THE ROLE OF NITRIC OXIDE SIGNALLING IN IMPULSIVE BEHAVIOURS IN MICE

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INTRODUCTION. Nitric oxide (NO) is a highly diffusible gaseous messenger molecule that has many important functions in various tissues. Abnormalities in NO signalling have been implicated in almost all major neuropsychiatric disorders. NO synthesis is catalyzed by three different isoforms of nitric oxide synthase (NOS), of which especially the neuronal isoform, NOS-I, has been shown to be involved in various human behaviours such as cognitive functioning, aggressiveness, and impulsivity (Reif et al., 2009; 2011; Kopf et al., 2011). Knockout animals are valuable tools to identify both the behavioural impact of a given gene, as well as subsequent neurobiological changes. Here we have investigated impulsivity-related behaviours – spontaneous locomotor activity and discriminative performance in the 5-hole box – and tissue concentrations of monoamines and their metabolites in *Nos1*-knockout mice. These mice have a targeted deletion of exon I of the *Nos1* gene which results in a residual brain NOS activity of ca 7% of wild-type controls. The *Nos1*-knockout mice have been previously described as highly aggressive (Nelson et al., 1995), a behavioural phenotype however not observed in the *Nos1*-knockout animals in our laboratory, and exhibiting impaired performance in a spatial learning task as well as slightly decreased anxiety as measured in the elevated plus-maze (Wultsch et al., 2007).

METHODS. Male and female wild-type control, heterozygous, and homozygous *Nos1*-knockout mice aged between 2 and 6 months were used. All animals had the same genetic background (C56BL/6) and were singly housed during the experiments. Spontaneous locomotor activity testing was conducted in both male and female animals in an open field (50 x 50 cm) divided into a central area and the surrounding periphery. Total distance travelled, distance travelled and time spent in the centre area, number of rearings, number of entries into the centre area, and latency entering the centre area were continuously recorded for 60 min. Five-choice visual discriminative performance was tested in the 5-hole box in female mice. The animals were food restricted to obtain a reduction in body weight to ca 85% of the free-feeding weight prior to the training for the task . The training started with 5 habituation sessions (all five holes illuminated and baited with a sugar pellet (20mg)), and proceeded with 7 autoshaping sessions in which the animals were trained to nose poke into an illuminated hole to receive the pellet. The parameters for the experimental phase were as follows: 30 trials per session, intertrial interval (ITI) 5 s, stimulus duration (SD) 20 s or 10 s, limited hold 5 s. Nose poke into wrong hole was punished with a 5s timeout during which the overhead light in the chamber was switched on. After completion of 5 sessions with SD 20 s and subsequent 4 sessions with SD 10 s, a session with increased ITI of 8 s was carried out. Monoamines and metabolites were measured in the striatum of the male mice using HPLC with electrochemical detection.

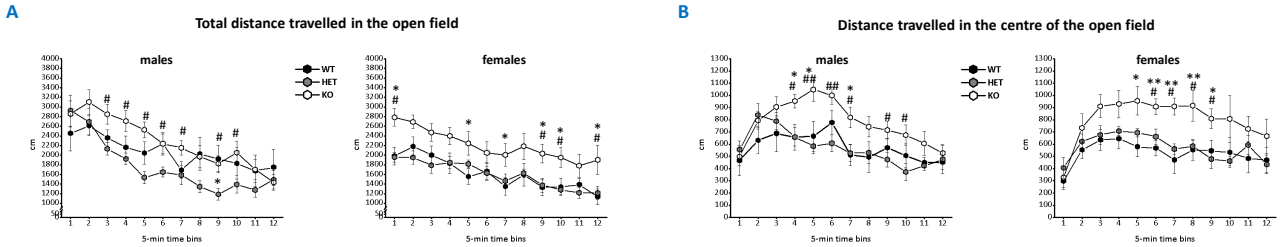

Figure 1. Both male and female homozygous *NOS1*-knockout mice display behavioural hyperactivity in the open field (OF) as measured by total distance travelled during the 1-hour experiment (A), which is mainly due to increased distance travelled in the centre area of the OF (B). The latter effect however was only observed after the animals had habituated to the OF. As the heightened locomotor activity in the homozygous *Nos1*-knockout mice was especially pronounced in the centre area of the OF, considered to be more aversive or 'unsafe' for the animal, it could be argued to reflect an impulsive tendency. (WT – wild-type C57BL/6 control; HET – heterozygous *NOS1*-knockout; KO – homozygous *NOS1*-knockout; * - p<0,05; ** - p<0,01 vs WT; # - p<0,05; ## - p<0,01 vs HET). p<0,05; ## - p<0,01 vs HET).

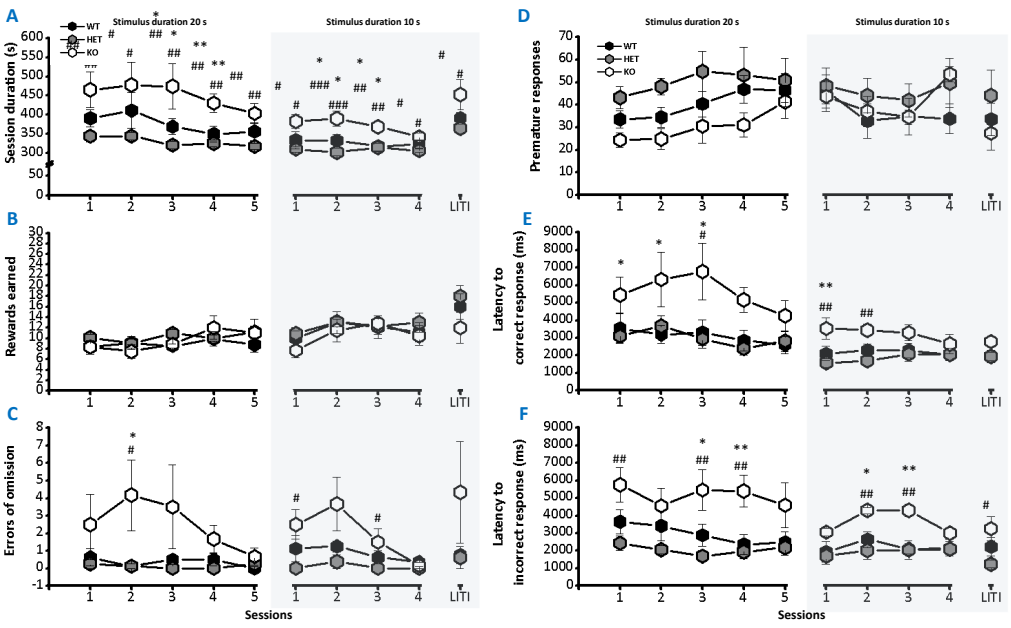

Figure 3. The discriminative performance in the 5-hole box. Female homozygous *NOS1*-knockout mice spend more time to complete the trials within sessions (A), make more errors of omission (C), and have increased latency to respond to the presented visual stimuli (E and F). As the accuracy of responding in these animals is not affected – the number of rewards earned within the session is not different between the genotypes (B) – these results suggest not a cognitive deficit but rather a motivational deficit in the female homozygous *NOS1*-knockout mice. The number of premature responses (D), a typical measure of impulsive responding in the 5-choice visual discrimination task, did not differ between the genotypes. (WT – wild-type C57BL/6 control; HET – heterozygous *NOS1*-knockout; KO – homozygous *NOS1*-knockout; * - p<0,05; ** - p<0,01 vs WT; # - p<0,05; ## - p<0,01 vs HET). LITI – long intertrial interval (8 s) session.

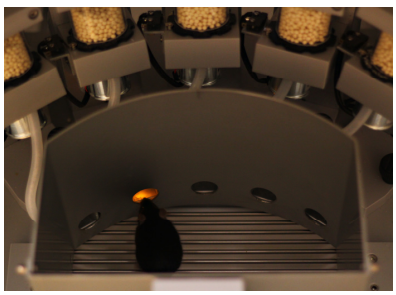

Figure 2. A female heterozygous *NOS1*-knockout mouse performing the visual discrimination task in the 5-hole box.

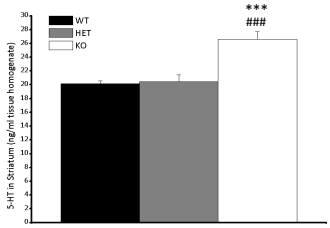

Figure 4. Striatal serotonin (5-HT) tissue levels are elevated in the homozygous *NOS1*-knockout mice while no changes in the 5-HT metabolite 5-HIAA, noradrenaline or its metabolites were found (not shown).

DISCUSSION. Nitric oxide signalling appears to have a role in regulating motor impulsivity as evidenced by the increased locomotor activity in the open field in homozygous *Nos1*-knockout mice. Interestingly, we did not find increased impulsive responding in the visual discrimination task in the 5-hole box in female homozygous *Nos1*-knockout mice, as the changes in the performance in these animals are more readily attributable to reduced motivation. Activation of ventral striatal 5-HT1/7 receptors has recently been shown to reduce motivation for food reward (Pratt et al., 2012); elevated 5-HT levels in the striatum - where there is an abundance of 5-HT1B receptors (Middlemiss and Hutson, 1990) - could thus contribute to the hypomotivational behavioural phenotype in the *Nos1*-knockout mice. The exact nature of the interplay between NO signalling and serotonergic neurotransmission in regulating these behaviours remains to be elucidated.

Kopf J, Schecklmann M, Hahn T, Dresler T, Dieler AC, Herrmann MJ, Fallgatter AJ, Reif A (2011) NOS1 ex1f-VNTR polymorphism influences prefrontal brain oxygenation during a working memory task. Neuroimage 57: 1617-1623.
Middlemiss DN, Hutson PH (1990) The 5-HT_{1B} receptors. In: Whitaker-Azmitia PM, Peroutka SJ (eds) The neuropharmacology of serotonin. New York Academy of Sciences, New York, pp 132-148.
Nelson RJ, Demas GE, Huang PL, Fishman MC, Dawson VL, Dawson TM, Snyder SH (1995) Behavioral abnormalities in male mice lacking neuronal nitric oxide synthase. Nature 378: 383-386.
Pratt WE, Schall MA, Choi E (2012) Selective serotonin receptor stimulation of the medial nucleus accumbens differentially affects appetitive motivation for food on a progressive ratio schedule. Neurosci Lett. 511: 84-88.
Reif A, Jacob CP, Rujescu D, Herterich S, Lang S, Gutknecht L, Baehne CG, Strobel A, Freitag CM, Ciegling I, Romanos M, Hartmann A, Rösler M, Renner TJ, Fallgatter AJ, Retz W, Ehlis AC, Lesch KP (2009) Influence of functional variant of neuronal nitric oxide synthase on impulsive behaviors in humans. Arch Gen Psychiatry 66: 41-50.
Reif A, Kiive E, Kurrikoff T, Paaver M, Herterich S, Konstabel K, Tulviste T, Lesch KP, Harro J (2011) A functional NOS1 promoter polymorphism interacts with adverse environment on functional and dysfunctional impulsivity. Psychopharmacology (Berl) 214: 239-248.
Wultsch T, Chourbaji S, Fritzen S, Kittel S, Grünblatt E, Gerlach M, Gutknecht L, Chizat F, Golfier G, Schmitt A, Gass P, Lesch KP , Reif A (2007)Behavioural and expressional phenotyping of nitric oxide synthase-I knockdown animals. J Neural Transm Suppl 72: 69-85.

This study was supported by the DFG Grant RE1632/1-1, /1-3 and /5 and KFO 125