





AFFILIATIONS

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

Taygun C. Uzuneser ^{1,4,5}, Matthew J. Jones ^{1,4,5}, Mohammed H. Sarikahya ^{1,4,5}, Dana Gummerson ^{1,4,5}, Emma Proud ^{1,4,5}, Hehe Wang ^{6,7}, Iwao Ojima ^{6,7}, Daniel B. Hardy ^{2,4,5}, Walter J. Rushlow ^{1,4,5} and Steven R. Laviolette ^{1,3,4,5}

¹ Dept. of Anatomy and Cell Biology, ² Dept. of Physiology and Pharmacology and Obstetrics and Gynaecology, ³ Dept. of Psychiatry, ⁴ Division of Maternal, Fetal and Newborn Health, Children's Health Research Institute (CHRI), Western University, London, ON, Canada.

⁵ St. Josephs Health Care, Lawson Health Research Institute, London, ON, Canada.

⁶ Institute of Chemical Biology and Drug Discoveries, ⁷ Department of Chemistry, Stony Brook University, Stony Brook, NY, United States.

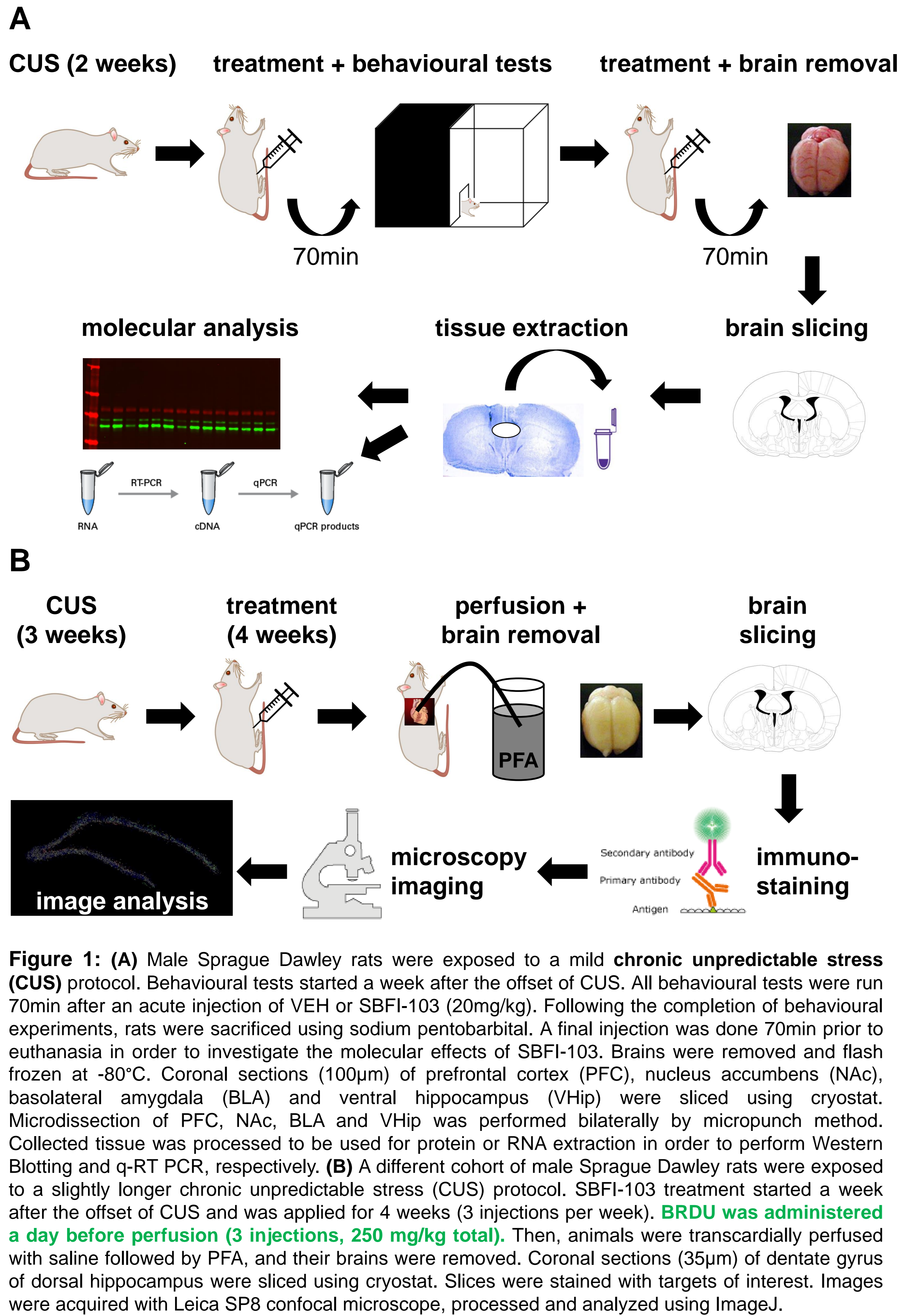
BACKGROUND

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).

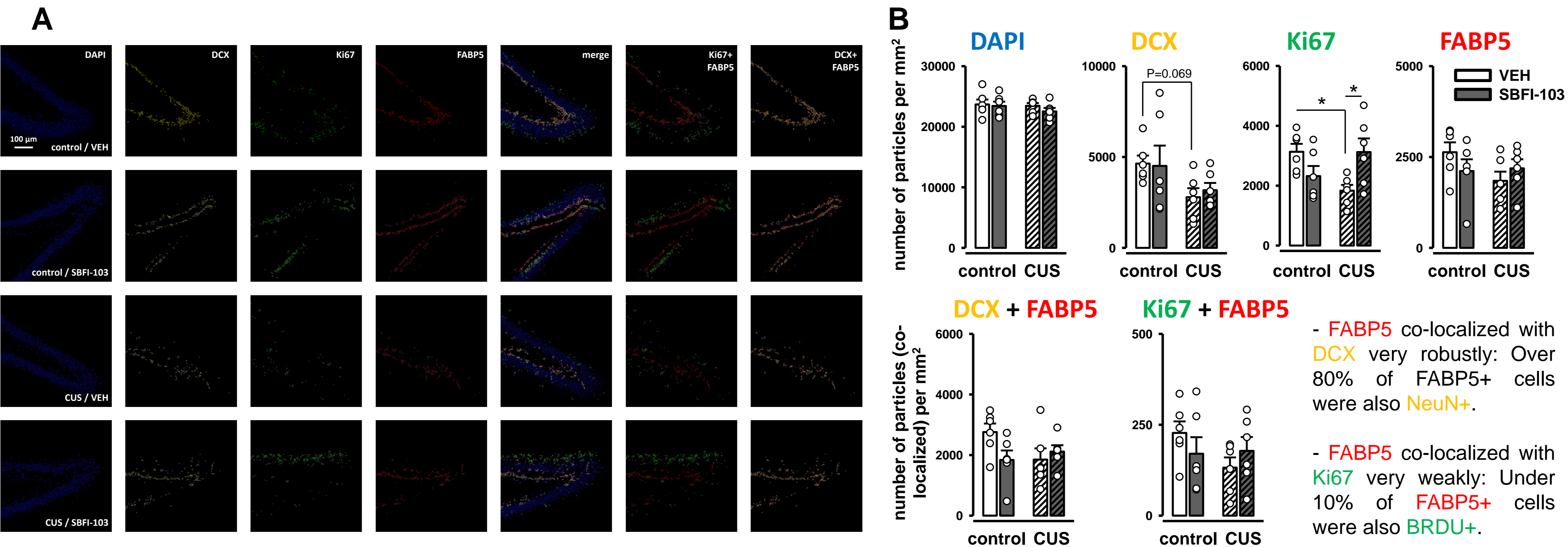
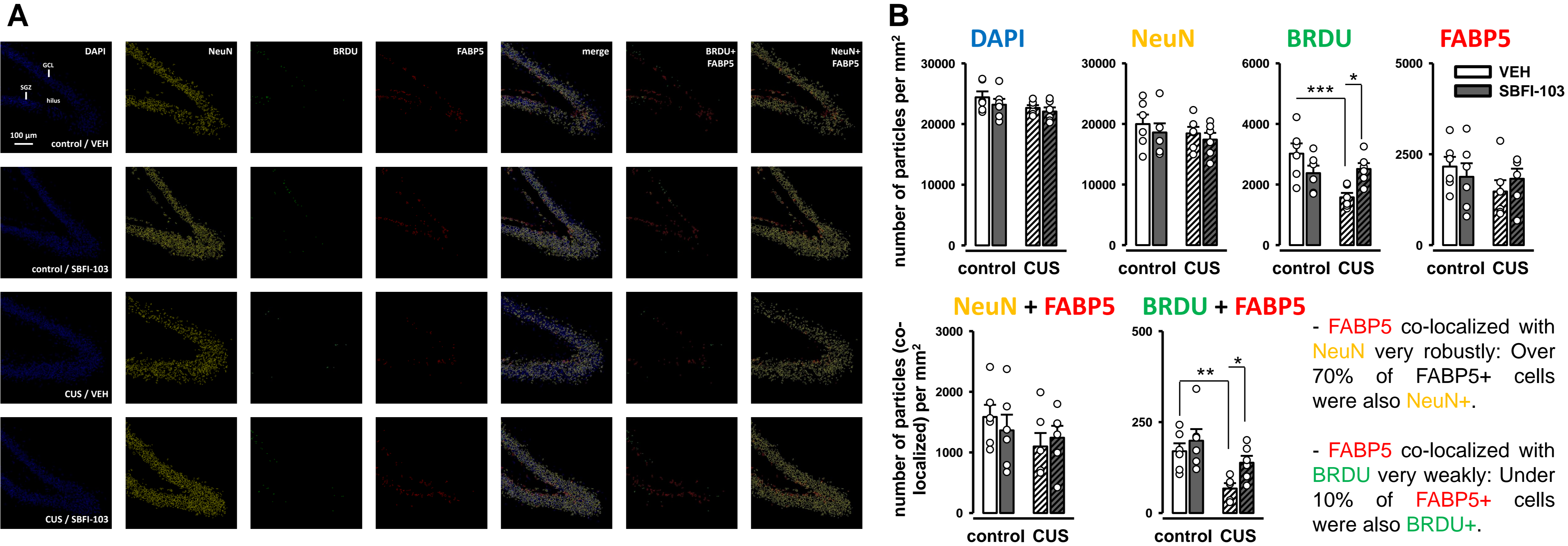
Adult neurogenesis is the process in which newly formed nerve cells are continuously added to the already existing neural network during adulthood. As pro-neurogenic effects were observed following treatment with different classes of antidepressants (7), and as chronic stress was shown to heavily disrupt 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.

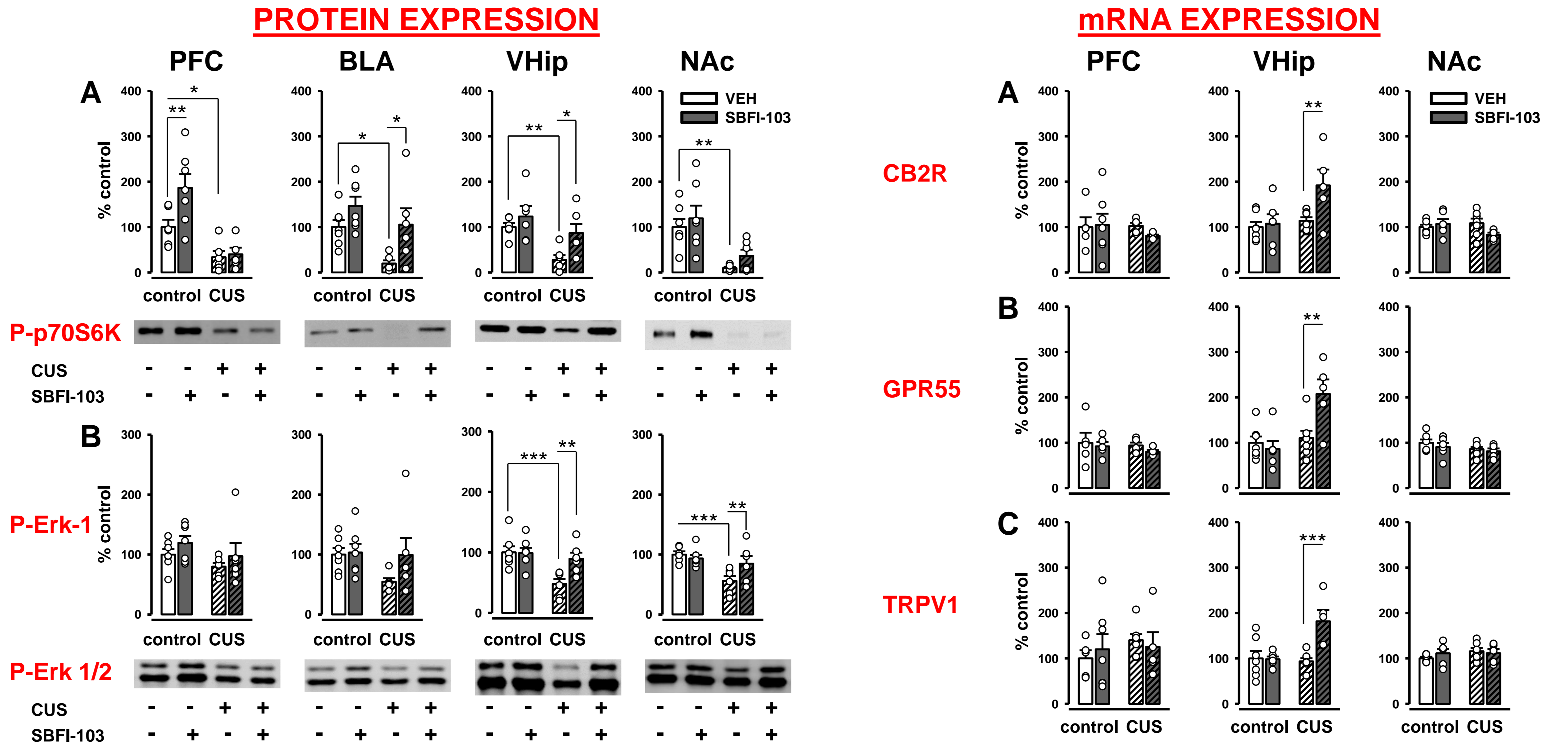
METHODS



RESULTS - IMMUNOSTAINING



RESULTS - MOLECULAR



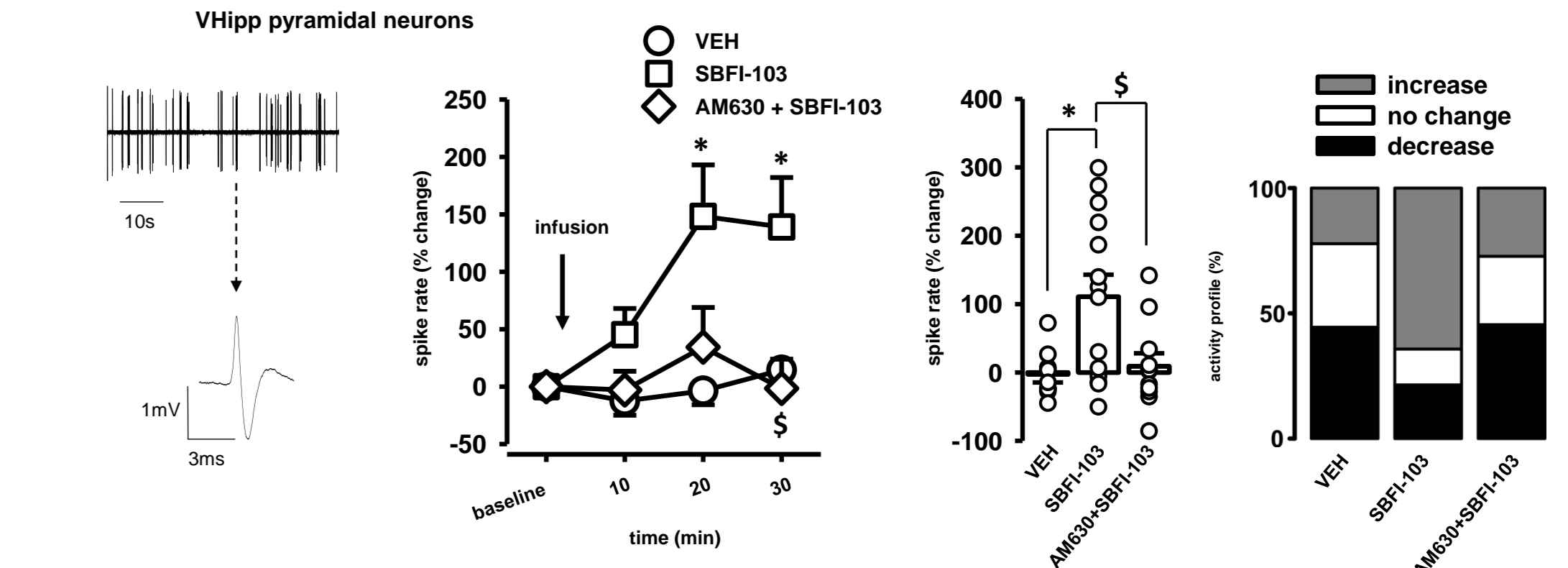
RESULTS - BEHAVIOURAL

BEHAVIOURAL TEST	BEHAVIOUR INVESTIGATED	CUS EFFECT	SBFI-103 EFFECT
Light-dark box	General anxiety	YES ↑	FULL REVERSAL
Elevated plus maze	General anxiety	YES ↑	PARTIAL REVERSAL
Open field	Locomotion	YES ↓	NO EFFECT
Sucrose preference	Anhedonia (depression)	YES ↑	FULL REVERSAL
Forced swim test	Stress-coping, despair (depression)	NO ↔	INCREASED COPING STRATEGIES
Novelty-suppressed feeding	Conflict (depression)	YES ↑	FULL REVERSAL
Social motivation	Social anxiety	NO ↔	NO EFFECT
Social discrimination	Social memory (cognition)	NO ↔	NO EFFECT
Spontaneous alternation	Spatial working memory (cognition)	NO ↔	NO EFFECT

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.**

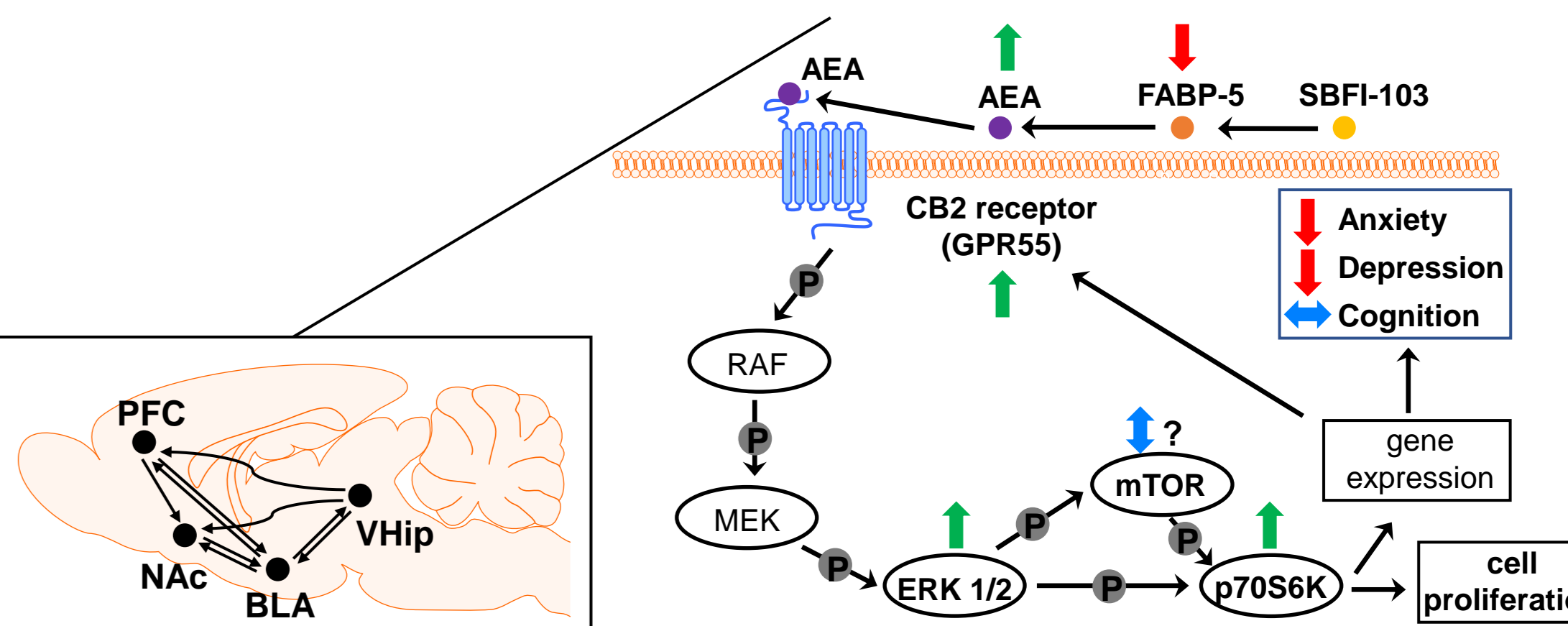


Figure 6: Proposed mechanism of action of SBFI-103.

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