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Running reduces stress and enhances cell genesis in aged mice

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Abstract

Cell proliferation and neurogenesis are diminished in the aging mouse dentate gyrus. However, it is not known whether isolated or social living affects cell genesis and stress levels in old animals. To address this question, aged (17–18 months old) female C57Bl/6 mice were single or group housed, under sedentary or running conditions. We demonstrate that both individual and socially housed aged C57Bl/6 mice have comparable basal cell proliferation levels and demonstrate increased running-induced cell genesis. To assess stress levels in young and aged mice, corticosterone (CORT) was measured at the onset of the active/dark cycle and 4 h later. In young mice, no differences in CORT levels were observed as a result of physical activity or housing conditions. However, a significant increase in stress in socially housed, aged sedentary animals was observed at the onset of the dark cycle; CORT returned to basal levels 4 h later. Together, these results indicate that voluntary exercise reduces stress in group housed aged animals and enhances hippocampal cell proliferation.

Keywords

Adult neurogenesis; Aging; Exercise; Hippocampus; Social environment; Stress

1. Introduction

The aging process is often associated with decreased physical capacity and cognitive decline. The function of the hippocampus, a brain region associated with learning and memory, is reduced in aged humans and animals; including deficits in synaptic plasticity

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Conflict of interest

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(Bach et al., 1999), synaptogenesis (Geinisman et al., 1992), and adult neurogenesis (Ben Abdallah et al., 2010; Kuhn et al., 1996; van Praag et al., 2005). In addition, age-associated neurodegenerative disorders such as Alzheimer's disease affect hippocampal function (Laakso et al., 1996). Much effort has been spent developing pharmacological therapies that may rescue cognition (Bach et al., 1999; Barad et al., 1998; Ingram et al., 1994), however, exercise may prevent or ameliorate age-related deficits. Physical activity is beneficial for cognitive function in elderly humans and has been shown to prevent brain atrophy (Colcombe et al., 2003; for reviews, see Heyn et al., 2004; Kramer et al., 2006; Rolland et al., 2008). In addition, running aged rodents demonstrate an increase in hippocampal cell proliferation (Kim et al., 2004; Blackmore et al., 2009; Kronenberg et al., 2006), neurogenesis and spatial memory function (van Praag et al., 2005). Less is known about other environmental factors that may regulate hippocampal function and in particular cell genesis in old animals, including social housing conditions.

Voluntary exercise increases cell proliferation in the young mouse dentate gyrus (van Praag et al., 1999) regardless of whether mice are housed individually or in groups (Kannangara et al., 2008). However, it remains unknown whether housing can influence the effects of voluntary exercise on cell genesis in aged mice. Isolated housing could be considered as a stressor, and therefore a negative regulator of neurogenesis. Increased corticosterone (CORT) levels due to isolation stress (Gould et al., 1998), exposure to a predator (Tanapat et al., 1998) and aging (Cameron and McKay, 1999) have been associated with decreased cell proliferation. Furthermore, in spontaneously hypertensive rats, the effect of exercise on cell proliferation is reduced (Naylor et al., 2005). With aging basal corticosterone levels are elevated (Sapolsky, 1992). High corticosterone levels could result in hippocampal damage and memory impairments (Lupien et al., 1994, 1998), which may be exacerbated by social isolation. Indeed, a study in adult rats suggested that social isolation may delay the onset of exercise induced cell genesis (Stranahan et al., 2006). Therefore, in the present study, we investigated whether housing can regulate the effects of voluntary exercise on hippocampal cell proliferation and CORT levels in the aged mouse.

2. Methods

Young (2-month-old) and aged (17–18-month-old) female C57BL/6J mice (Harlan Laboratories, Indianapolis, IN) were habituated to the laboratory environment for 1 week in standard conditions. Mice had a reversed day-night 12-h cycle (onset of dark cycle: 10 a.m.) with food and water provided *ad libitum*. Following habituation, animals were housed either individually, or in groups (3 mice per cage), with or without access to a running wheel, generating the following experimental groups: individual sedentary controls (I-CON); individual runners (I-RUN); social sedentary controls (S-CON); social runners (S-RUN). Distance run over 11 days was monitored electronically (Lafayette Instrument, Lafayette, IN).

2.1. BrdU injections

To label proliferating cells in the dentate gyrus, young and aged mice (young: I-CON, $n = 8$; I = RUN, $n = 8$; SCON, $n = 9$; S-RUN, $n = 9$; aged: I-CON, $n = 16$; I-RUN, $n = 16$; S-CON,

$n = 17$; S-RUN, $n = 18$) were administered 10 daily intraperitoneal (i.p.) injections of the mitotic marker bromodeoxyuridine (BrdU; 50 mg/kg dissolved in 0.9% NaCl, 50 μ g/g body weight, 10mg/mL; Sigma, St. Louis, MO), starting on the first day of running. One day after the last BrdU injection (day 11), mice were anesthetised with ketamine/xylazine (3:1). Mice underwent transcardial perfusions with 0.9% saline, followed by 4% paraformaldehyde and rapid decapitation. Brains were removed and placed in 4% paraformaldehyde for 24 h, followed by 30% buffered sucrose. Coronal sections (40 μ m) were obtained from the entire hippocampus using a Leica VT1000 vibratome (Nussloch, Germany) and stored in 0.1 M Tris-buffered saline (TBS; 0.15 M NaCl, 0.1 M Tris-HCl, pH 7.5).

2.2. Corticosterone assays

Corticosterone (CORT) levels were assessed in young mice (young: I-CON, $n = 8$; I-RUN, $n = 8$; S-CON, $n = 9$; S-RUN, $n = 9$), and a subset of aged mice (aged: I-CON, $n = 8$; I-RUN, $n = 8$; S-CON, $n = 9$; S-RUN, $n = 9$). Blood samples were taken via eye bleeds at two time points: at the onset of the mouse active cycle (10 a.m.) on days 10, and 11 of the experiment and 4 h into the active cycle (2 p.m.) on days 8, and 9 of running. The groups were counterbalanced so that half of the animals in each group were tested on a given day and each individual procedure was less than 1 min. Additional animals served as internal controls for the potential stress induced by repeated injections, and did not receive BrdU injections and were left undisturbed until blood retrieval (group housed internal sedentary controls; $n = 9$). CORT levels were measured with a radioimmunoassay kit according to the manufacturer's protocol (MP Biomedicals, Irvine, CA).

2.3. Immunocytochemistry

Immunohistochemical identification of BrdU-labelled cells in a subset of aged mice (I-CON, $n = 8$; I-RUN, $n = 8$; S-CON, $n = 8$; S-RUN, $n = 9$) was conducted as previously described (Farmer et al., 2004; Eadie et al., 2005; Kannangara et al., 2008; Redila and Christie, 2006). Briefly, a 1:12 series of free floating slices was washed in 0.1 M TBS, followed by immersion in 0.6% H_2O_2 in TBS for 30 min. Sections were placed in 50% formamide/2 $\times$ SSC (0.3 M NaCl and 0.3 M sodium citrate) at 65 $^{\circ}$ C, rinsed for 5 min in 2 $\times$ SSC, incubated in 2N HCl at 37 $^{\circ}$ C for 30 min and placed in 0.1 M boric acid (pH 8.5) for 10 min. Tissue was rinsed six times in TBS for a total of 90 min and incubated in TBS++ (0.1% Triton X-100 and 3% normal donkey serum in 0.1 M TBS). Sections were then incubated overnight with a biotinylated anti-BrdU primary antibody (1:100, MAB3262B, Chemicon, Temucula, CA). Tissue was rinsed in TBS the following day, and an avidin-biotin-peroxidase complex system (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA) was applied for 2 h. Tissue was rinsed and treated with a peroxidase detection kit that uses diaminobenzidine (DAB) as a chromogen (DAB Kit, Vector Laboratories) according to manufacturers' directions. The sections were mounted on 2% gelatin coated slides and cover-slipped for microscopic analysis. Quantification of cells labelled with BrdU was conducted as previously described (Kannangara et al., 2008). Briefly, the number of BrdU-immunopositive cells in the entire hippocampus was assessed by manually counting all positive cells in the granular cell layer (GCL) of the dentate gyrus in both the left and right hemispheres using an Olympus BX50 microscope with a 100 $\times$ objective lens (Center Valley, PA). Cells within the subgranular zone, defined as the area within two cell bodies ($\sim$ 20 μ m)

of the inner edge of the GCL, were combined with the GCL for quantification. Split nuclei cells that had not finished cytokinesis were counted as one cell for a conservative estimate. The distance between the sections was approximately 440 μm . A modified stereological approach was employed to estimate the total number of BrdU-positive cells/hippocampus.

2.4. Statistical analysis

Data are presented as means $\pm$ standard error of the mean (SEM). Differences between mean values of experimental groups were analyzed using two-way and three-way analyses of variance (ANOVA), followed by Fisher's post hoc t-tests as appropriate. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Hippocampal cell proliferation in the aged mice

As we recently showed that cell genesis is enhanced in young mice with exercise, regardless of housing conditions (Kannangara et al., 2008), we investigated the effects of exercise and housing conditions on cell genesis only in the 17–18-month-old female mice. A daily i.p. injection of BrdU for 10 days at a dose of 50 mg/kg (Fig. 1A) was employed to reliably obtain sufficient BrdU-labeling in older animals. Main effects were observed of both exercise ($F_{(1,27)} = 18.5$, $p < 0.0002$) and housing condition ($F_{(1,27)} = 39.9$, $p < 0.000001$), as well as an interaction between these two variables ($F_{(1,27)} = 9.6$, $p < 0.004$). Exercise increased cellular proliferation in the aged individual runners as compared to the aged individual sedentary control group (I-CON: 559.8 ± 50.13 , $n = 8$; I-RUN: 1191.4 ± 98.02 , $n = 6$; $p < 0.000001$; Fig. 1C). A smaller but significant difference was also observed between aged social runners and social sedentary controls (S-CON: 479.9 ± 45.85 , $n = 8$; S-RUN: 695.3 ± 74.70 , $n = 9$; $p < 0.02$). In addition, less BrdU-positive cells were observed in aged social runners in comparison to aged individual runners ($p < 0.00002$; Fig. 1C).

3.2. Corticosterone levels in young and aged mice

Corticosterone levels (CORT) were obtained in young and aged mice to assess stress levels at two time points. Mice were given daily BrdU injections for 10 days. As daily administration of BrdU might be stressful, a separate set of 3-month-old socially housed internal control (IC) animals ($n = 9$) that did not receive the multiple BrdU injections was also used to obtain CORT levels at active cycle onset (156.01 ± 24.72 ng/mL) and 4h later (26.96 ± 2.97 ng/mL). IC CORT levels did not differ from young BrdU injected mice at either time point ($p > 0.05$; Fig. 2).

3.2.1. Onset of the active cycle—At the onset of the active cycle a significant interaction between age, exercise and housing was observed ($F_{(1,59)} = 13.95$, $p < 0.0004$). In addition, there were main effects of exercise ($F_{(1,59)} = 4.86$, $p < 0.03$) and housing ($F_{(1,59)} = 5.02$, $p < 0.03$), but not age ($F_{(1,59)} = 0.02$, $p > 0.89$). Specific comparisons in the young animals revealed no differences between the groups (I-CON: 170.80 ± 35.65 ng/mL, $n = 7$; I-RUN: 133.24 ± 38.01 ng/mL, $n = 8$; S-CON: 137.26 ± 36.66 ng/mL, $n = 9$; S-RUN: 242.22 ± 47.21 ng/mL, $n = 9$; Fig. 2A). In the individually housed aged mice controls and runners had similar levels of CORT (I-CON: 124.55 ± 30.39 ng/mL, $n = 8$; I-RUN: 118.69 ± 30.22

ng/mL, $n = 8$; Fig. 2C). However, socially housed, aged sedentary animals (S-CON: 402.25 ± 84.14 ng/mL, $n = 9$) had significantly higher levels of CORT in comparison to all aged groups at the onset of the active cycle ($p < 0.01$). Interestingly, in aged group housed runners (S-RUN: 55.46 ± 18.01 ng/mL, $n = 9$) CORT levels were significantly lower than in their young counterparts (S-RUN: 242.22 ± 47.21 ng/mL, $n = 9$; $p < 0.004$), suggesting that exercise may benefit stress reduction more in aged than young mice.

3.2.2. Four hours into the active cycle—Levels of CORT were also assessed 4h into the mouse active cycle. There was no significant interaction between age, exercise and housing ($F_{(1,59)} = 1.22$, $p > 0.27$). In addition, no main effect of age ($F_{(1,59)} = 1.73$, $p > 0.19$), exercise ($F_{(1,59)} = 0.54$, $p > 0.46$) and housing condition ($F_{(1,59)} = 0.01$, $p > 0.93$) was observed in young mice (I-CON: 30.50 ± 6.96 ng/mL, $n = 7$; I-RUN: 37.41 ± 6.73 ng/mL, $n = 8$; S-CON: 46.91 ± 12.62 ng/mL, $n = 9$; S-RUN: 70.50 ± 20.54 ng/mL, $n = 9$; Fig. 2B) and aged mice (I-CON: 63.50 ± 23.31 ng/mL, $n = 8$; I-RUN: 81.86 ± 21.31 ng/mL, $n = 8$; S-CON: 57.60 ± 15.69 ng/mL, $n = 9$; S-RUN: 42.27 ± 9.94 ng/mL, $n = 9$; Fig. 2D).

3.3. Running distance in young and aged mice

We quantified the amount of running in individual and social running groups in both young and aged animals to determine if the differences observed in BrdU-labeling in social runners and individual runners were due to discrepant running wheel utilization. Both individual and social running groups made use of the running wheel to a large extent (average total distance run over 11 day exercise period: young I-RUN: 53.57 ± 5.92 km/mouse, $n = 8$ animals; young S-RUN: 91.69 ± 1.87 km/group, $n = 3$ cages, 3 animals/cage ($n = 9$); aged I-RUN: 34.76 ± 3.71 km/mouse, $n = 16$ animals; aged S-RUN: 54.62 ± 4.01 km/group, $n = 6$ cages, 3 animals/cage ($n = 18$). Main effects were observed for housing condition ($F_{(1,29)} = 15.67$, $p < 0.0004$) and age ($F_{(1,29)} = 16.92$, $p < 0.0003$), although no interaction was observed between these two factors ($F_{(1,29)} = 2.20$, $p > 0.15$). The average distance run per cage for aged individual runners was significantly less than young individual runners ($p < 0.0009$) and aged socially housed runners ($p < 0.0006$). Aged socially housed runners ran significantly less than young socially housed runners ($p < 0.006$). It is difficult to determine the amount of running each mouse completed in social running groups as mice run together and share the running wheel; however this experiment demonstrates that animals in both individual and social housing conditions do utilize the running wheel (Fig. 1D).

4. Discussion

The present study demonstrates that in both individual and social housing conditions, exercise increases cellular proliferation in the aged hippocampus. These results are consistent with the previous finding that treadmill training can enhance proliferation in aged rats (Kim et al., 2004) and that voluntary wheel running enhanced neurogenesis in individually housed 18-month-old mice (van Praag et al., 2005). Furthermore, these findings extend our previous research in young animals (Kannangara et al., 2008), demonstrating that irrespective of housing condition, voluntary exercise increases hippocampal cell proliferation in aged mice. These data show that social isolation (Stranahan et al., 2006) does not delay the onset of the beneficial effect of exercise on hippocampal cell genesis.

We observed a low basal level of cellular proliferation in aged animals. While cell proliferation in the adult dentate gyrus is robust in young animals (van Praag et al., 1999; Kannangara et al., 2008), the amount of proliferation decreases at an exponential rate after one month of age (Ben Abdallah et al., 2010). Indeed, our observation of reduced proliferation in 17–18-month-old mice is in accordance with previous experiments conducted in rats (Kuhn et al., 1996) and mice (Ben Abdallah et al., 2010). Interestingly, individually and socially housed mice showed comparable basal levels of cell proliferation, suggesting that housing does not influence cell division. Many groups include social housing as part of environmental enrichment paradigms (Kempermann et al., 1997; Pamplona et al., 2009; Zhu et al., 2005). In this study, we did not observe an effect of social housing on cell proliferation, suggesting that, at least in aged mice, social housing alone may not act as a strong form of environmental enrichment.

Wheel running increased proliferation in both housing conditions, but less so in aged social runners than aged individual runners. The average distance ran per cage for individual and social housed runners appear to reflect the rate of proliferation in the respective groups. It must be considered, however, that it is difficult to determine how much each mouse ran. If each group housed mouse did in fact run less than individually housed mice, this observation may in part be due to less access to the single running wheel, and having to share the wheel with two other animals. In addition, socially housed animals have the opportunity either to use the running wheel or to interact with their cage mates, which may lessen the degree to which the running wheel is employed. In support of this possibility, previous experiments have demonstrated that mice prefer social housing conditions to standard forms of environmental enrichment (Van Loo et al., 2004).

Corticosterone (CORT) is considered as an important modulator of adult neurogenesis. It has been hypothesized that the age-related decline of adult cell proliferation and neurogenesis (Kuhn et al., 1996) could be tightly correlated to the age-related increase in CORT (Sapolsky, 1992; Sapolsky et al., 1991), an idea supported by research showing that low levels of cell proliferation observed in aged rats can be increased through adrenalectomy (Cameron and McKay, 1999). We examined CORT levels at two different time points: at the onset of the mouse active cycle and 4 h into the active cycle. These two time points in our study were matched precisely to Stranahan et al. (2006). At the onset of the mouse active cycle, aged single housed sedentary and running animals showed comparable levels of CORT. Interestingly, aged, social housed, sedentary animals had the highest CORT levels. Running resulted in a very significant reduction of CORT levels in group housed aged animals. These levels were lower than in young group housed runners, suggesting that exercise is particularly effective in reducing stress in old animals. In young animals CORT levels did not change due to exercise or housing condition after 11 days. These results are in contrast to research that suggests that short-term running increases CORT levels in adult rats at the onset of the active cycle (Stranahan et al., 2006). Others have reported a similar increase in mice, but only after 4 weeks of running (Droste et al., 2003). The differential onset of stress responses in mice and rats associated with exercise may be the result of species-dependent differences (Snyder et al., 2009). It is of interest, that CORT levels in group housed young runners were significantly higher than in the aged runners. It remains unclear whether this indicates that cognitive function is more protected by running in aged

animals, as plasma corticosterone levels do not necessarily correlate with those measured in the hippocampus (Droste et al., 2009).

Four hours after the onset of the active cycle, we observed that CORT levels in all groups had dropped. This is in agreement with previous studies showing that CORT levels are highest at the onset of the active cycle (Malisch et al., 2008). In addition to the decrease of CORT levels across groups, we also demonstrated no significant differences between any of the housing or exercise conditions. This is in contrast to Stranahan et al. (2006), who observed that socially housed animals show a reduction in CORT, regardless of exercise condition. Again, this may result from a species-dependent difference (Snyder et al., 2009).

It is conceivable that the increase in cell genesis in aged female runners could be associated with improvements in memory function. In running individually housed 18-month-old male C57Bl/6 mice both spatial memory and neurogenesis were enhanced in (van Praag et al., 2005), whereas in very aged (22-month-old) mice cell genesis and memory improvement became refractory to exercise (Creer et al., 2010). It would be of interest to investigate whether there are differences between individual and social housing in this regard. However, in the present study animals were running for 11 days. While this is sufficient to enhance cell proliferation it does not indicate whether there are changes in neurogenesis. It takes newly born cells one month to become functional (van Praag et al., 2002) and to be recruited into circuits supporting spatial memory (Kee et al., 2007). Thus, studies with longer exercise duration will be needed to determine whether there is an interaction between effects of housing and exercise on cognition in aged animals.

In conclusion, exercise enhances proliferation in the aged mouse dentate gyrus without delay and regardless of housing condition. Moreover, running significantly lowers stress in socially housed aged mice. Further investigation into the influence of other environmental enrichment factors on cell proliferation will help to identify the mechanisms that underlie this process.

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References

- Bach ME, Barad M, Son H, Zhuo M, Lu YF, Shih R, Mansuy I, Hawkins RD, Kandel ER. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway Proc. Natl. Acad. Sci. U.S.A. 1999; 96:5280. [PubMed: 10220457]
- Barad M, Bourtoouladze R, Winder DG, Golan H, Kandel E. Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory Proc. Natl. Acad. Sci. U.S.A. 1998; 95:15020. [PubMed: 9844008]
- Ben Abdallah NM, Slomianka L, Vyssotski AL, Lipp HP. Early age-related changes in adult hippocampal neurogenesis in C57 mice Neurobiol. Aging. 2010; 31:151. [PubMed: 18455269]

- Blackmore DG, Golmohammadi MG, Large B, Waters MJ, Rietze RL. Exercise increases neural stem cell number in a growth hormone-dependent manner, augmenting the regenerative response in aged mice *Stem Cells*. 2009; 27:2044. [PubMed: 19544415]
- Cameron HA, McKay RD. Restoring production of hippocampal neurons in old age *Nat. Neurosci*. 1999; 2:894. [PubMed: 10491610]
- Colcombe SJ, Erickson KI, Raz N, Webb AG, Cohen NJ, McAuley E, Kramer AF. Aerobic fitness reduces brain tissue loss in aging humans *J. Gerontol. A: Biol. Sci. Med. Sci*. 2003; 58:176. [PubMed: 12586857]
- Creer DJ, Romberg C, Saksida LM, van Praag H, Bussey TJ. Running enhances spatial pattern separation in mice *Proc. Natl. Acad. Sci. U.S.A.* 2010 www.pnas.org/cgi/doi/10.1073/pnas.0911725107.
- Droste SK, Gesing A, Ulbricht S, Muller MB, Linthorst AC, Reul JM. Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis *Endocrinology*. 2003; 144(7):3012. [PubMed: 12810557]
- Droste SK, Collins A, Lightman SL, Linthorst AC, Reul JM. Distinct, time-dependent effects of voluntary exercise on circadian and ultradian rhythms and stress responses of free corticosterone in the rat hippocampus *Endocrinology*. 2009; 150(9):4170. [PubMed: 19477935]
- Eadie BD, Redila VA, Christie BR. Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density *J. Comp. Neurol*. 2005; 486(1):39. [PubMed: 15834963]
- Farmer J, Zhao X, van Praag H, Wodtke K, Gage FH, Christie BR. Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo *Neuroscience*. 2004; 124:71. [PubMed: 14960340]
- Geinisman Y, deToledo-Morrell L, Morrell F, Persina IS, Rossi M. Structural synaptic plasticity associated with the induction of long-term potentiation is preserved in the dentate gyrus of aged rats *Hippocampus*. 1992; 2:445. [PubMed: 1308201]
- Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress *Proc. Natl. Acad. Sci. U.S.A.* 1998; 95:3168. [PubMed: 9501234]
- Heyn P, Abreu BC, Ottenbacher KJ. The effects of exercise training on elderly persons with cognitive impairment and dementia: a meta-analysis *Arch. Phys. Med. Rehabil*. 2004; 85:1694. [PubMed: 15468033]
- Ingram DK, Spangler EL, Iijima S, Kuo H, Bresnahan EL, Greig NH, London ED. New pharmacological strategies for cognitive enhancement using a rat model of age-related memory impairment *Ann. N. Y. Acad. Sci.* 1994; 717:16. [PubMed: 8030831]
- Kannangara TS, Webber A, Gil-Mohapel J, Christie BR. Stress differentially regulates the effects of voluntary exercise on cell proliferation in the dentate gyrus of mice *Hippocampus*. 2008; 19:889.
- Kee N, Teixeira CM, Wang AH, Frankland PW. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus *Nat. Neurosci*. 2007; 10:355. [PubMed: 17277773]
- Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment *Nature*. 1997; 386:493. [PubMed: 9087407]
- Kim YP, Kim H, Shin MS, Chang HK, Jang MH, Shin MC, Lee SJ, Lee HH, Yoon JH, Jeong IG, Kim CJ. Age-dependence of the effect of treadmill exercise on cell proliferation in the dentate gyrus of rats *Neurosci. Lett*. 2004; 355:152. [PubMed: 14729257]
- Kramer AF, Erickson KI, Colcombe SJ. Exercise, cognition, and the aging brain *J. Appl. Physiol*. 2006; 101:1237. [PubMed: 16778001]
- Kronenberg A, Bick-Sander E, Bunk C, Wolf D, Ehninger G, Kempermann. Physical exercise prevents age-related decline in precursor cell activity in the mouse dentate gyrus *Neurobiol. Aging*. 2006; 27:1505. [PubMed: 16271278]
- Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation *J. Neurosci*. 1996; 16:2027. [PubMed: 8604047]

- Laakso MP, Partanen K, Riekkinen P, Lehtovirta M, Helkala EL, Hal-likainen M, Hanninen T, Vainio P, Soininen H. Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: an MRI study *Neurology*. 1996; 46:678. [PubMed: 8618666]
- Lupien S, Lecours AR, Lussier I, Schwartz G, Nair NP, Meaney MJ. Basal cortisol levels and cognitive deficits in human aging *J. Neurosci.* 1994; 14:2893. [PubMed: 8182446]
- Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NP, Thakur M, McEwen BS, Hauger RL, Meaney MJ. Cortisol levels during human aging predict hippocampal atrophy and memory deficits *Nat. Neurosci.* 1998; 1:69. [PubMed: 10195112]
- Malisch JL, Breuner CW, Gomes FR, Chappell MA, Garland T Jr.. Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior *Gen. Comp. Endocrinol.* 2008; 156:210. [PubMed: 18329645]
- Naylor AS, Persson AI, Eriksson PS, Jonsdottir IH, Thorlin T. Extended voluntary running inhibits exercise-induced adult hippocampal progenitor proliferation in the spontaneously hypertensive rat *J. Neurophysiol.* 2005; 93:2406. [PubMed: 15615829]
- Pamplona FA, Pandolfo P, Savoldi R, Prediger RD, Takahashi RN. Environmental enrichment improves cognitive deficits in spontaneously hypertensive rats (SHR): relevance for attention deficit/hyperactivity disorder (ADHD) *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 2009; 33:1153. [PubMed: 19549550]
- Redila VA, Christie BR. Exercise-induced changes in dendritic structure and complexity in the adult hippocampal dentate gyrus *Neuroscience.* 2006; 137:1299. [PubMed: 16338077]
- Rolland Y, Abellan van Kan G, Vellas B. Physical activity and Alzheimer's disease: from prevention to therapeutic perspectives *J. Am. Med. Dir. Assoc.* 2008; 9:390. [PubMed: 18585641]
- Sapolsky RM. Do glucocorticoid concentrations rise with age in the rat? *Neurobiol. Aging.* 1992; 13:171. [PubMed: 1542376]
- Sapolsky RM, Zola-Morgan S, Squire LR. Inhibition of glucocorticoid secretion by the hippocampal formation in the primate *J. Neurosci.* 1991; 11:3695. [PubMed: 1744687]
- Snyder JS, Choe JS, Clifford MA, Jeurling SI, Hurley P, Brown A, Kamhi JF, Cameron HA. Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice *J. Neurosci.* 2009; 29:14484. [PubMed: 19923282]
- Stranahan AM, Khalil D, Gould E. Social isolation delays the positive effects of running on adult neurogenesis *Nat. Neurosci.* 2006; 9:526. [PubMed: 16531997]
- Tanapat P, Galea LA, Gould E. Stress inhibits the proliferation of granule cell precursors in the developing dentate gyrus *Int. J. Dev. Neurosci.* 1998; 16:235. [PubMed: 9785120]
- Van Loo PL, Van de Weerd HA, Van Zutphen LF, Baumans V. Preference for social contact versus environmental enrichment in male laboratory mice *Lab. Anim.* 2004; 38:178. [PubMed: 15070458]
- van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus *Nat. Neurosci.* 1999; 2:266. [PubMed: 10195220]
- van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus *Nature.* 2002; 415:1030. [PubMed: 11875571]
- van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice *J. Neurosci.* 2005; 25:8680. [PubMed: 16177036]
- Zhu J, Apparsundaram S, Bardo MT, Dwoskin LP. Environmental enrichment decreases cell surface expression of the dopamine transporter in rat medial prefrontal cortex *J. Neurochem.* 2005; 93:1434. [PubMed: 15935059]

Fig. 1.

Voluntary exercise promotes cellular proliferation, irrespective of housing condition, in the aged mouse dentate gyrus. (A) Schematic diagram of the experimental paradigm and BrdU injection protocol. Exercising mice were allowed access to running wheels for 11 days. BrdU (50 mg/kg) was administered daily and mice were perfused on day 11. (B) Representative pictures of BrdU-positive cells in the dentate gyrus of individual sedentary controls (I-CON), individual runners (I-RUN), social sedentary controls (S-CON), and social runners (S-RUN). Scale bar: 50 μ m. (C) Individual and social exercising groups (black bars) demonstrate increases in the amount of BrdU-positive cells in comparison to their sedentary controls (white bars). (*) Denotes significance ($p < 0.05$) in comparison to sedentary controls; (**) denotes significance ($p < 0.05$) in comparison to all other experimental

groups. (D) Average running distance per cage during the 11-day exercise period for young and aged, individually and socially housed running animals. (*) and (**) denote significance ($p < 0.05$) in comparison to young individual runners and young social runners, respectively. Error bars represent S.E.M.

Fig. 2.

Corticosterone levels of young and aged mice. (A, B) In young mice no change in CORT was observed as a result of housing or exercise conditions. (C) Aged, socially housed, sedentary mice, have significantly higher stress levels than all other aged mice at active cycle onset. In addition, aged group housed runners have lower CORT levels than their young counterparts. (D) Four hours into active cycle, CORT levels of aged animals show no change as a result of exercise or housing condition. CORT levels in young Internal Controls that did not receive BrdU injections, are depicted with both young (A, B) and aged (C, D) groups. Error bars represent S.E.M. (**) Denotes significance in comparison to all other aged groups at the onset of the active cycle; (*) denotes a significant difference between young and aged grouped runners ($p < 0.05$).