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Research report

# Nrf2 deletion results in imparied performance in memory tasks and hyperactivity in mature and aged mice

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## HIGHLIGHTS

- Deletion of NRF2 resulted in enhanced motor performance.
- Deletion of NRF2 resulted in impaired cognitive performance with age.
- Deletion of NRF2 resulted in subtle changes in brain monoamines.

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## ABSTRACT

Oxidative stress has been implicated in both the functional and cognitive decline associated with neuropsychiatric diseases and aging. A master regulator of the body's defense mechanism against oxidative stress is nuclear factor erythroid 2-related factor (*NRF2*). Here we investigated the effects of NRF2 deletion on motor and cognitive performance in “Aged” mice (17–25 months old) as compared to “Mature” mice (3–15 months old). We observed that the Aged Nrf2$^{-/-}$ mice were hyperactive and exhibited impaired acquisition of an active avoidance response. Furthermore, the Mature mice also displayed a hyperactive phenotype and had impaired working memory in the probe trial of the water radial arm maze. Overall, it appears that NRF2 may be implicated in memory and activity functions and its deletion exacerbates deficits associated with aging. These observations provide a model for assessing the role of oxidative stress in age-related disorders.

## 1. Introduction

Oxidative stress has been implicated in several disease states ranging from cancer to neurodegenerative disorders. Endogenously produced reactive oxygen species (ROS), the oxidative stressors, have the potential to cause dysfunction in various mechanisms of cellular activity and, therefore, require regular removal. A key mechanism for ROS clearance is via nuclear factor erythroid 2-related factor (*NFE2L2*) encoding a protein, NRF2, which functions as a leucine-zipper transcription factor responsible for the downstream expression of multiple antioxidant proteins that detoxify ROS molecules (Lee and Johnson, 2004; Lambros and Plafker, 2015; Itoh et al., 1997; Hayes and Dinkova-Kostova, 2014; Wang et al., 2017). NRF2 binds to antioxidant response elements (ARE) which encode for genes such as NADPH:quinone oxidoreducatase-1, superoxide dismutase, heme oxygenase-1, catalase, sulforedoxin, thioredoxin, peroxiredoxin, and glutathione enzymes, all of which combat ROS (Lee and Johnson, 2004; Itoh et al., 1997; Hayes and Dinkova-Kostova, 2014). Under reduced conditions, NRF2 is sequestered in a complex in the cytoplasm by Kelch-like ECH associated protein 1 (Keap1) and Phosphoglycerate Mutase Family Member 5 (PGAM5) and is marked for degradation by ubiquitination, giving it a half-life of only 15 min (Lee and Johnson, 2004; Lo and Hannink, 2008; Kobayashi et al., 2004; Itoh et al., 1997; Hayes and Dinkova-Kostova, 2014). In the presence of ROS, Keap1 dissociates from NRF2, thereby allowing its translocation into the nucleus where it forms a heterodimer with small Maf proteins and binds to the ARE promoters initiating transcription of antioxidant genes (Itoh et al., 1997; Hayes and Dinkova-Kostova, 2014).

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NRF2 has been previously implicated in neuropsychiatric diseases such as depression, autism, and Alzheimer's disease (Bouvier et al., 2016; Furnari et al., 2014; Lipton et al., 2016; Prasad, 2016; Xu et al., 2017). For example, NRF2 deletion altered neurobehavioral development following exposure to valproic acid in motor tasks and has been shown to result in an increased depression-like phenotype (Bouvier et al., 2016; Furnari et al., 2014). NRF2 has also been linked to reducing neuroinflammation and dendritic spine loss (Buendia et al., 2016; Martín-de-Saavedra et al., 2013). Deletion of NRF2 or inhibition of the pathway results in reduced dopamine and serotonin levels in the prefrontal cortex, as well as, vascular endothelial growth factor (VEGF) reductions in the hippocampus (Martín-de-Saavedra et al., 2013). Additionally, in a chronic stress paradigm, NRF2 was linked to a mechanism for antidepressant response following fluoxetine treatment (Tritschler et al., 2015). Furthermore, activation of the NRF2 pathway through compounds like sulforaphane can reduce amyloid plaque accumulation and reduce the working memory deficits in Alzheimer's models (Lipton et al., 2016; Prasad, 2016; Xu et al., 2017). Interestingly, a novel role of NRF2 in neurogenesis and neural cell fate was recently identified where NRF2 deletion resulted in impaired long-term potentiation, reduced neurogenesis, and neural differentiation (Robledinos-Antón et al., 2017).

Oxidative stress has been implicated in functional deficits associated with aging (Muller et al., 2007). There is an age-dependent reduction in NRF2 and a resulting reduction of downstream antioxidant genes with increases in oxidative damage in proteins and DNA, culminating in apoptosis (Shih and Yen, 2006; Miller et al., 2012; Ames et al., 1993). Using accelerated aging mouse models (SAMP8, SOD1), oxidative stress accumulation increased circulating levels of pro-inflammatory cytokines (IL-6), activation of Nf-kappaB pathway, and cell senescence (Zhang et al., 2017; Farr et al., 2012). Furthermore, SAMP8 mice showed working memory deficits and reduced NRF2 levels in the brain, demonstrating the link between oxidative stress and cognitive function. However, both the number of mouse models of aging and understanding how neural effects contribute to deterioration of cognitive function is lacking. The present study further investigates the role of NRF2 in motor and cognitive function as well as a potential age-dependent phenotype.

## 2. Results

### 2.1. Mature & aged mice

There was no statistical difference in the spread of age ranges between Mature Nrf2$^{+/+}$ (9.82 ± 2.40 mnths, mean & stdev. respectively) and Nrf2$^{-/-}$ (11.4 ± 2.45 mnths, mean & stdev. respectively) ($t_{(36)} = 1.82$, $p = 0.0768$). Similar was true for Aged Nrf2$^{+/+}$ (23.6 ± 2.32 mnths, mean & stdev. respectively) and Nrf2$^{-/-}$ (22.5 ± 2.9 mnths, mean & stdev. respectively) ($t_{(21)} = 0.945$, $p = 0.355$).

### 2.2. Rotarod

Nrf2$^{+/+}$ and Nrf2$^{-/-}$ mice exhibited differential performance on the rotarod over the three days of training ($F_{(2,\ 116)} = 6.742$, $p = 0.002$; Fig. 1), with differences between each group ($F_{(3,\ 58)} = 18.23$, $p < 0.001$; Fig. 1) and an interaction effect between days of training and group ($F_{(6,\ 116)} = 4.566$, $p < 0.001$). Furthermore, the Mature Nrf2$^{-/-}$ mice exhibited a significantly longer latency to fall as compared to the Mature Nrf2$^{+/+}$ counterparts on day 1 ($t_{(1\ 7\ 4)} = 3.935$, $p = 0.002$, Bonferroni-corrected), as well as Mature Nrf2$^{-/-}$ mice compared to Aged Nrf2$^{+/+}$mice ($t_{(1\ 7\ 4)} = 6.431$, $p < 0.001$, Bonferroni-corrected) and Aged Nrf2$^{-/-}$ ($t_{(1\ 7\ 4)} = 6.34$, $p < 0.001$, Bonferroni-corrected) mice. On the second day of training an age effect was observed between Mature Nrf2$^{+/+}$ and Aged Nrf2$^{+/+}$ ($t_{(1\ 7\ 4)} = 3.431$, $p = 0.014$, Bonferroni-corrected), and Mature and Aged Nrf2$^{-/-}$ mice ($t_{(1\ 7\ 4)} = 3.498$, $p = 0.011$, Bonferroni-corrected), and lastly an age and genotype difference was found between Mature Nrf2$^{-/-}$ and Aged Nrf2$^{+/+}$ ($t_{(5.734)} = 5.734$, $p < 0.001$, Bonferroni-corrected) mice. Mature Nrf2$^{-/-}$ mice had the best performance over each day, but declined in performance over days whereas all other groups increased performance over days. Aged Nrf2$^{-/-}$ mice did not show this pattern which suggests an age-dependent phenotype.

### 2.3. Mature Adult mice

#### 2.3.1. Motor activity

To test whether Nrf2$^{-/-}$ mice have general higher locomotor activity levels, they were assessed in an activity chamber. Nrf2$^{-/-}$ mice demonstrated higher locomotor activity for horizontal movements ($t_{(47)} = 3.361$, $p = 0.002$) than Nrf2$^{+/+}$ mice (Fig. 2A). Additionally, Nrf2$^{-/-}$ mice displayed more stereotypic movements as compared to controls ($t_{(47)} = 3.717$, $p < 0.001$; Fig. 2B). Therefore, Nrf2$^{-/-}$ mice likely performed better than controls in the rotarod test because of a general higher activity rather than a learned motor ability over the trials. Furthermore, since Nrf2$^{-/-}$ mice demonstrated higher stereotyped movements (i.e. fewer new directional movements were made) these data appear to reflect a hyperactive state.

#### 2.3.2. Anxiogenic Phenotyping

Open Field: There were no group differences in terms of time spent in the periphery or open areas of the open field chamber (Fig. 3A) between Mature Nrf2$^{+/+}$ and Nrf2$^{-/-}$ mice.

Elevated Plus Maze: There were no group differences in terms of percent of time spent in either arm or number of entries (Fig. 3B & 3C). These two behavioral measures suggest that the hyperactivity seen in previous testing was not due to a more anxiogenic phenotype.

#### 2.3.3. Spatial navigation learning Tasks

Morris Water Maze (MWM): Since Nrf2$^{-/-}$ mice appeared to have higher activity states compared to Nrf2$^{+/+}$ mice, we sought to determine whether their learning was similar or impaired compared to Nrf2$^{+/+}$ mice in the MWM. During the hidden platform trials, both Nrf2$^{+/+}$ and Nrf2$^{-/-}$ mice were able to learn the task and showed significantly improved performance over four days ($F_{(3,\ 93)} = 26.41$, $p < 0.001$, Fig. 3A), with no differences between groups. However, during visible platform trials Nrf2$^{-/-}$ mice performed better than the Nrf2$^{+/+}$ mice (unpaired two-tailed *t*-test, $t_{(31)} = 2.623$, $p = 0.013$; Fig. 3B), which likely is the result of their hyperactive state. Both Nrf2$^{+/+}$ and Nrf2$^{-/-}$ mice were able to learn the maze and Nrf2$^{-/-}$ mice were better than Nrf2$^{+/+}$ mice in finding the visible platform, which suggests there was no impairment in spatial navigation.

Water Radial Arm Maze (wRAM): Over the ten days of hidden platform trials all mice demonstrated ability to learn the task with decreased latency to find the platform ($F_{(9,\ 225)} = 11.38$, $p < 0.001$), with only a statistical difference between genotypes on day 7 ($t_{(2\ 5\ 0)} = 2.837$, $p = 0.049$, Bonferroni corrected; Fig. 4E). Additionally, there was no statistically significant differences when comparing distance traveled over the trials between groups (Fig. 4D). During visible platform trials both groups were able to find the platform with no difference between groups (Fig. 4C). However, Nrf2$^{-/-}$ mice spent significantly less time in the goal arm (previously containing the hidden platform) during the probe trial ($t_{(17)} = 2.231$, $p = 0.039$; Fig. 4F), which suggests that Nrf2$^{-/-}$ mice may be randomly sampling each arm looking for the hidden platform, rather than using the contextual clues for spatial navigation.

#### 2.3.4. Passive avoidance

There was no significant difference between raw calculated Nrf2$^{+/+}$ and Nrf2$^{-/-}$ latencies during either the training or test trial (despite Nrf2$^{+/+}$ mice having a higher test latency) in passive avoidance learning between first and second trial (Fig. 5A). However, upon further