Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Effects of isoflurane or propofol on postnatal hippocampal neurogenesis in young and aged rats

Diana M. Erasso[a,1], Enrico M. Camporesi[b], Devanand Mangar[c], Samuel Saporta[a,*]

[a]Department of Pathology and Cell Biology/Neuroscience, School of Medicine, University Of South Florida, Tampa, FL, USA
[b]Department of Molecular Pharmacology and Physiology, School of Medicine, University of South Florida, and Florida Gulf-to-Bay Anesthesiology Associates, Tampa General Hospital, Tampa, FL, USA
[c]Florida Gulf-to-Bay Anesthesiology Associates, Tampa General Hospital, Tampa, FL, USA

ARTICLE INFO

Article history:
Accepted 20 July 2013
Available online 24 July 2013

Keywords:
Isoflurane
Propofol
Hippocampal neurogenesis

ABSTRACT

An increasing number of in vitro and in vivo studies suggest that anesthesia and surgery could be risk factors for later cognitive impairment in the young and aged brain. General anesthesia has been shown to impair spatial memory in rats and this performance is dependent on hippocampal function and postnatal hippocampal neurogenesis. Anesthetic induced alteration of one or more stages of postnatal hippocampal neurogenesis may in part explain this cognitive impairment following anesthesia. Three different populations of proliferating cells in the dentate gyrus (DG) were labeled with different thymidine analogs (EdU, IdU, and CldU) at 4, 8, and 21 days, respectively, in young (3-month-old) and aged (20-month-old) rats prior to a 3 h exposure to isoflurane, control, propofol, or 10% intralipid. 24 h following general anesthesia, brains were collected for analysis. The number of cells co-localized with neuronal differentiation and maturation labels with each of the thymidine analogs was quantified. In addition, new cell proliferation 24hr following anesthesia was assessed with anti-Ki67. The effect of anesthesia on astrocytes was also assessed with anti-S100β. Isoflurane or propofol did not affect new cell proliferation, as assessed by Ki67, in the DG of young or aged rats. However, propofol significantly decreased the number of differentiating neurons and increased the number of astrocytes in the DG of young, but not aged, rats. Isoflurane significantly decreased the number of maturing neurons and increased the number of astrocytes in the DG of aged, but not young, rats. Isoflurane and propofol anesthesia altered postnatal hippocampal neurogenesis in an age and agent dependent matter.

*Correspondence to: University of South Florida, 12901 Bruce B. Downs Blvd, MDC 11, Tampa, FL 33612, USA. Fax: +1 813 974 5536.
E-mail address: ssaporta@health.usf.edu (S. Saporta).
[1]Current affiliation: University of Miami, USA.

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.07.035

1. Introduction

A rising number of clinical studies suggest that anesthesia and surgery could be risk factors for later cognitive impairment, especially in the young (Johnson et al., 2002; Loepke and Soriano, 2008; Sun, 2010) and aged brain (Abildstrom et al., 2000; Moller et al., 1998, Canet et al., 2003; Monk et al., 2008). Moreover, recent in vitro (Liang et al., 2008; Xie et al., 2007; Xie and Xu, 2012) and in vivo (Popic et al., 2012; Yu et al., 2013; Zhao et al., 2010) studies suggest an anesthetic-induced neurotoxic effect on both of these age groups (Culley et al., 2007). However, it remains unclear whether these cognitive changes are due to the effects of surgery or anesthesia.

Postnatal generation of neurons occurs throughout life in two brain regions (Altman and Das, 1965; Kempermann and Gage, 2000), the subventricular zone of the lateral ventricle, where it has been suggested that neurons migrate into the olfactory bulb and are involved in olfactory memory (Pignatelli and Belluzzi, 2010; Sultan et al., 2010); and the subgranular zone of the dentate gyrus (DG) of the hippocampus where neurons appear to be involved in spatial learning and memory (Dupret et al., 2008; Kempermann, 2002). Generation of new neurons in the DG has been shown of key importance to hippocampal function, and has been shown to be involved in spatial memory (Broadbent et al., 2004; Moscovitch et al., 2005) suggesting that anesthetics may act on hippocampal neurogenesis to affect hippocampal dependent cognitive functions. Recently, there has been an increase in studies looking at the anesthetics effects on neurogenesis in vivo (Stratmann et al., 2009, 2010; Zhu et al., 2010) and in vitro (Culley et al., 2011; Sall et al., 2009; Zhao et al., 2013), but results differ from each other mainly because of anesthetic agent, dose, and age group being studied. Consequently, it is critical to gain more insight on the effects of anesthetics on hippocampal neurogenesis.

A time line of neuronal precursor maturation in the DG has been suggested by Ming and Song (2005), while details regarding this differentiation and maturation remain to be resolved, the timing of differentiation and maturation, based on developmental markers, appears to be consistent (Bonaguidi et al., 2012; Ming and Song, 2005). Postnatal hippocampal neurogenesis is a multistep process that involves proliferation of neural progenitor cells, followed by the differentiation to a neuronal phenotype, migration during the late phase of differentiation, neuronal maturation and synaptic integration of the these cells into the existing hippocampal circuitry (Ming and Song, 2005; Piatti et al., 2006). These developmental stages can be identified by cell morphology and the expression of developmentally regulated markers (Kempermann et al., 2004; Ming and Song, 2005). Some nascent cells also differentiate into astrocytes that populate the DG (Palmer et al., 2000), and stimuli that affect postnatal neurogenesis also affect gliogenesis (Kempermann et al., 2002).

Based on the suggested timeline of neuronal precursor development in the DG (Ming and Song, 2005); in the present study, we investigated the effects of two commonly used anesthetics, the inhaled anesthetic isoflurane and the intravenous anesthetic propofol, on nascent cells undergoing proliferation, early (4-day-old cells), late (8-day-old cells) differentiation, and maturation (21-day-old cells) in the DG of young (3 mo) and aged (21 mo) rats at the time of exposure to anesthesia. Anesthetic-induced alteration of any stage of postnatal hippocampal neurogenesis may, in part explain, the resulting cognitive impairment surgery and anesthesia.

2. Results

The results of these experiments are presented in Table 1 (isoflurane) and Table 2 (propofol). Young animals had a statistically greater amount of cell proliferation in the DG than aged animals ($F_{3,7}=8.57$, $p<0.0001$ and $F_{3,7}=28.93$, $p<0.0001$ for isoflurane and propofol, respectively). Similarly, we found a decrease in the number of nascent cells undergoing early differentiation ($F_{2,11}=16.61$, $p<0.0001$ and $F_{2,11}=59.75$, $p<0.0001$ for isoflurane and propofol, respectively), late differentiation ($F_{7,11}=70.18$, $p<0.0001$ and $F_{7,11}=73.02$, $p<0.0001$ for isoflurane and propofol, respectively) and maturation ($F_{7,11}=16.48$, $p<0.0001$ and $F_{7,11}=52.65$, $p<0.0001$ for isoflurane and propofol, respectively) in aged rats as compared to young rats.

2.1. *Cell proliferation in the DG (Ki67+) is not altered 24 h after isoflurane or propofol*

Neither isoflurane (Table 1) nor propofol (Table 2) anesthesia altered new cell proliferation in the subgranular zone of the DG 24 h following anesthesia as assessed by Ki67 ($F_{3,7}=0.51$, $p=0.818$ and $F_{3,7}=0.98$, $p=0.47$, respectively).

2.2. *Propofol altered nascent cells undergoing early differentiation (EdU+) in the DG of young rats*

Isoflurane did not alter the number of nascent cells undergoing early differentiation in the DG of young or aged rats ($F_{2,11}=16.61$ $p=0.41$ and $p=0.16$, respectively) (Table 1). Conversely, propofol significantly decreased the number of these cells in young (Fig. 1A) (EdU, $F_{2,11}=59.75$ $p=0.034$), but not in aged (Fig. 1B), rats. Propofol specifically altered nascent cells differentiating into neurons (EdU/DCX, $p=0.023$), but did not alter the cells differentiating into astrocytes (EdU/S100β, $p=0.260$) (Fig. 1A and B).

2.3. *Propofol altered nascent cells undergoing late differentiation (IdU^+) in the DG of young rats*

Isoflurane did not alter the number of nascent cells undergoing differentiation in the DG of young or aged rats ($F_{7,11}=70.18$ $p=0.423$ and $p=0.273$, respectively) (Table 1). Conversely, propofol significantly decreased the number of these same cells in young (IdU, $F_{7,11}=73.02$ $p=0.034$) (Fig. 2A), but not in aged rats ($p=0.20$) (Fig. 2B). Propofol specifically altered nascent cells differentiating into neurons (IdU/DCX, $p=0.047$) and astrocytes (IdU/S100 β, $p=0.0001$) (Fig. 2A and B).

Table 1 – Mean number of labeled cells (mean ± SEM) in the dentate gyrus for each developmental and phenotypic marker following isoflurane anesthesia or control. Comparisons were made between the treatment group and its respective control.

Isoflurane

		Ki67	EdU			IdU			CldU		
			EdU	EdU/DCX	EdU/S100β	IdU	IdU/DCX	IdU/S100β	CldU	CldU/NeuN	CldU/S100β
Young	Control (N=8)	952±122	496±84.6	282.7±66.8	21.3±10.7	3090±307.8	2154±245.1	472±24	1740±373	326±68.6	281±17.5
	Isoflurane (N=8)	814±155	464±106.5	250.7±69.3	26.7±5.3	3164±213.1	2028±283.9	426.7±16.7	1590±332.4	368±99.9	308±22.9
Aged	Control (N=8)	237±0.3	197.3±55.7	10.67±5.3	21.3±14.1	178±22.7	68±10.4	64±9.2	166±20.5	110±20.2	48±9.24
	Isoflurane (N=8)	280±0.3	117.3±45.6	21.3±5.3	26.7±10.7	208±43	92±23	74.7±10.7	**106±17.9***	**41.9 ±14.8****	**101.3±19.2***

* $p \leq 0.05$.
** $p \leq 0.01$.

Table 2 – Mean number of labeled cells (mean ± SEM) in the dentate gyrus for each developmental and phenotypic marker following propofol anesthesia or intralipid (control). Comparisons were made between the treatment group and its respective control.

Propofol

		Ki67	EdU			IdU			CldU		
			EdU	EdU/DCX	EdU/S100β	IdU	IdU/DCX	IdU/S100β	CldU	CldU/NeuN	CldU/S100β
Young	Intralipid (N=8)	1372±152	624±42.3	298.7±21.3	21.3±5.3	3470±189.3	1962±213	300±17.4	1932±207.2	374±61.1	246±19.3
	Propofol (N=8)	1126±149	**453.3±54.1***	**197.3±18.2***	26.7±5.3	**2768±299.8***	**1386±254.7***	**566.4±18****	1762±241.8	348±35.1	248±31.3
Aged	Intralipid (N=8)	198±60.9	181.3±37.3	10.67±5.3	26.7±5.3	189±47.72	114.7±44	64±9.2	152±45.8	80±25.1	64±9.2
	Propofol (N=8)	176±61.5	96±27.7	5.3±5.3	16±9.2	136±39.79	48±11.68	74.7±10.7	90.7±15.8	40±13	80±16

* $p \leq 0.05$.
** $p \leq 0.01$.

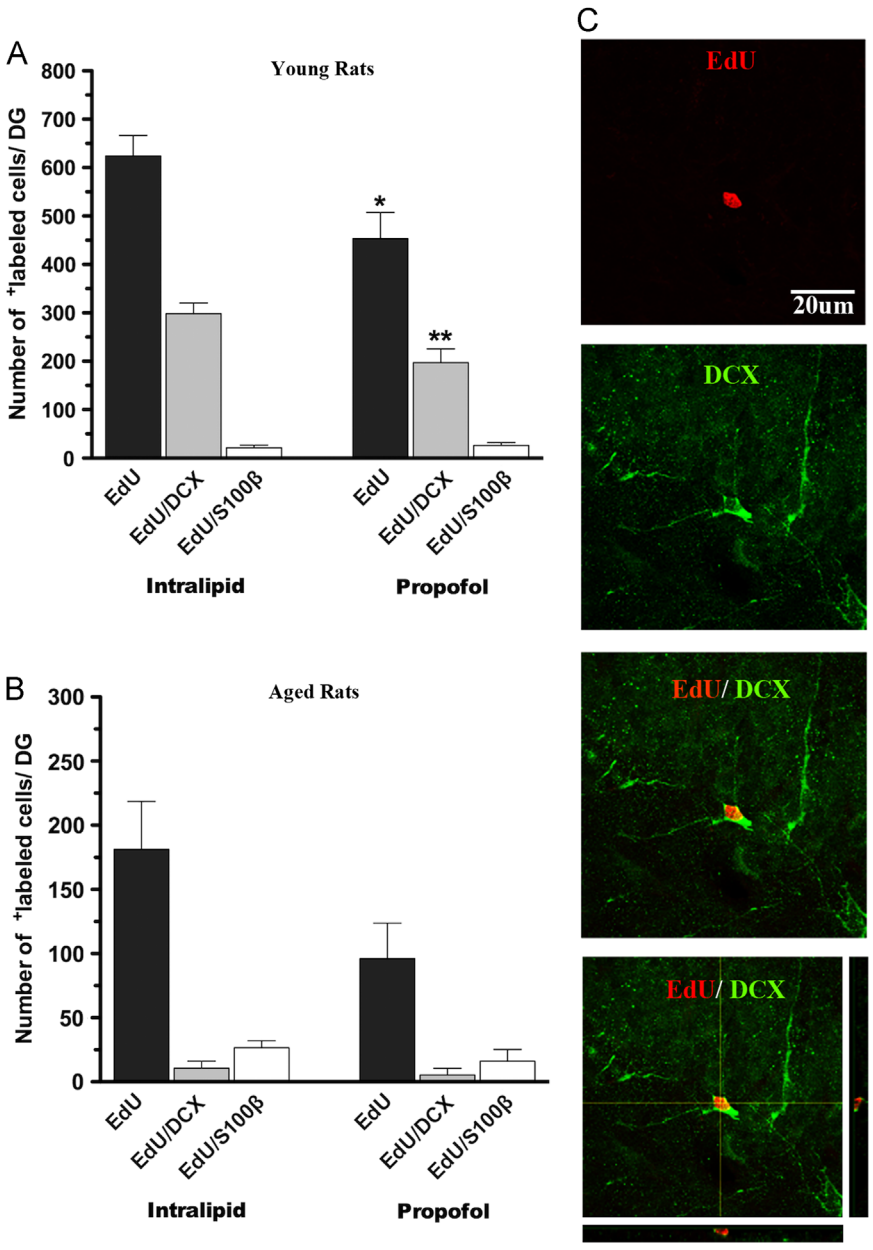

Fig. 1 – Quantification of EdU$^+$ cells (right column), EdU$^+$/DCX$^+$ cells (middle column), and EdU$^+$/S100β$^+$cells (left column) in the DG of young (A) and aged (B) rats after propofol or intralipid infusion ($n=8$ per group). Propofol significantly decreased the number of EdU$^+$ cells (*$P=0.034$) and EdU$^+$/DCX$^+$ cells ($P=0.023$) in young rats, but did not alter the number of EdU$^+$/S100β$^+$cells ($P=0.260$). (C) Confocal image confirming co-localization of EdU (red) and DCX (green) in a cell of the DG indicating differentiation into a neuronal phenotype.**

2.4. *Isoflurane altered nascent cells undergoing maturation (CldU+) in the DG of aged rats*

Isoflurane significantly decreased the number of nascent cells undergoing maturation (CldU+) in the DG of aged ($F_{7,11}=16.48$ $p=0.022$) (Fig. 3B), but not in young ($p=0.384$) rats (Fig. 3A). In addition, isoflurane specifically decreased the number of maturing neurons (CldU/NeuN, $p=0.008$), and increased the number of maturing astrocytes (CldU/S100 β, $p=0.033$). Conversely, propofol did not alter the number or phenotype of nascent cells undergoing maturation in the DG of young ($p=0.301$) or aged ($p=0.117$) rats (Table 2).

3. Discussion

The experiments reported here showed an agent and age dependent effect of isoflurane and propofol on nascent cells in the DG of young and aged rats. Specifically, isoflurane decreased the number of maturing neurons in the DG born 21 days prior to anesthesia exposure in aged, but not young, rats, while propofol decreased the number of differentiating neurons in the DG born 4 and 8 days prior to anesthesia exposure in young, but not aged, rats (see Fig. 4). We also confirmed previously published reports showing that young animals have a significantly greater amount of cell

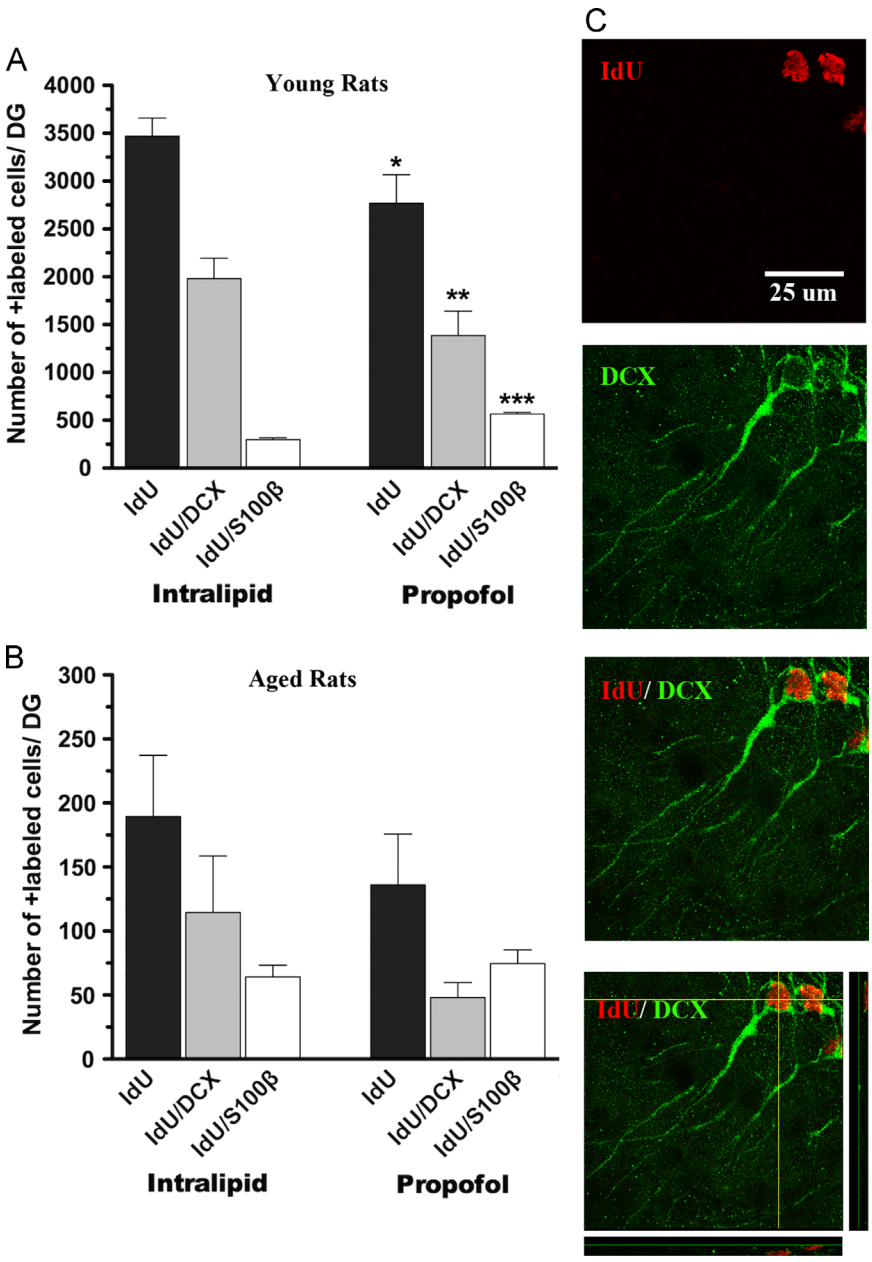

Fig. 2 – Quantification of IdU$^+$ cells (right column), IdU$^+$/DCX$^+$ cells (middle column), and IdU$^+$/S100β$^+$cells (left column) in the DG of young rats (A) and aged rats (B) after propofol or intralipid infusion ($n=8$ per group). Propofol significantly decreased the number of IdU$^+$ cells (*P=0.034), IdU$^+$/DCX$^+$ cells (P=0.047), but increased the number of IdU$^+$/S100β$^+$cells (***P<0.0001) in the DG of young rats. (C) Confocal imaging confirming co-localization of IdU$^+$ (red) and DCX$^+$ (green) indicating a neuronal phenotype.**

proliferation in the DG than aged animals (Galvan and Jin, 2007; Kuhn et al., 1996; Lazarov et al., 2010; Seki and Arai, 1995; Shruster et al., 2010). In addition, we found that the number of dividing, Ki-67-positive, cells in the DG of young or aged rats is not affected immediately after receiving isoflurane or propofol anesthesia, which is consistent with previous reports showing no effect of anesthesia on stem cell proliferation in the DG immediately after anesthesia (Tung et al., 2008). While other studies have used multiple S-phase markers to assess neurogenesis, to our knowledge, the current report is the first study using timed injections of multiple thymidine analogs in the same animal to assess the effects of anesthesia on multiple populations of postnatal hippocampal neurons at different stages of their development.

3.1. *Control groups*

Comparison of inhaled (isoflurane) and intravenous (propofol) anesthetics posses some significant technical challenges. Induction of anesthesia using isoflurane subjects the animal to significantly less stress than placing the rat in a restrainer. Similarly, controls for each anesthetic (room air vs. intralipid)

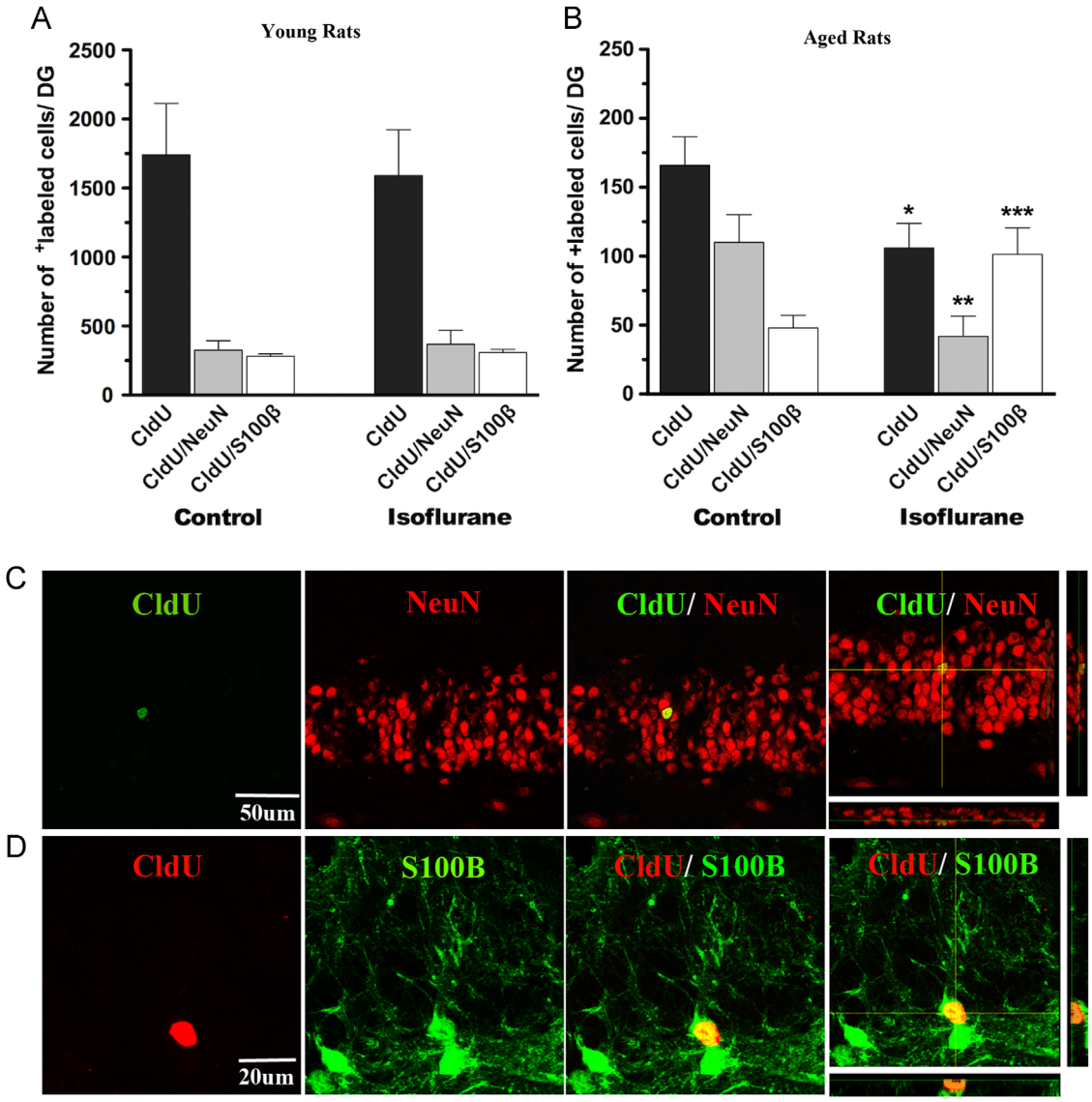

Fig. 3 – Quantification of CldU$^+$ cells (right column), CldU$^+$/NeuN$^+$ cells (middle column), and CldU$^+$/S100β$^+$cells (left column) in the DG of young (A) and aged (B) rats after isoflurane or control exposure ($n=8$ per group). Isoflurane significantly decreased the number of CldU$^+$ cells (*P=0.02), and the number of CldU$^+$/NeuN$^+$ cells (P=0.008), but increased the number of CldU$^+$/S100β$^+$cells (***P=0.033). (C) Confocal imaging demonstrating co-localization of CldU$^+$ (green) and NeuN$^+$ (red) indicating a neuronal phenotype. (D) Confocal imaging demonstrating co-localization of CldU$^+$ (red) and S100β$^+$(green) indicating an astrocytic phenotype.**

are, of necessity, treated differently. While direct comparison of the effects of isoflurane to those of propofol are not possible, some cautious conclusions can be drawn regarding the effect of each of these anesthetics on nascent cells in the DG of young and aged rats.

3.2. *Isoflurane anesthesia specifically affected 21-day-old neurons and astrocytes in the DG of aged rats*

Twenty-one day old neurons in the DG are maturing and functionally integrating into the hippocampus (Duan et al., 2008; Ming and Song, 2005). Therefore, our findings that isoflurane anesthesia decreased the number of maturing neurons in the aged rat brain suggests that impairments of learning and/or memory reported following isoflurane anesthesia (Culley et al., 2003, 2004) may be the result of a decrease in the number of mature hippocampal neurons. Although, a direct cognitive assessment is not included in our present study, recent studies published by other groups have suggested that cognitive impairment occurs in parallel to a decrease in hippocampal neurogenesis (Stratmann et al., 2009; Zhu et al., 2010).

Isoflurane has been shown to affect neuroprogenitor cells in a time, dose and age dependent way in vitro (Culley et al., 2011; Sall et al., 2009; Zhao et al., 2013) and in vivo (Stratmann et al., 2009; Zhu et al., 2010). For instance, exposure of neuroprogenitor cells to 2.4%, but not 1.2% isoflurane has been shown to decrease new cell proliferation in vitro. While,

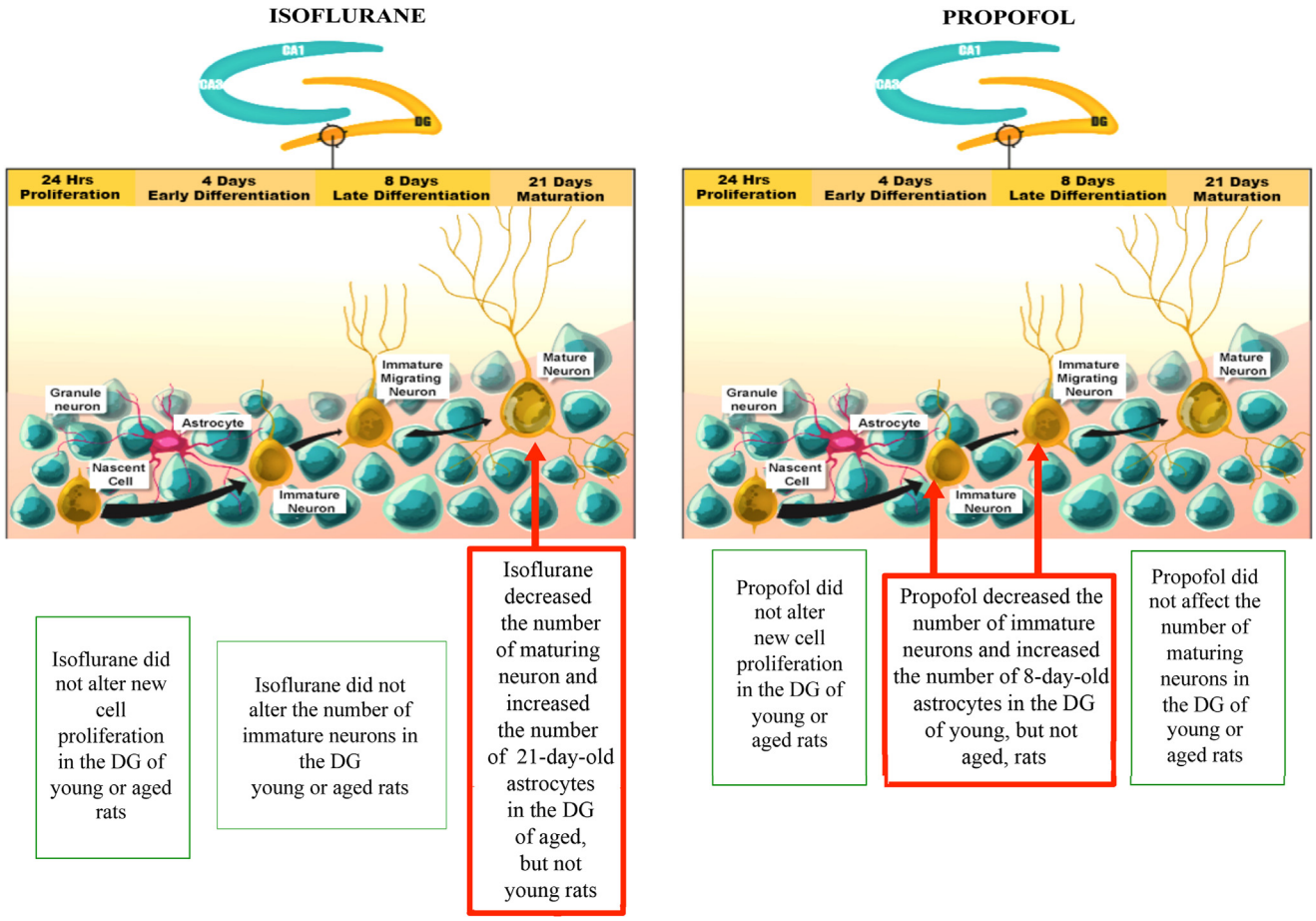

Fig. 4 – Cartoon summarizing the effect of isoflurane or propofol anesthesia on nascent cells undergoing development in young and aged rats. Black arrows in both panels represent the normal development (~21 days) through which nascent cell differentiate, become immature neurons, migrate and mature into functional neurons that synaptically integrate into the hippocampal circuitry. The left panel represents the significant differences found after isoflurane anesthesia, which are specific to maturing neurons and astrocytes. The right panel represents the significant differences found after propofol anesthesia, which are specific to the immature neurons and astrocytes.

repeated (Zhu et al., 2010) or single (Stratmann et al., 2009) exposure of postnatal, but not young adult rats, to 1% or more of isoflurane has been shown to decrease hippocampal new cell proliferation. Even though, the above mentioned studies do not directly apply to the two age populations being considered in our present study, a clear suggestion can be made about the deleterious effects of some general anesthetics on hippocampal new cell proliferation (Erasso et al., 2012) and neurogenesis at the extremes of ages. A particular point to take into consideration is that our present study focuses on the cells born previous to anesthesia, rather than on the cells born post-anesthesia, possibly accounting for some contradictory results of other groups that found no difference in new cell proliferation, differentiation or neuronal maturation in the DG of sixteen month-old rats (Stratmann et al., 2010).

The decrease in the number of 21-day-old maturing neurons at the time of isoflurane anesthesia in this study suggests that this decrease in the number of neurons that are integrating within the DG may have an effect on cognitive function. A number of studies examining the role of the DG on learning and memory formation (Kempermann, 2002a; Leuner et al., 2006; Nakashiba et al., 2008) suggest that when neurogenesis in the hippocampus decreases, spatial memory is impaired (Clelland et al., 2009). Thus, supporting the idea that maturing DG neurons are important to learning and memory (Deng et al., 2009; Jessberger et al., 2009) and that neurogenesis is a required determinant of dentate gyrus-dependent information processing and memory. Therefore, it seems reasonable to infer from our results that the isoflurane specific decrease in the number of maturing neurons in the DG of aged rats could result in later cognitive impairment.

We also found an increase in the number of 21-day-old astrocytes with isoflurane anesthesia in aged rats as assessed by S100β, a calcium binding protein localized and secreted by astrocytes (Donato, 1999; Donato et al., 2013; Schafer and Heizmann, 1996). In small concentrations, S100β acts as a growth factor for neurons and glia, but at high concentrations it may have deleterious effects and may induce apoptosis due to an increase in proinflammatory cytokines expression (Rothermundt et al., 2003; Steiner et al., 2007). Accordingly, S100β has been used as a neurodegenerative biomarker (Cata et al., 2011; Steiner et al., 2011) and an increase in S100β label in our present study suggest an activation of astrocytes by a

promotion of the glial cell fate, proposing a low level of inflammation in the DG as a result of anesthesia. Recent laboratory studies have examined anesthetic induced neuroinflammation and have shown that clinically relevant concentrations of isoflurane induce increased levels of the proinflammatory cytokines (TNF- α, IL-6, and IL-1β) in vitro and in the brains of mice in vivo (Wu et al., 2010). Moreover, animal studies have shown that inflammatory cytokines are involved in cognitive impairment (Wan et al., 2007). Consequently, it is possible that an anesthetic-induced inflammatory response may also contribute to the development of cognitive impairment (Xie et al., 2009). However, the effects of anesthesia on astrogliosis in the DG have not been sufficiently investigated, and it is unclear whether increased astrogliosis plays a role in neuronal loss and/or the cognitive impairment following anesthesia. Consequently, more studies assessing the effects of anesthetics on astrogliosis are needed.

3.3. *Propofol anesthesia specifically affected 4 and 8-day-old immature neurons and astrocytes in the DG of young rats*

Four and eight-day old nascent cells in the DG are differentiating into neurons or astrocytes (Duan et al., 2008; Ming and Song, 2005). Therefore, our findings that propofol decreased the number of 4 and 8 day old neurons and increased the number of astrocytes in the young rat brain; similar to the previously discussed isoflurane data on the aged rat brain, suggests not only that this may produce an effect on learning/memory, but also that an inflammatory response may be taking place. Although, using animals at different ages and different anesthetic agents, similar studies looking at the anesthetic effects on learning and memory from the behavioral (Jevtovic-Todorovic et al., 2003) and cellular point of view (Li et al., 2007; Stratmann et al., 2009) have reported deficits in learning and memory after exposure to anesthesia.

3.4. *Isoflurane and propofol anesthesia affected nascent cells undergoing development in an age- and agent-dependent manner*

In this study, isoflurane specifically affected the aged rat brain, whereas propofol specifically affected the young rat brain suggesting an age and agent dependent effect. A large body of clinical and laboratory data indicate that hippocampal function is higher during younger years and gradually declines with age (Plassman et al., 2008). During young development, the brain is undergoing different brain process such as neurogenesis at a higher rate possibly becoming vulnerable to anesthetic agents. Then, with advancing age, the proliferative activity of hippocampal neural stem cells and neuronal differentiation capacity decline, leading to a dramatic, approximately ten-fold, reduction in neurogenesis between the age of 2–24 months in a rodent's life (Gage, 2002; Kuhn et al., 1996; Shruster et al., 2010; van Praag et al., 2005). Interestingly, at least in some studies, the reduction of neurogenesis appears to be correlated with age-associated cognitive deficits (Bizon and Gallagher, 2003; Bizon et al., 2004; Lazarov et al., 2010). Consequently, the young and aged brain may be more susceptible to insults that would go unnoticed at an adult age and may both be more vulnerable to anesthetics (Culley et al., 2007).

Additionally, the present study suggests that isoflurane and propofol have different effects, which may be attributed to different molecular mechanism of intracellular calcium release of both anesthetics. A recent in vitro study, on human neuroprogenitor cell exposure to isoflurane demonstrated the crucial role of differential regulation of intracellular calcium on the promotion or inhibition of neurogenesis (Zhao et al., 2013). Zhao et al. particularly found that 24-h exposure to isoflurane inhibits neuronal cell fate, but stimulates glial cell fate by excessive intracellular calcium release resulting in abnormally elevated cytosolic calcium concentrations. Neuroprogenitor cells are regulated by GABA and intracellular calcium transport so that calcium signal triggers the immediate early genes responsible for stimulating resting cells to re-enter the cell cycle, promotes DNA synthesis initiation and contribute to cell cycle completion (Ben-Ari, 2002; Berridge, 1995; Ge et al., 2007; LoTurco et al., 1995). Thus, it seems reasonable to speculate that isoflurane and propofol may affect intracellular calcium levels differently consequently affecting neurogenesis also differently. Nevertheless, more studies on this mechanism are needed in order to make an accurate conclusion.

In summary, our experiments showed an age and agent dependent effect of isoflurane and propofol on nascent cells in the DG of young and aged rats. Specifically, isoflurane affected 21-day-old maturing neurons and astrocytes in the DG of aged rats, whereas propofol affected 4 and 8-day-old differentiating neurons and astrocytes in the DG of young rats. These results suggest a possible mechanism for the impairment of cognitive function reported after exposure to anesthetics. Clearly, questions regarding the exact mechanism of anesthetic induced neuronal loss and astrocytes proliferation remain to be elucidated

4. Experimental procedure

4.1. *Animals*

Young (3 month-old) and aged (20 month-old) Male Fisher 344 (F344) rats (Harlan, Indianapolis, IN) were used in this study. Rats were pair-housed in environmentally controlled conditions (12:12 h light: dark cycle at 21±1 °C) and provided food and water ad lib. This study was conducted in accordance with the National Institute of Health Guide and Use of Laboratory Animals, and was approved by the Institutional Animal Care and Use committee of the University of South Florida, College of Medicine.

4.2. *Experimental design*

Three different populations of proliferating cells in the DG were labeled with three different thymidine analogs (EdU, IdU, and CldU) that were intraperitoneally injected into all animals at 4, 8, and 21 days, respectively, prior to the anesthetic or control exposure (Fig. 5). The effect of general anesthesia on differentiation and maturation of these cells

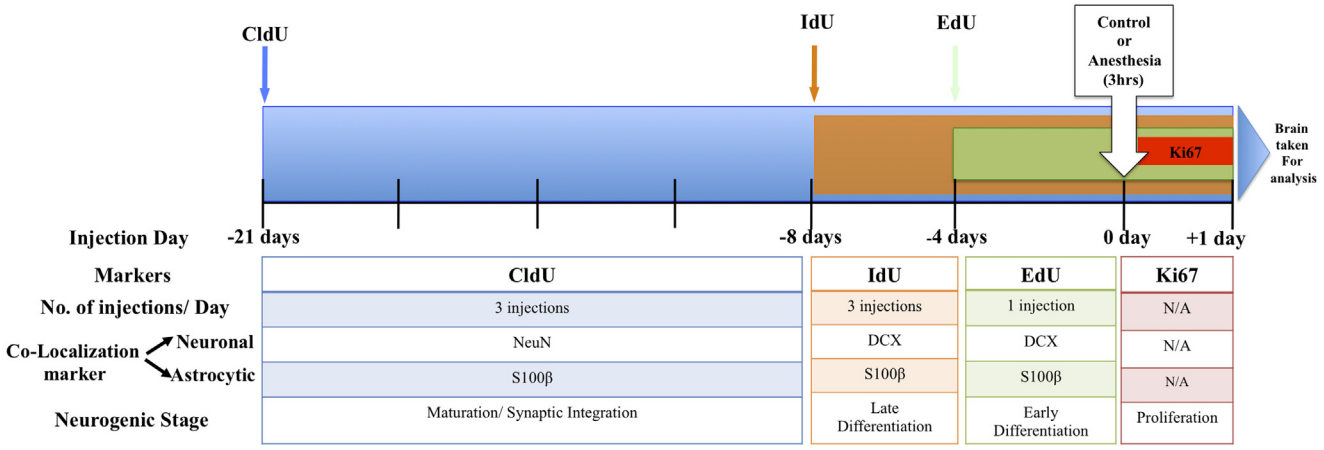

Fig. 5 – Three different thymidine analogs and one endogenous marker were used to assess the effects of isoflurane or propofol on each stage of new cell development in the DG of young and aged rats. Rats in all groups ($n=8$ per group) were injected 21, 8, and 4 days before control or anesthesia exposure, with CldU, IdU and EdU, respectively. Immunohistochemically labeled cells in brain sections were co-localized with the following antibodies: DCX (doublecortin), NeuN or S100β in order to detect immature neurons (EdU/DCX, IdU/DCX), mature neurons (CldU/NeuN) and astrocytes (EdU/S100β, IdU/ S100β, CldU/ S100β). The endogenous marker Ki67 was used to identified new cell proliferation.

was assessed by quantifying the number of cells co-localized with labels of neuronal differentiation and maturation with each of the thymidine analogs injected. In addition, the number of thymidine-positive cells co-localized with an astrocyte-specific (S100β) label was assessed. The effect of anesthesia on cell proliferation was assessed with the endogenous cell proliferation marker anti-Ki67 (Scholzen and Gerdes, 2000), an endogenous nuclear protein that is expressed in all actively dividing cells (Scholzen and Gerdes, 2000).

4.3. *Preparation and administration of thymidine analogs*

Solutions of 5-Chloro-2′-deoxyuridine (CldU; Sigma #C6891 at 17 mg/ml) and 5-Iodo-2′-Deoxyuridine (IdU; MP#100357 at 23 mg/ml) were prepared in sterile saline and administered by intraperitoneal (IP) injection (Vega and Peterson, 2005). Because cell division is a rare event in the subgranular zone of the DG of aged rats, and because many of the nascent cells do not survive (Cameron and McKay, 2001; Kempermann, 2002b), we were concerned that one injection of thymidine analog would not yield a sufficient number of labeled nascent cells for a meaningful analysis. Therefore, all animals received three IP injections of CldU and IdU administered at 4-h intervals, as shown in Fig. 1. Animals in all groups were injected with CldU (42.5 mg/Kg) 21-days before, and IdU (57.5 mg/Kg) 8-days before anesthesia or control exposure. A solution of 5-ethynyl-2′-deoxyuridine (EdU; cat# E10187, Invitrogen, Carlsbad, CA) was prepared at a concentration of 60 mg/ml in sterile saline. Animals in all groups received a single IP injection of EdU (160 mg/Kg) 4 days before anesthesia or control exposure.

4.4. *General anesthesia*

4.4.1. *Isoflurane*

One cohort of rats was randomly assigned to one of four different groups ($n=8$/group): young rats and aged rats exposed to 1.5% isoflurane delivered in 2 L/min O_2 via nose cone for 3 h, and young rats and aged rats exposed to a mixture of air and 2 L/min O_2 in their home cage for 3 h (control for isoflurane). Isoflurane (Forane, Ohmeda Caribe, NJ, USA) was delivered via a standard anesthesia vaporizer.

4.4.2. *Propofol*

A second cohort of rats was randomly assigned to four different groups ($n=8$/group): young rats and aged rats that received 35 mg/kg/h of propofol and young rats and aged rats that received 10% intralipid (control for propofol). Unanesthetized rats were placed in a restrainer, and a tail vein catheter attached to a syringe pump inserted. Propofol (Disoprivan; AstraZeneca) or 10% intralipid (Fresenius Kabi; Sweden), which served as the vehicle control for propofol (Diprivan Inc, 2004), was continuously administered using a syringe pump (model 11 Plus; Harvard Apparatus) for 3 h via the tail vein catheter. Rats receiving intralipid remained partially restrained for 3 h.

4.5. *Physiological measurements during anesthesia*

Rectal temperature of anesthetized rats was maintained at 37 °C with a thermostatically controlled heating pad and monitored using a thermalet-monitoring thermometer (Physitemp instrument Inc, Clifton, NJ, USA). Hemoglobin oxygen saturation (SpO_2) and heart rate (HR) of anesthetized rats were recorded during anesthesia using the SurgiVet multi parameter monitor (Smiths medical, Dublin, OH, USA). Diastolic, systolic and mean arterial pressures of anesthetized rats were measured every 30 min throughout the anesthesia period by tail cuff using the CODA non-invasive blood pressure system for rats (CODA2, Kent Scientific Corporation). Similar physiological measurements were not made on control animals. All physiological measurements of anesthetized rats remained within normal physiological limits (Konze, 2007). Following anesthesia, rats were returned their cages and monitored until they were fully alert, ambulatory and showed no signs of discomfort.

4.6. Tissue collection and processing

Twenty-four hours after anesthesia or control exposure, rats were deeply anesthetized with pentobarbital (50 mg/Kg, IP) and transcardially perfused with saline followed by ice-cold 4% paraformaldehyde in 0.1 M PBS. Brains were removed, postfixed in the same fixative solution at 4 °C overnight, transferred to 20% sucrose in PBS until equilibrated, and frozen with dry ice. Sagittal cryosections (30 μm) through the entire DG were collected serially.

4.7. Immunohistochemistry and labeling protocol

For Ki67 immunohistochemistry, sections were pretreated with 1X Saline-Sodium Citrate (SSC) at 80 °C, and endogenous peroxidase activity quenched with 0.6% H_2O_2 solution in PBS. Sections were blocked in 2% normal goat serum and 0.25% Triton X-100 in PBS (PBS-TS), and incubated overnight at 4 °C with a rabbit polyclonal antibody against human Ki67 (NCL-Ki67p; Novocastra Laboratories/Vision BioSystems, Newcastle upon Tyne, UK) at a dilution of 1:2000 in PBS-TS. The following day, sections were washed in PBS, incubated in biotinylated secondary antibody (goat anti-rabbit IgG rat adsorbed 1:1000; BA-1000 Vector Laboratories, Burlingame, CA) in PBS-TS, and washed in PBS before incubation in avidin–biotin substrate (ABC kit cat no. PK-6100, Vector Laboratories, Burlingame, CA). Sections were then washed in PBS, and reacted with 3,3′-diaminobenzidine tetrahydrochloride (DAB) solution (cat no.1856090, Thermo Scientific, Rockford, IL).

For CldU and IdU immunohistochemistry, sections were washed three times for 10 min in PBS before being treated with 0.2 N HCL at 37 °C. Sections were then washed with borate buffer (pH 8.5), followed by three washes of PBS, incubated in 5% normal goat serum and 0.25% Triton X-100 in PBS (PBS-TS), and for 24 h at 4 °C free-floating sections were exposed to rat anti-BrdU (Accurate cat# OBT-0030; clone BU1/75) at 1:250 for CldU and to mouse anti-BrdU (Becton Dickinson cat# 347580; clone B44) at 1:500 for IdU (Vega and Peterson, 2005). All secondary antibodies were used at 1:300 and conjugated to Alexa 594 or Alexa 488 fluorophore.

EdU labeling (Zeng et al., 2010) was performed using Click-iT™ EdU imaging kit (cat# C10339, Invitrogen, Carlsbad, CA). The manufacturer's protocol, which is normally intended for use in cell culture, was adapted for free-floating brain tissue sections. Sections were washed three times in PBS, twice with 3% bovine serum albumin (BSA) in PBS, permeabilized with 0.5% Triton X-100 in PBS, washed twice with 3% BSA in PBS and incubated with the Click-iT™ reaction cocktail (CuSO4, Alexa Fluor 594 Azide, and manufacture's reaction buffer additive). Sections were then washed once more with 3% BSA in PBS, mounted and coverslipped with Vectashield mounting medium (cat# H-1000 Vector Laboratories, Burlingame, CA, USA).

4.8. Thymidine analog co-localization with histological markers

The phenotypes of CldU, IdU and EdU labeled cells were determined using double immunofluorescent staining. Antibodies against doublecortin (DCX), neuronal nuclei (NeuN) and calcium binding protein S100β were used to detect immature neurons, mature neurons and astrocytes, respectively. Following treatment with HCL for thymidine analog immunohistochemistry, an antibody cocktail of polyclonal antibody raised against human DCX (1:200, cat# 4604 Cell Signaling technology, Inc. Danvers, MA, USA) and IdU antibody was used to assess whether IdU^+ cells were immature neurons. Similarly, a monoclonal antibody against NeuN (MAB377 Millipore, Billerica, MA, USA) was used at a concentration of 1:500 in a cocktail with the CldU antibody to assess whether CldU+ cells were mature neurons, and a monoclonal antibody raised against S100β (ab52642 abcam, Cambridge, MA, USA) was used at a concentration of 1:3000 in an antibody cocktail with CldU or IdU to assess whether CldU+ or IdU^+ were astrocytes. For double labeling of EdU/DCX and EdU/S100β, EdU staining was performed first, followed by incubation with DCX or S100β.

4.9. Microscopy and cell counting

A modified unbiased stereology method was used to estimate the number of positive labeled cells in the DG for each thymidine analog and ki-67 marker (Mouton, 2002; Schmitz and Hof, 2005). Twelve sections spaced 360 μm apart throughout the entire medial-lateral extent of the DG were collected per animal. Because labeled cells in the DG are a rare event, all positively labeled cells contained within the DG were counted in each of the twelve sections per animal. The number of labeled cells in each DG examined was summed for individual animals and the sum from each animal multiplied by the sections spacing to estimate the total number positive cells in the DG (Mouton, 2002). Sections were imaged on an Olympus FV1000 laser-scanning microscope with a 40X objective (1330X final magnification), and Z stacks were created at 0.5 μm intervals throughout the 30 μm section with a guard region of 2 μm excluded from top and bottom to confirm that cells were double labeled.

4.10. Statistical analyses

All data are presented as mean ± SEM and analyzed using a one-way Analysis of variance (ANOVA) followed by a Bonferroni *post-hoc* test. Probability values less than 0.05 were considered statistically significant. Statistical comparison of the data was performed using GraphPad Prism version 5.00 for Mac (GraphPad Software, San Diego California USA, http://www.graphpad.com).

REFERENCES

Abildstrom, H., Rasmussen, L.S., Rentowl, P., Hanning, C.D., Rasmussen, H., Kristensen, P.A., Moller, J.T., 2000. Cognitive dysfunction 1-2 years after non-cardiac surgery in the elderly. ISPOCD group. International study of post-operative cognitive dysfunction. Acta Anaesthesiol. Scand. 44, 1246–1251.

Altman, J., Das, G.D., 1965. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J. Comp. Neurol. 124, 319–335.

Ben-Ari, Y., 2002. Excitatory actions of gaba during development: the nature of the nurture. Nature reviews. Neuroscience 3, 728–739.

Berridge, M.J., 1995. Calcium signalling and cell proliferation. BioEssays: News Rev Mol Cell Dev Biol 17, 491–500.
Bizon, J.L., Gallagher, M., 2003. Production of new cells in the rat dentate gyrus over the lifespan: relation to cognitive decline. Eur. J. Neurosci. 18, 215–219.
Bizon, J.L., Lee, H.J., Gallagher, M., 2004. Neurogenesis in a rat model of age-related cognitive decline. Aging Cell 3, 227–234.
Bonaguidi, M.A., Song, J., Ming, G.L., Song, H., 2012. A unifying hypothesis on mammalian neural stem cell properties in the adult hippocampus. Curr. Opin. Neurobiol. 22, 754–761.
Broadbent, N.J., Squire, L.R., Clark, R.E., 2004. Spatial memory, recognition memory, and the hippocampus. Proc. Natl. Acad. Sci. USA 101, 14515–14520.
Cameron, H.A., McKay, R.D., 2001. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J. Comp. Neurol. 435, 406–417.
Canet, J., Raeder, J., Rasmussen, L.S., Enlund, M., Kuipers, H.M., Hanning, C.D., Jolles, J., Korttila, K., Siersma, V.D., Dodds, C., Abildstrom, H., Sneyd, J.R., Vila, P., Johnson, T., Munoz Corsini, L., Silverstein, J.H., Nielsen, I.K., Moller, J.T., 2003. Cognitive dysfunction after minor surgery in the elderly. Acta Anaesthesiol. Scand. 47, 1204–1210.
Cata, J.P., Abdelmalak, B., Farag, E., 2011. Neurological biomarkers in the perioperative period. Br. J. Anaesth. 107, 844–858.
Clelland, C.D., Choi, M., Romberg, C., Clemenson Jr., G.D., Fragniere, A., Tyers, P., Jessberger, S., Saksida, L.M., Barker, R. A., Gage, F.H., Bussey, T.J., 2009. A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science 325, 210–213.
Culley, D.J., Baxter, M., Yukhananov, R., Crosby, G., 2003. The memory effects of general anesthesia persist for weeks in young and aged rats. Anesth. Analg. 96, 1004–1009 (table of contents).
Culley, D.J., Baxter, M.G., Crosby, C.A., Yukhananov, R., Crosby, G., 2004. Impaired acquisition of spatial memory 2 weeks after isoflurane and isoflurane–nitrous oxide anesthesia in aged rats. Anesth. Analg. 99, 1393–1397 (table of contents).
Culley, D.J., Xie, Z., Crosby, G., 2007. General anesthetic-induced neurotoxicity: an emerging problem for the young and old?. Curr. Opin. Anaesthesiol. 20, 408–413.
Culley, D.J., Boyd, J.D., Palanisamy, A., Xie, Z., Kojima, K., Vacanti, C.A., Tanzi, R.E., Crosby, G., 2011. Isoflurane decreases self-renewal capacity of rat cultured neural stem cells. Anesthesiology 115, 754–763.
Deng, W., Saxe, M.D., Gallina, I.S., Gage, F.H., 2009. Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. J. Neuroscience: Off. J. Soc. Neurosci. 29, 13532–13542.
Donato, R., 1999. Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. Biochim. Biophys. Acta 1450, 191–231.
Donato, R., Cannon, B.R., Sorci, G., Riuzzi, F., Hsu, K., Weber, D.J., Geczy, C.L., 2013. Functions of S100 proteins. Curr. Mol. Med. 13, 24–57.
Diprivan Inc, A.C., 2004. DIPRIVAN® 1% w/v propofol injection 10 mg/mL.
Dupret, D., Revest, J.M., Koehl, M., Ichas, F., De Giorgi, F., Costet, P., Abrous, D.N., Piazza, P.V., 2008. Spatial relational memory requires hippocampal adult neurogenesis. PLoS One 3, e1959.
Duan, X., Kang, E., Liu, C.Y., Ming, G.L., Song, H., 2008. Development of neural stem cell in the adult brain. Curr. Opin. Neurobiol. 18, 108–115.
Erasso, D.M., Chaparro, R.E., Quiroga Del Rio, C.E., Karlnoski, R., Camporesi, E.M., Saporta, S., 2012. Quantitative assessment of new cell proliferation in the dentate gyrus and learning after isoflurane or propofol anesthesia in young and aged rats. Brain Res. 1441, 38–46.
Galvan, V., Jin, K., 2007. Neurogenesis in the aging brain. Clin. Interventions Aging 2, 605–610.
Gage, F.H., 2002. Neurogenesis in the adult brain. J Neurosci. 22, 612–613.
Ge, S., Pradhan, D.A., Ming, G.L., Song, H., 2007. GABA sets the tempo for activity-dependent adult neurogenesis. Trends Neurosci. 30, 1–8.
Jessberger, S., Clark, R.E., Broadbent, N.J., Clemenson Jr., G.D., Consiglio, A., Lie, D.C., Squire, L.R., Gage, F.H., 2009. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. Learn. Mem. 16, 147–154.
Jevtovic-Todorovic, V., Hartman, R.E., Izumi, Y., Benshoff, N.D., Dikranian, K., Zorumski, C.F., Olney, J.W., Wozniak, D.F., 2003. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci. 23, 876–882.
Johnson, T., Monk, T., Rasmussen, L.S., Abildstrom, H., Houx, P., Korttila, K., Kuipers, H.M., Hanning, C.D., Siersma, V.D., Kristensen, D., Canet, J., Ibanaz, M.T., Moller, J.T., 2002. Postoperative cognitive dysfunction in middle-aged patients. Anesthesiology 96, 1351–1357.
Kempermann, G., Gage, F.H., 2000. Neurogenesis in the adult hippocampus. In: Proceedings of the Novartis Foundation Symposium, vol. 231, pp. 220–235. (Discussion 235–241, 302–306).
Kempermann, G., 2002. Why new neurons? Possible functions for adult hippocampal neurogenesis. J. Neurosci.: Off. J. Soc. Neurosci. 22, 635–638.
Kempermann, G., 2002a. Why new neurons? Possible functions for adult hippocampal neurogenesis. J. Neurosci. 22, 635–638.
Kempermann, G., 2002b. Neuronal stem cells and adult neurogenesis. Ernst Schering Res. Found. Workshop, 17–28.
Kempermann, G., Gast, D., Gage, F.H., 2002. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann. Neurol. 52, 135–143.
Kempermann, G., Jessberger, S., Steiner, B., Kronenberg, G., 2004. Milestones of neuronal development in the adult hippocampus. Trends Neurosci. 27, 447–452.
Konze, A., 2007. Humane Endpoints (in laboratory animal experimentation).
Kuhn, H.G., Dickinson-Anson, H., Gage, F.H., 1996. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J. Neurosci. 16, 2027–2033.
Lazarov, O., Mattson, M.P., Peterson, D.A., Pimplikar, S.W., van Praag, H., 2010. When neurogenesis encounters aging and disease. Trends Neurosci. 33, 569–579.
Leuner, B., Gould, E., Shors, T.J., 2006. Is there a link between adult neurogenesis and learning?. Hippocampus 16, 216–224.
Li, Y., Liang, G., Wang, S., Meng, Q., Wang, Q., Wei, H., 2007. Effects of fetal exposure to isoflurane on postnatal memory and learning in rats. Neuropharmacol. 53, 942–950.
Liang, G., Wang, Q., Li, Y., Kang, B., Eckenhoff, M.F., Eckenhoff, R. G., Wei, H., 2008. A presenilin-1 mutation renders neurons vulnerable to isoflurane toxicity. Anesth. Analg. 106, 492–500 (table of contents).
Loepke, A.W., Soriano, S.G., 2008. An assessment of the effects of general anesthetics on developing brain structure and neurocognitive function. Anesth. Analg. 106, 1681–1707.
LoTurco, J.J., Owens, D.F., Heath, M.J., Davis, M.B., Kriegstein, A.R., 1995. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron 15, 1287–1298.
Ming, G.L., Song, H., 2005. Adult neurogenesis in the mammalian central nervous system. Annu. Rev. Neurosci. 28, 223–250.
Moller, J.T., Cluitmans, P., Rasmussen, L.S., Houx, P., Rasmussen, H., Canet, J., Rabbitt, P., Jolles, J., Larsen, K., Hanning, C.D., Langeron, O., Johnson, T., Lauven, P.M., Kristensen, P.A., Biedler, A., van Beem, H., Fraidakis, O., Silverstein, J.H.,

Beneken, H., Gravenstein, J.S., 1998. Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. International Study of Post-Operative Cognitive Dysfunction. Lancet 351, 857–861.
Monk, T.G., Weldon, B.C., Garvan, C.W., Dede, D.E., van der Aa, M. T., Heilman, K.M., Gravenstein, J.S., 2008. Predictors of cognitive dysfunction after major noncardiac surgery. Anesthesiology 108, 18–30.
Moscovitch, M., Rosenbaum, R.S., Gilboa, A., Addis, D.R., Westmacott, R., Grady, C., McAndrews, M.P., Levine, B., Black, S., Winocur, G., Nadel, L., 2005. Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. J. Anat. 207, 35–66.
Mouton, P.R., 2002. In: Principles and Practices of Unbiased Stereology: An Introduction for Bioscientistss. The Johns Hopkins University Press, Baltimore, Maryland.
Nakashiba, T., Young, J.Z., McHugh, T.J., Buhl, D.L., Tonegawa, S., 2008. Transgenic inhibition of synaptic transmission reveals role of CA3 output in hippocampal learning. Science 319, 1260–1264.
Palmer, T.D., Willhoite, A.R., Gage, F.H., 2000. Vascular niche for adult hippocampal neurogenesis. J. Comp. Neurol. 425, 479–494.
Piatti, V.C., Esposito, M.S., Schinder, A.F., 2006. The timing of neuronal development in adult hippocampal neurogenesis. Neurosci.: Rev. J. Bringing Neurobiol. Neurol. Psychiatry 12, 463–468.
Pignatelli, A., Belluzzi, O., 2010. Neurogenesis in the Adult Olfactory Bulb. In: Menini, A. (Ed.), The Neurobiology of Olfaction. Frontiers in Neuroscience.
Plassman, B.L., Langa, K.M., Fisher, G.G., Heeringa, S.G., Weir, D.R., Ofstedal, M.B., Burke, J.R., Hurd, M.D., Potter, G.G., Rodgers, W. L., Steffens, D.C., McArdle, J.J., Willis, R.J., Wallace, R.B., 2008. Prevalence of cognitive impairment without dementia in the United States. Ann. Intern. Med. 148, 427–434.
Popic, J., Pesic, V., Milanovic, D., Todorovic, S., Kanazir, S., Jevtovic-Todorovic, V., Ruzdijic, S., 2012. Propofol-induced changes in neurotrophic signaling in the developing nervous system in vivo. PLoS One 7, e34396.
Rothermundt, M., Peters, M., Prehn, J.H., Arolt, V., 2003. S100B in brain damage and neurodegeneration. Microsc. Res. Tech. 60, 614–632.
Sall, J.W., Stratmann, G., Leong, J., McKleroy, W., Mason, D., Shenoy, S., Pleasure, S.J., Bickler, P.E., 2009. Isoflurane inhibits growth but does not cause cell death in hippocampal neural precursor cells grown in culture. Anesthesiology 110, 826–833.
Schmitz, C., Hof, P.R., 2005. Design-based stereology in neuroscience. Neuroscience 130, 813–831.
Schafer, B.W., Heizmann, C.W., 1996. The S100 family of EF-hand calcium-binding proteins: functions and pathology. Trends Biochem. Sci. 21, 134–140.
Scholzen, T., Gerdes, J., 2000. The Ki-67 protein: from the known and the unknown. J. Cell. Physiol. 182, 311–322.
Seki, T., Arai, Y., 1995. Age-related production of new granule cells in the adult dentate gyrus. Neuroreport 6, 2479–2482.
Shruster, A., Melamed, E., Offen, D., 2010. Neurogenesis in the aged and neurodegenerative brain. Apoptosis 15 (11), 1415–1421.
Steiner, J., Bernstein, H.G., Bielau, H., Berndt, A., Brisch, R., Mawrin, C., Keilhoff, G., Bogerts, B., 2007. Evidence for a wide extra-astrocytic distribution of S100B in human brain. BMC Neurosci. 8, 2.
Steiner, J., Bogerts, B., Schroeter, M.L., Bernstein, H.G., 2011. S100B protein in neurodegenerative disorders. Clin. Chem. Lab. Med.: CCLM/FESCC 49, 409–424.
Stratmann, G., Sall, J.W., May, L.D., Bell, J.S., Magnusson, K.R., Rau, V., Visrodia, K.H., Alvi, R.S., Ku, B., Lee, M.T., Dai, R., 2009. Isoflurane differentially affects neurogenesis and long-term neurocognitive function in 60-day-old and 7-day-old rats. Anesthesiology 110, 834–848.
Stratmann, G., Sall, J.W., Bell, J.S., Alvi, R.S., May, L.V., Ku, B., Dowlatshahi, M., Dai, R., Bickler, P.E., Russell, I., Lee, M.T., Hrubos, M.W., Chiu, C., 2010. Isoflurane does not affect brain cell death, hippocampal neurogenesis, or long-term neurocognitive outcome in aged rats. Anesthesiology 112, 305–315.
Sun, L., 2010. Early childhood general anaesthesia exposure and neurocognitive development. Br. J. Anaesth. 105, I61–I68.
Sultan, S., Mandairon, N., Kermen, F., Garcia, S., Sacquet, J., Didier, A., 2010. Learning-dependent neurogenesis in the olfactory bulb determines long-term olfactory memory. FASEB J.: Off. Publ. Fed. Am. Soc. Exp. Biol. 24, 2355–2363.
Tung, A., Herrera, S., Fornal, C.A., Jacobs, B.L., 2008. The effect of prolonged anesthesia with isoflurane, propofol, dexmedetomidine, or ketamine on neural cell proliferation in the adult rat. Anesth. Analg. 106, 1772–1777.
van Praag, H., Shubert, T., Zhao, C., Gage, F.H., 2005. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci. 25, 8680–8685.
Vega, C.J., Peterson, D.A., 2005. Stem cell proliferative history in tissue revealed by temporal halogenated thymidine analog discrimination. Nat. Methods 2, 167–169.
Wan, Y., Xu, J., Ma, D., Zeng, Y., Cibelli, M., Maze, M., 2007. Postoperative impairment of cognitive function in rats: a possible role for cytokine-mediated inflammation in the hippocampus. Anesthesiology 106, 436–443.
Wu, X., Lu, Y., Dong, Y., Zhang, G., Zhang, Y., Xu, Z., Culley, D.J., Crosby, G., Marcantonio, E.R., Tanzi, R.E., Xie, Z., 2010. The inhalation anesthetic isoflurane increases levels of proinflammatory TNF-alpha, IL-6, and IL-1beta. Neurobiol. Aging.
Xie, G., Zhang, W., Chang, Y., Chu, Q., 2009. Relationship between perioperative inflammatory response and postoperative cognitive dysfunction in the elderly. Med. Hypotheses 73, 402–403.
Xie, Z., Dong, Y., Maeda, U., Moir, R.D., Xia, W., Culley, D.J., Crosby, G., Tanzi, R.E., 2007. The inhalation anesthetic isoflurane induces a vicious cycle of apoptosis and amyloid beta-protein accumulation. J. Neurosci. 27, 1247–1254.
Xie, Z., Xu, Z., 2012. General anesthetics and beta-amyloid protein. Prog Neuropsychopharmacol Biol Psychiatry.
Yu, D., Jiang, Y., Gao, J., Liu, B., Chen, P., 2013. Repeated exposure to propofol potentiates neuroapoptosis and long-term behavioral deficits in neonatal rats. Neurosci. Lett. 534, 41–46.
Zeng, C., Pan, F., Jones, L.A., Lim, M.M., Griffin, E.A., Sheline, Y.I., Mintun, M.A., Holtzman, D.M., Mach, R.H., 2010. Evaluation of 5-ethynyl-2′-deoxyuridine staining as a sensitive and reliable method for studying cell proliferation in the adult nervous system. Brain Res. 1319, 21–32.
Zhao, Y., Liang, G., Chen, Q., Joseph, D.J., Meng, Q., Eckenhoff, R. G., Eckenhoff, M.F., Wei, H., 2010. Anesthetic-induced neurodegeneration mediated via inositol 1,4,5-trisphosphate receptors. J. Pharmacol. Exp. Ther. 333, 14–22.
Zhao, X., Yang, Z., Lianq, G., Wu, Z., Peng, Y., Joseph, DJ., Inan, S., Wei, H., 2013. Dual effects of isoflurane on proliferation, differentiation, and survival in human neuroprogenitor cells. Anesthesiology 118, 537–549.
Zhu, C., Gao, J., Karlsson, N., Li, Q., Zhang, Y., Huang, Z., Li, H., Kuhn, H.G., Blomgren, K., 2010. Isoflurane anesthesia induced persistent, progressive memory impairment, caused a loss of neural stem cells, and reduced neurogenesis in young, but not adult, rodents. J. Cereb. Blood Flow Metab. 30, 1017–1030.