Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 86 (2007) 327–333
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Neurogenesis decreases during brain maturation from adolescence to adulthood

Jun He, Fulton T. Crews *

Bowles Center for Alcohol Studies, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States

Received 19 June 2006; received in revised form 27 October 2006; accepted 1 November 2006
Available online 13 December 2006

Abstract

Adolescence is an important stage of brain development. Recent studies have indicated that neurogenesis in the brain occurs throughout life prompting comparisons of adolescent and adult neurogenesis. Since insulin-like growth factor 1 (IGF-1) has been implicated in promoting neurogenesis we investigated the levels of neurogenesis in adolescents (PND30) and adults (PND120) using IGF-1 over-expressing mice and IGFBP-1 (IGF binding protein-1) over-expressing mice. Proliferation and differentiation of neuroprogenitors were determined using bromodeoxyuridine (BrdU)- and doublecortin (DCX)-labeling. High levels of neurogenesis were found in both the hippocampal dentate gyrus (DG) and the forebrain subventricular zone (SVZ) of the adolescents as compared with the adults. Both adolescent IGF-1 and IGFBP-1 transgenic mice as well as their wildtype controls have significantly higher expression of BrdU and DCX in the hippocampus and SVZ when compared with their adult counterparts. However, no significant differences on BrdU-labeling were found when either of transgenic mice were compared with their wildtype littermates in both age groups. These studies indicate that adolescent mice have high levels of neurogenesis compared to adults suggesting a dramatic loss of neurogenesis during the transition from adolescence to adulthood. However, the role of IGF-1 during adolescent development is still unclear.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Neurogenesis; IGF-1; Adolescent; IGF binding protein-1

1. Introduction

Human adolescence is an important stage for both neuromaturation, particularly in cortical and limbic brain regions, and behavioral maturation, especially in complex learning such as social interaction skills. Adolescence in humans and other vertebrate species is defined by characteristic behaviors that include high levels of risk-taking, high exploration, novelty and sensation seeking, social interaction, high activity and play behaviors that likely promote the acquisition of skills for maturation and independence (Spear, 2000). Adolescence is also characterized by marked changes in hormones and growth factors. The significant neurochemical and neuroanatomical remodeling during this highly plastic postnatal development shapes neuronal networks and behavioral characteristics that persist into adulthood. On one hand, various studies using adolescent rodent models have shown that overproduction of axons and synapses occurs during early puberty and is followed by a rapid pruning later in adolescence (Giedd et al., 1999; Andersen et al., 2000; Andersen and Teicher, 2004), which likely contributes to maturation of the brain and the transition to adult. On the other hand, neurogenesis (generation of new neurons) in forebrain subventricular zone (SZV) and hippocampal dentate gyrus (DG) continues throughout life in both humans and rodents (Kempermann et al., 2003). Therefore, we proposed to determine if neurogenesis changes during the transition from adolescence to adulthood.

Insulin-like growth factor 1 (IGF-1) is an important growth factor during postnatal development known to enhance neurogenesis in developing mice (Aberg et al., 2000). Studies have found that insulin-like growth factor 1 (IGF-1) promotes the survival and proliferation of neuroprogenitor cells postnatally leading to increased neurogenesis and synaptogenesis in both *in vitro* and *in vivo* models (Aberg et al., 2000; Anderson

* Corresponding author. Tel.: +1 919 966 5678, +1 919 966 0501; fax: +1 919 966 5679.
E-mail address: ftcrews@med.unc.edu (F.T. Crews).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.11.003

et al., 2002; Perez-Martin et al., 2003; Poulsen et al., 2005). Interestingly, exercise-induced enhancement in adult neurogenesis has been suggested to be secondary to increased brain IGF-1 (Ding et al., 2006). During brain development IGF-1 expression occurs in coordination with rapid neuronal growth consistent with a large body of evidence that supports a role for IGF-1 in promoting neuroprogenitor cell proliferation, survival and differentiation in fetal development and during the brain growth spurt that occurs just after birth in humans and rodents (Ye and D'Ercole, 2006). Transgenic mice that over-express IGF-1 exhibit overgrowth of the brain resulting in higher brain weight as compared to the wildtype controls (Ye et al., 1995). In contrast, the transgenic mice that over-express IGF binding protein-1 (IGFBP-1), whose activation inhibits IGF-1 bioactivity, reduces neurogenesis leading to brain growth retardation (Ye et al., 1995). These findings prompted the current investigation of neurogenesis in adolescents and adults using the transgenic mice with over-expression of either heterozygote IGF-1 or IGFBP-1 and their wildtype controls (Wt).

In order to accurately measure the level of neurogenesis, both exogenous mitotic marker bromodeoxyuridine (BrdU) and endogenous neuronal marker doublecortin (DCX) were used in the current study. As a uridine analog, BrdU is incorporated into all proliferating cells shortly after its administration. It is a widely used marker for studying neurogenesis, particularly proliferation of neuroprogenitors as used in this study (Kempermann et al., 1997; Cameron and McKay, 2001; Crews et al., 2004). In addition, DCX is a cytoskeletal protein that expresses transiently in newborn neurons only (Brown et al., 2003) and is a marker of progenitors that are differentiating into neurons. Therefore, we and others use DCX to directly quantify neurogenesis (Brown et al., 2003). As an endogenous marker, DCX can overcome the limitations of bromodeoxyuridine (BrdU) labeling (Palmer et al., 2000; Kuan et al., 2004) and provide an independent validation on progenitors and neurogenesis.

2. Materials and methods

2.1. Subject and design

Transgenic IGF-1 and IGFBP-1 over-expressing mice with C57BL6 background were kindly provided by Dr. D'Ercole at UNC-Chapel Hill (Ye et al., 1995). Both IGF-1 and IGFBP-1 transgenic (Tg) mice were then bred with wildtype (Wt) C57BL6 mice in order to obtain heterozygotes of the target transgene as well as their non-transgenic littermate mice, which serve as wildtype controls. All mice were fed standard laboratory chow and maintained in a light-, temperature- and humidity-controlled environment. The animal protocols were approved by the University of North Carolina Institutional Animal Care and Use Committee.

The genotypes of mice were determined by polymerase chain reaction (PCR) of mouse-tail genomic DNA using REDExtract-N-Amp Tissue PCR kit (Sigma, St. Louis, MO). Both IGF-1 and IGFBP-1 primers were kindly provided by Dr. D'Ercole's lab (Ye et al., 1995). For the adolescent group, 30-day-old (PND30) mice with each transgene (either IGF-1 or IGFBP-1) as well as their wildtype littermate controls were randomly chosen ($n=5$–6) for the study. Likewise, 120-day-old (PND120) mice were used as adult groups for each transgene.

2.2. BrdU injections

Bromodeoxyuridine (BrdU, Sigma; St. Louis, MO, USA) was dissolved in 0.9% saline and administered intraperitoneally once a day at a dose of 300 mg/kg/day for 2 days. Animals were killed at 24 h after the last injection.

2.3. Histological procedures

Animals were deeply anesthetized with ketamine and transcardially perfused with 0.1 M phosphate-buffered saline (PBS, pH=7.4) followed by 4% paraformaldehyde in PBS. The brains were then cut 40 μm thick by vibratome (Leica, Wetzlar, Germany).

For BrdU staining, we followed the protocol described by Kuhn et al. 1996. Following DNA denaturing, anti-mouse BrdU antibody (Chemicon MAB3424, Temecula, CA, USA) was used at a dilution of 1:2000 with overnight incubation at 4 °C. Sections were then rinsed with Tris-buffered saline (TBS), and incubate with biotinylated horse anti-mouse (rat adsorbed) secondary antibody (Vector Laboratories, Burlingame, CA, USA) at a dilution of 1:200 at room temperature for 1 h. Subsequently, avidin–biotin–peroxidase complex (ABC Elite Kit, Vector Laboratories) was applied for 1 h at room temperature. Finally, the BrdU-positive cells were visualized using nickel-enhanced diaminobenzidine (DAB) as a chromagen. A similar method was used for DCX labeling, omitting the DNA denaturing steps. The tissue was incubated with anti-goat DCX (1:400, Catalog number: SC8066, Santa Cruz, CA, USA) for 48 h at 4 °C.

2.4. Microscopic quantification

$BrdU^+$ cells were quantified using profile counting in the granule cell layer and subgranular zone (SGZ) of the dorsal dentate gyrus (Bregma −1.46 to −2.3) as described previously (Crews et al., 2004). The SGZ was defined as an approximate 50 μm ribbon between the granule cell layer and hilus. All $BrdU^+$ cells within the dorsal dentate gyrus were counted (5–6 sections per animal) at 600× with an oil immersion lens (Olympus Plan Apo 60X oil, numerical aperture=1.4). The number of the $BrdU^+$ cells within each section was then averaged since there is no significant difference in the number of $BrdU^+$ cells across this structure (Herrera et al., 2003). Thus the average number of $BrdU^+$ cells per section derived from 5–6 sections per animal is compared across groups. Previous studies in our laboratory have found that this profile counting method yields results essentially identical to those found using a non-biased stereological method (Crews et al., 2004). The BrdU-IR (immunoreactivity) in subventricular zone (SVZ) and DCX-IR was measured using image analysis software (Bioquant Nova Advanced Image Analysis, R&M Biometric, Nashville, TN). Images were captured on an Olympus BX50 microscope and

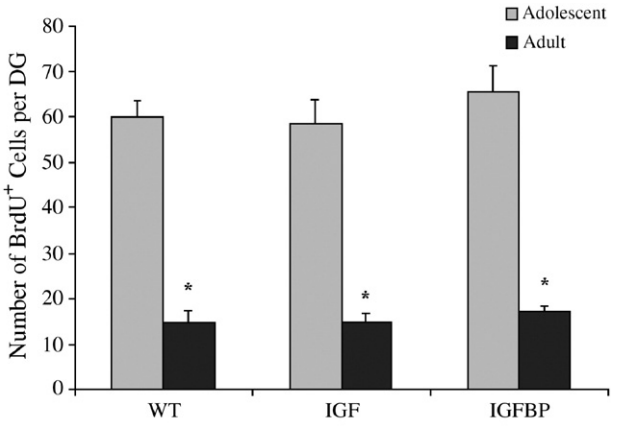

Fig. 1. The number of $BrdU^+$ cell in the hippocampus of adolescent and adult brains. The number of $BrdU^+$ cell per dentate gyrus section is significantly higher in adolescent hippocampus than that of adults (*, $p<0.05$) regardless of the genetic background of strains (IGF-1 transgenic mice, IGFBP-1 transgenic mice, and wildtype littermates).

Sony DXC-390 video camera at 400× for SVZ BrdU-IR and 64× for dentate DCX-IR. Light levels were normalized to preset levels and the microscope, camera, and software were background corrected to ensure reliability of image acquisition (Crews et al., 2004). For the SVZ, the BrdU-IR was measured with a 50 μm box at the dorsolateral corner of the lateral ventricle of each section within Bregma 1.2 to 0.3 of each brain. For dentate DCX-IR, the granule cell layer and SGZ of the dentate gyrus were outlined within each section from Bregma −1.46 to −2.3. The staining density was measured in pixels for the defined area (pixels/mm^2).

2.5. Statistics

All of the statistical analyses were conduced using SPSS 11.5. All values were reported as mean±SEM, and analyzed using ANOVA (Univariate analysis of variance). Follow-up Posthoc analyses were conducted using LSD when needed. Differences were considered significant if the p was smaller than 0.05.

3. Results

To investigate the role of IGF-1 on neurogenesis during adolescence and adulthood, the expression of neurogenic markers BrdU and DCX were quantified in adolescent mice (postnatal day 30, PND30) and adult mice (PND120) with IGF-1 transgene, IGFBP-1 transgene and their wildtype controls. Interestingly, adolescent hippocampus and SVZ had higher levels of neurogenic capacity as compared with adult brains in all three strains.

3.1. Increased neurogenesis in the hippocampus of the adolescents compared with adults

In the dentate gyrus of the hippocampus, BrdU-labeling was significantly higher in adolescent compared with adult brains (Figs. 1 and 2). The number of $BrdU^+$ cells was 61.2±2.9 and 15.7±1.2 per dentate gyrus in the adolescent and adult groups

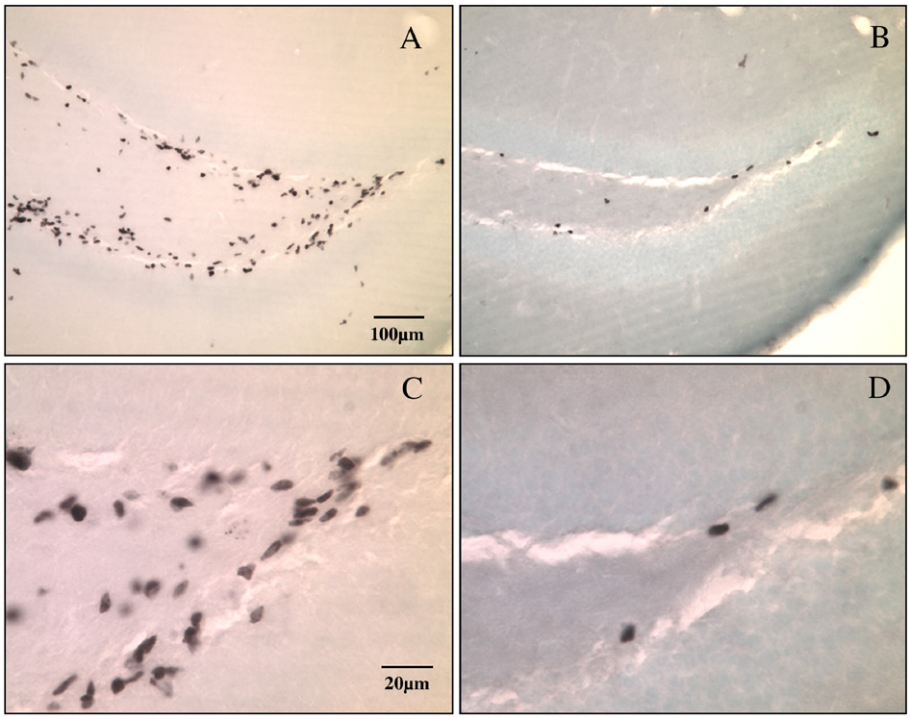

Fig. 2. Representative pictures of $BrdU^+$ cell in the dentate gyrus of the hippocampus. A. Adolescent hippocampus in 10×. B. Adult hippocampus in 10×. C. Adolescent hippocampus in 60×. D. Adult hippocampus in 60×.

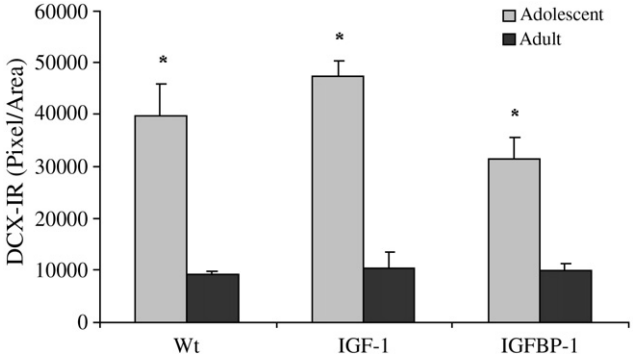

Fig. 3. The expression of DCX (doublecortin) in the hippocampus of adolescent and adult brains. The DCX immunoreactivity (IR, in pixel per mm^3) is significantly higher in adolescent hippocampus than those of adults (*, $p<0.05$) in IGF-1 transgenic, IGFBP-1 transgenic mice, and their littermate controls.

respectively ($p<0.05$). Surprisingly, no statistical differences among the transgenic groups were found ($F_{(2,24)}=0.76$, $p>0.05$) using ANOVA. There is no significant interaction between age and transgenic modification either ($F_{(2,24)}=1.6$, $p>0.05$) suggesting the higher BrdU-labeling in all adolescent groups regardless of the genetic background. Thus, adolescent mice (PND 30) regardless of their genetic background (IGF-1, IGFBP-1 or wildtype) have a significantly increased proliferation of neuroprogenitors compared to adults (PND 120) in the hippocampal dentate gyrus.

Using the endogenous neurogenic marker doublecortin (DCX) to overcome the limitations related to BrdU-labeling (Balajee et al., 1999; Palmer et al., 2000; Kuan et al., 2004), we further demonstrated the increased DCX immunoreactivity in Adolescent hippocampus as compared with Adults (Figs. 3 and 4). DCX immunoreactivity was 41±3.0 in adolescent DG and 9.8±1.0 (pixel density $\times 10^{-3}$ per mm^2, $p<0.001$) in adults when all strains are combined. ANOVA reveal significant effects on age (adolescent vs. adult, $F_{(1,24)}=138.6$, $p<0.001$). Although the effects of transgenic strains (IGF-1, Wt, and IGFBP-1) on DCX expression did not reach the cutoff of statistical significance ($F_{(2,24)}=3.2$ $p=0.057$), we still can see a trend of increase in DCX expression in IGF-1 adolescent group. Thus, further pairwise comparisons in the adolescent groups reveal a significant difference on DCX expression between IGF-1 and IGFBP-1 transgenic groups ($p<0.05$) in adolescents. However, when these two transgenic groups were compared with their wildtype littermate respectively, neither group reached statistical significance (both p values are smaller than 0.05). Based on these data, we conclude that doublecortin is expressed significantly higher in the dentate gyrus of the hippocampus of adolescent brains than that of adult brains. The DCX expression was significantly increased in the IGF-1 over-expressing transgenic mice when compared with the IGFBP-1 transgenic mice ($p<0.05$) among the adolescent groups, but not adults (Figs. 3 and 4). However, neither group was significantly different from their wildtype littermates suggesting only a moderate influence of IGF-1 on adolescent neurogenesis. In sum, IGF-1 over-expressing transgenic mice had higher DCX expression than the IGFBP-1 over-expressing mice (lower IGF-1), however the most robust differences occurred between adolescent and adult mice of all 3 strains.

3.2. Increased BrdU-labeling in the forebrains of adolescents as compared with adults

We further analyzed the forebrain neurogenesis by measuring BrdU-immunoreactivity (BrdU-IR) in the subventricular

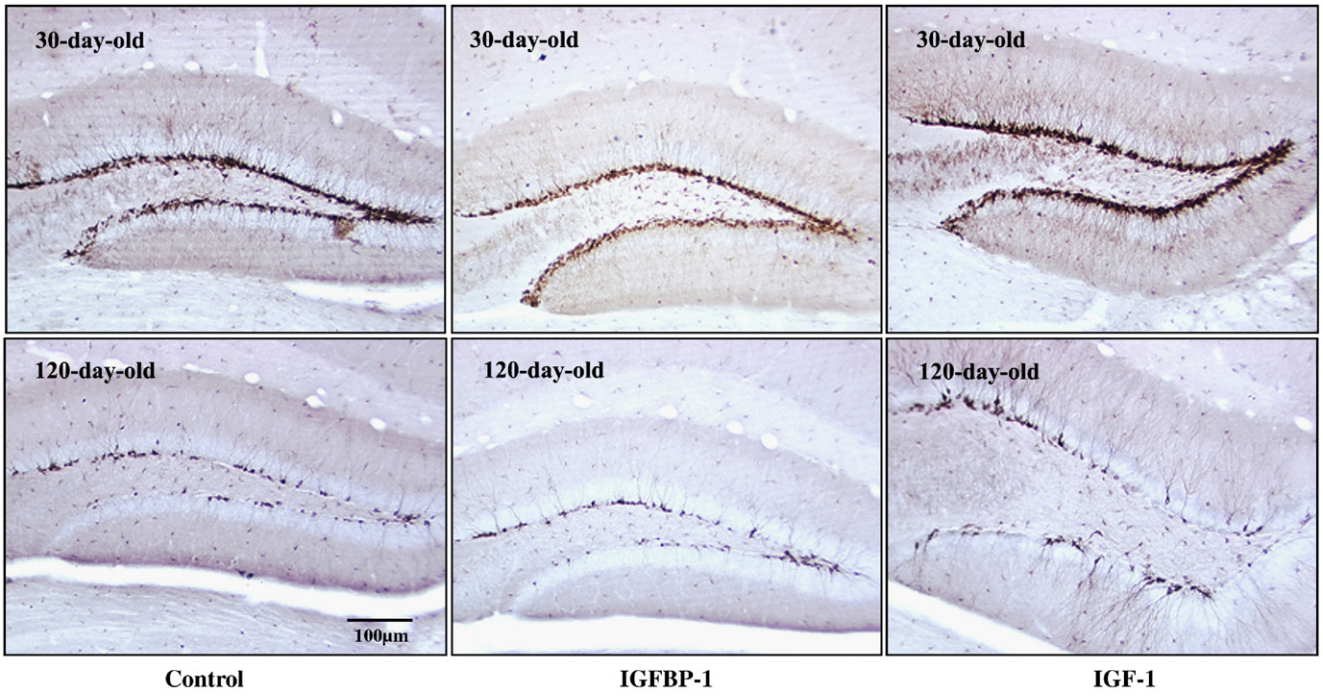

Fig. 4. Representative pictures of DCX expression in the hippocampus of adolescent and adult brains. Adolescent mice were 30-day-old and adult mice were 120-day-old.

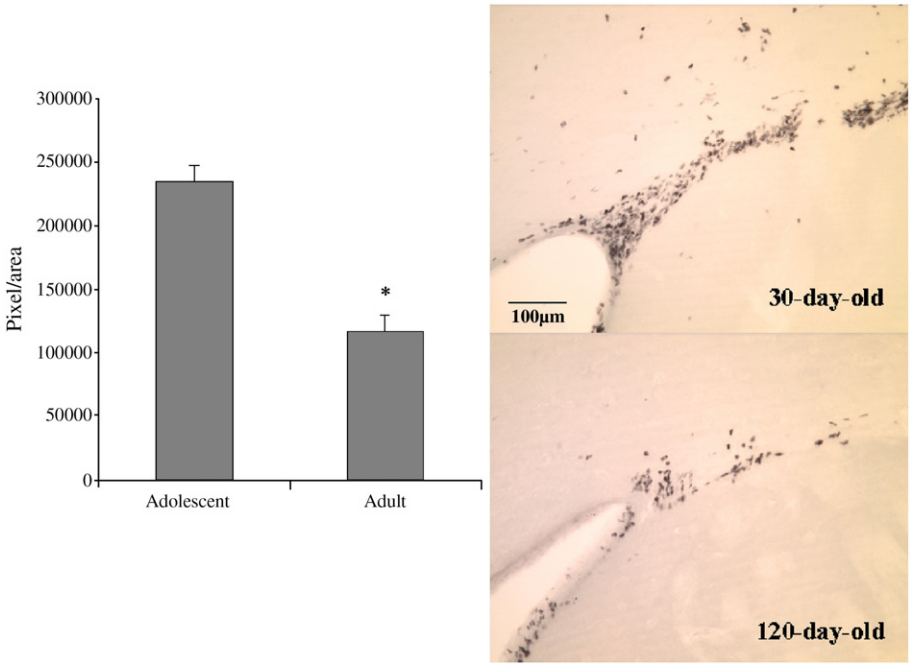

Fig. 5. Enhanced BrdU-immunoreactivity in the forebrain subventricular zone (SVZ) of the adolescents as compared with adults. BrdU-labeling is significantly higher in the SVZ of the adolescents than that of the adults (*, $p<0.05$). The representative pictures are shown on the right (adolescents are 30-day-old and adults are 120-day-old).

zone (SVZ) of adolescent and adult mice using Bioquant system (Fig. 5). No differences were found among strains in forebrain SVZ, however ANOVA reveals a significant effect on age (adolescent vs. adults, $F_{(1,24)}=14.9$, $p<0.01$) on BrdU-IR was 235±20 in adolescent groups combined vs. 117±1.9 (pixel density $\times 10^{-3}$ per mm^2; $F_{(1,24)}=14.9$, $p<0.01$) in adult groups combined. There are no statistical differences between the different transgenic groups (IGF-1, IGFBP-1, and Wt, $F_{(2,24)}=3.1$, $p>0.05$). No interaction between the age and transgenic groups was found either ($F_{(2,24)}=0.85$, $p>0.05$) in this region. Thus, both forebrain and hippocampal neurogenesis is markedly reduced during brain maturation from adolescence to adults, but IGF-1 does not appear to make a major contribution to the decreases in neurogenesis that occurs in the maturation from adolescents to adults.

4. Discussion

The most significant finding of the current study is that neurogenesis in both forebrain and hippocampus decreases dramatically from adolescent to adult. Neurogenesis across the dorsal and ventral regions of the hippocampus are fairly constant (Herrera et al., 2003), however, we measured the dorsal two-thirds of the hippocampus and cannot rule out differences in the ventral region. Neurogenesis starts when proliferating neuroprogenitors of the dentate gyrus exit cell cycle and begin expressing neuronal markers, such as DCX. Within approximately one month, these cells migrate into the granule cell layer, extend dendritic and axonal processes and show electrophysiological characteristics of new born neurons that make appropriate synaptic connections to carry information to and from other parts of the brain (Gross, 2000). In rats, quantitative studies have found that hundreds of thousands of new granule cells are formed each month representing up to 6% of hippocampal granule cells formed each month (Cameron and McKay, 2001). The high level of neurogenesis in adolescent brain found in this study could be reflective of the high level of neuroplasticity during adolescence. Although the direct link between neurogenesis and learning remain elusive (Leuner et al., 2006), studies have suggested that neurogenesis is at least partially involved in learning and memory (Kempermann et al., 1997; van Praag et al., 1999; Shors et al., 2001; Kempermann, 2002). Animals in an enriched environment show improved learning skills on tasks as well as an increase in neurogenesis (Kempermann et al., 1997). Improved learning itself is associated with increased neurogenesis (Gould et al., 1999) as well. Further, inhibition of neurogenesis has been found to block associative memory (Shors et al., 2001). Human adolescent brain has previously been found to have somewhat larger gray matter areas than adults (Paus, 2005). During maturation of the brain from adolescence to adults the human and rodent cortices shrink with synaptic pruning leading to more cortical activation and reduced plasticity (Spear, 2004). Forebrain neurogenesis represent a plastic process, which is important in forming neurons and synapses that allow discrimination of distinct odors (Rochefort et al., 2002; Wilson et al., 2004). Thus, the high levels of neurogenesis in adolescence compared to adults may reflect the highly plastic nature of the adolescent brain and its ability to learn.

A variety of data supported our initial hypothesis that IGF-1 would increase neurogenesis (Aberg et al., 2000). Several studies have shown that IGF-1 over-expressing transgenic

mice have increased brain growth and increased number of hippocampal DG neurons (Ye et al., 1995; O'Kusky et al., 2000). In contrast, IGFBP-1 transgenic mice showed retarded brain growth (Ye et al., 1995). Exercise has been found to increase neurogenesis in both rats and mice (Carro et al., 2000; Carro et al., 2001; Crews et al., 2004) apparently through increasing IGF-1 transport into the brain. However, the current study did not show a robust influence of IGF-1 on adolescent neurogenesis. No differences were found in BrdU-labeling between IGF-1 and IGFBP-1 transgenic mice in both adolescents and adults. Even though a statistically significant increase in DCX expression was found in IGF-1 mice when directly compared with IGFBP-1 mice, neither was different from their littermate controls since the magnitude of effect was small.

In the current study, we had expected to see an increase in neurogenesis in IGF-1 transgenic animals, however, our results suggest that most of the increased growth probably occurred before PND 30, at which age our study was conducted. Additionally, we used BrdU-labeling, which directly measures the number of newly proliferating neurons at the time of BrdU injection, either PND30 (adolescents) or PND120 (adults), allowing us to precisely follow neurogenesis within the period of adolescent development. Thus, the similar levels of BrdU-labeling among the IGF-1, IGFBP-1 and wildtype mice in this study indicate that the rate of cell proliferation was not significantly different after PND30, which is supported by the study showing that growth curves reach a plateau before adolescence (O'Kusky et al., 2000). It may be that the total number of hippocampal neurons (O'Kusky et al., 2000) in IGF-1 transgenic mice is greater than controls, but the rate of proliferation at the time of BrdU administration (after PND30) is not different. Together, our results suggest that the pronounced influence of IGF-1 on neurogenesis probably occurs before the animals reach adolescence.

In addition, although adolescents have greater exploratory activity than adults (Spear, 2000), which could be a contributing factor for the enhanced neurogenesis, such difference is unlikely to explain the robust increases in adolescent neurogenesis we saw in the current study. Studies have shown that the baseline activity in the home caged laboratory animals are not markedly different between adolescents and adults (Rezvani and Levin, 2004; Maldonado and Kirstein, 2005). Exercise has been shown to promote neurogenesis when the mice were running on wheels for about 5 km per day (Crews et al., 2004). The simple home caged adolescent mice in the current study are not likely to have high enough activity to alter neurogenesis. Therefore, the enhanced neurogenesis in adolescents is mostly likely due to the developmental processes.

Taken together, the current study clearly demonstrated the significant decline on neurogenesis from adolescent to adult indicating a critical window of opportunity where the neuronal circuitry is still adjustable for better adaptation. On one hand, any manipulation during this critical period could lead to more pronounced damage in adulthood. For instance, one study from our laboratory has shown that adolescents are more vulnerable to alcohol-induced reduction in neurogenesis in both forebrain SVZ and hippocampal DG (Crews et al., 2006). On the other hand, the highly plastic period of adolescence could result in better functional compensation and recovery. A study has shown that adolescents are relatively insensitive to the locomotor effects of amphetamine (Brunell and Spear, 2006). Adolescents also differ in response to nicotine-induced plasticity-related gene expression (arc, c-fos, etc.) from adults (Schochet et al., 2005). In sum, adolescent brain development is a window of both vulnerabilities and opportunities (Dahl, 2004). It is supported by the current findings on the decline of neurogenesis from adolescents to adults, which open a new arena of research on the role of neurogenesis during this critical window of adolescent brain remodeling.

Acknowledgements

The authors wish to thank Dr. J. D'Ercole and Dr. P. Ye for providing breeding pair of the transgenic mice. Also thanks to Ms. M. Mann for assistance with manuscript preparation. Supported by NIH-NIAAA.

References

Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. J Neurosci 2000;20:2896–903.

Andersen SL, Teicher MH. Delayed effects of early stress on hippocampal development. Neuropsychopharmacology 2004;29:1988–93.

Andersen SL, Thompson AT, Rutstein M, Hostetter JC, Teicher MH. Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. Synapse 2000;37:167–9.

Anderson MF, Aberg MA, Nilsson M, Eriksson PS. Insulin-like growth factor-I and neurogenesis in the adult mammalian brain. Brain Res Dev Brain Res 2002;134:115–22.

Balajee AS, May A, Bohr VA. DNA repair of pyrimidine dimers and 6–4 photoproducts in the ribosomal DNA. Nucleic Acids Res 1999;27:2511–20.

Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG. Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 2003;467:1-10.

Brunell SC, Spear LP. Effects of acute ethanol or amphetamine administration on the acoustic startle response and prepulse inhibition in adolescent and adult rats. Psychopharmacology (Berl) 2006;186:579–86.

Cameron HA, McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 2001;435:406–17.

Carro E, Nunez A, Busiguina S, Torres-Aleman I. Circulating insulin-like growth factor I mediates effects of exercise on the brain. J Neurosci 2000;20: 2926–33.

Carro E, Trejo JL, Busiguina S, Torres-Aleman I. Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. J Neurosci 2001;21:5678–84.

Crews FT, Nixon K, Wilkie ME. Exercise reverses ethanol inhibition of neural stem cell proliferation. Alcohol 2004;33:63–71.

Crews FT, Mdzinarishvili A, Kim D, He J, Nixon K. Neurogenesis in adolescent brain is potently inhibited by ethanol. Neuroscience 2006;137:437–45.

Dahl RE. Adolescent brain development: a period of vulnerabilities and opportunities. Keynote address. Ann N Y Acad Sci 2004;1021:1-22.

Ding Q, Vaynman S, Akhavan M, Ying Z, Gomez-Pinilla F. Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. Neuroscience 2006;140:823–33.

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, et al. Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 1999;2:861–3.

Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in the hippocampal formation. Nat Neurosci 1999;2:260–5.

Gross CG. Neurogenesis in the adult brain: death of a dogma. Nat Rev Neurosci 2000;1:67–73.

Herrera DG, Yague AG, Johnsen-Soriano S, Bosch-Morell F, Collado-Morente L, Muriach M, et al. Selective impairment of hippocampal neurogenesis by chronic alcoholism: protective effects of an antioxidant. Proc Natl Acad Sci U S A 2003;100:7919–24.

Kempermann G. Regulation of adult hippocampal neurogenesis — implications for novel theories of major depression. Bipolar Disord 2002;4:17–33.

Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature 1997;386:493–5.

Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH. Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. Development 2003;130:391–9.

Kuan CY, Schloemer AJ, Lu A, Burns KA, Weng WL, Williams MT, et al. Hypoxia–ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. J Neurosci 2004;24:10763–72.

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;16:2027–33.

Leuner B, Gould E, Shors TJ. Is there a link between adult neurogenesis and learning? Hippocampus 2006;16:216–24.

Maldonado AM, Kirstein CL. Cocaine-induced locomotor activity is increased by prior handling in adolescent but not adult female rats. Physiol Behav 2005;86:568–72.

O'Kusky JR, Ye P, D'Ercole AJ. Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development. J Neurosci 2000;20:8435–42.

Palmer TD, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 2000;425:479–94.

Paus T. Mapping brain maturation and cognitive development during adolescence. Trends Cogn Sci 2005;9:60–8.

Perez-Martin M, Cifuentes M, Grondona JM, Bermudez-Silva FJ, Arrabal PM, Perez-Figares JM, et al. Neurogenesis in explants from the walls of the lateral ventricle of adult bovine brain: role of endogenous IGF-1 as a survival factor. Eur J Neurosci 2003;17:205–11.

Poulsen FR, Blaabjerg M, Montero M, Zimmer J. Glutamate receptor antagonists and growth factors modulate dentate granule cell neurogenesis in organotypic, rat hippocampal slice cultures. Brain Res 2005;1051:35–49.

Rezvani AH, Levin ED. Adolescent and adult rats respond differently to nicotine and alcohol: motor activity and body temperature. Int J Dev Neurosci 2004;22:349–54.

Rochefort C, Gheusi G, Vincent JD, Lledo PM. Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. J Neurosci 2002;22:2679–89.

Schochet TL, Kelley AE, Landry CF. Differential expression of arc mRNA and other plasticity-related genes induced by nicotine in adolescent rat forebrain. Neuroscience 2005;135:285–97.

Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memories. Nature 2001;410:372–6.

Spear L. Modeling adolescent development and alcohol use in animals. Alcohol Res Health 2000;24:115–23.

Spear LP. Adolescent brain development and animal models. Ann N Y Acad Sci 2004;1021:23–6.

van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 1999;2:266–70.

Wilson DA, Best AR, Sullivan RM. Plasticity in the olfactory system: lessons for the neurobiology of memory. Neuroscientist 2004;10:513–24.

Ye P, D'Ercole AJ. Insulin-like growth factor actions during development of neural stem cells and progenitors in the central nervous system. J Neurosci Res 2006;83:1–6.

Ye P, Carson J, D'Ercole AJ. In vivo actions of insulin-like growth factor-I (IGF-I) on brain myelination: studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice. J Neurosci 1995;15:7344–56.