$GABA_{B(1)}$ receptor subunit isoforms differentially mediate susceptibility to depression-related behaviour following early-life stress

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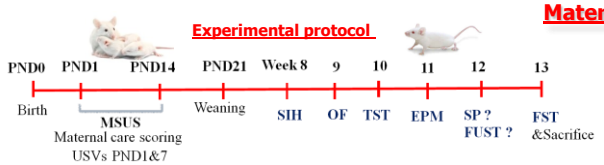
Introduction

- 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[1].
- $GABA_B$ receptors have been implicated in the pathophysiology of depression and anxiety disorders [2]. The $GABA_B$ receptor is an heterodimer composed of a $GABA_{B(1)}$ subunit and a $GABA_{B(2)}$ subunit with the $GABA_{B(1)}$ receptor subunit existing as two isoforms, $GABA_{B(1a)}$ and $GABA_{B(1b)}$. The generation of mice lacking either one of these isoforms has allowed the elucidation of specific roles for these isoforms in physiology and behaviour[3]. 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 $GABA_{B(1)}$ receptor subunit isoforms in susceptibility to the negative behavioural effects of early-life stress. To this end, wildtype, $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice were maternally separated (MS) for 3 hours daily from postnatal day (PND) 1 to 14 or left undisturbed in their homecage (nonseparated, NS). Maternal behaviours were monitored during the first 2 weeks after delivery. Ultrasonic vocalizations (USVs) of the pups were measured during separation from the dam on PND1 and PND7 to assess anxiety. Upon reaching adulthood, anxiety- and depression-related behaviours were assessed using a battery of tests including stress induced hyperthermia (SIH), saccharin preference test/female urine sniffing test (FUST), tail suspension test (TST) and forced swim test (FST). Locomotor activity was also assessed by the open field (OF).

Materials & Methods

Experimental protocol



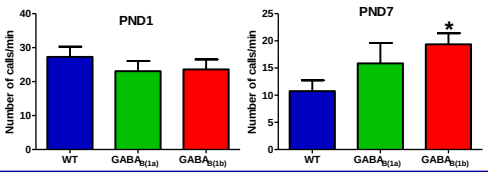
Behavioural Tests

Anxiety	Depression	Anhedonia
Ultrasonic Vocalizations (USVs)	Tail suspension test (TST)	Saccharin preference test (SP)
Stress induced hyperthermia (SIH)	Forced swim test (FST)	Female urine sniffing test (FUST)

Experimental protocol: WT, $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice underwent the MSUS (unpredictable MS combined with unpredictable maternal stress) from PND1-14 or were left undisturbed. The MS paradigm was conducted as described by Franklin and colleagues but with some modifications[4]. Maternal care behaviours were analyzed from PND1 to PND14. Ultrasonic vocalizations (USVs) were measured on PND1 and PND7. Animals were weaned on PND21 and once weaned 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 $p < 0.05$.

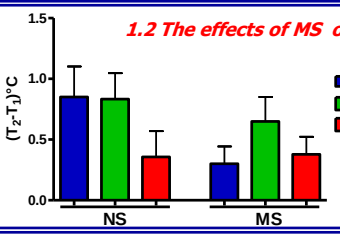
1. Anxiety-Related Behaviours

1.1 $GABA_{B(1b)}^{-/-}$ mice exhibit increased Ultrasonic Vocalizations



MS $GABA_{B(1b)}^{-/-}$ pups displayed increased ultrasonic vocalizations on PND7 ($p<0.05$). *Significantly different to WT mice; *p< 0.05 according to Fisher's LSD posthoc test.

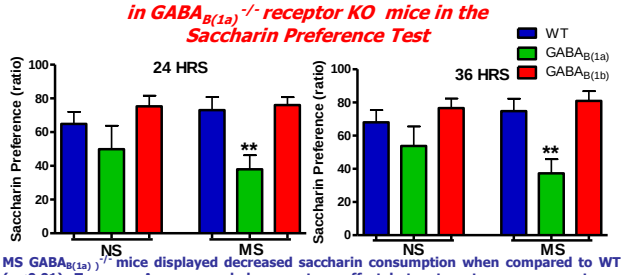
1.2 The effects of MS on SIH responses



Two-way ANOVA revealed no effects of MS or genotype effect in the SIH test0

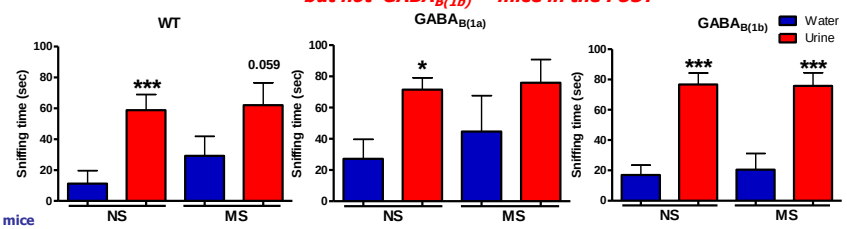
2. Anhedonic-like Behaviours

2.1 MS induced Anhedonic-like behaviour in $GABA_{B(1a)}^{-/-}$ receptor KO mice in the Saccharin Preference Test



MS $GABA_{B(1a)}^{-/-}$ mice displayed decreased saccharin consumption when compared to WT mice ($p<0.01$). Two-way Anova revealed a genotype effect but not a stress or a genotype-stress interaction. *Significantly different to the WT group **p< 0.01 according to Fisher's LSD posthoc test.

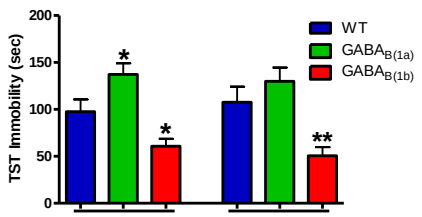
2.2 MS induced anhedonic-like behaviour in $GABA_{B(1a)}^{-/-}$ but not $GABA_{B(1b)}^{-/-}$ mice in the FUST



MS WT and MS $GABA_{B(1a)}^{-/-}$ mice but not MS $GABA_{B(1b)}^{-/-}$ mice spent less time in sniffing urine than water. *Significantly different from water sniffing time duration ***p<0.001, *p<0.05 according to t-test analysis.

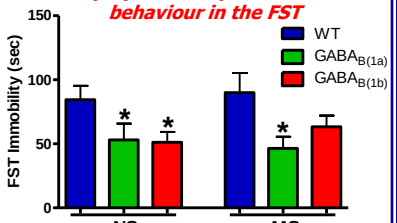
3. Antidepressant-like Behaviours

3.1 Differential role of $GABA_{B(1a)}$ and $GABA_{B(1b)}$ receptor subunit in the TST



NS $GABA_{B(1a)}^{-/-}$ mice exhibited increased immobility ($p<0.05$). NS and MS $GABA_{B(1b)}^{-/-}$ mice exhibited decreased immobility. *Significantly different from WT mice **p<0.01, *p<0.05 according to Fisher's LSD posthoc test.

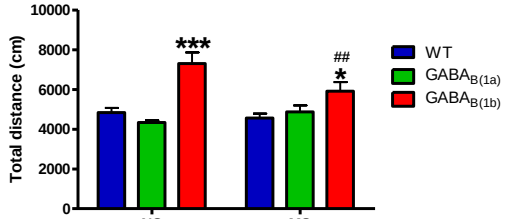
3.2 $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice displayed antidepressant-like behaviour in the FST



NS and MS $GABA_{B(1a)}^{-/-}$ mice and NS $GABA_{B(1b)}^{-/-}$ mice exhibited decreased immobility when compared to the WT mice ($p<0.05$). *Significantly different from WT mice *p<0.05 according to Fisher's LSD posthoc test.

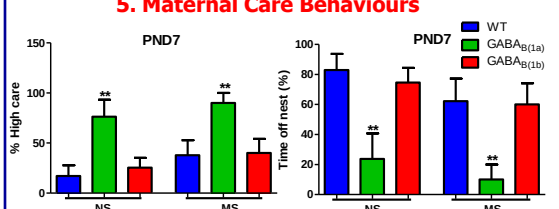
4. Locomotor Activity

4.1 $GABA_{B(1b)}^{-/-}$ mice exhibit increased locomotor activity in the OF



NS $GABA_{B(1b)}^{-/-}$ mice displayed increased locomotor activity ($p<0.001$), this was attenuated by MS. Two-way Anova analysis revealed a genotype effect and a stress-genotype interaction. *Significantly different to WT mice; #Significantly different to NS $GABA_{B(1b)}^{-/-}$ mice *p< 0.05 ***p< 0.001 according to Fisher's LSD posthoc test.

5. Maternal Care Behaviours



$GABA_{B(1a)}^{-/-}$ dams spent more time actively nursing ($p<0.01$) and less time off nest ($p<0.01$) than WT mice during the PND7. *Significantly different to WT mice; **p<0.01 according to Fisher's LSD posthoc test.

Conclusions

•In this study, we investigated the role of the $GABA_{B(1a)}$ and $GABA_{B(1b)}$ 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 $GABA_{B(1b)}^{-/-}$ pups emitted more USVs on PND7 when compared to the WT mice, thus 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 $GABA_{B(1a)}^{-/-}$ mice but not in WT or $GABAB_{(1b)}^{-/-}$ mice thus suggesting an early-life stress x $GABA_{B(1a)}^{-/-}$ interaction in a model of anhedonia. In the FUST test of anhedonia, MS decreased reward-seeking behaviour in both WT and $GABA_{B(1a)}^{-/-}$ mice while $GABA_{B(1b)}^{-/-}$ mice were resistant to the anhedonic effects of MS in this test. Moreover, $GABA_{B(1b)}^{-/-}$ mice showed an antidepressant-like phenotype in both the TST and FST, while $GABA_{B(1a)}^{-/-}$ mice exhibited a depressive-like phenotype in the TST but an antidepressant-like phenotype in the FST.

•$GABA_{B(1b)}^{-/-}$ mice exhibited hyperactivity in the open field when compared to WT and $GABA_{B(1a)}^{-/-}$ mice. Intriguingly, this hyperactivity was attenuated by MS.

•The analysis of maternal care behaviours on the PND7 revealed that $GABA_{B(1a)}$ 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 $GABA_{B(1)}$ isoforms may play differential roles in susceptibility to early-life stress. Specifically, mice lacking the $GABA_{B(1b)}$ receptor isoform appear to be resilient to early life stress-induced anhedonia, while mice lacking the $GABA_{B(1a)}$ receptor isoform are more susceptible to early-life stress induced anhedonia in adulthood.

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References: **1.** Caspi A. et al., Science, 2003. 301(5631): p. 386-9; **2.** Cryan, J.F. and K. Kaupmann, Trends Pharmacol Sci, 2005. 26(1): p. 36-43. **3.** Jacobson, L.H., et al., J Neurosci, 2006. 26(34): p. 8800-3; **4.** Franklin T.B. , et al., Biol Psychiatry. 2010 Sep 1;68(5):408-15.