THE BIO-BEHAVIOURAL PROFILE OF THE NDUFS4 MOUSE AS A PLATFORM FOR NEUROPSYCHIATIC RESEARCH INTO MOOD DISORDERS

Daniel J. van Rensburg, Zander Lindeque, Brian H. Harvey, Stephanus F. Steyn*
Center of Excellence for Pharmaceutical Sciences, Faculty of Health Sceinces, North-West University, Potchefstroom, South Africa
Correspondence: Stephan.Steyn@nwu.ac.za

INTRODUCTION

Conclusive etiological and pathological mechanisms involved in mood disorders are yet to be elucidated. Given the multitude of existing hypotheses, it is clear that the answer will not be found within a single hypothesis but rather when these hypotheses are puzzled together. Therefor the answer cannot be found without all the pieces. A growing body of work suggests that mitochondrial disfunction forms part of the puzzle that is mood disorders[1,2]. There is corelative evidence linking mitochondrial dysfunction to mood disorders[3,4] that is supported by preclinical models of depression and bipolar disorder[5,6]. Consequently, there is need for a validated preclinical model with mitochondrial dysfunction as construct to further investigate the aforementioned correlation.

METHODS

• **Animals**
Pre-pubertal (Male and Female) Ndufs4 and C57/bl mice were exposed to a battery of behavioural tests from postnatal day 28 (PND 28) to PND 35 after which the animals were harvested on PND 36. The Ndufs4 mice were genotyped on PND 21 to identify the 3 different genotypes i.e., Wild type (WT), Heterozygous (HET), and Knockout (KO). As before[7], the genotyping was done using conventional PCR amplification and agarose gel electrophoresis.

•**Behavioural tests**
Open Field[8] (PND 28-30), Tail suspension[9]- (PND 31), Forced swim-test[8] (PND35), Elevated plus maze[10] (PND 33), and Mirror box[11] (PND34) data were measured as previously described and analysed with EthoVision XT12 and FST Scoreboard 2.0.

• **Neurochemical analysis**
Neurochemical concentrations will be measured with a liquid chromatography tandem mass spectrometry (LC–MS/MS) procedure, based on previously described methods[12].

• **Statistical analysis**
A one-way Welch (with Dunnett's T3 post-hoc test) ANOVA (analysis of variances) was used to analyze group differences between genotypes (regardless of homogeneity of variance), whereas a Kruskal-Wallis H-test (with Dunn's post-hoc test) was performed in instances where the assumption for normality of distribution was not true. For the latter, the results 17 of the Levene's test (homogeneity of variances) are reported in the text. To control for the influence of locomotor activity on behavior, a one-way ANCOVA (analysis of co-variance) was performed with distance moved parameters used as the co-variant for time spent immobile. In all instances, significance was accepted as $p < 0.05$ and reported as Bonferroni-adjusted values.

CONCLUSION

The *Ndufs4*$^{-/-}$ Whole-body Knockout (KO) mouse has a comparable phenotype to the Ndufs4$^{+/-}$ Heterozygous (HET) and *Ndufs4*$^{+/+}$ Wild-Type (WT) until ~*PND30* - *PND35*[13]. It is theorised that high energy demanding phases (i.e., neurodevelopment) increases tissue specific mitochondrial disfunction[13], due to the bioenergetic system being unable to the allostatic load[14]. The KO mice show an overall decrease in mobility during the aforementioned age followed by a drastic decrease after *PND36*[15]. The KO mice displayed lower baseline mobility during the open field test and the elevated plus maze (data not shown). Therefore, we used this downwards trend as covariant in the ANCOVA analysis of the tail suspension test and forced swim test. The KO mice displayed depressive-like behaviour on *PND31*, compared to the HET and WT cohorts (Figure A). Thereafter, the forced swim test yielded no significant results, indicating that the depressive-like behaviour was not sustained. Interestingly, the KO mice presented with risk resilience type behaviour in the mirror box test (Figure B). To the best of our knowledge, transient depressive-like behaviour and risk resilience has never before been reported in the Ndufs4 model, although it does align with behavioural shifts reported in other mitochondrial dysfunctional models[4]. We suspect that the behavioural shift is caused by the observed hyperserotonergic state, as measured on *PND36* (Figure C & D). The surge in serotonin, altered kynurenine turnover (Figure E & F) and overall redox dysregulation (data not shown) might further hint towards the underlying mechanism that initiates the phenotypic and neurological decline, typically seen from ~*PND40*. Interestingly, the HET mice presented with increased oxidative stress (lower GSH/GSSG ratio) as indicated by large effect sizes (Figure G & H), which might mean that the heteroplasmy causes a stress sensitivity. **In conclusion**, the *Ndufs4* KO mice undergo a drastic behavioural shift at a point where existing mitochondrial function is not sufficient to support normal developing biological systems. The biobehavioural profile of the *Ndufs4* KO mouse aligns with other models that are being investigated as suitable models for bipolar disorder. The *Ndufs4* model may be a useful animal model to support the development of (other) preclinical models for psychiatric diseases, and further our understanding of mechanisms underpinning bipolar disorder.

RESULTS

Tail suspension test (Figure A)
On PND31, there were statistical differences between genotypes for time spent immobile In the tail suspension test (Figure A), after controlling for distance moved in the open field test ($F_{2, 29}$ = 6.84, p = 0.004; η_p^2 = 0.3). Of note, the mean distance moved had a significant effect on time spent immobile in the tail suspension test ($F_{1, 29}$ = 14.72, p = 0.001; η_p^2 = 0.3).

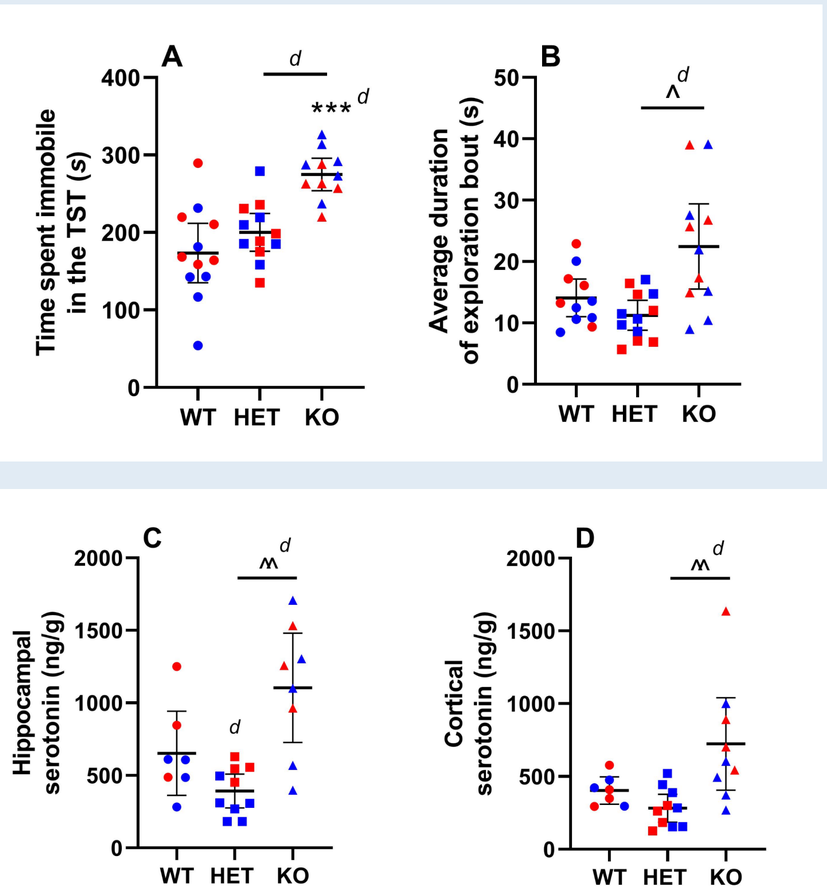
Mirror box test (Figure B)
In Figure B, there were statistical differences between genotypes for average duration per exploration bout in the mirror box test ($F_{2, 18.5}$ = 5.93, p = 0.01; η^2 = 0.2 [0.0; 0.4]),

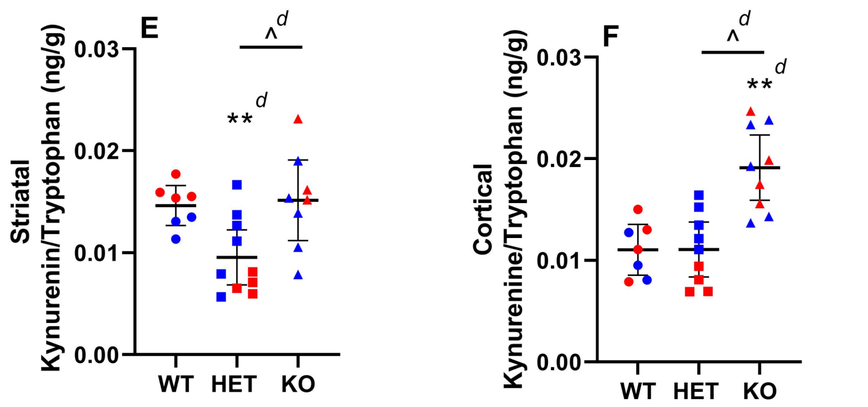
Serotonin levels (Figure C & D)
There were statistical differences between genotypes for hippocampal (Figure C; $F_{2, 10.8}$ = 9.60, p = 0.004; η^2 = 0.5 [0.2; 0.7]) and cortical (Figure D; $F_{2, 14.2}$ = 5.53, p = 0.02; η^2 = 0.4 [0.1; 0.6]) serotonin values.

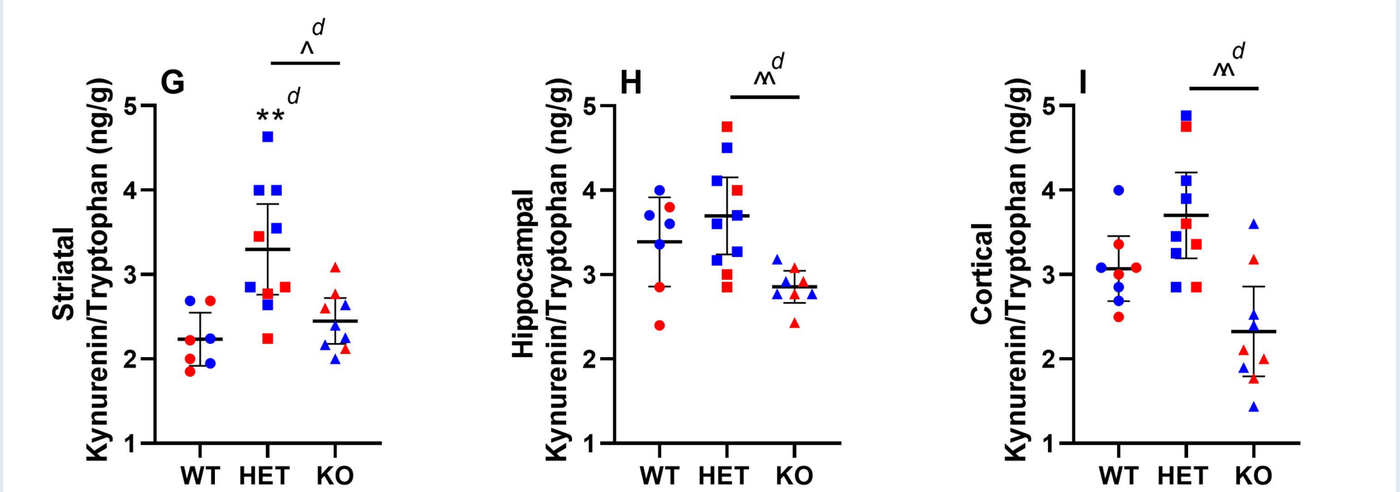
Kynurenine/ Tryptophan levels (Figure E & F)
There were statistical differences between genotypes for hippocampal (Figure C; $F_{2, 10.8}$ = 9.60, p = 0.004; η^2 = 0.5 [0.2; 0.7]) and cortical (Figure D; $F_{2, 14.2}$ = 5.53, p = 0.02; η^2 = 0.4 [0.1; 0.6]) serotonin values.

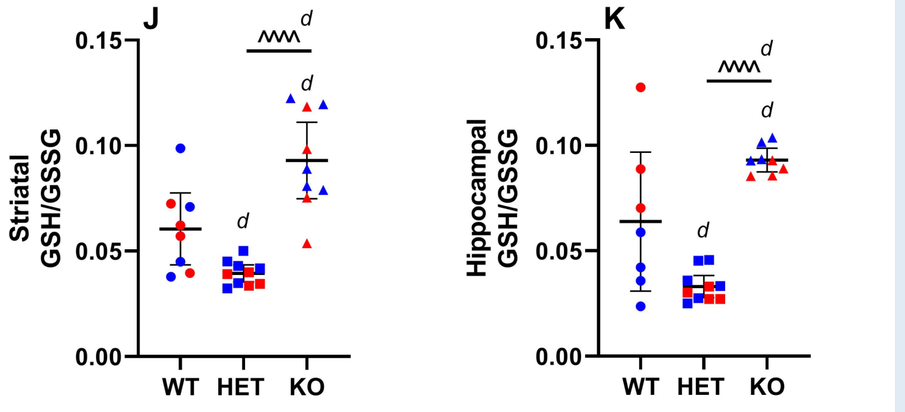
Kynurenine/ Tryptophan levels (Figure G, H & I)
There were statistical differences between genotypes for striatal (Figure G; $F_{2, 14.8}$ = 7.42, p = 0.006; η^2 = 0.5 [0.1; 0.6]), hippocampal (Figure H; $F_{2, 11.8}$ = 8.6, p = 0.005; η^2 = 0.3 [0.03; 0.5]) and cortical (Figure I; $F_{2, 15.8}$ = 8.76, p = 0.0027; η^2 = 0.5 [0.1; 0.6]) kynurenic acid/ kynurenine values.

GSH/GSSG ratios (Figure J & K)
There were statistical differences between genotypes for striatal (Figure J; $F_{2,11}$ = 23.6, $p \leq$ 0.0005; η^2 = 0.6 [0.3; 0.8]) and hippocampal (Figure 3-5B; $F_{2, 6.85}$ = 154, $p \leq$ 0.0005; η^2 = 0.5 [0.1; 0.6]) GSH/GSSG values.









1) Sharma, S. and R.S. Akundi, 2019.Curr. Neuropharmacol., 17(6): 550-562.
2) Kato, T., 2017. Schizophr. Res., 187: 62-66.
3) Giménez-Palomo, A., et al., 2021. Front. Psychiatry, 12: 977.
4) van Rensburg, D., et al., 2022. Mitochondrion, 64: 82-102.
5) Chen, F., et al., 2013. Synapse 6,7(3): 127-134.
6) Frey, B.N., et al., 2006. Bipolar disorders,8(3): 275-280.
7) Terburgh, K., et al., 2019. Biochim. Biophys. Acta - Mol., 1865(1): 98-106.
8) Steyn, S.F., et al., 2018. Behav. Brain Res., 351: 49-62.
9) Can, A., et al., 2011. J. Vis. Exp., (58).
10) Regenass, W., el al., 2018. J. Psychopharmacol.,, 32(2): 134-145.
11) Wolmarans, D.W., et al.,, 2022. Neurosci., 177: 85-93.
12) Karsten, L., et al., 2021. in Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences.
13) Kruse, S.E., et al., 2008. Cell metabolism, 7(4): 312-320.
14) Allen, J., et al., 2021. Mitochondrion, 56: 111-117.
15) Van De Wal, M.A.E., et al., 2022. Brain, 145(1): 45-63.

NORTH-WEST UNIVERSITY NOORDWES-UNIVERSITEIT YUNIBESITI YA BOKONE-BOPHIRIMA
Pharmacen
SOUTH AFRICAN MEDICAL RESEARCH COUNCIL