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Isoflurane Exposure did not Adversely Affect Recognition Memory or Decrease Hippocampal Brain Derived Neurotrophic Factor Expression in the 17 Day Old Rat Pup

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Abstract

A previous study showed that hippocampal BDNF mRNA decreased in 17 day old (D17) rats, relative to age-matched naïve rats, at day 1, 3, 7 and 14 after sham surgery in a traumatic brain injury model. The anesthetic isoflurane activates GABA and inhibits NMDA receptor currents, both of which are known to decrease Brain-Derived Neurotrophic Factor (BDNF) mRNA in rat hippocampi. Hippocampal BDNF is necessary for normal cognitive function. Effects of isoflurane alone on hippocampal BDNF are not known. We hypothesized that, in D17 rat pups, isoflurane would decrease hippocampal mRNA/protein levels of BDNF and Synapsin I (a downstream target of BDNF important for cognitive function) and impair performance on the Novel Object Recognition Test (NOR). ISOF BDNF and Synapsin I mRNA decreased relative to Naïve at day 1 and 8 after exposure, but not at day 14. Isoflurane exposure did not decrease hippocampal protein levels of BDNF or Synapsin I and did not impair NOR performance. In contrast to the neonatal rat pup, anesthetic exposure did not impair cognitive function. We speculate that adverse effects of anesthetics on rat pup cognitive function and BDNF expression are highly dependent on age at exposure.

Keywords: Anesthesia; Development; Cognition; Sham surger

Introduction

Sham surgery for traumatic brain injury decreased hippocampal Brain Derived Neurotrophic Factor (BDNF) mRNA in 17 day old (D17) male rats, relative to age and gender-matched Naïves, at days 1, 3, 7 and 14 after sham surgery [1]. In contrast, mRNA levels of other neurotrophins did not differ between Naïve and Sham rats. Sham surgery involved a craniotomy, maternal separation and exposure to the anesthetic, isoflurane. The effects of isoflurane alone on hippocampal BDNF expression are not known. Given the importance of BDNF in normal learning and memory, and the growing concern that anesthetic exposure during brain development may adversely affect cognitive function, we asked whether isoflurane exposure alone could decrease hippocampal BDNF expression and impair cognitive function in the D17 rat.

Endogenous hippocampal BDNF is required for normal cognitive function [2,3]. Hippocampus-dependent learning was associated with selective induction of hippocampal BDNF mRNA [4] while inhibition of BDNF expression impaired learning and memory in rodents [5,6]. BDNF regulates Synapsin I, a regulator of synaptic transmission [7-9]. Interference with BDNF action impairs normal learning and memory, in part by disrupting synaptic plasticity [10,11].

Hippocampal BDNF expression decreased in response to agents that enhance gamma aminobutyric acid (GABA) activity or antagonize the n-methyl-d-aspartate receptor (NMDAR) [12,13]. Since isoflurane both enhances GABA activity and inhibits the NMDAR, [14,15] isoflurane exposure alone may decrease hippocampal BDNF expression.

Experimental and clinical data suggest that exposure to anesthetics during development adversely affects cognitive function. D7 Rats exposed to anesthesia developed neuronal death [16,17] and learning and memory impairments [18]. Epidemiological studies suggest that repeated exposure to anesthesia during childhood is an independent risk factor for acquiring learning disabilities [19,20].

We hypothesized that isoflurane exposure at D17 would decrease rat hippocampal BDNF and Synapsin I mRNA and protein levels on post exposure day (PED) 1, 8 and 14, and that repeated isoflurane exposures would impair performance on the Novel Object Recognition (NOR) test, a test of recognition memory, at PED8.

Materials and Methods

Animal procedures and tissues

Experimental protocols were approved by the Animal Care and Use Committee at the University of Utah, in accordance with US NIH guidelines. Male Sprague—Dawley rats were obtained from Charles Rivers Laboratories (Raleigh, NC) on D7-10 of life. Littermates were randomized to generate 6-7 rats per group per time point. All rats were allowed free access to food and water, and kept in a temperature- and light-controlled environment.

17 day old rat littermates were exposed to 2.5% isoflurane for 45 minutes and allowed to recover for 15 minutes (ISOF), or were removed from their dams for 60 minutes (Naive) to match the duration of anesthesia exposure and maternal separation experienced by Sham rats. Recovery and induction chambers were actively warmed; rectal temperatures were obtained at 60 minutes of exposure.

Based on reports of adverse neurologic outcome after repetitive

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anesthetic exposure [19] we repeated the experimental paradigm on three consecutive days (ISOF3 and Naïve3). We had found no difference between Sham and Naïve rats' cognitive performance at 14 days after injury, [21] so NOR was performed at PED8.

Hippocampi were collected at PED 1, 8 and 14. Rats were anesthetized with xylazine (8 mg/kg) and ketamine (40 mg/kg). The hippocampus was dissected on ice and quickly frozen to -80°C.

Molecular studies

Hippocampal BDNF and Synapsin I mRNA levels were measured by real-time RT-PCR as previously described [21] using primers and probes for BDNF, Rn 02531967_ s1 and for Synapsin I, Rn00569468_ m1 with Taqman GAPDH (PE Applied Biosystems) as internal control.

Immunoblotting was performed as previously described [21]. Proteins were extracted using ice-cold lysis buffer with protein inhibitors (Roche Applied Science, IN). Protein concentration was determined by BCA (Pierce Protein Research Products, IL) for equal protein loading. Proteins were separated by SDS PAGE using Criterion XT Precast 4-12% Bis-Tris Gels (Bio-Rad, CA), followed by transfer to PVDF membranes and blocking. Overnight incubation with antibodies against BDNF (1:600 SC-549 Santa Cruz Biotechnology, Inc, Santa Cruz CA) and Synapsin-1 D12G5 (1:1200 5297 Cell Signaling Technology, Inc, Danvers, MA) was followed by incubation with secondary antibody (1:4,000 goat anti-rabbit HRP, Cell Signaling Technology MA). Signals were quantified relative to GAPDH (1:4000 14C10 Cell Signaling Technology, MA) by densitometry on a Kodak Image Station 2000R (Eastman Kodak/SIS, NY).

Cognitive testing

As previously described [22], NOR testing was performed using a 32X 52X 30 cm^3 chamber. Tracking and data collection were accomplished using the Ethovision XT® (Noldus Inc, VA) system and by manual timing of videotaped rats by blinded observers. The rat encountered two objects (familiarization), and one novel and one familiar object when returned to the chamber 20 minutes later (testing). Normal memory results in novel exploration time that is greater than 50% of total.

Statistical Analyses

All data were analyzed using one-way ANOVA. Post Hoc testing was done using Fisher's Protected Least Significant Difference and $p<0.05$ was defined as the cutoff for statistical significance, using Statview® (SAS institute, NC) software.

Results

There were no temperature differences between Naïve and ISOF rats (36.2 ± 0.2°C vs. 35.9 ± 0.2°C, p=0.3). We have previously demonstrated that Sham rats maintain normal oxygenation and ventilation during isoflurane exposure [21]. Naïve and ISOF rats weights were not different at PED 1, 3, 6, 8 or 14.

ISOF decreased BDNF mRNA at PED1 (41 ± 7% of Naïve, $p<0.01$) and 8 (37 ± 11% of Naïve, $p<0.05$) and decreased Synapsin I mRNA at PED 1 (79 ± 5% of Naïve, $p<0.01$) and 8(74 ± 3% of Naïve, $p<0.001$) (Figure 1). ISOF did not affect BDNF or Synapsin I mRNA at PED 14.

BDNF protein did not change at PED 1, 8 or 14 (Figure 2). Synapsin I protein did not differ between ISOF and Naïve groups at PED1 (1.65 ± 0.6 vs. 1.95 ± 1.2, p=0.8) or 8 (0.5 ± 0.16 vs. 0.4 ± 0.07, p=0.5). Both ISOF3 and Naïve 3 groups performed normally on the NOR (ISOF3=68 ± 4% vs. Naïve 3=58 ± 2% total exploration.

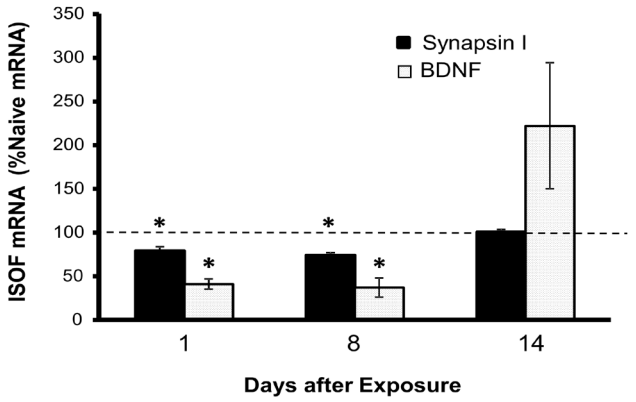

Figure 1: BDNF and Synapsin I mRNA. The graph depicts hippocampal Synapsin I (black bars) and BDNF(dotted white bars) mRNA levels in ISOF rats as a percentage of Naïve at 1, 8 and 14 days after exposure, ±SEM, n=6 *p<0.05 relative to Naïve.

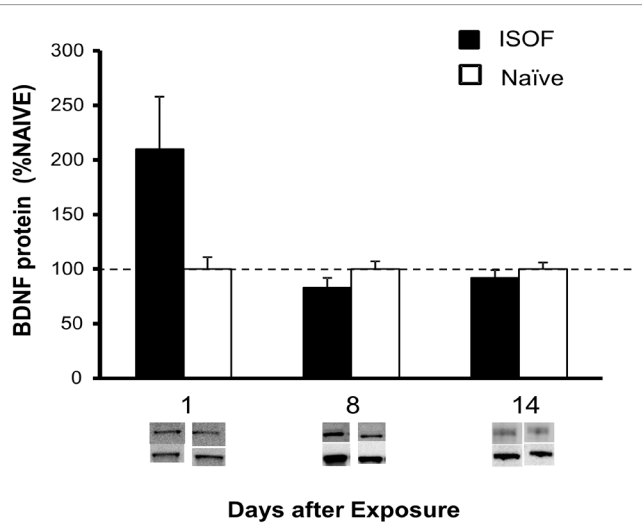

Figure 2: BDNF protein. The graph depicts hippocampal BDNF protein levels in ISOF (black bars) and Naïve rats (white bars) as densitometry units relative to GAPDH and normalized to Naïve at day 1, 8 and 14 after exposure, ±SEM, n=6 *p<0.05 relative to Naïve; representative blots from Naïve and ISOF are shown.

Discussion

In summary, isoflurane exposure at D17 decreased rat pup hippocampal BDNF and Synapsin 1 mRNA, but not protein, on PED 1 and 8. In contrast to sham surgery, isoflurane exposure did not affect BDNF mRNA at PED14. Finally, repetitive isoflurane exposures did not impair NOR performance on PED8. These data suggest that isoflurane effects on BDNF expression and memory depend on age at exposure.

The discrepancies between BDNF mRNA and protein levels in our study are not unexpected. First, BDNF protein levels reflect not only production but the degradation of BDNF and cleavage of pro-BDNF. Second, the rat BDNF gene encodes at least 11 mRNA variants [23] but only one BDNF protein. Given expected differences in translational efficiency between mRNA variants, overall translation could be unaffected despite decreased total BDNF mRNA.

Isoflurane is an NMDAR antagonist and GABA agonist. While NMDAR antagonists decrease BDNF mRNA levels in adult and immature hippocampi [12,13], the effect of GABA agonists on BDNF depends on developmental age. GABA agonists decrease BDNF mRNA in adult rat hippocampi, [13] but increase it in immature neurons [12,24]. This maturational difference likely corresponds to the switch in GABA action from excitatory to inhibitory at D14 [25]. Our mRNA results suggest that pharmacologic modulation of BDNF production in the D17 rat brain resembles that of the adult.

There are two reports of isoflurane effects on cerebral BDNF protein. At two hours after exposure, isoflurane decreased BDNF in neonatal rat thalami [26] and isoflurane (combined with nitrous oxide) decreased BDNF in adult rat hippocampi [27]. In both reports, BDNF protein levels rose over time after anesthetic exposure, suggesting that BDNF depression was transient. If so, these reports could be consistent with unchanged hippocampal BDNF protein days after isoflurane exposure.

Isoflurane decreased Synapsin I mRNA, but not protein, at PED1 and 8. Unchanged Synapsin I is expected in light of unchanged BDNF protein, since Synapsin I is a downstream target of BDNF [9,28]. Discrepancies between Synapsin I mRNA and protein, specific to rat hippocampus, were previously reported [29] and thought to reflect regional differences in plasticity.

Repeated exposure to isoflurane at D17 did not impair NOR testing at PED8. This contrasts with reports of impaired cognitive function after anesthetic exposure in the neonatal rat [18]. Those rats were younger (D7) and exposed to a far greater inhibition of NMDARs and activation of GABA receptors than the rat pups in our study. Reports of adverse effects of isoflurane on cognitive function are limited to rats exposed at D14 or younger [30]. This age-dependency may reflect the developing brain's greater susceptibility to neuronal death [31] and/or synaptogenesis. Given the growing evidence that anesthetics disrupt synaptogenesis [32], anesthetics could have greater adverse effects at D7, the peak of synaptogenesis, than at later time points such as D14 [16,33].

Our results are limited to male rats in the first fourteen days after isoflurane exposure and functional findings are limited to short term memory. We conclude that isoflurane does not alter hippocampal BDNF protein days after exposure. We speculate that isoflurane effects on BDNF expression and memory in the rat pup depend on age at exposure, and that isoflurane does not adversely affect cognitive function in the D17 rat.

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References

1. Schober ME, Block B, Requena DF, Hale MA, Lane RH (2012) Developmental traumatic brain injury decreased brain derived neurotrophic factor expression late after injury. Metab Brain Dis 27: 167-173.
2. Binder DK, Scharfman HE (2004) Brain-derived neurotrophic factor. Growth Factors 22: 123-131.
3. Numakawa T, Suzuki S, Kumamaru E, Adachi N, Richards M, et al. (2010) BDNF function and intracellular signaling in neurons. Histol Histopathol 25: 237-258.
4. Hall J, Thomas KL, Everitt BJ (2000) Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. Nat Neurosci 3: 533-535.
5. Linnarsson S, Björklund A, Ernfors P (1997) Learning deficit in BDNF mutant mice. Eur J Neurosci 9: 2581-2587.
6. Alonso M, Vianna MR, Depino AM, Mello e Souza T, Pereira P, et al. (2002) BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation. Hippocampus 12: 551-560.
7. Knapek S, Gerber B, Tanimoto H (2010) Synapsin is selectively required for anesthesia-sensitive memory. Learn Mem 17: 76-79.
8. Jovanovic JN, Czernik AJ, Fienberg AA, Greengard P, Sihra TS (2000) Synapsins as mediators of BDNF-enhanced neurotransmitter release. Nat Neurosci 3: 323-329.
9. Griesbach GS, Hovda DA, Gomez-Pinilla F (2009) Exercise-induced improvement in cognitive performance after traumatic brain injury in rats is dependent on BDNF activation. Brain Res 1288: 105-115.
10. Lu Y, Christian K, Lu B (2008) BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? Neurobiol Learn Mem 89: 312-323.
11. Nagappan G, Lu B (2005) Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. Trends Neurosci 28: 464-471.
12. Lindholm D, Castrén E, Berzaghi M, Blöchl A, Thoenen H (1994) Activity-dependent and hormonal regulation of neurotrophin mRNA levels in the brain--implications for neuronal plasticity. J Neurobiol 25: 1362-1372.
13. Zafra F, Castren E, Thoenen H, Lindholm D (1991) Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. Proc Natl Acad Sci U S A 88: 10037-10041.
14. Ming Z, Knapp DJ, Mueller RA, Breese GR, Criswell HE (2001) Differential modulation of GABA- and NMDA-gated currents by ethanol and isoflurane in cultured rat cerebral cortical neurons. Brain Res 920: 117-124.
15. Brosnan RJ (2011) GABA(A) receptor antagonism increases NMDA receptor inhibition by isoflurane at a minimum alveolar concentration. Vet Anaesth Analg 38: 231-239.
16. Yon JH, Daniel-Johnson J, Carter LB, Jevtovic-Todorovic V (2005) Anesthesia induces neuronal cell death in the developing rat brain via the intrinsic and extrinsic apoptotic pathways. Neuroscience 135: 815-827.
17. Nikizad H, Yon JH, Carter LB, Jevtovic-Todorovic V (2007) Early exposure to general anesthesia causes significant neuronal deletion in the developing rat brain. Ann N Y Acad Sci 1122: 69-82.
18. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, et al. (2003) Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 23: 876-882.
19. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, et al. (2009) Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology 110: 796-804.
20. Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, et al. (2011) Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. Pediatrics 128: e1053-1061.
21. Schober ME, Block B, Beachy JC, Statler KD, Giza CC, et al. (2010) Early and sustained increase in the expression of hippocampal IGF-1, but not EPO, in a developmental rodent model of traumatic brain injury. J Neurotrauma 27: 2011-2020.
22. Reger ML, Hovda DA, Giza CC (2009) Ontogeny of Rat Recognition Memory measured by the novel object recognition task. Dev Psychobiol 51: 672-678.
23. Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T (2007) Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res 85: 525-535.
24. Berninger B, Marty S, Zafra F, da Penha Berzaghi M, Thoenen H, et al. (1995) GABAergic stimulation switches from enhancing to repressing BDNF expression in rat hippocampal neurons during maturation in vitro. Development 121: 2327-2335.
25. Khazipov R, Khalilov I, Tyzio R, Morozova E, Ben-Ari Y, et al. (2004) Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus. Eur J Neurosci 19: 590-600.
26. Lu LX, Yon JH, Carter LB, Jevtovic-Todorovic V (2006) General anesthesia activates BDNF-dependent neuroapoptosis in the developing rat brain. Apoptosis 11: 1603-1615.

27. Cho HJ, Sung YH, Lee SH, Chung JY, Kang JM, et al. (2013) Isoflurane Induces Transient Anterograde Amnesia through Suppression of Brain-Derived Neurotrophic Factor in Hippocampus. J Korean Neurosurg Soc 53: 139-144.

28. Valente P, Casagrande S, Nieus T, Verstegen AM, Valtorta F, et al. (2012) Site-specific synapsin I phosphorylation participates in the expression of post-tetanic potentiation and its enhancement by BDNF. J Neurosci 32: 5868-5879.

29. Melloni RH Jr, Hemmendinger LM, Hamos JE, DeGennaro LJ (1993) Synapsin I gene expression in the adult rat brain with comparative analysis of mRNA and protein in the hippocampus. J Comp Neurol 327: 507-520.

30. Zhu C, Gao J, Karlsson N, Li Q, Zhang Y, et al. (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.

31. Wise-Faberowski L, Zhang H, Ing R, Pearlstein RD, Warner DS (2005) Isoflurane-induced neuronal degeneration: an evaluation in organotypic hippocampal slice cultures. Anesth Analg 101: 651-657, table of contents.

32. Briner A, De Roo M, Dayer A, Muller D, Habre W, et al. (2010) Volatile anesthetics rapidly increase dendritic spine density in the rat medial prefrontal cortex during synaptogenesis. Anesthesiology 112: 546-556.

33. Rice D, Barone S Jr (2000) Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108: 511-533.