# SELECTIVE ABLATION OF GLUCOCORTICOID RECEPTOR IN NORADRENERGIC SYSTEM OF MICE: THE INFLUENCE ON BRAIN NORADRENERGIC RECEPTORS

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***Introduction:*** Stress induced hyperactivity of hypothalamic-pituitary-adrenal system (HPA) is believed to be one of the major contributors to the pathology of depression. The HPA exerts its action through glucocorticoid receptors (GR). Noradrenergic system is also reported to play a significant role in depression while its neurons are regulated by GR. Moreover, we have reported that modulation of alpha1–adrenoceptors (alpha1-AR) after some antidepressant treatments concerned mainly the alpha1A subtype [1].

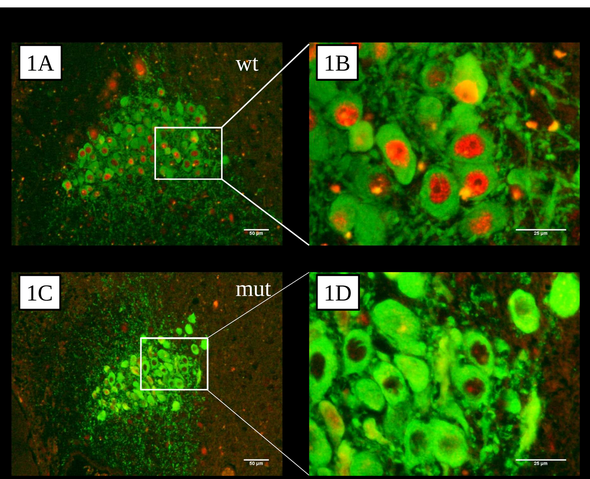
***Aims:*** The aim of this study was to investigate effects of conditional inactivation of the GR in noradrenergic neurons of mice – line $GR^{DBHCre}$ on behavior and adrenergic receptors expression in their brains.

***Methods:*** Mutant C56BL/6 mice ($GR^{DBHCre}$) were generated by crossing transgenic mice hosting the Cre recombinase under the dopamine beta-hydroxylase (DBH) promoter with animals harboring the floxed GR gene [2] (Fig 3).

Immunohistochemistry (IHC) and immunofluorescence (IF) studies were performed on 6 µm thick paraffin sections of brains fixed in 4% paraformaldehyde. Sections were incubated with primary antibodies (anti-GR, 1:50, Abcam; anti-TH, 1:1000, Millipore, NeuN 1:3000 Millipore) and subsequently with proper secondary IgG.

Depressive like behavior was measured by the Tail Suspension Test (TST), Forced Swimming Test (FST) and Open Field Test (OFT). 12 mutant (mut) and 13 wild type (wt) mice were habituated in experimental room and subjected subsequently to OFT, TST and FST, with 3 day intervals between each test. Animals were videorecorderd and behavior was assessed with use of video tracking software Noldus EhtoVision XT8. In TST and FST animals were tracked for 6 minutes, and behavior was analyzed both during total test time and splitted into 1 minute intervals. OFT duration was 60 min, animals were placed in 40x40cm arena, with central zone defined as 24x24cm square area in the middle and the data were analyzed both for total time and splitted into 10 min intervals.

Expression of mRNA encoding adrenergic receptors and housekeeping genes was assessed in prefrontal cortex (PFC) by Real-Time PCR utilizing TaqMan probes (Applied Biosystems).



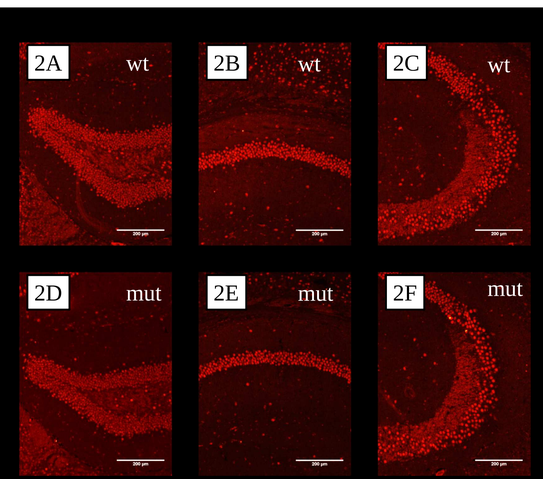




Fig 1. Image of Locus Coeruleus showing GR immunoreactivity (red signal) in TH positive cells (green signal) in control mouse (A and B). GR signal is lost in TH positive LC neurons in mutant mouse (C and D).

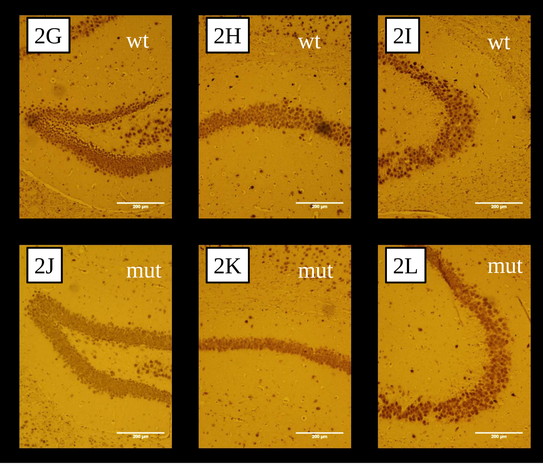



Fig 2. Images from hippocampal regions CA1 (2C;F;I;L), CA3 (2B;E;H;K) and Dentate Gyrus (2A;D;G;J). Images show similar pattern of GR (A-F) and NeuN (G-L) expression in control and mutant mice.

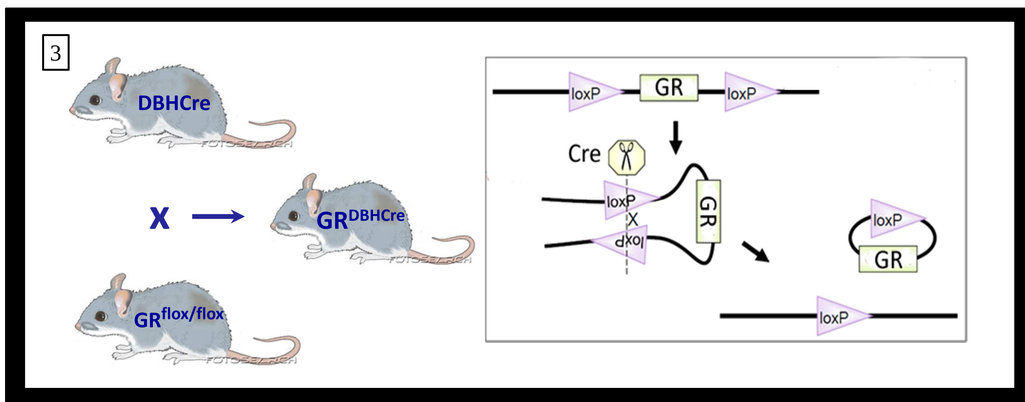
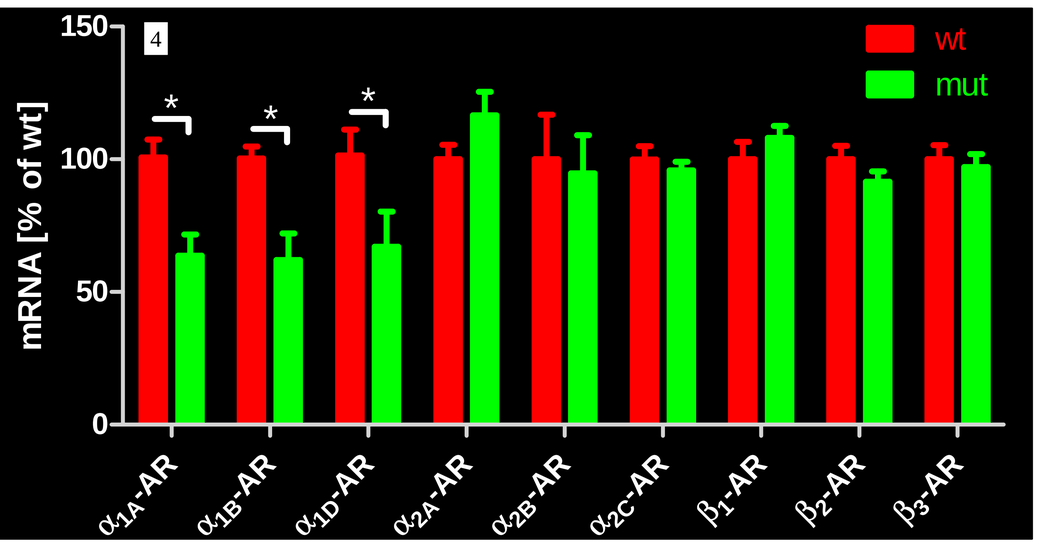



Fig 3. Schematic representation of Cre/loxP system used to generate $GR^{DBHCre}$ mice line

Fig 4. Graph presenting levels of mRNA of adrenergic receptors (AR) in PFC assessed by Real-Time PCR. Changes were confined to alpha1-AR subtypes showing decrease of these receptors expression in mutant mice. *p<0.05



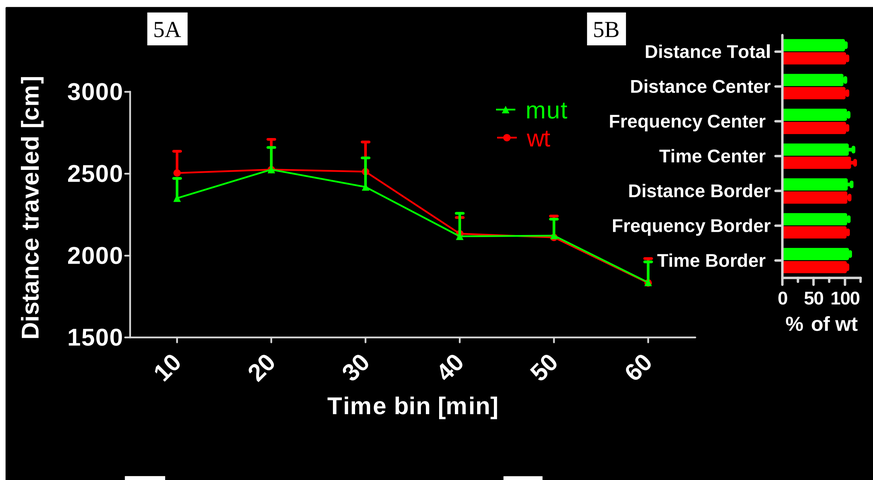







Fig 5. Mice behavior in the Open Field Test: distance traveled in entire arena splitted into 10 min time intervals (Fig5A); distance moved, time spent and number of entries (frequency) to border and center zones from entire 60 min of test, as well as total distance traveled in entire arena (Fig 5B)

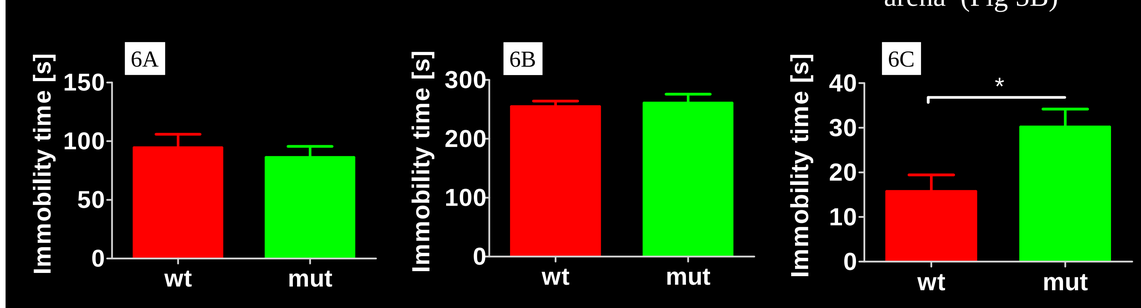



Fig 6. GRDBHCre mice behavior in the Forced Swim Test and Tail Supsension Test: immobility time within the last 4 min of FST (Fig 6A), full duration of TST (Fig 6B) and first minute of TST (Fig 6C). *p<0.05

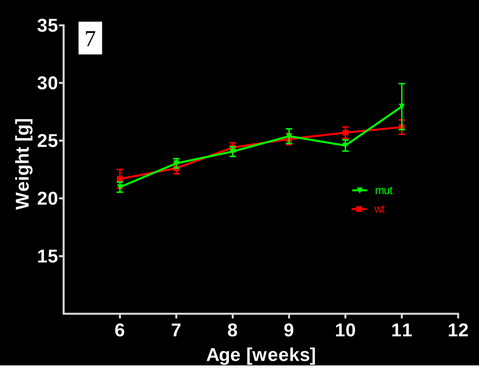



Fig 7. Weight of animals at different age. Both w/t and mutant mice show similar weight gain. n = 12

***Conclusions*** $GR^{DBHCre}$ mice display selective ablation of GR receptor in noradrenergic neurons of LC (Fig 1), while GR expression in other brain areas such as PFC (not shown) and hippocampus (Fig 2A-F) remains unchanged. Importantly, the mutation itself does not seem to induce any physical impairment: both w/t and mutant animals showed similar weight gain (Fig 7) and locomotor activity in the OFT (Fig 5A), thus making the model useful for further unbiased behavioral study. Furthermore, mutant animals did not show any signs of neuronal cell loss as assessed by NeuN staining in PFC (not shown) and hippocampus (Fig 2G-L).

Animals did not show any marked difference in depressive-like behavior (Fig 6A, B) apart from significantly increased immobility time in the first minute of TST. However, whether this effect has any physiological meaning has to be further investigated.

It remains to be answered whether downregulation of all alpha1-AR subtypes detected by us in PFC may contribute to the impairment of noradrenergic transmission. Although changes in alpha1-adrenergic transmission would be expected to cause tendencies toward depressive-like behavior as shown by other authors [3]. We propose that GRDBHCre mice may represent an interesting new tool to study the role of stress in depression in context of noradrenergic system which is among targets of antidepressant drugs action

***References:***

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