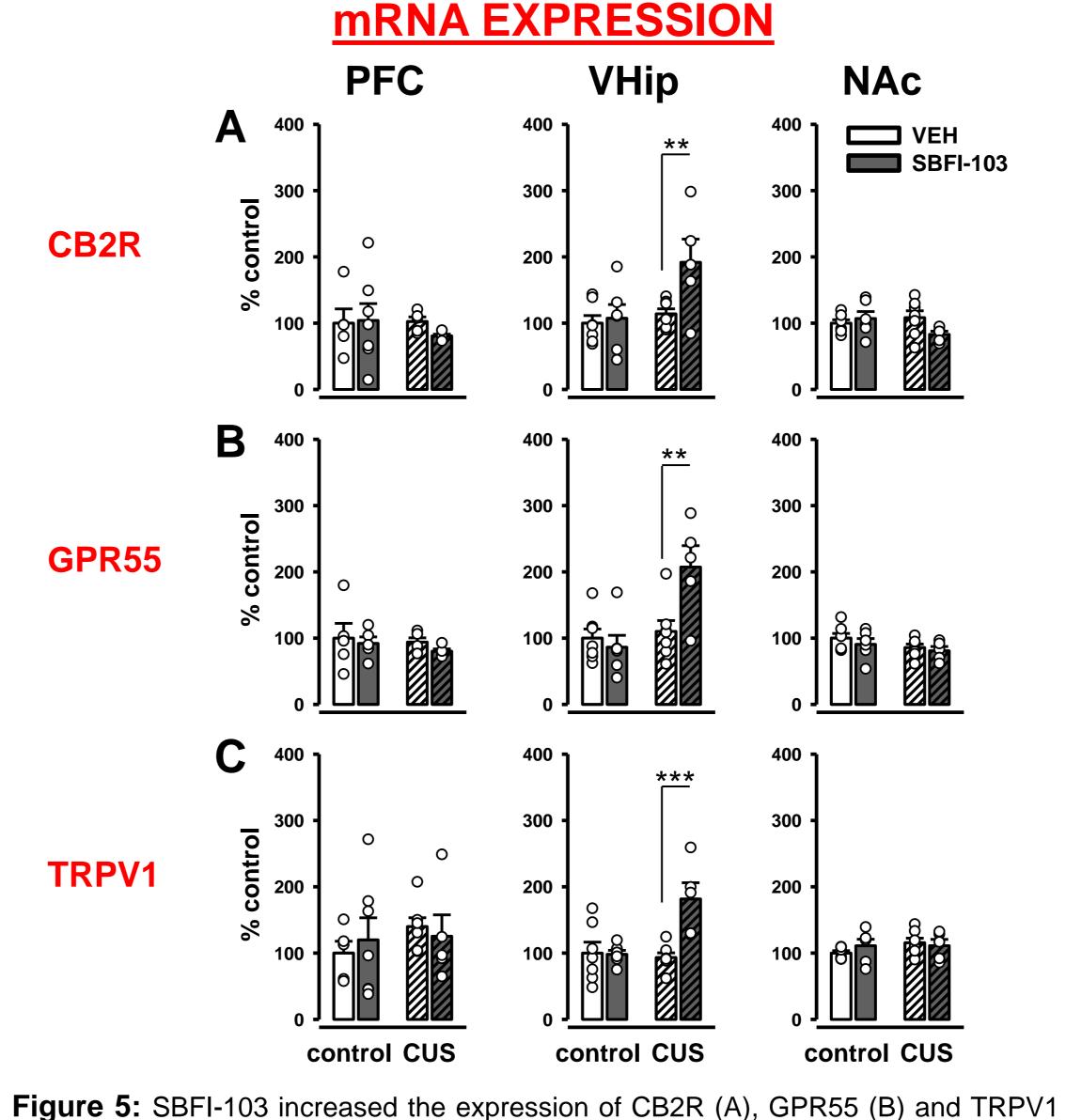
Figure 3: (A) Representative immunofluorescent images from dentate gyrus of dorsal hippocampus, including hilus, SGZ and GCL. (B) Analyzed images were quantified for each target protein (DAPI, doublecortin (DCX), Ki67 and FABP5, top). Number of Ki67+ and DCX+ cells were significantly reduced by stress. SBFI-103 reversed Ki67 levels to control. Colocalization of FABP5 with DCX and Ki67 were assessed (bottom).

# **RESULTS - MOLECULAR**



PROTEIN EXPRESSION

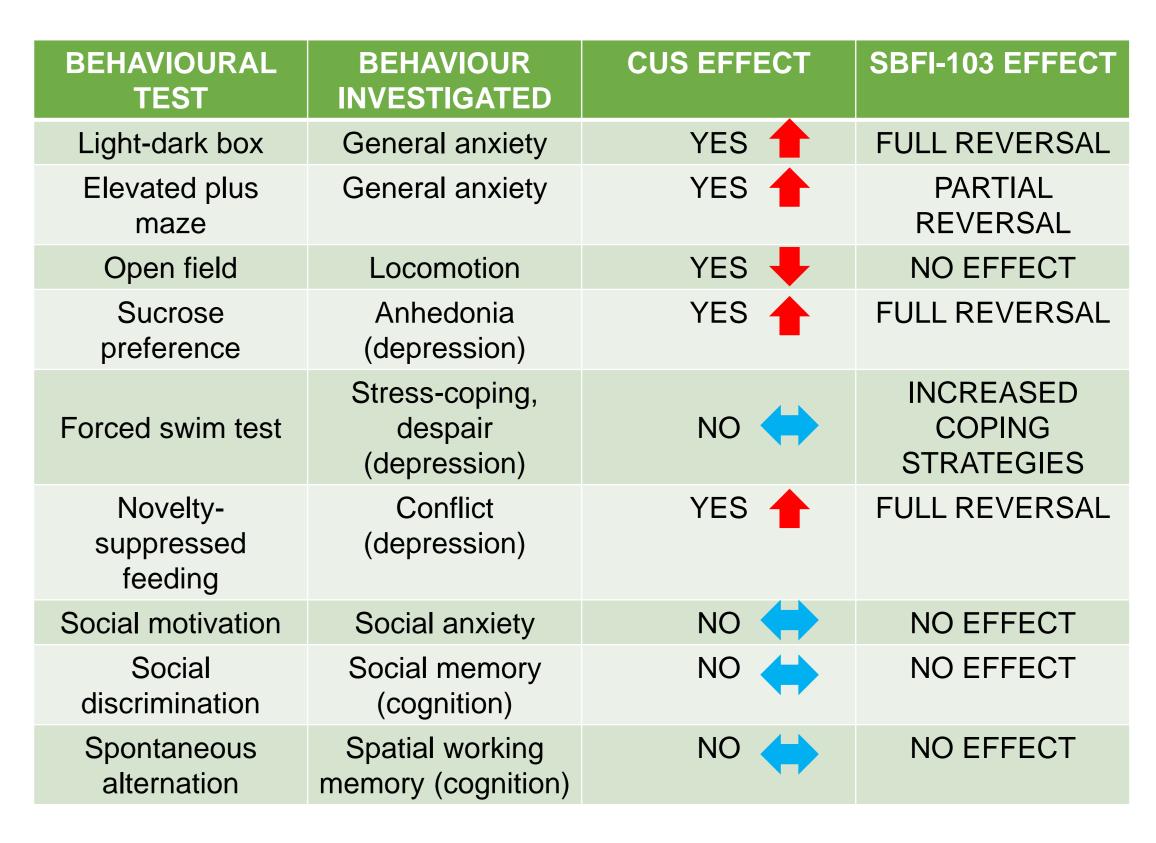
**Figure 4:** Stress caused reduced expression of phosphorylated p70S6K (A) and Erk 1/2 (B, Erk-2 not shown but is similar to Erk-1), established molecular markers that control emotional behaviour. Reductions were fully or partially reversed by SBFI-103, most robustly in the VHip.



were also BRDU+.

(C) mRNA levels of the stressed animals in the VHip. No effects detected for CB1R, NAPE-PLD, FAAH, DAGL-alpha, MAGL, BDNF, PPAR-alpha or -gamma.

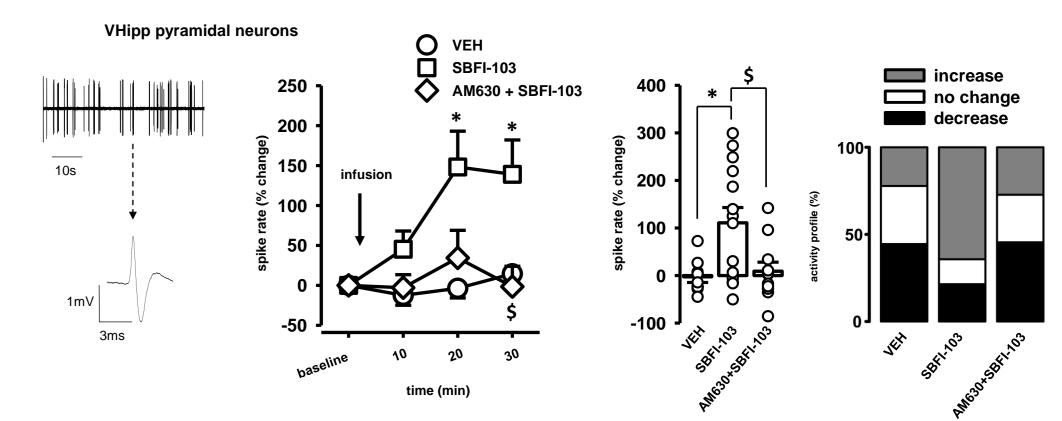
### **RESULTS - BEHAVIOURAL**



**Table 1:** A table summarizing the effects of CUS on anxiety, cognition, locomotion and depression-like behaviour, and the efficacy of SBFI-103 in terms of its reversal potential on these behaviours. **Stress-induced anxiogenic behaviour** (tested by light-dark box and elevated plus maze tests) **and depression-like behaviours** (tested by sucrose preference, novelty-suppressed feeding and forced swim tests) **were robustly ameliorated by acute SBFI-103 administration** 70 min before the onset of the test.

### CONCLUSIONS

- Chronic inhibition of FABP5 restored stress-induced reductions of phosphorylated Erk1-2 and p70S6 kinase, key molecules that modulate emotional behaviour, in the ventral hippocampus.
- Chronic inhibition of FABP5 increased the mRNA levels of CB2R and GPR55 in stressed animals, consistent with our behavioural and physiological findings (9).



- Chronic inhibition of FABP5 reversed stress-induced reductions of proliferation markers of adult hippocampal neurogenesis.
- Inhibition of FABP5 ameliorated stress-induced anxiogenic and depression-like phenotype in rats.
- Systemic inhibition of FABP5 is a novel pharmacotherapeutic approach that exhibits promising efficacy against neuropsychiatric disorders: Reversal effects against stress were detected on behavioural, molecular and neurogenic aspects.

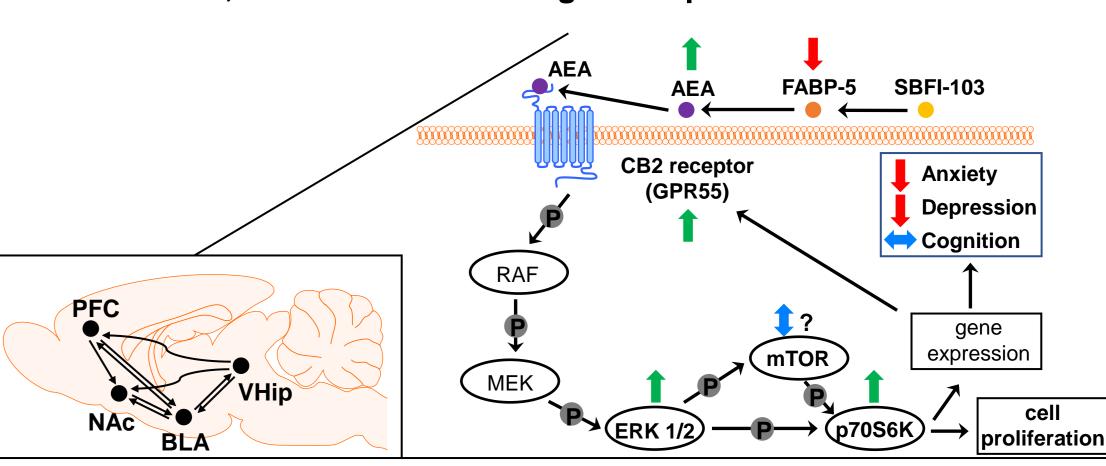


Figure 6: Proposed mechanism of action of SBFI-103.

### REFERENCES

- 1- Gunduz-Cinar O, MacPherson KP, Cinar R, et al., 2013. Mol Psychiatry 18:813-823. 2- Chevalier G, Siopi E, Guenin-Mace et al., 2020. Nat Commun 11:6363.
- 3- Kaczocha M, Glaser ST, Deutsch DG, 2009. Proc Natl Acad Sci USA. 106:6375-6380.
  4- Kaczocha M, Rebecchi MJ, Ralph BP, et al., 2014. PLoS One. 9:1-10.
  5- Shimamoto C, Ohnishi T, Maekawa M, et al., 2014. Hum Mol Genet 23:6495-6511.
- 5- Shimamoto C, Ohnishi T, Maekawa M, et al., 2014. Hum Mol Genet 23.6495-6511.
  6- Hamilton J, Koumas C, Clavin BH, et al., 2018. Behav Pharmacol. 29:503-508.
  7- Duman RS, Nakagawa S, Malberg J. 2001. Neuropsychopharmacology 25:836-844
  8- Egeland M, Zunszain PA, Pariante CM. 2015. Nat Rev Neurosci. 16:189-200.
- 9- Uzuneser TC, Szkudlarek HJ, Jones MJ, et al., 2023. Cereb Cortex. 33:2470-2484. 10- Jones MJ, Uzuneser TC, Clement T, et al. 2023. Psychopharmacology. 11- Yan S, Elmes MW, Tong S, et al., 2018. Eur J Med Chem 154:233-252.