Introduction

Recent evidence suggests that interactions between genetic risk factors and adverse environmental conditions, particularly during early-life, are important risk factors for the development of depression and anxiety disorders.

GABA<sub>0.07</sub> receptors have been implicated in the pathophysiology of depression and anxiety disorders<sup>2</sup>. The GABA<sub>0.07</sub> receptor is a heterodimer composed of a GABA<sub>0.07</sub> subunit and a GABA<sub>3</sub> subunit with the GABA<sub>0.07</sub>/GABA<sub>3</sub> receptor subunit existing as two isoforms, GABA<sub>0.07a</sub> and GABA<sub>0.07b</sub>. The generation of mice lacking either one of these isoforms has allowed the elucidation of specific roles for these isoforms in physiology and behaviour<sup>3</sup>. It is currently unclear whether either isoform plays a role in determining susceptibility or resilience to the development of depression- and anxiety-related behaviours following early-life stress.

The aim of the present study was to determine the role of the GABA<sub>0.07</sub> receptor subunits in susceptibility to the negative behavioural effects of early-life stress. To this end, wildtype, GABA<sub>0.07a</sub> and GABA<sub>0.07b</sub> mice were maternally separated (MS) for 3 hours daily from postnatal day (PND) 1 to 14 or left undisturbed in their homecage (non-maternally-separated, NMS). Ultrasonic vocalizations (USVs) were measured on PND4 and PND7. Animals were weaned on PND21 and once weaned were grouped in 3-4 mice per cage. In the experimental protocol 1, at 8 weeks of age the behaviour of mice was assessed using a battery of behavioural tests. In the experimental protocol 2, at 9 weeks of age WT and GABA<sub>0.07b</sub> mice were injected with 0.07 mg/kg, 40 mg/kg, and 80 mg/kg of saccharin (6% saccharin in water) two hours later in order to assess the survival of newly-born cells. The brains collected were stained also for K<sub>17</sub>, a marker of cell proliferation. Data were analysed using either one-way or two-way ANOVA followed by Fisher’s LSD post-hoc test. The criteria for statistical significance was set at p<0.05.

Materials & Methods

Experimental protocol 1

1.1 GABA<sub>0.07a</sub>-/- mice exhibit increased Ultrasonic Vocalizations

1.2 The effects of MS on SIH response

3.1 MS induced anhedonic-like behaviour in GABA<sub>0.07a</sub>-/- mice in the SPT

3.2 MS induced anhedonia in GABA<sub>0.07b</sub>-/- mice in the FST

4.1 GABA<sub>0.07a</sub> and GABA<sub>0.07b</sub> mice displayed antidepressant-like behaviour in the FST

4.2 Differential role of GABA<sub>0.07a</sub> and GABA<sub>0.07b</sub> receptor subunit in the FST

5.1 GABA<sub>0.07a</sub>-/- mice exhibit increased cell proliferation in the ventral dentate gyrus

5.2 GABA<sub>0.07a</sub>-/- mice exhibit increased cell proliferation in the dorsal DG

6.1 GABA<sub>0.07a</sub>-/- mice exhibit increased survival of newly-born cells in the dorsal dentate gyrus

Conclusions

• MS GABA<sub>0.07a</sub>-/- mice exhibit anhedonia and increased vocalizations in early-life stress conditions. However, in adulthood neither MS nor genotype affected anxiety levels as measured by the SIH test, a physiological measure of anxiety. In the SPT, MS decreased saccharin consumption in GABA<sub>0.07a</sub>-/- mice but not in WT or GABA<sub>0.07b</sub>-/- mice suggesting an early-life stress x GABA<sub>0.07a</sub>-/- interaction in a model of anhedonia. The SPT and SIH test are positively correlated in mice, and it is possible that the depression-like phenotype of GABA<sub>0.07a</sub>-/- mice is resilient to anhedonia of this kind. Moreover, GABA<sub>0.07a</sub>-/- mice showed an antidepressant-like phenotype in both the SPT and SIH test, while GABA<sub>0.07b</sub>-/- mice exhibited a depressive-like phenotype in the SPT but an antidepressant-like phenotype in the SIH test. GABA<sub>0.07a</sub>-/- mice exhibited increased locomotion in the open field when compared to WT and GABA<sub>0.07b</sub>-/- mice. Intriguingly, this hyperactivity was attenuated by MS. GABA<sub>0.07a</sub>-/- mice displayed increased cell proliferation in the adult hippocampus, suggesting that the modulation of neurogenesis could be a plausible mechanism underlying the antidepressant-like phenotype of GABA<sub>0.07a</sub>-/- mice. Specifically, GABA<sub>0.07a</sub>-/- mice exhibited increased cell proliferation in the ventral but not dorsal hippocampus. It has been suggested that the ventral hippocampus plays a preferential role in regulating stress responses and anxiety, while the dorsal hippocampus has preferentially inhibitory roles in spatial learning and memory<sup>4</sup>. Moreover, the MS attenuated the increase of cell proliferation in the GABA<sub>0.07b</sub>-/- mice. Under basal conditions GABA<sub>0.07a</sub>-/- mice exhibited increased survival of newly-born cells in the dorsal dentate gyrus. Intriguingly, under stress conditions the GABA<sub>0.07a</sub>-/- mice exhibited increased survival of newly-born cells in the ventral hippocampus, underlining the importance of the ventral hippocampus in stress responses.

Taken together, the data presented suggests that the difference GABA<sub>0.07a</sub>-/- isoforms may differentially modulate the behavioural effects of early-life stress. Specifically, mice lacking the GABA<sub>0.07b</sub>-/- receptor isoform appear to be resilient to stress-induced anhedonia, while mice lacking the GABA<sub>0.07a</sub>-/- receptor isoform are more susceptible to early-life-stress-induced anhedonia in adulthood.

References