Deletion of Tlx and social isolation impairs exercise-induced neurogenesis in the adolescent hippocampus

Danka A. Kozareva, Olivia F. O'Leary, John F. Cryan and Yvonne M. Nolan*

Department of Anatomy and Neuroscience, University College Cork, Ireland

APC Microbiome Institute, University College Cork, Ireland

To whom correspondence should be addressed:

*Yvonne M. Nolan, Department of Anatomy and Neuroscience, University College Cork, Ireland

Tel: (353) 21-420 5476

Fax: (353) 21-427 3518

email: y.nolan@ucc.ie

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Abstract

Adolescence is a sensitive period of neurodevelopment during which life experiences and the surrounding environment can have profound effects on the brain. Neurogenesis is a neurodevelopmental process of generating functional neurons from neural stem cells. Hippocampal neurogenesis occurs throughout the lifespan and has been shown to play a role in learning, memory and in mood regulation. In adulthood it is influenced by extrinsic environmental factors such as exercise and stress. Intrinsic factors that regulate hippocampal neurogenesis include the orphan nuclear receptor TLX (Nr2e1) which is primarily expressed in the neurogenic niches of the brain. While mechanisms regulating adult hippocampal neurogenesis have been widely studied, less is known on how hippocampal neurogenesis is affected during adolescence. Thus, the aim of this study was to investigate the influence of both TLX and isolation stress on exercise-induced increases in neurogenesis in running and sedentary conditions during adolescence. Single- (i.e. isolation stress) wild type and $Nr2e1^{-/-}$ or pair-housed wild type mice were housed in sedentary conditions or allowed free access to running wheels for 3 weeks during the adolescent period. A reduction of neuronal survival was evident in mice lacking TLX, and exercise did not increase hippocampal neurogenesis in these $Nr2e1^{-/-}$ mice. This suggests that TLX is necessary for the pro-neurogenic effects of exercise during adolescence. Interestingly, although social isolation during adolescence did not affect hippocampal neurogenesis, it prevented an exercise-induced increase in neurogenesis in the ventral hippocampus. Together these data demonstrate the importance of intrinsic and extrinsic factors in promoting an exercise-induced increase in neurogenesis at this key point in life.

Key words: adult neurogenesis, adolescence, stress, exercise, TLX

Adolescence is a critical phase of development associated with plasticity-driven organization of neural circuits in the hippocampus, prefrontal cortex and amygdala (Pattwell et al., 2011; Selemon, 2013). It is also a key period for susceptibility to stress and the emergence of neurobiological disorders such as schizophrenia, depression and anxiety (Green and Nolan, 2014; Hueston et al., 2017; O'Connor and Cryan, 2014; Paus et al., 2008). By approximately postnatal day (P) 30 in rodents, dentate gyrus (DG) formation, cerebellar neurogenesis and myelogenesis are completed, and neurogenesis (the birth of new neurons) is restricted to the niches of the brain where the process persists through adulthood – the subgranular zone (SGZ) of the DG of the hippocampus and the subventricular zone (Lemasson et al., 2005; Li et al., 2009).

Hippocampal neurogenesis has been widely studied in the adult brain and is known to be regulated by several extrinsic and intrinsic factors (Aimone et al., 2014; Gregoire et al., 2014). For example, extrinsic factors such as stress and exercise have been shown to decrease or increase adult hippocampal neurogenesis, respectively (Levone et al., 2015; van Praag et al., 1999a). However, comparatively fewer studies have investigated the impact of these extrinsic factors on hippocampal neurogenesis in the adolescent brain (Abel and Rissman, 2013; Kirshenbaum et al., 2014; Wei et al., 2011). In adult rodents, exercise has been shown to enhance learning and memory (Creer et al., 2010; Marlatt et al., 2012; van Praag et al., 1999a), protect against stress-induced depression and anxiety-like behaviours (Duman et al., 2008; Grippo et al., 2014) and protect against cognitive deficits in neurodegenerative disorders (Barbour et al., 2007; Cotman et al., 2007; Ryan and Nolan, 2016). How exercise can facilitate processes as diverse as spatial learning and memory, anxiety and responses to stress is not yet clear. However, accumulating evidence suggests that the hippocampus is functionally segregated along its dorsoventral axis in rodents such that the dorsal

hippocampus (dHi) plays a predominant role in spatial learning and memory while the ventral hippocampus (vHi) plays a predominant role in anxiety and the stress response (Bannerman et al., 2004; Fanselow and Dong, 2010). Similarly, there is an emerging view that neurogenesis might also be similarly functionally segregated along this axis (O'Leary and Cryan, 2014; Tanti and Belzung, 2013). Intrinsic factors that control hippocampal neurogenesis include the orphan nuclear receptor TLX (Nr2e1) which is primarily expressed in the neurogenic niches of the postnatal brain and has been implicated as an important regulator of neural stem cells by maintaining them in their proliferative and non-differentiated state (Roy et al., 2004; Shi et al., 2004). In a spontaneous deletion mouse model, adult TLX knock out ($Nr2e1^{-/-}$) mice display altered neurogenesis and synaptic plasticity, as well as an impairment of dendritic structures in the DG. These mice also present with an aggressive phenotype and display cognitive impairments in hippocampal-dependent tasks (Christie et al., 2006; Young et al., 2002). Interestingly, some of these effects are apparent in adolescence (O'Leary et al., 2016a; O'Leary et al., 2016b).

Taken together, while mechanisms regulating adult hippocampal neurogenesis have been thoroughly investigated, less is known about how neurogenesis is affected during the adolescent period. Moreover, the impact of facilitators and impeders of neurogenesis, such as exercise and stress, on the adolescent brain has yet to be established. This is surprising given that adolescence is a critical period for the maturation of neurons as well as a time during which profound social and physiological change occurs. Thus, the aim of this study was to investigate (1) the impact of exercise during adolescence on hippocampal neurogenesis; (2) the role of the intrinsic factor TLX on exercise-induced changes in hippocampal neurogenesis and (3) whether social isolation stress influences exercise-induced changes in hippocampal neurogenesis. Given the growing evidence of segregated effects on neurogenesis along the

dorsoventral axis of the hippocampus, we examined the impact of exercise, TLX, and social isolation on neurogenesis in the dorsal versus ventral hippocampus.

This animal study was conducted in strict compliance with the European Directive 2010/63/EU, and under an authorization issued by the Health Products Regulatory Authority Ireland and approved by the Animal Ethics Committee of University College Cork (UCC). Breeding pairs of Nr2e1$^{-/-}$ mice exhibiting a spontaneous deletion of TLX were kindly provided by Prof. Elizabeth Simpson (University of British Colombia) and were generated as previously described (Wong et al., 2010). Male Nr2e1$^{-/-}$ mice and wild type (WT) littermate controls on a BL6129 background were singly-housed upon weaning and given *ad libitum* access to food and water under a 12-12 h light/dark cycle. At 4 weeks of age (P28), mice received 4 x bromodeoxyuridine (BrdU; 75mg/kg, i.p., Sigma Cat# B5002) injections at 2-hour intervals to label newly-born cells. Half of the animals from each genotype were then housed with free access to a running wheel for 3 weeks (Med Associates Inc Cat# ENV-044), thus there were 4 experimental groups (WT sedentary, WT running, Nr2e1$^{-/-}$ sedentary; Nr2e1$^{-/-}$ running; n = 5-6 per group; **Fig. 1**). Male mice were collected from 11 litters in total. For welfare reasons Nr2e1$^{-/-}$ mice have to be singly housed due to their aggressive phenotype (Young et al., 2002). Thus, their corresponding wild type littermates were also singly housed. Since single housing is a social isolation stressor, we sought to examine whether this chronic stress influenced the effect of exercise on hippocampal neurogenesis in WT mice (**Fig. 1**).

Three weeks following BrdU administration and initiation of exercise, mice (P49) were anesthetized with Euthasol (1.0 mL/kg, i.p.) and transcardially perfused with phosphate buffered saline (PBS) solution followed by 4.0% paraformaldehyde (PFA). Brains were postfixed overnight, cryoprotected in 30% sucrose and subsequently flash frozen. Coronal

sections (40 μm) through the entire hippocampus were collected onto slides in a 1:6 series and surviving cells and surviving newly born neurons were identified using immunohistochemistry for BrdU alone, and colocalization of BrdU and the neuronal marker NeuN, respectively. Briefly, sections were incubated in HCl (2M; 37°C, 45 mins), renatured in 0.1M sodium tetraborate (pH 8.5) and then blocked in 3% normal donkey serum (NDS; Sigma Cat# D9663). Sections were incubated with rat anti-BrdU antibody (Abcam Cat# AB6326; 1:250; overnight at 4°C), followed by AlexaFluor594 donkey anti-rat (Abcam Cat# ab150156; 1:500; 2 hours at room temperature) and mouse anti-NeuN (Millipore Cat# MAB377; 1:100; overnight at 4°C). Sections were then incubated in AlexaFluor488 donkey anti-mouse (Abcam Cat# ab150105 1:500; 2 hours at room temperature). To assess cell death, immunohistochemistry for the apoptosis marker caspase-3 was performed. Briefly, sections were incubated in H_2O_2 (in 1% methanol, 40 mins at room temperature) and then blocked in 10% normal goat serum (NGS; Sigma Cat# G9023). Sections were incubated with rabbit anti-active caspase-3 antibody (Promega Cat# G7481; 1:250; overnight at 4°C), followed by sequential incubations with the streptavidin-biotin immunoenzymatic antigen detection system (Abcam, Cat# ab64261). Images were obtained using an Olympus BX533 upright microscope coupled to an Olympus DP72 camera and Olympus FV1000 scanning laser confocal system (BioSciences Imaging Centre, UCC). Immunofluorescent Z-stack images with a 4.4 μm step size were collected using a 10x objective, while DAB staining was analysed at 20x magnification with bright field. Systematic random sampling was employed through the whole DG by counting the cells on both hemispheres of each section in 1:6 series (240 μm apart). Cell quantification was performed using the image processing software package, ImageJ v 1.43m. All cell numbers are expressed as an average per section. The dorsal DG was defined as AP: - 0.94 to AP: - 2.30 and the ventral DG as AP: - 2.46 to AP: - 3.80 as described previously (O'Leary et al., 2012; Paizanis et al., 2010).

Exercise significantly increased the number of surviving new cells in the whole DG of WT mice (exercise effect: $F_{1, 19} = 7.915$, $p = 0.011$), but not in Nr2e1$^{-/-}$ mice (exercise x genotype interaction: $F_{1, 19} = 6.747$, $p = 0.018$; **Fig. 2A**). Furthermore, both sedentary and running Nr2e1$^{-/-}$ mice exhibited a reduction in the number of surviving new cells compared to their WT littermates (genotype effect: $F_{1, 19} = 244.002$, $p < 0.001$; **Fig. 2A**). These changes in cell survival were driven by an exercise-induced increase in the number of surviving new neurons (neurogenesis) in the DG of WT mice (exercise effect: $F_{1,19} = 6.024$, $p = 0.024$), but not in Nr2e1$^{-/-}$ mice (exercise x genotype interaction: $F_{1,19} = 5.704$, $p = 0.027$; **Fig. 2B**). Additionally, sedentary Nr2e1$^{-/-}$ mice also exhibited reduced hippocampal neurogenesis compared to their WT littermates (genotype effect: $F_{1,19} = 130.096$, $p < 0.001$; **Fig. 2B)**. Upon subdivision of the DG into dorsal (dDG) and ventral (vDG) regions, this genotype effect was apparent in both subregions for both new cell (BrdU+) and new neuron (BrdU+NeuN+) survival (**BrdU+:** dDG: $F_{1,19} = 98.723$, $p < 0.001$; vDG: $F_{1,19} = 92.748$, $p < 0.001$; **Fig 2A**; **BrdU+NeuN+:** dDG: $F_{1,19} = 69.635$, $p < 0.001$; vDG: $F_{1,19} = 88.056$, $p < 0.001$; **Fig. 2B**) . However, the exercise-induced increase in new cell survival and neurogenesis was only apparent in the dDG (exercise x genotype interaction: **BrdU+:** $F_{1,19} = 4.078$, $p = 0.058$; **BrdU+NeuN+:** $F_{1,19} = 4.502$; $p = 0.047$) but not in the vDG of WT mice (**Fig. 2)**. No difference was observed in the percentage of surviving new cells that adopted a neuronal fate in the DG of WT animals; almost all BrdU+ cells were immunopositive for NeuN and there was no effect of exercise on cells adopting a neuronal fate. In the DG of Nr2e1-/- mice, however, a significantly smaller percentage of surviving cells matured into neurons in both sedentary and exercise conditions (genotype effect: $F1,19 = 31.530$, $p < 0.001$; % BrdU+NeuN+ cells/BrdU+ cells: WT sedentary: $M = 70.156$, $SD = 4.065$; WT

running: M = 74.398, SD = 9.338; Nr2e1-/- sedentary: M = 53.839, SD = 9.130; Nr2e1-/- running: M = 51.151, SD = 9.257).

Since exercise increased neurogenesis in the dorsal but not ventral DG of WT mice, we decided to investigate whether the stress of this social isolation during adolescence could explain the lack of effect of exercise-induced increases in neurogenesis in the vDG. Thus we compared the effect of running on cell survival and hippocampal neurogenesis in single-housed compared to pair-housed WT mice (**Fig. 3**). Two way ANOVA revealed that exercise significantly increased cell survival in paired- but not single-housed mice in the whole DG, and this effect persisted in both dDG and vDG (exercise x stress interaction: total DG: $F_{1,17} = 7.420$, $p = 0.014$; dDG: $F_{1,17} = 5.911$, $p = 0.026$; vDG: $F_{1,17} = 5.825$, $p = 0.027$; **Fig. 3A**). Interestingly, exercise significantly increased neurogenesis of both paired and single-housed mice who had access to a running wheel for three weeks compared to their sedentary counterparts (exercise effect: DG: $F_{1,17} = 34.054$; $p < 0.001$). However, the exercise-induced increase in neurogenesis was significantly lower in single-housed animals than in pair-housed animals (exercise x stress interaction: DG: $F_{1,17} = 6.866$; $p = 0.018$). Moreover, upon analysis of the dorsal and ventral regions of the DG, we found that although exercise increased neurogenesis in the dDG in both pair-housed and single-housed mice (dDG: $F_{1,17} = 29.350$; $p < 0.001$; **Fig. 3B**), this effect was attenuated by the stress of single housing (exercise x stress interaction dDG: $F_{1,17} = 5.239$, $p = 0.035$). Additionally, the exercise-induced increases in neurogenesis observed in the vDG of pair-housed mice (exercise effect: vDG: $F_{1,17} = 15.421$; $p = 0.001$) was prevented in singly housed mice (exercise x stress interaction: vDG: $F_{1,17} = 6.933$, $p = 0.017$; **Fig. 3B**).

There was a trend towards a reduction of caspase-3+ cells in the vDG of Nr2e1$^{-/-}$ mice with access to a running wheel, compared to their sedentary and WT running counterparts (exercise x genotype interaction: $F_{1,12} = 3.8$, $p = 0.077$; **Fig. 4A)**. There was a significant reduction in the number of apoptotic cells in the vDG but not dDG or whole DG in pair-housed mice with access to a running wheel compared to their single-housed or pair-housed sedentary littermates (exercise x stress interaction: vDG: $F_{1,12} = 5.770$, $p < 0.05$; **Fig. 4B**).

Our results indicate that the well-known pro-neurogenic effect of exercise observed during adulthood (van Praag et al., 1999a; van Praag et al., 1999b) also occurs in adolescent male mice. This finding corroborates the effects of exercise on cognitive function observed in adolescent rats (Hopkins et al., 2011). Mice exposed to exercise during adolescence have increased hippocampal levels of pro-neurogenic brain-derived neurotrophic factor (BDNF) (Gallego et al., 2015) and enhanced expression of synaptic plasticity genes (Abel and Rissman, 2013). Exercise has also been shown to rescue alcohol-induced deficits in cell proliferation in adolescent rats (Helfer et al., 2009). Interestingly, a physical skills training task has recently been reported to increase cell survival in the DG of adolescent rats (DiFeo and Shors, 2017). However, training on this type of tasks involves physical exercise and learning, both of which increase neurogenesis and so a definitive conclusion on the effects of exercise alone during adolescence on hippocampal neurogenesis cannot be determined from this study. The present report is the first to demonstrate that voluntary running increases the survival of newly born cells and neurons in the DG of adolescent mice.

In the absence of TLX, a key regulator of adult neurogenesis, we found no pro-neurogenic effect of exercise. It has been previously shown that deletion of TLX leads to significant reduction in adult neurogenesis, synaptic plasticity and impaired dendritic structure in the DG

of adult mice (Christie et al., 2006). Similarly, we show reduced survival of newborn cells and neurons in the DG of sedentary Nr2e1$^{-/-}$ adolescent mice, an effect which was not mitigated by exercise. This positions TLX as a regulator of exercise-induced increases in neurogenesis during adolescence. Further studies will determine whether TLX mitigates the pro-neurogenic effects of exercise in adulthood and indeed throughout the lifespan. We have previously shown that a lack of TLX is associated with impairments in hippocampal-related cognitive and anxiety behaviours during adolescence. Specifically, adolescent but not adult Nr2e1$^{-/-}$ mice showed deficits in spatial working memory, contextual fear conditioning and cued fear conditioning (O'Leary et al., 2016a). The fact that a lack of TLX expression from birth through adulthood did not persistently induce the same impairments during adolescence and adulthood may point towards compensatory mechanisms occurring past the adolescent period, which ameliorate to some degree the deficits caused by deletion of TLX, independent of neurogenesis. It is worth noting that in contrast to our data with TLX deficiency, ablation of neurogenesis through irradiation, another model of reduced hippocampal neurogenesis, has been shown to be sensitive to the rescue effects of exercise (Clark et al., 2008; Naylor et al., 2008). However, the effects of adolescent hippocampal irradiation on neuronal survival remain unknown.

We observed an exercise-induced increase in neurogenesis, but not cell survival, in the DG of singly-housed adolescent mice which is consistent with previous reports using adult mice (Dostes et al., 2016; Gregoire et al., 2014; Kannangara et al., 2009). It is worth noting however that single housing has been shown to blunt cell proliferation in running rats (Leasure and Decker, 2009; Stranahan et al., 2006) but these differences have not been reconciled in adolescent rodents. To date, the effect of exercise and housing conditions on neurogenesis across the septo-temporal axis of the hippocampus has not been examined. This

is interesting in light of the emerging view that the ventral hippocampus may be the predominant sub-region involved in stress responses (Bannerman et al., 2004; O'Leary and Cryan, 2014; Tanti and Belzung, 2013). Hence, the conflict in findings on hippocampal neurogenesis from group-housed and single-housed rodents may be a function of the differential effects of exercise on the dorsal and ventral hippocampus. Our results demonstrate that social isolation prevents the pro-neurogenic effects of exercise in the vDG but not the dDG. Specifically, exercise increased the survival of new neurons in the dDG of both single- and pair-housed mice, but only in the vDG of pair-housed mice. In addition, the exercise-induced increase in neurogenesis in the dDG of both single and pair-housed mice was significantly attenuated by single housing. Importantly, the studies to date report on exercise-induced increases in neurogenesis in the whole DG, which may explain why studies employing single-housed mice consistently replicate the pro-neurogenic effects of exercise (Mustroph et al., 2012) observed in group-housed mice (van Praag et al., 1999a). Here we show that social isolation during adolescence acts as differential regulator of exercise across the distinct anatomical regions of the DG and propose that neurogenesis in both the dDG and vDG should be taken into consideration when investigating the role of hippocampal neurogenesis in exercise and stress-induced changes in behaviour. Nonetheless, the effects reported here may be specific to the adolescent period. Thus an examination of the effect of social isolation stress during other time periods of the lifespan on any potential exercise-induced changes in neurogenesis in the subregions of the DG is warranted in future studies.

The mechanisms underlying the differential effect of social isolation stress on exercise-induced changes in neurogenesis in the dDG and vDG during adolescence remain unclear. It has been reported that unpredictable chronic mild stress in adult mice preferentially decreased the survival of new neurons in the vDG (Elizalde et al., 2010; Tanti et al., 2012), supporting

the view that neurogenesis in the ventral pole of the DG may be more susceptible to the effects of stress. Interestingly, environmental enrichment, which includes exercise, promoted neurogenesis only in the dorsal hippocampus (Tanti et al., 2012). The effects of stress on neurogenesis are thought to be mediated by the glucocorticoid (GR) and mineralocorticoid (MR) receptors (Saaltink and Vreugdenhil, 2014), which are highly expressed in the hippocampus of rodents (Montaron et al., 2003). However, there are some preliminary and inconclusive findings regarding the difference in expression levels of the GR receptor in the dDG and vDG (Lin et al., 2012; Robertson et al., 2005), while MR receptor expression has been shown to be more concentrated in the vDG, at least in the rat brain (Robertson et al., 2005). Notwithstanding, both receptors have been shown to have a distinct activation pattern across the septo-temporal axis of the DG in response to acute stress (Caudal et al., 2014; Dorey et al., 2012). Moreover, a recent report has demonstrated that exercise increased GR expression in the hippocampus in single-housed but not pair-housed adult mice (Pan-Vazquez et al., 2015). Whether adolescent social isolation stress can affect the pro-neurogenic effect of exercise through the differential expression and activation of GR and MR in the vDG remains to be investigated. Another potential vDG-mediated mechanism underlying the attenuation of exercise-induced increase in neurogenesis by stress is through changes in the pro-neurogenic plasticity molecule BDNF (Berchtold et al., 2005; Chen and Russo-Neustadt, 2009; Ploughman et al., 2007; Tang et al., 2008). This is due to the fact that a stressful spatial navigation task has previously been shown to differentially affect the expression of BDNF in the dorsal (increased expression) and ventral (decreased expression) subregions of the DG (Hawley et al., 2012). The N-methyl-D-aspartate (NMDA) receptor activation pathway has also been implicated in both stress- and exercise-related changes in hippocampal neurogenesis. Specifically, exercise induced a robust increase in the activation of NMDA receptor albeit in cortical mouse tissue (Dietrich et al., 2005) and NMDA receptors have been

reported to operate downstream of the stress hormone, corticosterone to regulate hippocampal neurogenesis (Cameron et al., 1998). Given that there is a lower density of binding sites for NMDA receptors in the vDG compared to dDG (Pandis et al., 2006), it could be speculated that the two stimuli (isolation stress and exercise) compete for activation of the same pathway. Finally, isolation stress and exercise may also differentially impact upon cell death in the dorsal and ventral DG. In the current study, we show a significant decrease in the number of apoptotic cells in the vDG of pair housed mice with access to a running wheel. This may reflect a protective mechanism of exercise against cell death, which is attenuated by isolation-induced stress. However, it is important to note that the apoptosis measured here accounted for all cells in the vDG, hence we cannot conclude that the effects of isolation stress and exercise on apoptosis are specific to newly generated neurons.

It is surprising that we found no differences in the survival of newborn neurons of either area of the DG in single- and pair- housed sedentary adolescent mice. Studies conducted during adulthood have reported that social isolation results in anxiety- and depression-like behaviours in mice along with a reduction in levels of neuroplasticity genes (Berry et al., 2012; Ieraci et al., 2016). Social isolation during adolescence in non-human primates (marmosets) also impaired hippocampal neurogenesis in time-dependent manner (Cinini et al., 2014). Moreover, social isolation during adulthood has been shown to delay the pro-neurogenic effects of exercise in rats (Stranahan et al., 2006). Interestingly, evidence from the Pereira lab suggests that an enriched environment is necessary to promote neurogenesis in single- housed adult mice (Monteiro et al., 2014). It is possible that in the absence of other external stimuli, social condition does not affect neurogenesis during the adolescent period, possibly due to the high basal rate of neurogenesis that occurs during adolescence compared to adulthood (He and Crews, 2007). Alternatively, the mouse strain used in the current study

(generated on a BL6129 background) may have been a confounding factor by potentially limiting our ability to detect any downregulation of new neurons. In several independent studies examining the role of genetic influence on the baseline level of hippocampal neurogenesis, the B6129SF1 and 129Sv were among the strains showing the lowest levels of newborn neurons (Clark et al., 2011; Kempermann et al., 1997; Merritt and Rhodes, 2015).

In conclusion, our results demonstrate that social isolation stress during adolescence attenuates an exercise-induced increase in neurogenesis. We show that this effect is most pronounced in the ventral hippocampus, a brain sub-region which plays a predominant role in anxiety and in regulating the stress response. Adolescence is a critical period for susceptibility to stress-related disorders as well as a time during which remodeling of hippocampal connectivity, including neurogenesis occurs. Thus the impact of stress during adolescence on hippocampal neurogenesis and associated behaviours may be particularly potent. We also show that TLX is necessary for the pro-neurogenic effects of exercise during adolescence and have previously shown that the role of TLX in anxiety-related behaviours is most apparent during adolescence. TLX is thus an important intrinsic regulator of exercise-induced changes in neurogenesis and may be a key target in understanding the interaction between positive and negative modifiable lifestyle factors such as stress and exercise on hippocampal neurogenesis and associated behaviours during adolescence.

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Figure Captions

Figure 1: Experimental design.

Outline of the experimental groups and timeline illustrating the duration of the experiment.

Figure 2: Nr2e1 is necessary for the pro-neurogenic effect of exercise to occur.

Mean number of BrdU+ **(A)** and BrdU+NeuN+ **(B)** cells per section in the whole, dorsal and ventral hippocampus of singly-housed wild type or Nr2e1$^{-/-}$ adolescent mice with or without access to running wheels. Data are expressed as mean ± SEM. *** $p < 0.001$ compared to WT counterparts; ++ $p < 0.01$, + $p < 0.05$ compared to WT sedentary mice; (Two-way ANOVA, Fisher's LSD), n = 5-6. Representative confocal images through the DG from WT sedentary (**C**) and running **(D)** and Nr2e1$^{-/-}$ sedentary (**E**) and running **(F)** mice. Immunohistochemical staining shows BrdU+ (red), NeuN+ (green) and BrdU+NeuN+ (orange) cells at 10X magnification. Scale bar = 100 μm. Higher magnification images depict immunopositive cells in the DG for BrdU (**C' – F'**), NeuN (**C'' – F''**) and merged channels (**C''' – F'''**). Scale bar = 25 μm.

Figure 3: Differential modulation of neurogenesis by isolation stress and exercise across the septo-temporal axis of the DG in adolescent mice.

Mean number of BrdU+ (**A**) and BrdU+NeuN+ (**B**) cells per section in the whole, dorsal and ventral hippocampus of singly- or pair- housed adolescent mice with or without access to running wheels. Data are expressed as mean ± SEM. * $p < 0.05$, *** $p < 0.001$ compared to single- or pair- housed sedentary counterparts; + $p < 0.05$, ++ $p < 0.01$ compared to pair-housed running mice; (Two-way ANOVA, Fisher's LSD), n = 4-6. Representative confocal images of coronal sections through the dDG and vDG immunohistochemically stained with BrdU (red) and NeuN (green) from pair-housed sedentary **(**dDG: **C,** vDG: **G)**, pair-housed

running **(**dDG: **D,** vDG: **H)**, single-housed sedentary **(**dDG: **E,** vDG: **I)** and single-housed running **(**dDG: **F,** vDG: **J)** mice. Images were taken at 10X magnification. Scale bar = 100 μm. Higher magnification images depict immunopositive cells in the DG for BrdU (dDG: **C' – F'**, vDG: **G' – J'**), NeuN (dDG: **C'' – F'',** vDG: **G'' – J''**) and merged channels (dDG: **C''' – F''',** vDG: **G''' – J'''**). Scale bar = 25 μm.

Figure 4: Differential modulation of apoptosis by isolation stress and exercise across the septotemporal axis of the DG in WT but not Nr2e1$^{-/-}$ adolescent mice.

Mean number of active caspase-3+ cells per section in whole, dorsal and ventral hippocampus of singly-housed wild type or Nr2e1$^{-/-}$ adolescent mice (**A**) and singly- or pair-housed wild type adolescent mice with or without access to running wheels (**B**). Data are expressed as mean ± SEM. * $p < 0.05$, compared to single- or pair- housed sedentary counterparts; + $p < 0.05$, compared to single-housed running mice; (Two-way ANOVA, Fisher's LSD), n = 4. Representative bright field images of coronal sections through the hippocampus immunocytochemically stained with active caspase-3 (dark brown; black arrows) from a wild type control (**C**) and Nr2e1$^{-/-}$ (**D**) mouse. Images were taken at 20X magnification. Scale bar = 100 μm.

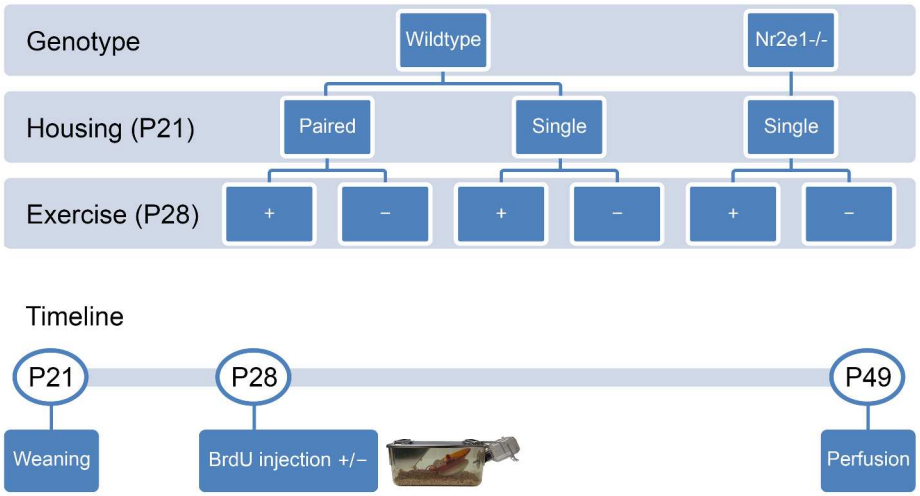

Figure 1: Experimental design.

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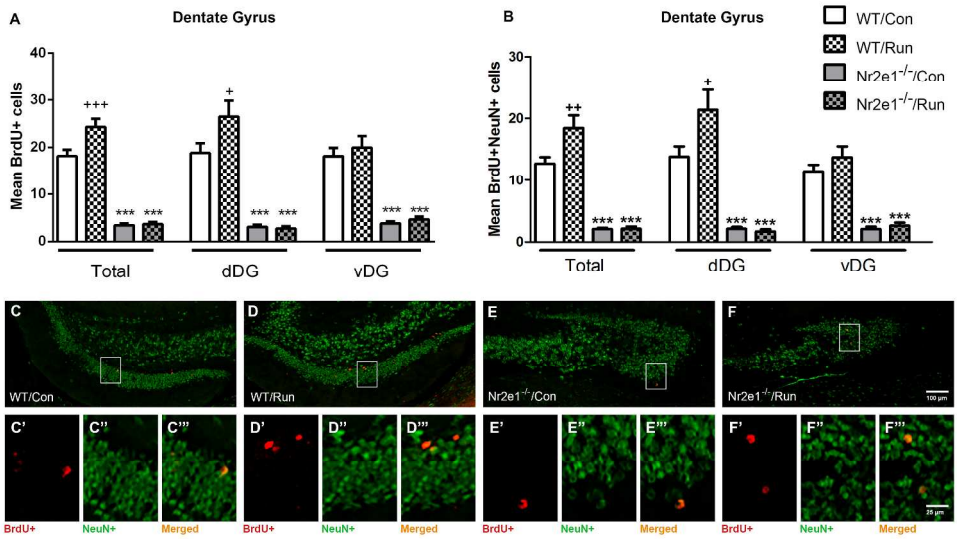

Figure 2: Nr2e1 is necessary for the pro-neurogenic effect of exercise to occur.

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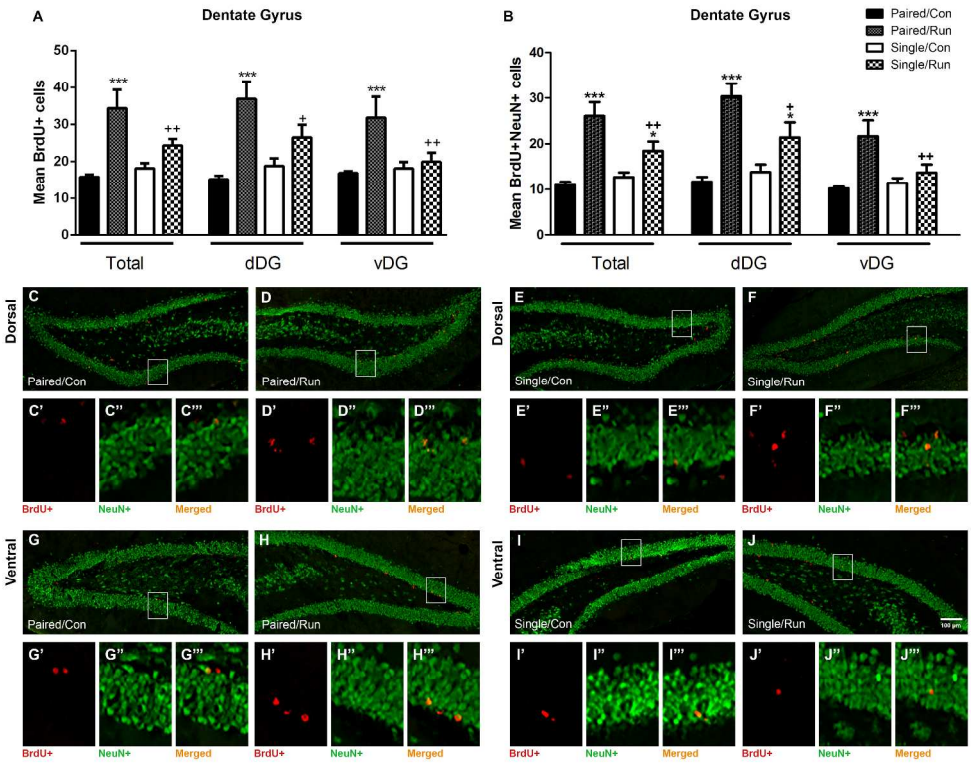

Figure 3: Differential modulation of neurogenesis by isolation stress and exercise across the septo-temporal axis of the DG in adolescent mice.

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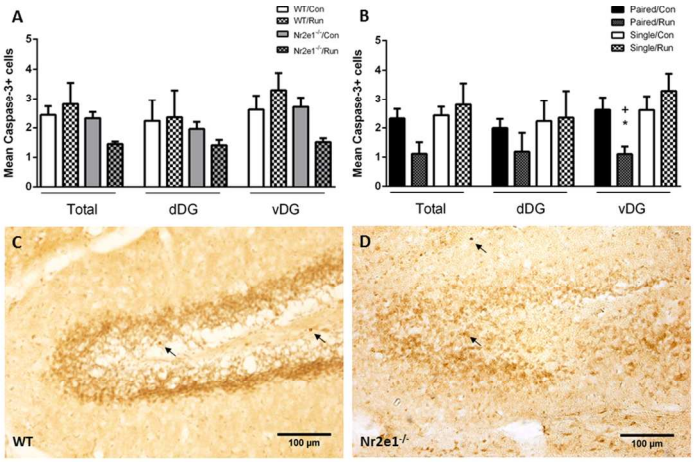

Figure 4: Differential modulation of apoptosis by isolation stress and exercise across the septotemporal axis of the DG in WT but not Nr2e1-/- adolescent mice.

338x190mm (96 x 96 DPI)