Published in final edited form as:

Neurotoxicol Teratol. 2017 ; 60: 82–86. doi:10.1016/j.ntt.2016.11.001.

NEONATAL INHIBITION OF Na⁺-K⁺-2Cl⁻-COTRANSPORTER PREVENTS KETAMINE INDUCED SPATIAL LEARNING AND MEMORY IMPAIRMENTS

Ryan A. Stevens¹, Brandon D. Butler¹, Saurabh S. Kokane², Andrew W. Womack², and Qing Lin^{2,*}

¹Department of Biology, College of Science, the University of Texas at Arlington, Arlington, Texas, USA

²Department of Psychology, College of Science, the University of Texas at Arlington, Arlington, Texas, USA

Abstract

Prolonged ketamine exposure in neonates at anesthetic doses is known to cause long-term impairments of learning and memory. A current theoretical mechanism explains this phenomenon as being neuro-excitotoxicity mediated by compensatory upregulation of *N*-methyl-*D*-aspartate receptors (NMDARs), which then initiates widespread neuroapoptosis. Additionally, the excitatory behavior of GABAergic synaptic transmission mediated by GABA_A receptors (GABA_ARs), occurring during the early neuronal development period, is proposed as contributing to the susceptibility of neonatal neurons to ketamine-induced injury. This is due to differential developmental expression patterns of Na⁺-K⁺-2Cl⁻ co-transporter (NKCC1) and K⁺-Cl⁻ co-transporter. Studies have shown that bumetanide, an NKCC1 inhibitor, allows neurons to become inhibitory rather than excitatory early in development. We thus hypothesized that bumetanide co-administration during ketamine treatment would reduce over excitation and protect the neurons from excitotoxicity. In this initial study, the Morris Water Maze test was used to assess the effects of co-administration of ketamine and bumetanide to neonatal Sprague-Dawley rats on long-term learning and memory changes seen later in life. It was revealed that bumetanide, when co-treated with ketamine neonatally, significantly impeded behavioral deficits typically seen in animals exposed to ketamine alone. Therefore, these findings suggest a new mechanism by which neonatal ketamine induced learning impairments can be prevented.

Keywords

anesthetic; bumetanide; GABA_AR; ketamine; NKCC1; neurotoxicity

*Correspondence should be addressed to Qing Lin M.D., Ph.D., Department of Psychology, College of Science, The University of Texas at Arlington, 313 Life Science building, 501 South Nedderman Drive, Arlington, Texas 76019. qilin@uta.edu.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. INTRODUCTION

Over the past decade, concern regarding potential neurological damage caused by pediatric anesthetic use has become more notable (Bong, Allen, & Kim, 2013; DiMaggio, Flick et al., 2011; Ing et al., 2012). Both preclinical and population based retrospective studies of individuals who had received anesthetics during early childhood report a possible relationship between anesthetic exposure during early development and long term cognitive and behavioral damage (Jevtovic-Todorovic, 2013; Reddy, 2012; Wang, Xu, & Miao, 2014; Wilder et al., 2009). Ketamine hydrochloride, known simply as ketamine, a non-competitive *N*-methyl-*D*-aspartate receptor (NMDAR) antagonist, is commonly used as a pediatric anesthetic. However, multiple animal studies have conclusively shown that prolonged neonatal exposure to ketamine, or its congener, caused widespread neuroapoptosis that ultimately manifested in deficits of learning and memory and impairments in long-term synaptic plasticity in adulthood (Huang, Liu, Jin, Ji, & Dong, 2012; Jevtovic-Todorovic et al., 2003; Wang et al., 2014; Womack et al., 2013). A compensatory upregulation of the NMDARs develops significantly when ketamine is withdrawn, which triggers neuronal excitotoxicity leading to neuroapoptosis (Kokane & Lin, 2016; Slikker et al., 2007; Zou et al., 2009).

In immature neurons, the inhibitory neurotransmitter, γ -aminobutyric acid (GABA), produces excitatory action mediated by GABA_A receptors (GABA_ARs) (Ben-Ari, 2002; Owens & Kriegstein, 2002). This is due to the fact that differential expression patterns of Na⁺-K⁺-2Cl⁻-cotransporter (NKCC1) and K⁺-Cl⁻-cotransporter (KCC2) in the neurons of the neonatal brain flip the Cl⁻ electrochemical gradient, maintaining elevated intracellular levels of Cl⁻ (Clayton, Owens, Wolff, & Smith, 1998). Bumetanide, an inhibitor of NKCC1, has been of great interest to researchers investigating potential therapeutics towards alleviating cognitive and memory deficits in Down's syndrome (Deidda et al., 2015) and in reducing autistic-like behaviors (Hadjikhani et al., 2015). Bumetanide inhibition of NKCC1 in neurons during neonatal ages allows for intracellular Cl⁻ concentrations to decrease (Brandt, Nozadze, Heuchert, Rattka, & Löscher, 2010). Thus, we proposed that depolarization occurring due to GABA_AR-mediated excitatory action contributes to the increase in the susceptibility of immature neurons to neuro-excitotoxic injury following neonatal exposure to ketamine.

Since earlier studies have already looked at the effects of neonatal administration of ketamine on learning and memory (Huang et al., 2012; Paule et al., 2011; Womack et al., 2013), it was important to replicate this model, with modification, to test the impact of bumetanide co-treatment during ketamine exposure. To accomplish this, the Morris Water Maze (MWM) test was used to assess spatial learning and memory acquisition, and short-term recall (Vorhees & Williams, 2006). In this initial study, we reported novel observations by providing behavioral evidence to indicate that blockade of NKCC1 by bumetanide, as a prophylactic, during ketamine exposure plays a protective role in preventing ketamine induced long-term cognitive and learning deficits. We proposed that the underlying mechanism would be to restrict GABA_AR-mediated excitatory action and lower the

excitotoxic insult. Thus, these behavioral data have laid the groundwork for future studies of underlying mechanisms at cellular and molecular levels.

2. MATERIALS AND METHODS

2.1 Animals

All experiments were carried out according to the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas at Arlington. Sprague-Dawley rat pups, male and female, in age groups of postnatal day seven (PND 7) and three to five weeks-old were randomly sampled. All animals were housed in a 12-12-hour constant light/dark cycle at controlled temperature (22–25°C) and humidity (55–60%). Additionally, food and water was provided ad libitum.

2.2 Drug administration

Ketamine hydrochloride (AmTech Group, Inc.) was diluted in saline solution and administered subcutaneously (SC). A dose of 20 mg/kg ketamine, six times, at 2 hour intervals was administered to rat pups at age PND 7. Bumetanide was solvated in saline containing dimethylsulfoxide (DMSO) at a ratio of 1:49 (DMSO:saline) and was administered intracerebroventricularly (ICV). ICV injections were given with the goal of delivering bumetanide into the right lateral ventricle (RLV) by following previously established methods for neonatal rats at PND 7. Briefly, bumetanide was delivered 2 mm into the RLV through the skull exterior by using a 31 gauge, 6 mm in length syringe (BD Ultra-Fine). The insertion site was 1.5 mm lateral and 2 mm rostral with coordinates measured in respect to the bregma (Han & Holtzman, 2000; Kim, Cho, Nelson, Zipfel, & Han, 2014). Directly following drug administration, animals presenting with signs of intracranial hemorrhage were excluded from the study and euthanized by following an IACUC approved protocol. In an effort to maintain statistical consistency, sampling and drug administration were performed until each group totaled an $n = 10$.

A dose of 0.02 mg/kg bumetanide, 3 times at 4 hour intervals, was administered to rat pups separately or concomitantly with ketamine. Control animals were administered vehicle injections of either saline and/or saline-DMSO working solution at the same time points. The route of administration of the vehicle was kept consistent with the route of administration of the drugs. Animals were grouped according to drug administration regime and were as follows: DMSO-saline+saline (VEH+VEH, $n = 10$ animals), DMSO-saline+ketamine (VEH+KET, $n = 10$ animals), bumetanide+ketamine (BUM+KET, $n = 10$ animals), bumetanide+saline (BUM+VEH, $n = 10$ animals).

2.3 Spatial navigation training and MWM procedures

Spatial acquisition training and testing were executed utilizing the MWM to measure and compare spatial learning and short-term recall of spatial memory. All training and testing occurred during the age of adolescence – 4–5 weeks old. A pool, 160 cm in diameter, was filled with water, and was made opaque by addition of nontoxic acrylic paint, and kept at room temperature (~25°C). The quadrants of the circular pool were labeled NW, NE, SW, and SE. Distinct visual cues were placed on the walls surrounding the MWM to allow for

spatial navigation. A hidden platform was placed in one specific target quadrant – the NW quadrant was selected – subsurface within the pool, and was not moved during the entire training phase. Spatial acquisition software (EthoVision XT by Noldus) was used to monitor all movements within the pool during training and probe trials. Performing two trials per day, per animal, each group underwent a training phase for five days in the water maze. Additionally, each trial consisted of a different start location per animal – for example, Trial-one: North entry, Trial-two: East entry. Each animal was allowed a maximum of 120 s to find the hidden platform during each trial of the training phase. If the animal was unable to find the platform during this time, they were either guided or placed on the platform for 10 s. Following the training phase, 24 hours after, all animals were administered a probe test. For the probe test, the hidden platform was removed from the pool in order to test for memory recall. Each animal was allowed one probe test lasting 60 s with a start location differing from the training start locations (e.g. Probe Trial: South entry).

2.4 Statistical analysis

Statistical significance for performance in the water maze was computationally analyzed (SPSS Inc.) via repeated measures ANOVA followed by Bonferroni post hoc analysis against the control group, VEH+VEH. $n = 20$ trials each group per day for the training phase, and $n = 10$ trials per group for the probe phase. Plots were produced to illustrate these findings (Systat Software Inc.).

3. RESULTS

3.1 Training phase: average time taken to locate a hidden escape platform

The average latencies from the training phase of the MWM task are illustrated in Figure 1A. Learning and memory were assessed through analysis of the average latencies for each group across five days of training. The VEH+VEH (control) group displayed an average decrease in latency in finding the platform which showcased learning throughout the training phase. Linearly independent pairwise comparisons found that there were significant differences among the groups on day four, $F(3,36) = 3.60$, $p = .02$, and day five, $F(3,36) = 11.23$, $p < .001$. The VEH+KET group exhibited a greater average in latency to find the escape platform as compared to the VEH+VEH group on day five, which was also found to be significant, $p < .001$. These results lend support to previous research findings that conclude that early ketamine exposure does exhibit a negative effect on spatial learning and memory (Huang et al., 2012; Womack et al., 2013). Furthermore, in order to assess the role of bumetanide pre-treatment in the prevention of ketamine-induced learning deficits, the BUM+KET group was compared to the VEH+KET group. These data show that prolonged latencies to finding the hidden platform on days four and five were greatly corrected, $p = .04$, $p < .001$, respectively. In addition, latencies between the BUM+KET and VEH+VEH (control) did not measure any significant results, further in support of the hypothesis, illustrating that bumetanide co-treatment demonstrates neuroprotection against ketamine's negative effects on spatial learning and memory.

3.2 Training phase: ratio of target quadrant duration over total time in water maze

Figure 1B displays the average ratio of the time spent in the target quadrant divided by the total time spent in the MWM for each treatment group over five training days. This measurement was performed to rule out the possibility of outliers who might have located the platform without using memory dependent search strategies. Because some animals required less time to find the target quadrant and subsequent hidden escape platform than others, this ratio was necessary to accurately determine these effects rather than simply measuring the total time - or latency in this instance - spent within the target quadrant. Repeated measures ANOVA found that there were significant differences among the groups on day three, $F(3,36) = 5.72$, $p = .003$, day four, $F(3,36) = 5.39$, $p = .004$, and day five, $F(3,36) = 9.64$, $p < .001$. Post hoc analysis revealed that the VEH+VEH group showed, to a greater degree, a statistically significant duration ratio in the target quadrant compared to the VEH+KET group on day five, $p = .02$. In order to test the effects of bumetanide on preventing ketamine-induced learning deficits, the VEH+KET group was compared to the BUM+KET group. The comparison showed that the BUM+KET group showed a greater average duration ratio compared to the VEH+KET group, $p < .001$. Importantly, the BUM+VEH group exhibited no significant differences compared to the VEH+VEH group. These data were in concordance with those exhibited in Figure 1A and provided further evidence that spatial learning and memory impairments induced by ketamine can be prevented via co-treatment with bumetanide.

3.3 Probe test phase: time spent in target quadrant

Figure 2 exhibits the average duration spent in the target quadrant during the probe trial which was conducted 24 h after the fifth day of training. One-way ANOVA revealed that there was a significant difference among treatment groups, $F(3,36) = 5.09$, $p = .01$. Post hoc analysis revealed that the VEH+KET group showed a significantly lower duration in the target quadrant as compared to the VEH+VEH group, demonstrating a significant decrease in memory retention for the location of the escape platform of the VEH+KET group, $p = .01$. The effects of bumetanide co-administration on ketamine-induced memory retention deficits were then tested by comparing the BUM+KET group to the VEH+KET group. These data showed an increased average duration in the target quadrant in BUM+KET treated rats, $p = .01$. Furthermore, there was no significant difference between the VEH+VEH, BUM+KET, and BUM+VEH groups. These data lend support to those in Figure 1A and B and to previously mentioned findings, that have shown ketamine's negative effects on spatial learning and memory. More importantly, these findings demonstrate that ketamine-induced deficits of spatial learning and memory could be prevented via co-treatment with bumetanide.

3.4 Probe test phase: swim velocity

Total velocity among all treatment groups were measured with no significant differences found among the groups, with mean (M) and standard deviation (SD) given as follows: VEH+VEH ($M = 23.78$ cm/s, $SD = 3.47$, $n = 20$), VEH+KET ($M = 22.90$ cm/s, $SD = 3.50$, $n = 20$), BUM+KET ($M = 23.40$ cm/s, $SD = 3.32$, $n = 20$), and BUM+VEH were ($M = 23.37$ cm/s, $SD = 2.59$, $n = 20$). These measurements confirm that the differences among the

treatment groups were indicative of spatial memory recall and learning variances. A postulate of whether or not water maze variations among treatment groups resulted from changes in motor coordination or physical ability can be ruled out based on these findings.

4. DISCUSSION

Using the MWM behavioral task, in combination with pharmacological manipulations, the present study tested whether potentially neuronal hyperactive excitatory-GABAergic synaptic signaling in the developing brain was involved in ketamine-induced learning and memory deficits in neonates. Consistent with previous studies by our and other groups (Huang et al., 2012; Jevtovic-Todorovic et al., 2003; Womack et al., 2013), we have successfully replicated the spatial learning and memory deficit paradigm by using a rat model that received prolonged ketamine exposure at neonatal ages. Such subsequent deficits have been strongly suggested to be associated with the excitotoxicity-related neuroapoptosis in several brain regions, including the hippocampus, in the developing brain (Huang et al., 2012; Jevtovic-Todorovic et al., 2003; Liu et al., 2011; Sliker et al., 2007; Womack et al., 2013; Zou et al., 2009). The most popular mechanism proposed for this is due to excitotoxicity caused by the compensatory upregulation of NMDARs during ketamine withdrawal. The development of NMDAR upregulation results in toxic levels of intracellular Ca^{2+} concentrations after ketamine washout, which in turn triggers neuroapoptotic cascades (Kokane & Lin, 2016; Liu, Paule, Ali, & Wang, 2011; Shi et al., 2010).

During early brain development, GABA – the primary inhibitory neurotransmitter in the adult brain – depolarizes and excites targeted neurons by an efflux of Cl^- through the opening of GABA_A channels. This is due to high expression of the NKCC1 and weak expression of KCC2 in cell membranes, maintaining elevated intracellular levels of Cl^- , thereby making GABA_A -mediated action excitatory (Clayton et al., 1998). GABA_A -mediated depolarization has been suggested to increase the susceptibility of neonatal neurons to neurotoxic injury induced by GABA_A agonist-like anesthetics (Patel & Sun, 2009). Furthermore, prolonged neonatal ketamine exposure leads to over-stimulation of NMDAR-mediated synaptic transmission and enhanced excitatory GABA_A -mediated synaptic transmission. Therefore, this unique property of immature neurons makes them more vulnerable to the excitotoxic effects of ketamine.

Blockade of NKCC1 via bumetanide has been of great interest to researchers investigating potential neonatal epileptic therapeutics, which have shown that bumetanide treatment decreased GABA excitability in neonatal neurons (Dzhala et al., 2005; Dzhala, Brumback, & Staley, 2008). We thus hypothesized that upregulation of NMDARs due to prolonged ketamine exposure resulted secondarily in subsequent hyperactivity of GABAergic neurons, which mediated hyper-excitation via GABA_A s. If the functioning of NKCC1 was blocked, the hyper-excitation would be prevented as a result of lowering intracellular Cl^- levels. In order to test this potential mechanism that contributes to learning and memory deficits caused by hyperactive GABAergic-signaling excitotoxicity, we examined whether or not selective blockage of NKCC1 via use of bumetanide, as a prophylactic, could prevent spatial learning and memory deficits induced by ketamine exposure at neonatal ages. The resulting co-administration of bumetanide with ketamine demonstrated protection by preventing the

accumulation of deficits during early ketamine exposure. Inferences were based on the findings that ketamine did cause long lasting deficits in spatial learning and memory as seen in the form of significant increases in the latency to find the hidden escape platform, and decreased time spent within the target quadrant during the probe. In contrast, ketamine co-treatment with bumetanide was able to prevent these deficits from occurring. It became evident that by blockade of NKCC1 via bumetanide in neonates, learning and memory deficiencies can be prevented, resulting in memory retention similar to the control. Thus, these initial observations support our hypothesis that GABA_AR-mediated depolarization of neurons in the developing brain is augmented by ketamine exposure, which is linked to the previously described neuro-excitotoxic mechanism in immature brain neurons. Future studies by our group will aim at investigating the underlying mechanisms at cellular and molecular levels.

As interest has increased regarding the neurotoxic impact of anesthetics on the developing brain, some limitations have been identified, regarding our group's desire to initially measure the effects of bumetanide co-treatment with ketamine at multiple doses of bumetanide. It was found that other groups reported difficulties with bumetanide's ability to cross the blood-brain barrier when administered systemically (Brandt et al., 2010; Töllner et al., 2014). Because of this information, we decided to deliver bumetanide ICV by following established methods. However, due to bumetanide's solubility at physiological pH, we were unable to produce multiple deliverable doses due to bumetanide dose-volume limitations for the ICV route. Related to this issue, another group has produced various pro-bumetanide synthetics which appear to have promising effects as tested in an epileptic model (Töllner et al., 2014). Because blockade of NKCC1 could potentially allow for a deeper understanding of the neurochemical processes at play, we are currently studying the effects of systemic pro-bumetanide administration in the neonatal ketamine animal model at multiple doses. In spite of the limitations mentioned, inferences that we have made are based on known mechanisms effecting learning, memory, and behavior. Understanding the behavioral impact of early ketamine exposure as it relates to known neurochemical mechanisms will enhance the paradigm by which anesthetic research is viewed and how it potentially relates to public health and patient safety.

In conclusion, the results from the present study support the claim that neonatal ketamine administration is linked to long-lasting spatial learning and memory deficits seen later in life. Importantly, our study indicates that GABA_AR-mediated excitatory action may contribute distinctly to these neuronal excitotoxic effects of ketamine on immature neurons in the developing brain. These findings illuminate a new mechanism of learning and memory deficits caused by neonatal administration of ketamine.

Acknowledgments

The research outlined within this study was supported by NIH R01 grant NS 040723 to Q. Lin and the Ronald E. McNair Post Baccalaureate Achievement Program at UT Arlington to R. Stevens. Additional support was awarded to R. Stevens via the UT Arlington Honors College through an Undergraduate Research Fellowship. A special thank you is given to all undergraduate researchers in Dr. Qing Lin's Laboratory, and to Dr. Perry Fuchs and his group for sharing their laboratory space for behavioral testing.

References

- Ben-Ari Y. Excitatory actions of GABA during development: the nature of the nurture. *Nature Reviews Neuroscience*. 2002; 9:728–739. <http://doi.org/10.1038/nrn920>.
- Bong CL, Allen JC, Kim JTS. The effects of exposure to general anesthesia in infancy on academic performance at age 12. *Anesthesia and Analgesia*. 2013; 6:1419–1428. <http://doi.org/10.1213/ANE.0b013e318299a7c2>.
- Brandt C, Nozadze M, Heuchert N, Rattka M, Löscher W. Disease-modifying effects of phenobarbital and the NKCC1 inhibitor bumetanide in the pilocarpine model of temporal lobe epilepsy. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2010; 25:8602–8612. <http://doi.org/10.1523/JNEUROSCI.0633-10.2010>.
- Clayton G, Owens G, Wolff J, Smith R. Ontogeny of cation Cl^- cotransporter expression in rat neocortex. *Developmental Brain Research*. 1998; 109:281–292. [http://dx.doi.org/10.1016/S0165-3806\(98\)00078-9](http://dx.doi.org/10.1016/S0165-3806(98)00078-9). [PubMed: 9729431]
- Deidda G, Parrini M, Naskar S, Bozarth IF, Contestabile A, Cancedda L. Reversing excitatory GABAAR signaling restores synaptic plasticity and memory in a mouse model of Down syndrome. *Nature Medicine*. 2015; 4:318–26. <http://doi.org/10.1038/nm.3827>.
- DiMaggio C, Sun L, Li G. Early childhood exposure to anesthesia and risk of developmental and behavioral disorders in a sibling birth cohort. *Anesthesia & Analgesia*. 2011; 5:1143–1151. <http://doi.org/10.1016/j.neuroscience.2014.03.029>.
- Dzhala VI, Brumback AC, Staley KJ. Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. *Annals of Neurology*. 2008; 2:222–235. <http://doi.org/10.1002/ana.21229>.
- Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, ... Staley KJ. NKCC1 transporter facilitates seizures in the developing brain. *Nature Medicine*. 2005; 11:1205–1213. <http://doi.org/10.1038/nm1301>.
- Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, Olson MD, ... Warner DO. Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. *Pediatrics*. 2011; 5:e1053–e1061. <http://doi.org/10.1542/peds.2011-0351>.
- Hadjikhani N, Zurcher NR, Rogier O, Ruest T, Hippolyte L, Ben-Ari Y, Lemonnier E. Improving emotional face perception in autism with diuretic bumetanide: A proof-of-concept behavioral and functional brain imaging pilot study. *Autism*. 2015; 2:149–157. <http://doi.org/10.1177/1362361313514141>.
- Han BH, Holtzman DM. BDNF protects the neonatal brain from hypoxic-ischemic injury in vivo via the ERK pathway. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2000; 15:5775–5781. <http://doi.org/10.1523/JNEUROSCI.2015-5775>.
- Huang L, Liu Y, Jin W, Ji X, Dong Z. Ketamine potentiates hippocampal neurodegeneration and persistent learning and memory impairment through the PKC γ -ERK signaling pathway in the developing brain. *Brain Research*. 2012; 1476:164–171. <http://doi.org/10.1016/j.brainres.2012.07.059>. [PubMed: 22985497]
- Ing C, DiMaggio C, Whitehouse A, Hegarty MK, Brady J, von Ungern-Sternberg BS, ... Sun LS. Long-term differences in language and cognitive function after childhood exposure to anesthesia. *Pediatrics*. 2012; 3:e476–e485. <http://doi.org/10.1542/peds.2011-3822>.
- Jevtovic-Todorovic V. Functional implications of an early exposure to general anesthesia: Are we changing the behavior of our children? In *Molecular Neurobiology*. 2013; 2:288–293. <http://doi.org/10.1007/s12035-013-8488-5>.
- Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, ... Wozniak DF. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2003; 3:876–882. <http://doi.org/10.1097/00008506-200307000-00029>.
- Kim GS, Cho S, Nelson JW, Zipfel GJ, Han BH. TrkB agonist antibody pretreatment enhances neuronal survival and long-term sensory motor function following hypoxic ischemic injury in neonatal rats. *PLoS ONE*. 2014; 2:1–9. <http://doi.org/10.1371/journal.pone.0088962>.

- Kokane, SS., Lin, Q. Impact of early life ketamine exposure in the developing brain and cognitive sequelae: A discussion of apoptotic neurodegeneration mechanisms. In: Preedy, V., editor. *Neuropathology of Drug Addictions and Substance Misuse*. Waltham: Academic Press; 2016. p. 581-592.
- Liu F, Paule MG, Ali S, Wang C. Ketamine-induced neurotoxicity and changes in gene expression in the developing rat brain. *Current Neuropharmacology*. 2011; 1:256–261. <http://doi.org/10.2174/157015911795017155>.
- Owens DF, Kriegstein AR. Is there more to GABA than synaptic inhibition? *Nature Reviews Neuroscience*. 2002; 9:715–727. <http://doi.org/10.1038/nrn919>.
- Patel P, Sun L. Update on neonatal anesthetic neurotoxicity: insight into molecular mechanisms and relevance to humans. *Anesthesiology*. 2009; 4:703–708. <http://doi.org/10.1097/ALN.0b013e31819c42a4>.
- Paule MG, Li M, Allen RR, Liu F, Zou X, Hotchkiss C, ... Wang C. Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. *Neurotoxicology and Teratology*. 2011; 2:220–230. <http://doi.org/10.1016/j.ntt.2011.01.001>.
- Reddy S. Effect of general anesthetics on the developing brain. *Journal of Anaesthesiology Clinical Pharmacology*. 2012; 1:6–10. <http://doi.org/10.4103/09709185.92426>.
- Shi Q, Guo L, Patterson Ta, Dial S, Li Q, Sadovova N, ... Wang C. Gene expression profiling in the developing rat brain exposed to ketamine. *Neuroscience*. 2010; 3:852–863. <http://doi.org/10.1016/j.neuroscience.2010.01.007>.
- Slikker W, Zou X, Hotchkiss CE, Divine RL, Sadovova N, Twaddle NC, ... Wang C. Ketamine-induced neuronal cell death in the perinatal rhesus monkey. *Toxicological Sciences*. 2007; 1:145–158. <http://doi.org/10.1093/toxsci/kfm084>.
- Töllner K, Brandt C, Töpfer M, Brunhofer G, Erker T, Gabriel M, ... Löscher W. A novel prodrug-based strategy to increase effects of bumetanide in epilepsy. *Annals of Neurology*. 2014; 4:550–562. <http://doi.org/10.1002/ana.24124>.
- Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*. 2006; 2:848–858. <http://doi.org/10.1038/nprot.2006.116>.
- Wang R, Jin JH, Womack A, Lyu D, Kokane S, Tang N, ... Chen J. Neonatal ketamine exposure causes impairment of long-term synaptic plasticity in the anterior cingulate cortex of rats. *Neuroscience*. 2014; 268:309–317. <http://doi.org/10.1016/j.neuroscience.2014.03.029>. [PubMed: 24674848]
- Wang X, Xu Z, Miao CH. Current clinical evidence on the effect of general anesthesia on neurodevelopment in children: An updated systematic review with meta-regression. *PloS One*. 2014; 1:e85760. <http://doi.org/10.1371/journal.pone.0085760>.
- Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, ... Warner DO. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. *Anesthesiology*. 2009; 4:796–804. <http://doi.org/10.1097/SA.0b013e3181be865c>.
- Womack, AW., Wang, RR., Ortega, J., Perish, J., Lyu, D., Zou, X., ... Lin, Q. Neuroscience 2013. Society for Neuroscience; 2013. Neonatal ketamine exposure causes longlasting deficit of spatial learning associated with impairment of synaptic plasticity in the forebrain cortex of rats - Abstract.
- Zou X, Patterson TA, Sadovova N, Twaddle NC, Doerge DR, Zhang X, ... Wang C. Potential neurotoxicity of ketamine in the developing rat brain. *Toxicological Sciences*. 2009; 1:149–158. <http://doi.org/10.1093/toxsci/kfn270>.

Highlights

- Neonatal GABAergic excitation is proposed to enhance ketamine-induced neurotoxicity
- Blockade of NKCC1 is proposed to cause neonatal neurons to become more inhibitory
- Ketamine induced learning and memory deficits in neonatal animals were confirmed
- Co-administration with bumetanide prevents ketamine-induced learning and memory deficits
- GABAergic-excitatory synaptic signaling may be linked to ketamine's neurotoxic effect

Figure 1.

A) Average latency to find a hidden escape platform across treatment groups over five days of water maze training. On day five, there was a significant difference measured in the VEH +KET group when compared to the VEH+VEH group, *** $p < .001$. On days four and five, there was significance measured between groups of the VEH+KET and BUM+KET, + $p < .05$ and +++ $p < .001$, respectively. $n = 20$ trials each group per day. B) Average ratio of time spent within the target quadrant divided by the total time spent within a water maze over five days of training. On day five, there was a significant difference measured in the VEH+KET group when compared to the VEH+VEH group, * $p < .05$. On days three through five, BUM +KET group showed significance when compared to the VEH+KET group, ++ $p < .01$ and +++ $p < .001$, respectively.

Figure 2.

Average time spent within the target quadrant during a probe trial. During the probe trial, the VEH+KET group was found to have a significant decrease in the time spent within the target quadrant when compared to the VEH+VEH and BUM+KET groups, $*p = .01$, $^+p = .01$, respectively. Also, there was no significance between the BUM+VEH group when compared to the VEH+VEH, demonstrating that bumetanide alone did not have an effect on spatial memory. $n = 10$ trials per group.