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Research report

Exercise related anxiety-like behaviours are mediated by TNF receptor signaling, but not depression-like behaviours

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ABSTRACT

Depression can involve disrupted pro-inflammatory TNF signaling via the TNF receptors TNFR1 and TNFR2, or the soluble TNF receptors sTNFR1 and sTNFR2. However, exercise might attenuate pro-inflammatory signaling in depression and related anxiety. We hypothesized that six months voluntary wheel running exercise would improve depression-like and anxiety-like behaviours in WT and TNFR1$^{-/-}$ mice, but not in TNF$^{-/-}$ and TNFR2$^{-/-}$ mice compared to their respective control mice.

Methods: We investigated the effects of six months voluntary wheel running exercise on open field (OF) and elevated zero maze (EZM) anxiety-like behaviours, and forced swim test (FST) depression-like behaviours in control and exercise WT, TNF$^{-/-}$, TNFR1$^{-/-}$, and TNFR2$^{-/-}$ mice with two-way ANOVAs.

Results: Exercise reduced of anxiety-like behaviours in TNFR2$^{-/-}$ exercise mice compared to their respective controls. Compared to WT control mice, WT exercise mice displayed significantly reduced EZM anxiety-like behaviours. There were no exercise related changes in FST immobility time. Between-strains analyses found WT control and exercise mice displayed reduced EZM anxiety-like behaviours compared to TNF$^{-/-}$ and TNFR1$^{-/-}$ control and exercise mice, and WT exercise mice displayed reduced anxiety-like behavior compared to TNFR2$^{-/-}$ exercise mice.

Discussion: Exercise associated TNFR1 and TNFR2 signaling in concert in WT exercise mice mediated reductions in aspects of anxiety-like behaviours. These findings are consistent with the current view that imbalances in TNF signaling are involved in disrupted affect. Additional studies are needed to further explore the roles of exercise related TNFR1 and TNFR2 signaling in anxiety-like and depression-like behaviours.

1. Introduction

Tumor necrosis factor alpha (TNF) signaling via the membrane bound TNF receptors tumor necrosis factor receptor 1 (TNFR1) and tumor necrosis factor receptor 2 (TNFR2), and the soluble TNF receptors soluble TNFR1 (sTNFR1) and soluble TNFR2 (sTNFR2) are implicated in the pathophysiology of depression and related anxiety (Kohler et al., 2017; Rizavi et al., 2016; Smith, 1991). Patients with depression and/or anxiety have demonstrated significant changes in the levels of TNF and the TNF receptors in cells, plasma, and serum (Dowlati et al., 2010; Dunjic-Kostic et al., 2013; Himmerich et al., 2008; Kohler et al., 2017; Liu et al., 2012; Maes et al., 1998; Muthuramalingam et al., 2016; Vieira et al., 2010), and whilst no differences have also been found (Euteneuer et al., 2012), these findings are in the minority. Recent meta-analyses have shown chronically elevated TNF was associated with treatment non-response (Strawbridge et al., 2015) and TNF has consequently become a potential biomarker for depression (Martinez-Cengotitabengoa et al., 2016).

Preclinical studies have investigated the roles of TNF and the TNF receptors in depression-like and anxiety-like behaviours. The administration of exogenous TNF increased immobility time in the Porsolt forced swim test (Kaster et al., 2012; Palin et al., 2009), whilst mouse models of TNF receptor deficit utilizing TNFR1$^{-/-}$ or TNFR2$^{-/-}$ mice have shown reductions in immobility time in the forced swim test (FST) in both TNFR1$^{-/-}$ (Kaster et al.,

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2012; Simen et al., 2006) and TNFR2$^{-/-}$ mice (Simen et al., 2006), suggesting that TNF signaling via both receptors might be involved in the etiology of depression-like behaviours. TNF signaling via TNFR1 and TNFR2 is also associated with increased time spent in the centre of the open field (OF), suggestive of reductions in anxiety-like behaviours in the context of TNF receptor deficits (Patel et al., 2010). Collectively the disruption of TNF and the TNF receptors in patients with depression and the involvement of TNF signaling via the TNFR1 and TNFR2 in anxiety-like and depression-like behaviours suggests significant involvement of TNF signaling via both the TNFR1 and TNFR2 in these behaviours.

TNFR1 and TNFR2 have distinctive expression and roles. TNFR1 is expressed on most cells, and is involved in the negative regulation of cellular proliferation, and the activation of cellular apoptosis (Baune et al., 2012; Cui et al., 2011; Grell et al., 1995; Iosif et al., 2006; Wajant et al., 2003). In contrast, TNFR2 is only expressed on endothelial cells and cells of haematopoietic lineage and microglia, oligodendrocytes, and neuronal subtypes in the brain (Arnett et al., 2001; Dopp et al., 2002; Grell et al., 1995; McCoy and Tansey, 2008; Yang et al., 2002). TNFR2 is considered to have a neuroprotective role through inhibiting caspase initiated apoptosis and by the protection of neurons from glutamate related excitotoxicity (Baune et al., 2012; Heir and Stellwagen, 2015; Wajant et al., 2003). In constitutional conditions, sTNFR1 has an antagonist function to pro-inflammatory TNF signaling via the TNFR1 (Pinckard et al., 1997). In health, TNF signaling that is balanced between the TNFR1 and TNFR2 is thought to contribute to the maintenance of normal cellular functioning, however a shift towards greater TNF signaling via the TNFR1 is considered to contribute to TNF associated neuropathology and neurodegeneration (Baune et al., 2012). This suggests the modulation of TNF signaling in depression is an important therapeutic aim.

Exercise is a therapy with few risks and side effects, and has significant effects on TNF and the TNF receptors, and may contribute to the modulation of altered TNF signaling in depression. During acute aerobic exercise, major working muscles exponentially increase the expression of interleukin 6 (Petersen and Pedersen, 2005; Petersen and Pedersen, 2006) that blunts TNF expression, with increases the soluble TNF receptors sTNFR1 and sTNFR2 (Petersen and Pedersen, 2005; Petersen and Pedersen, 2006). This results in a net increase in anti-inflammatory factors including IL1ra, IL10, and the sTNFR1, suggesting that chronic exercise could contribute to chronically reduced TNF levels. Preclinical studies of chronic exercise have shown evidence of this, with reductions in TNF levels following three or 16 weeks of voluntary wheel running (Liu et al., 2013; Pervaiz and Hoffman-Goetz, 2011) suggesting that chronic exercise could contribute to reducing TNF, and may alter TNF signaling via the TNF receptors with associated benefits for anxiety-like and depression-like behaviours. Indeed four weeks of wheel running increased the time spent in the central regions of the OF and open arms of elevated plus maze (EPM), suggesting a reduction in anxiety-like behaviours (Binder et al., 2004; Duman et al., 2008), whilst three to four weeks of running reduced immobility time in the FST (Cunha et al., 2013; Duman et al., 2008) and tail suspension test (Cunha et al., 2013). Considered together, the effects of exercise on TNF, the TNF receptors, and anxiety-like and depression-like behaviours suggest that exercise might confer benefits for anxiety-like and depression-like behaviours arising from altered TNF signaling via the TNF receptors. However, the question of whether TNF signaling via the TNF receptors mediates the effects of exercise on anxiety-like and depression-like behaviours remains unknown. This study therefore sought to investigate the roles of TNF signaling via the TNF receptors TNFR1 and TNFR2 in mediating the effects of exercise on depression-like and related anxiety-like behaviours. We utilized genetically modified mouse models with TNF and TNF receptor knockout; including wild type (WT), tumor necrosis factor alpha knockout (TNF$^{-/-}$), tumor necrosis factor alpha receptor 1 knockout (TNFR1$^{-/-}$), and tumor necrosis factor alpha receptor 2 knockout (TNFR2$^{-/-}$) mice compared to a WT control strain. We provided mice with *ad libitum* voluntary wheel running exercise from three months to nine months of age (six months). We hypothesized exercise would improve depression-like and anxiety-like behaviours in WT and TNFR1$^{-/-}$ mice arising from advantageous exercise associated TNF signaling via all TNF receptors in WT mice, and from the neuroprotective role of TNFR2 signaling in TNFR1$^{-/-}$ mice (Baune et al., 2012). However we anticipated no exercise related changes in TNF$^{-/-}$ and TNFR2$^{-/-}$ exercise mice because exercise has no effect on TNF in the absence of TNF signaling in TNF$^{-/-}$ mice, and because TNF signaling via the TNFFR1 involves the negative regulation of cellular proliferation and the activation of cellular apoptosis (Wajant et al., 2003).

2. Results

2.1. Mouse body weights

Examination of mouse body weights showed that all strains maintained healthy body weights over the experimental period, with WT and TNFR1$^{-/-}$ control and exercise mice, and TNF control mice displaying significant weight gain over the experimental period consistent with development from young adulthood to middle age (Table 2).

2.2. Exercise distances travelled

Two way repeated measures ANOVA analyses of the distances travelled over the experimental period showed there were no significant differences in the mean monthly distances travelled between strains (Fig. 1). All mice therefore ran comparable distances over the experiment, and these were consistent with the distances run by mice in previous studies (Marlatt et al., 2012; Morgan et al., 2017), with no differences between groups that might contribute to behavioral changes.

2.3. Behavioural assessments

2.3.1. Locomotor activity in the home cage

To determine whether TNF signaling via the TNF receptors affected baseline locomotor activity we quantified locomotor activity in the animals' home cages. Investigation into home cage locomotor activity utilizing two way ANOVA found significant effects of strain ($F(3, 96) = 5.277$, $P = 0.002$) and treatment ($F(1, 96) = 6.385$, $P = 0.013$), with no strain by treatment interaction ($F(3, 96) = 0.9103$, $P = 0.439$). Post hoc analyses with Sidak's' correction for multiple comparisons found TNFR1$^{-/-}$ exercise mice travelled significantly less distance in the home cage compared to TNFR1$^{-/-}$ control mice ($p = 0.014$), but there were no differences in the distances travelled between control and exercise mice in WT, TNF$^{-/-}$, or TNFR2$^{-/-}$ mice (Fig. 2A, Table 3). However, WT control mice

Table 1
Treatment group numbers for mouse strains.

Strain	Control n (males: females)	Exercise n (males: females)
WT	19 (9: 10)	17 (7: 10)
TNF$^{-/-}$	19 (9: 10)	15 (8: 7)
TNFR1$^{-/-}$	17 (10: 7)	16 (9: 7)
TNFR2$^{-/-}$	6 (5:1)	6 (6: 0)

Legend: WT = wild type; TNF$^{-/-}$ = tumor necrosis factor alpha knockout; TNFR1$^{-/-}$ = tumor necrosis factor alpha receptor 1 knockout; TNFR2$^{-/-}$ = tumor necrosis factor alpha receptor 2 knockout.