GABA<sub>B1</sub> receptor subunit isoforms differentially mediate susceptibility to depression-related behaviour following early-life stress

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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<sup>1</sup>
- GABA<sub>B</sub> receptors have been implicated in the pathophysiology of depression and anxiety disorders<sup>2</sup>. The GABA<sub>B1</sub> receptor is a heterodimer composed of a GABA<sub>B1a</sub> subunit and a GABA<sub>B1b</sub> subunit with the GABA<sub>B1</sub> receptor subunit existing as two isoforms, GABA<sub>B1aL</sub> and GABA<sub>B1b</sub>L<sup>3</sup>.The generation of mice lacking either one of these isoforms has allowed the elucidation of specific roles for these isoforms in physiology and behaviour. However, 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>B1a</sub> receptor subunit isoforms in susceptibility to the negative behavioural effects of early-life stress. To this end, wildtype, GABA<sub>B1a</sub><sup>−/−</sup> and GABA<sub>B1b</sub><sup>−/−</sup> mice were maternally separated (MS) for 3 hours daily from postnatal day (PND) 1 to 14 or left undisturbed in their homecage (NS). Maternal care behaviours were monitored during the first 3 days after delivery. Ultrasonic vocalisations (USVs) were measured on PND1 and PND7. Animals were weaned on PND11 and once-rafted were grouped in 3–4 mice per cage. At 8 weeks of age, the behaviour of mice was assessed using a battery of behavioural tests. Data were analyzed using either one-way or two-way ANOVA followed by Fisher’s LSD posthoc test. The criteria for statistical significance was set at p < 0.05.

Materials and Methods

Experimental protocol: WT, GABA<sub>B1a</sub><sup>−/−</sup> and GABA<sub>B1b</sub><sup>−/−</sup> mice underwent the MSUS (unpredictable MS combined with unpredictable maternal stress) from PND1 to PND14 or were left undisturbed. The MS paradigm was conducted as described by Franklin and colleagues with some modifications<sup>4</sup>. Maternal care behaviours were analyzed from PND1 to PND14. Ultrasonic vocalizations (USVs) were measured on PND1 and PND7. Animals were weaned on PND11 and once-rafted were grouped in 3–4 mice per cage. At 8 weeks of age, the behaviour of mice was assessed using a battery of behavioural tests. Data were analyzed using either one-way or two-way ANOVA followed by Fisher’s LSD posthoc test. The criteria for statistical significance was set at p < 0.05.

1.1 GABA<sub>B1a</sub>L<sup>−/−</sup> mice exhibit increased Ultrasonic Vocalizations

1. Anxiety-Related Behaviours

1.2 The effects of MS on SiH responses

2.1 MS induced Anhedonic-like behaviour in GABA<sub>B1a</sub>L<sup>−/−</sup> KO mice in the Saccharin Preference Test

2.2 MS induced anhedonic-like behaviour in GABA<sub>B1a</sub>L<sup>−/−</sup> but not GABA<sub>B1b</sub>L<sup>−/−</sup> mice in the FUST

3.1 Differential role of GABA<sub>B1a</sub> and GABA<sub>B1b</sub> receptor subunit in the TST

3.2 GABA<sub>B1a</sub>L<sup>−/−</sup> and GABA<sub>B1b</sub>L<sup>−/−</sup> mice displayed antidepressant-like behaviour in the FST

4.1 GABA<sub>B1a</sub>L<sup>−/−</sup> mice exhibit increased locomotor activity in the OF

5. Maternal Care Behaviours

Conclusion

- In this study, we investigated the role of the GABA<sub>B1a</sub>L and GABA<sub>B1b</sub>L receptor subunit isoforms in susceptibility to the behavioural effects of MS, an animal model of early-life stress. Upon reaching adulthood, both MS and NS mice underwent a battery of tests to assess anhedonia (a core symptom of depression), antidepressant-like and anxiety-related behaviours. MS GABA<sub>B1aL</sub> pups emitted more USVs on PND7 compared to the WT mice, suggesting an increase in anxiety-like behaviour. However, in adulthood, neither MS nor genotype affected anxiety levels as measured using the SiH test, a physiological measure of anxiety.
- In the saccharin preference test, MS decreased saccharin consumption in GABA<sub>B1bL</sub> mice but not in GABA<sub>B1aL</sub> mice thus suggesting an early-life stress x GABA<sub>B1a</sub>L interaction in a model of anhedonia. In the FUST test of anhedonia, MS decreased reward-seeking behaviour in both WT and GABA<sub>B1a</sub>L mice while GABA<sub>B1b</sub>L mice were resistant to the anhedonic effects of MS in this test. Moreover, GABA<sub>B1a</sub>L mice showed an antidepressant-like phenotype in both the TST and FST, while GABA<sub>B1b</sub>L mice exhibited a depressive-like phenotype in the TST but an antidepressant-like phenotype in the FST. GABA<sub>B1b</sub>L mice exhibited hyperactivity in the open field when compared to WT and GABA<sub>B1a</sub>L mice. Intriguingly, this hyperactivity was attenuated by MS.
- The analysis of maternal care behaviours on the PND7 revealed that GABA<sub>B1aL</sub> dams spent more time actively nursing and less time off nest than WT dams. Thus, suggesting that the observed effects on anhedonia, a core symptom of depression, are not a result of poor maternal care.
- Taken together, this study suggests that the different GABA<sub>B</sub> isoforms may play differential roles in susceptibility to early-life stress. Specifically, mice lacking the GABA<sub>B1a</sub>L receptor isoform appear to be resilient to early-life stress-induced anhedonia, while mice lacking the GABA<sub>B1b</sub>L receptor isoform are more susceptible to early-life stress induced anhedonia in adulthood.

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