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The blockade of NMDA receptor ion channels by ketamine is enhanced in developing rat cortical neurons

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Abstract

Ketamine is a non-competitive antagonist of NMDA receptors (NMDARs) commonly used as a dissociative anesthetic in many pediatric procedures. Ketamine acts primarily by blocking NMDA ligand-gated channels. Experimental studies indicate that ketamine administration used for inducing clinically relevant anesthesia can lead to neurotoxic effects, such as apoptosis, selectively on immature brain neurons. However, the underlying mechanisms remain unclear. This study used whole-cell patch-clamp recordings in an *in vitro* preparation of forebrain slices to analyze pharmacologically the differences in the effects of ketamine administration on the NMDAR channel activity between immature and mature neurons. NMDAR channel activity was recorded in the form of evoked NMDAR-mediated excitatory postsynaptic currents (eEPSCs) from the forebrain of both neonatal and adult rats. Results show that ketamine inhibited eEPSCs in a dose-dependent manner in both immature and mature neurons. However, at each concentration of ketamine applied to the brain slice, a more extensive inhibition could be seen in neonatal neurons than in adult neurons. Further, the blocking effect of ketamine on eEPSCs was measured during the period of 1, 3, and 6 h after ketamine washout. Inhibition of eEPSCs in immature neurons was still evident 6 h after washout. In contrast, the blockade of eEPSCs in mature neurons recovered completely from the inhibition by ketamine in a time-dependent manner. These results indicate that ketamine produces a greater and longer blocking effect on NMDAR channels in immature neurons than in mature neurons. This differential effect is likely to be a critical link to the higher vulnerability to ketamine-induced neurotoxicity in neurons of the developing brain.

Keywords

ketamine; NMDA-mediated currents; immature brain neuron

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1. Introduction

NMDARs (N-methyl-D-aspartate receptors) are ionotropic receptors (ligand-gated ion channels) gated by glutamate, distributed ubiquitously throughout the CNS. NMDARs mediate many neuronal functions during neuronal development in that glutamate plays an important role by regulating neuronal survival and migration, axonal and dendritic structure, synaptogenesis and synaptic plasticity [18,20,24]. Clinically, NMDARs are targets of several anesthetics. Ketamine, used as a dissociative anesthetic, acts primarily as the non-competitive antagonist by blocking NMDA ion channels and is commonly used for analgesia and anesthesia in a variety of pediatric procedures [7,12,17] because of its rapid onset and short acting followed by rapid recovery.

Over the past decade, however, increasing experimental evidence has shown that prolonged ketamine exposure used for inducing clinically relevant anesthesia may cause dose-dependent, widespread apoptosis of brain neurons (most prominent seen in the forebrain) in immature nonhuman primates and rodents [14,25,32,37,38]. The window of vulnerability is highly restricted to the brain growth spurt period. Recent studies further indicate that repeated ketamine exposure occurring during the period of brain development results in subsequent long-lasting cognitive deficits [22]. It is currently proposed that the apoptosis caused by ketamine is associated with a compensatory upregulation of NMDARs that would subsequently be over-stimulated by endogenous glutamate leading to dysregulation of Ca^{2+} signaling [19,27,28]. However, this cannot answer a key question why the neuroapoptosis occurs selectively in the immature brain. Most NMDARs in the mammalian CNS are comprised of two NR1 subunits and two NR2 subunits (NR2A-D). Accumulating evidence suggests that the molecular properties of NMDARs undergo postnatal changes to the adult form [2,26]. For example, NR2B subunits have a high expression in the early postnatal brain, and NR2A levels increase progressively with development [21,23]. These endow the receptor of immature neurons with distinct electrophysiological and pharmacological properties from mature neurons. We hypothesize that functional properties of NMDARs in the immature neurons, which are different from those in the mature brain evidenced as a difference in NR2A/NR2B ratio, are a major determinant of the NMDAR sensitivity to ketamine. Thus, comparing the differences in the pharmacological effects of ketamine on NMDAR-mediated channel activity between immature and mature neurons is a critical and fundamental issue in initiating the study of pathogenic mechanisms by which ketamine selectively triggers neuroapoptotic death in the developing brain.

This is an initial study using whole-cell patch-clamp electrophysiology in an *in vitro* preparation of forebrain slices to examine pharmacological differences in the effects of ketamine administration on the NMDAR channel activity between immature and mature neurons, in order to provide physiological evidence to support our hypothesis and to lay the groundwork for future studies.

2. Materials and methods

Rats (Sprague-Dawley, male and female) in age groups of postnatal 4–7 days (PND 4–7) and 3–4 weeks were used in this study. Rats were housed under a 12–12 h constant light/dark cycle in a temperature (22–25°C) and humidity (55–60%) controlled environment with free access to food and water. The study was carried out according to the protocols approved by the Institutional Animal Care and Use Committee of UT Arlington.

Brain slices preparation

The general procedures for preparing acute frontal cortex slices were similar to those described in our previously published articles [11,13,33]. Brain slices were obtained from

PND 4–7 and 3–4 week old rats. After decapitation under deep anesthesia with sodium pentobarbital (50 mg/kg, i.p.), the whole brain was carefully removed and quickly transferred to an ice-cold (0–4°C) bath of artificial cerebrospinal fluid (ACSF; composition in mM: NaCl 124, KCl 3.3, KH₂PO₄ 1.2, CaCl₂ 2H₂O 2.5, MgSO₄ 2.4, NaHCO₃ 26, and glucose 10). The bath was continuously bubbled with a 95% O₂/5% CO₂ gas mixture (pH 7.3–7.4). After cooling for about 1 min, appropriate portions containing frontal cortex were trimmed and the brain block was glued onto the stage of a vibrating tissue slicer (DTK-1000, Dosaka EM. CO. LTD., Japan), where 4–5 brain slices (350–400 µm), including the region of the frontal cortex were obtained and immediately transferred to a chamber with oxygenated (95% O₂ and 5% CO₂) ACSF. All electrophysiological patch-clamp recordings were done at room temperature (23–25 °C).

Whole-cell patch-clamp recordings of NMDAR-mediated currents

Recording procedures, data acquisition and equipment have been described in previously published work by our and other groups [11,36]. Briefly, patch electrodes were prepared from borosilicate glass (1.2 mm outside diameter, 0.69 mm inside) using a horizontal electrode puller (P-87, Sutter, USA) to produce tip openings of 1–2 µm (2–4 MΩ). A single brain slice was then held down in the recording chamber with an anchor and was immersed in oxygenated ACSF at a flow rate of 2.2–2.6 ml/min using a fast perfusion system (World Precision Instruments, USA). For excitatory postsynaptic currents (EPSCs) recordings, electrodes were filled with an internal solution containing (in mM): Cs₂SO₄, 110; CaCl₂, 0.5; MgCl₂, 2; EGTA, 5; HEPES, 5; tetraethylammonium-Cl, 5; with pH adjusted to 7.2–7.4 by CsOH, and had an osmolarity of 290–320 mOsm. Whole-cell patch-clamp recordings were made from pyramidal neurons across layers II/III under voltage-clamp mode at a holding membrane potential of –70 mV in order to maintain physiological conditions. Pyramidal neurons of the frontal cortex were recognized and identified by their morphology using a 40 X water-immersion lens. The neuron recorded was visualized with an infrared videomicroscopy (DAGE-MTI). NMDAR-mediated currents were recorded when the membrane potential was clamped at +40 mV [10,16,36]. EPSCs were recorded with an Axon 200B amplifier (Molecular Devices, USA) connected to a Digidata interface (Digidata 1440A, Molecular Devices, USA), and evoked electrically by delivering a single electrical pulse (0.3 ms, 0.25–0.5 mA) to the layer V of the frontal cortex. The evoked NMDAR-mediated EPSCs (abbreviated as eEPSCs) were pharmacologically isolated in ACSF containing: CNQX (from Sigma/Aldrich, 10 µM which blocks non-NMDA-mediated ionic currents) and bicuculline (from Sigma/Aldrich, 10 µM which blocks GABA-mediated ionic currents) [36]. The stimulus intensity used to eEPSCs was adjusted to produce 50% of maximal response as determined for each neuron recorded.

Experimental protocol

1) The effects of ketamine (from Medvet Inc.) on NMDAR-mediated channel activity were tested by recording eEPSCs while ketamine was being applied to slices at three different concentrations (1, 5, and 10 µM), respectively. Ketamine was bath applied at least for 5 min in order to achieve the largest inhibition of eEPSCs. Concentrations at which ketamine was administered were based on the pre-trial and references [1]. The baseline eEPSCs (pre-ketamine) was set as 100%, and percentage inhibition by ketamine was then determined. At each concentration, the maximal inhibition of eEPSCs by ketamine was chosen to determine the concentration-response relationship. 2) The recovery of inhibited NMDAR-mediated channel activity was examined by sampling eEPSCs at different time points after ketamine was washed out. To do this, slices were first bath-treated with 10 µM ketamine for 5 min and inhibited eEPSCs were sampled. eEPSCs were then sampled from these slices at 1, 3, and 6 h after washout of ketamine. eEPSCs sampled from the slices that were only incubated in oxygenated ACSF without ketamine treatment were set up as controls. As shown in Fig. 2A,

amplitudes of eEPSCs sampled at 1, 3 and 6 h, respectively, after slices were incubated in ACSF were not significantly different. Therefore, eEPSCs recorded at these time points could serve as controls (baseline) used for determining percent changes in eEPSCs caused by ketamine exposure in order to indicate the recovery of inhibited eEPSCs at different time points after washout of ketamine.

Data analysis and statistics

Data were digitized using pCLAMP 10.3 (Molecular Devices, USA). Statistical significance before and after drug application was analyzed using paired *t* tests. A grouped *t* test was used to compare the differences in responses between groups having different treatments. Data are reported as mean $\pm$ SEM with significance set at $P < 0.05$.

3. Results

eEPSCs were recorded from pyramidal neurons across layers II/III of the forebrain cortex in PND 4–7 and adult rats (Fig. 1A). The mean amplitudes of eEPSCs were 536.3 ± 33.3 pA in neonatal pups ($n=20$) and 526.8 ± 53.4 pA in adult rats ($n=18$). There was no statistical difference in the amplitude between two groups ($P=0.875$). However, the mean duration of eEPSCs (128.6 ± 10.0 ms) in neonatal rats ($n=20$) was significantly greater than that in adult rats (102.0 ± 5.8 , $n=18$, $P=0.043$).

The effects of ketamine on eEPSCs were tested in forebrain slices of both neonatal and adult rats. Ketamine was applied to slices for 5 min at doses of 1, 5, and 10 μ M, respectively, and the maximal inhibition of eEPSCs amplitude was measured. Ketamine inhibited eEPSCs in brain slices of both neonatal and adult rats in a dose-dependent manner (Fig. 1A). However, at each concentration, inhibition of eEPSCs was significantly greater in neonatal forebrain than in adult ones. As shown in Fig. 1B, the mean amplitudes of eEPSCs were $68.4 \pm 3.1\%$ of baseline in the neonatal group ($n=7$) in the presence of 1 μ M ketamine, compared to $84.3 \pm 3.8\%$ in the adult group ($n=7$). In the presence of 5 μ M ketamine, the amplitudes of eEPSCs were $55.6 \pm 3.4\%$ of baseline in neonatal forebrain slices ($n=8$) and $73.0 \pm 4.1\%$ of baseline in adult ones ($n=7$), respectively. When the concentration of ketamine was increased to 10 μ M, the amplitudes of eEPSCs were decreased to $25.0 \pm 3.1\%$ of baseline in the neonatal group ($n=9$) and $44.4 \pm 3.5\%$ of baseline in adult group ($n=8$), respectively.

The recovery of inhibited NMDAR channel activity was measured by sampling eEPSCs in the slices at different time points after ketamine was washed out. Baseline eEPSCs were sampled in the slices that were only incubated in oxygenated ACSF at 1, 3, and 6 h, respectively, to serve as controls (Fig. 2A). Brain slices were bath-treated with 10 μ M ketamine for 5 min and eEPSCs were sampled. eEPSCs were then sampled at 1 h after ketamine washout, and it was found that the inhibition of eEPSCs in both age groups was still obvious without significant difference when compared to the inhibition right after ketamine treatment ($23.5 \pm 3.2\%$ of control, $n=8$, neonatal and $44.9 \pm 3.7\%$ of control, $n=7$, adult). The amplitudes of eEPSCs were $25.0 \pm 2.4\%$ ($n=9$, neonatal) and $47.3 \pm 4.0\%$ ($n=5$, adult) of control levels, indicating that the blockade of eEPSCs induced by ketamine outlasted the ketamine infusion period for at least 1 h in both immature and mature slices. At 3 h after washout, the mean amplitudes of eEPSCs recorded from neonatal forebrain were $29.7 \pm 7.3\%$ ($n=7$). In contrast, the mean amplitudes of eEPSCs recorded from adult forebrain recovered greatly to $78.5 \pm 8.5\%$ of control level. At 6 h after washout, eEPSCs of adult neurons have completely recovered. However, eEPSCs were still remarkably inhibited in immature neurons ($35.2 \pm 1.5\%$ of control level, $n=7$) with a slight but significant recovery when compared to the inhibition caused by ketamine treatment (Ketamine, the first bar of each age group, Fig. 2B).

Thus, these results provide new evidence that NMDAR channels of immature forebrain are subject to a much more blockade by ketamine compared to those of mature forebrain.

4. Discussion

The main findings of this study are that we demonstrate for the first time that ketamine produces a greater inhibition of NMDAR-mediated channel activity in immature brains compared to that in mature ones. This inhibition is concentration-dependent and long-lasting. This differential pharmacological property of NMDARs in responses to ketamine in immature brains shed light on a potential link to the pathogenic mechanisms of ketamine-induced neurotoxicity in the developing brain.

NR1 subunits of the NMDAR are functional units that form an ionic channel opening for Na^+ and Ca^{2+} influx to produce EPSCs when activated by glutamate binding, whereas functional properties of NMDARs are critically determined and regulated by the changes in the composition of NR2s [2,21,23]. It has long been known that postnatal changes in the molecular properties of NMDARs occur majorly in alterations of the NR2 composition during development [9,30]. This developmental switch endows the receptor on neonatal brain neurons with pharmacological and kinetic properties distinct from that on adult neurons. NR2A-/NR2B-containing NMDAR subtypes populate the majority of spine synapses in glutamate neurons the brain and undergo a particularly well-characterized developmental shift in the composition of these two NR2 isoforms. NR2B subunits have a high expression in the early postnatal brain, and NR2A levels increase progressively with development [5,34,35]. Electrophysiological studies demonstrate that NR1/NR2A channels have higher open probability and faster deactivation than NR1/NR2B channels when responding to glutamate release, which results in the faster rise and decay times for NR2A-containing NMDARs [3,4,8]. However, NR1/NR2B channels carry more Ca^{2+} charge than NR1/NR2A channels even though they may have lower peak currents [8,29]. This may result from deactivation of NR1/NR2B receptors with slower decay time, which is slow enough to compensate for their lower open probability. A pharmacological study using NR2B selective antagonists indicates that NR2B-mediated synaptic transmission in young rats [6]. In the present study, NMDAR-mediated channel currents were evoked from both neonatal and adult forebrain slices by postsynaptic injection of depolarized currents. In neonatal slices, at the same duration (0.3 ms) of stimulus pulse, evoked NMDAR-mediated EPSCs lasted significantly longer than that seen in adult slices. This result supports partially the view that NMDAR channels in immature brains composed mainly of NR1/NR2B subunits respond to glutamate release in a slower onset and decay manner. Importantly, above electrophysiological and kinetic features may help account for the changes in the affinity of the receptor to drug binding. We have now performed the first study to examine the differential effect of ketamine on NMDAR-mediated channel activity in the neonatal forebrains and to evaluate if this effect is age-related by comparing it with the ketamine's effect on adult ones. We found that ketamine produced a greater inhibition of NMDAR-mediated EPSCs in a dose-dependent manner in forebrain slices of rats at ages of PND 4–7. Moreover, the significant inhibition of NMDAR-mediated channel activity lasted much longer in immature forebrain slices than in adult ones. In *in vivo* pharmacological studies, the competitive antagonist, AP5, and non-competitive antagonist, ketamine, produced more effective inhibition of nociception by blocking NMDARs in spinal dorsal horn neurons in rat pups [31]. We thus suggest that enhanced inhibition of NMDAR mediated channel activity induced by ketamine in immature neurons is closely associated with the electrophysiological and kinetic properties of NMDARs in immature forebrains, which are distinct from those in mature forebrains [5,35]. Future studies are clearly needed to design a series of experiments to investigate the relationship between molecular properties of the receptor and affinity of

the receptor to ketamine binding, particularly focusing on preferential effects of ketamine on the specific NR2B/NMDAR subtype in immature neurons.

Inappropriate or over-activation of NMDARs has been implicated in the etiology of several neurotoxic states, including apoptotic neuronal death. Excess extracellular glutamate release and/or compensatory up-regulation of NMDARs are major underlying mechanisms by which NMDARs are inappropriately or over activated [5,15,21]. *In vivo* studies have demonstrated that neurons in the immature brain begin to develop apoptosis following repeated ketamine exposure, and the apoptotic cell death reaches the peak at 6 h after the last dose of ketamine is systemically administered [14,37,38], which corresponds in the time-course to an up-regulation of NMDAR NR1s [19,27]. This supports the view that a compensatory enhanced NMDAR channel activity and subsequently over-stimulated glutamatergic transmission by endogenous glutamate play an important role in the ketamine-produced apoptosis in developing neurons. Based on our present findings that NMDAR-mediated EPSCs in immature brain neurons are subject to a stronger and more sustained block by ketamine, it is plausible to argue that a much more compensatory up-regulation of NMDARs would develop subsequently following the receptor blockade by ketamine. This should be a key element by which over-stimulated glutamatergic transmission triggers the apoptotic cascade. The data obtained from the present study have illuminated a novel mechanism and provide critical preliminary evidence for initiating a study focusing on the role of differential molecular and pharmacological properties of NMDARs in ketamine-induced apoptosis in the developing brain.

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References

1. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF, Kavalali ET, Monteggia LM. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*. 2011; 475:91–95. [PubMed: 21677641]
2. Bloodgood, BL.; Sabatini, BL. NMDA receptor-mediated calcium transients in dendritic spines. In: Dongen, V., editor. *Biology of the NMDA Receptor*. CRC Press, NCBI Bookshelf, NIH; 2009.
3. Burgard EC, Hablitz JJ. NMDA receptor-mediated components of miniature excitatory synaptic currents in developing rat neocortex. *J. Neurophysiol.* 1993; 70:1841–1852. [PubMed: 7507519]
4. Chen L, Cooper NG, Mower GD. Developmental changes in the expression of NMDA receptor subunits (NR1, NR2A, NR2B) in the cat visual cortex and the effects of dark rearing. *Mol. Brain Res.* 2000; 78:196–200. [PubMed: 10891601]
5. Cull-Candy S, Brichley S, Farrant M. NMDA receptor subunits: diversity, development and disease. *Curr Opin Neurobiol.* 2001; 11:327–335. [PubMed: 11399431]
6. de Armentia ML, Sah P. Development and subunit composition of synaptic NMDA receptors in the amygdala: NR2B synapses in the adult central amygdala. *J. Neurosci.* 2003; 23:6876–6883. [PubMed: 12890782]
7. Durrmeyer X, Vutskits L, Anand KJ, Rimensberger PC. Use of analgesic and sedative drugs in the NICU: integrating clinical trials and laboratory data. *Pediatr. Res.* 2010; 67:117–127. [PubMed: 20091937]
8. Erreger K, Dravid SM, Banke TG, Wyllie DJ, Traynelis SF. Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signaling profiles. *J. Physiol.* 2005; 563:345–358. [PubMed: 15649985]
9. Farrant M, Feldmeyer D, Takahashi T, Cull-Candy SG. NMDA-receptor channel diversity in the developing cerebellum. *Nature*. 1994; 368:335–339. [PubMed: 7907398]

10. Foster KA, McLaughlin N, Edbauer D, Phillips M, Bolton A, Constantine-Paton M, Sheng M. Distinct roles of NR2A and NR2B cytoplasmic tails in long term potentiation. *J Neurosci.* 2010; 30:2676–2685. [PubMed: 20164351]
11. Gong K, Cao F, He Y, Gao C, Wang D, Zhang F, An Y, Lin Q, Chen J. Enhanced excitatory and reduced inhibitory synaptic transmission contribute to persistent pain-induced neuronal hyper-responsiveness in anterior cingulate cortex. *Neuroscience.* 2010; 171:1314–1325. [PubMed: 20951771]
12. Hall RW, Shbarou RM. Drugs of choice for sedation and analgesia in the neonatal ICU. *Clin. Perinatol.* 2009; 36:215–226. [PubMed: 19559316]
13. He Y, Liu M, Gong K, Chen J. Differential effects of long and short train theta burst stimulation on LTP induction in rat anterior cingulate cortex slices: multi-electrode array recordings. *Neurosci. Bull.* 2009; 5:309–318. [PubMed: 19784087]
14. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovskaya V, Turski L, Olney JW. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science.* 1999; 283:70–74. [PubMed: 9872743]
15. Ikonomidou C. Trigger of apoptosis in the immature brain. *Brain Dev.* 2009; 31:488–492. [PubMed: 19307071]
16. Jeun SH, Cho HS, Kim KJ, Li QZ, Sung K-W. Electrophysiological Characterization of AMPA and NMDA Receptors in Rat Dorsal Striatum. *Korean J Physiol Pharmacol.* 2009; 13:209–214. [PubMed: 19885039]
17. Kohrs R, Durieux ME. Ketamine: teaching an old drug new tricks. *Anesth. Analg.* 1998; 87:1186–1193. [PubMed: 9806706]
18. Komuro H, Rakic P. Modulation of neuronal migration by NMDA receptors. *Science.* 1993; 260:95–97. [PubMed: 8096653]
19. Liu F, Paule MG, Ali S, Wang C. Ketamine-induced neurotoxicity and changes in gene expression in the developing rat brain. *Curr. Neuropharmacol.* 2011; 9:256–261. [PubMed: 21886601]
20. McDonald JW, Johnston MV. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res. Rev.* 1990; 15:41–70. [PubMed: 2163714]
21. Paoletti P, Neyton J. NMDA receptor subunits: function and pharmacology. *Curr. Opin. Pharmacol.* 2007; 7:39–47. [PubMed: 17088105]
22. Paule MG, Li M, Allen RR, Liu F, Zou X, Hotchkiss C, Hanig JP, Slikker W, Wang C. Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. *Neurotoxicol. Teratol.* 2011; 33:220–230. [PubMed: 21241795]
23. Rauner C, Kohr G. Triheteromeric NR1/NR2A/NR2B receptors constitute the major N-methyl-D-aspartate receptor population in adult hippocampus synapses. *J. Biol. Chem.* 2011; 286:7558–7566. [PubMed: 21190942]
24. Reiprich P, Kilb W, Luhmann HJ. Neonatal NMDA receptor blockade disturbs neuronal migration in rat somatosensory cortex in vivo. *Cereb Cortex.* 2005; 15:349–358. [PubMed: 15269112]
25. Scallet AC, Schmued LC, Slikker W Jr, Grunberg N, Faustino PJ, Davis H, Lester D, Pine PS, Sistare F, Hanig JP. Developmental neurotoxicity of ketamine: morphometric confirmation, exposure parameters, and multiple fluorescent labeling of apoptotic neurons. *Toxicol. Sci.* 2004; 81:364–370. [PubMed: 15254342]
26. Sheng M, Cummings J, Roldan LA, Jan YN, Jan LY. Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. *Nature.* 1994; 368:144–147. [PubMed: 8139656]
27. Shi Q, Guo L, Patterson TA, Dial S, Li Q, Sadavoda N, Zhang X, Hanig JP, Paule MG, Slikker W Jr, Wang C. Gene expression profiling in the developing rat brain exposed to ketamine. *Neuroscience.* 2010; 166:852–863. [PubMed: 20080153]
28. Sinner B, Friedrich O, Zink W, Zausig Y, Graf BM. The toxic effects of S(+)-ketamine on differentiating neurons in vitro as a consequence of suppressed neuronal Ca^{2+} oscillations. *Anesth. Analg.* 2011; 113:1161–1169. [PubMed: 21788311]
29. Sobczyk A, Scheuss V, Svoboda K. NMDA receptor subunit-dependent $[Ca^{2+}]$ signaling in individual hippocampal dendritic spines. *J. Neurosci.* 2005; 25:6037–6046. [PubMed: 15987933]

30. Tovar KR, Westbrook GL. The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *J. Neurosci.* 1999; 19:4180–4188. [PubMed: 10234045]
31. Urch CE, Rahman W, Dickenson AH. Electrophysiological studies on the role of the NMDA receptor in nociception in the developing rat spinal cord. *Dev. Brain Res.* 2001; 126:81–89. [PubMed: 11172889]
32. Walker SM, Westin BD, Deumens R, Grafe M, Yaksh TL. Effects of intrathecal ketamine in the neonatal rat. *Anesthesiology.* 2010; 113:147–159. [PubMed: 20526188]
33. Wang D, Li Z, Chang Y, Wang R, Chen X, Zhao Z, Cao F, Jin J, Liu M, Chen J. Neural circuits and temporal plasticity in hindlimb representation of rat primary somatosensory cortex: revisited by multi-electrode array on brain slices. *Neurosci. Bull.* 2010; 26:175–187. [PubMed: 20502495]
34. Williams K, Russell SL, Shen YM, Molinoff PB. Developmental switch in the expression of NMDA receptors occurs in vivo and in vitro. *Neuron.* 1993; 10:267–278. [PubMed: 8439412]
35. Yashiro K, Philpot BD. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. *Neuropharmacology.* 2008; 55:1081–1094. [PubMed: 18755202]
36. Zhao MG, Toyoda H, Wang YK, Zhuo M. Enhanced synaptic long-term potentiation in the anterior cingulate cortex of adult wild mice as compared with that in laboratory mice. *Mol. Brain.* 2009; 2:1–6. [PubMed: 19138433]
37. Zou X, Patterson TA, Divine RL, Sadovova N, Zhang X, Hanig JP, Paule MG, Slikker W Jr, Wang C. Prolonged exposure to ketamine increases neurodegeneration in the developing monkey brain. *Int. J. Devl. Neurosci.* 2009; 27:727–731.
38. Zou X, Patterson TA, Sadovova N, Twaddle NC, Doerge DR, Zhang X, Fu X, Hanig JP, Paule MG, Slikker W, Wang C. Potential neurotoxicity of ketamine in the developing rat brain. *Toxicol. Sci.* 2009; 108:149–158. [PubMed: 19126600]

Highlights

Effects of ketamine on NMDAR-mediated EPSCs are tested in neonatal and adult neurons

Ketamine blocks NMDAR-mediated EPSCs more greatly in neonatal neurons

Ketamine produces a longer blockage on NMDAR-mediated EPSCs in neonatal neurons

Higher vulnerable to ketamine-induced neurotoxicity in the developing brain is linked

Fig. 1. Concentration-dependent antagonism by ketamine of evoked NMDAR-mediated EPSCs (eEPSCs) in patches from forebrain slices of neonatal and adult rats. **A:** Increasing doses of ketamine antagonized eEPSCs dose-dependently seen in both neonatal and adult brain slices. eEPSCs prior to ketamine administration were indicated by gray traces, and during ketamine application were indicated by black traces. At each dose, ketamine produced a significantly greater reduction in eEPSCs in neonatal forebrains compared to that in adult ones. Each trace is an average of 5 sweeps. **B:** A summary of dose-dependent reduction produced by increasing doses of ketamine from forebrain cortical slices of neonatal and

adult brains. eEPSCs recorded prior to ketamine application was set as 100% and the percentage change after each dose of bath-applied ketamine indicates the reduction in eEPSCs. Statistically significant difference between groups of neonatal and adult rats at each dose of ketamine was determined and indicated as $P < 0.05$.

Fig. 2.

Time-course of changes in the ketamine-induced antagonism of eEPSCs after ketamine was washed out. **A:** eEPSCs sampled 1, 3, and 6 h after slices were incubated in ACSF (no ketamine exposure) were set as controls. There was no significant difference in the amplitude among different time points for both adult ($n=20$) and neonatal ($n=20$) neurons. The amplitudes of eEPSCs recorded from control slices (**A**) were normalized and set as 100%. **B:** The inhibition of eEPSCs was sampled right after slices were bath-treated with 10 μ M ketamine for 5 min. eEPSCs were then sampled at 1, 3, and 6 h after washout. Changes in eEPSCs due to ketamine exposure and after washout of ketamine were presented as

percentages of control levels of their corresponding time points, respectively. #: $P < 0.05$, ##: $P < 0.01$, and ###: $P < 0.001$, compared with the control set for each time point. *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$, compared with the ketamine-treated neurons (Ketamine, the first bar of each age group).