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NEONATAL KETAMINE EXPOSURE CAUSES IMPAIRMENT OF LONG-TERM SYNAPTIC PLASTICITY IN THE ANTERIOR CINGULATE CORTEX OF RATS

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Abstract

Ketamine, a dissociative anesthetic most commonly used in many pediatric procedures, has been reported in many animal studies to cause widespread neuroapoptosis in the neonatal brain after exposure in high doses and/or for prolonged period. This neurodegenerative change occurs most severely in the forebrain including the anterior cingulate cortex (ACC) that is an important brain structure for mediating a variety of cognitive functions. However, it is still unknown whether such apoptotic neurodegeneration early in life would subsequently impair the synaptic plasticity of the ACC later in life. In this study, we performed whole-cell patch-clamp recordings from the ACC brain slices of young adult rats to examine any alterations in long-term synaptic plasticity caused by neonatal ketamine exposure. Ketamine was administered at postnatal day 4–7 (subcutaneous injections, 20 mg/kg given six times, once every 2 h). At 3–4 weeks of age, long-term potentiation (LTP) was induced and recorded by monitoring excitatory postsynaptic currents from ACC slices. We found that the induction of LTP in the ACC was significantly reduced when compared to the control group. The LTP impairment was accompanied by an increase in the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated excitatory synaptic transmission and a decrease in GABA inhibitory synaptic transmission in neurons of the ACC. Thus, our present findings show that neonatal ketamine exposure causes a significant LTP impairment in the ACC. We suggest that the imbalanced synaptic transmission is likely to contribute to ketamine-induced LTP impairment in the ACC.

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CONFLICT OF INTEREST STATEMENT The authors declare that they have no conflict of interests

Keywords

ketamine; anterior cingulate cortex; long-term potentiation; excitatory synaptic transmission; inhibitory synaptic transmission

There is a growing concern that exposure to anesthetics during the neonatal period may cause neurotoxic injury in brain neurons that results in alterations of brain functions later in life. Both human and animal studies have indicated an association between early exposure to anesthetics and subsequent development of learning disabilities (Bouman et al., 1999; Wilder et al., 2009; Wang and Orser, 2011; Murphy and Baxter, 2013; Wang et al., 2013; Yu et al., 2013). Ketamine, a non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist, is commonly used for inducing anesthesia in a variety of pediatric procedures (Durrmeyer et al., 2010; Tsze et al., 2012; Asadi et al., 2013; Nielsen et al., 2013). Many animal studies have shown that ketamine can produce widespread neuroapoptosis in the neonatal brain after exposure in high doses and/or for prolonged period (Olney et al., 2002; Zou et al., 2009a,b; Paule et al., 2011; Huang et al., 2012). It is suggested pathophysiologically that the development of neuroapoptosis in the neonatal brain is caused by a compensatory up-regulation of NMDARs during the time when the ketamine exposure is withdrawn, which subsequently leads to Ca^{2+} overload in turn triggering the apoptotic cascade (Shi et al., 2010; Liu et al., 2011; Sinner et al., 2011). A study of neurodevelopment indicates that exposure to ketamine in high doses affects the neurogenesis of rat cortical neural stem progenitor cells that subsequently alters the normal brain development (Dong et al., 2012). Several studies by our and other groups also suggest that ketamine-induced apoptosis involves a pathological mechanism of learning and memory deficits that are associated with long-term anesthetic treatment (Viberg et al., 2008; Paule et al., 2011; Huang et al., 2012; Womack et al., 2013). Thus, these findings imply that the development of apoptotic neurodegeneration induced by ketamine exposure during the neonatal period may contribute the long lasting learning and memory deficits later in life.

According to the previous study, several brain regions develop widespread neuroapoptosis after neonatal exposure to ketamine (Ikonomidou et al., 1999; Zou et al., 2009b). Among them, the anterior cingulate cortex (ACC), a key structure of the forebrain region, is severely affected by neuroapoptotic injury (Ikonomidou et al., 1999; Zou et al., 2009b). Substantial evidence has been accumulated to support the important role of the ACC in learning and memory (Frankland et al., 2004; Goshen et al., 2011; Einarsson and Nader, 2012). Activity-dependent synaptic plasticity is commonly thought to play a pivotal role in various kinds of behaviors ranging from development to learning and memory (Martin et al., 2000; Malenka and Bear, 2004; Zhuo, 2008). It is well-established that NMDARs are critically involved in multiple forms of synaptic plasticity in the brain, including long-term potentiation (LTP) and long-term depression (LTD) (Liu et al., 2004; Berberich et al., 2007; Unoki et al., 2012; Volianskis et al., 2013). A previous study reported that neonatal NMDARs antagonist exposure results in a lasting reduction in synaptic strength in the hippocampus (Bellinger et al., 2002). However, there have been few studies documenting the long-lasting effect of early treatment with ketamine on ACC synaptic plasticity. Therefore, using a rat model of ketamine exposure that has been validated to cause neuroapoptosis in neonatal brains (Zou

et al., 2009b; Shi et al., 2010; Liu et al., 2011; Brambrink et al., 2012), the present study examined whether neuroapoptotic degeneration that develops in neonatal brains would subsequently produce long-term deleterious effect on synaptic plasticity in the ACC later in life. By performing *in vitro* whole-cell patch-clamp recordings from the pyramidal neurons of ACC slices, we found that the induction of LTP was profoundly suppressed in rats that were exposed to ketamine neonatally. To determine the mechanisms for this LTP impairment, we also addressed the changes in the intrinsic excitatory and inhibitory neurotransmission in the ACC following neonatal ketamine exposure. Our results demonstrate the imbalance of synaptic excitation and inhibition in the ACC of ketamine-treated rats.

EXPERIMENTAL PROCEDURES

Animals

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 the protocols approved by the Institutional Animal Care and Use Committee of University of Texas at Arlington.

Neonatal treatment with ketamine

According to the previous studies, the window of vulnerability to the neurotoxic effect of ketamine is restricted to the period of rapid synaptogenesis, which occurs at ages of PND 0–14 and peaks between 3–7 days after birth (Ikonomidou et al., 1999; Wang and Slikker, 2008). Thus, neonatal ketamine treatment was performed on the same day in PND 4–7 rat pups that were randomly assigned to groups of drug and saline treatments. For the drug treatment group, they were administered subcutaneously with ketamine for 6 times at 2-h intervals at the dose of 20 mg/kg per injection (Zou et al., 2009b). During drug treatment, animals were maintained at a light anesthetized level as evidenced by lack of voluntary movement, and minimal reaction to the physical stimulation. Animal in the control group were administered with saline for the same number of injections at the volume of 0.02 ml. Pups were returned to their dams to help maintain body temperature and reduce stress between each treatment. During drug treatment, body temperature was maintained at 37°C using a thermostatically heating blanket. Data obtained from arterial blood gas and glucose analysis by other groups in neonatal animals that were exposed to ketamine do not support that apoptotic degeneration was attributable to metabolic or respiratory distress (Jevtovic-Todorovic et al., 2003; Slikker et al., 2007). Further, several studies using the same protocol of ketamine administration have confirmed the apoptotic neurodegeneration in the neonatal rats brain (Ikonomidou et al., 1999; Zou et al., 2009b; Shi et al., 2010; Liu et al., 2011).

Brain slices preparation

At the age of 3–4 weeks, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and ACC slices were obtained according to previously-published procedures (He et al., 2009; Gong et al., 2010; Jin et al., 2013; Lu et al., 2013). Briefly, after decapitation, the whole brain was carefully removed and quickly transferred to an ice-cold 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, the anterior half of the brain was dissected, and the brain block was glued onto the stage of a vibrating tissue slicer (DTK1000, Dosaka EM. Co., LTD., Japan), where 4–5 coronal brain slices (350–400 µm), including the region of the ACC were obtained and immediately transferred to a holding chamber with oxygenated (95% O₂ and 5% CO₂) ACSF.

Whole-cell patch-clamp recordings

Patch electrodes (2–4 MΩ) were prepared using borosilicate glass (1.2 mm outside diameter, 0.69 mm inside) and made by a horizontal electrode puller (P-87, Sutter, USA). A single brain slice was then held down in the recording chamber with an anchor and was kept immersed in circulating oxygenated ACSF at a flow rate of 2.2–2.6 ml/min using a fast perfusion system (World Precision Instruments, USA). Recordings were done at room temperature (23–25 °C) (Zhao et al., 2005a, 2009; He et al., 2009; Gong et al., 2010; Jin et al., 2013).

Recording procedures, data acquisition and equipment have been described in previously published work by our and other groups (Zhao et al., 2009; Gong et al., 2010; Jin et al., 2013). Briefly, postsynaptic currents were recorded from layers II–III of pyramidal neurons of the ACC because layers II–III are reported to be the major location of intracortical horizontal pathways (Hess et al., 1994), and the superficial layers of the ACC received nucleus of somatosensory information (Zhuo, 2007). Under voltage-clamp mode, the membrane potential was clamped at –70 mV in order to maintain physiological conditions. Neurons of the ACC were recognized and identified by their morphology using a 40× water-immersion lens. The neuron recorded was visualized with an infrared video microscope (DAGE-MTI). For excitatory postsynaptic current (EPSC) 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 osmolality of 290–320 mOsm. Bicuculline (20 µM) was added to ACSF to block GABA_A receptor-mediated inhibitory synaptic currents. A bipolar tungsten stimulating electrode was positioned in the deep (V/VI) layer cortex of the ACC, and a single pulse was delivered at 0.025 Hz (0.3 ms, 0.25–0.5 mA) with a 0.05 mA stepwise increase. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)-mediated evoked EPSCs (eEPSCs) were evoked and then recorded. After obtaining stable eEPSCs for at least 10 min, the LTP was induced by Theta-burst Stimulation (TBS) with postsynaptic depolarization at +30 mV. The TBS consisted of 10 bursts, each containing 4 pulses at 100 Hz with an inter-burst interval of 200 ms. This stimulation protocol has been demonstrated to be more robust and stable than others (He et al., 2009). The stimulus strength was adjusted to induce 50% of the maximal magnitude of the eEPSCs. Under the voltage-clamp mode, miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs) were recorded, respectively, from ACC neurons. For recording AMPAR-mediated mEPSCs, the slices were perfused with ACSF containing tetrodotoxin (TTX, 1 µM), NMDAR inhibitor D-(–)-2-amino-5-phosphonopentanoic acid (D-APV, 50 µM), and bicuculline (20 µM) with a holding potential of –70 mV. NMDAR-mediated mEPSCs was

pharmacologically isolated in ACSF containing: TTX (1 μ M), CNQX (10 μ M) and bicuculline (20 μ M) at the holding potential of +40 mV. mIPSCs were recorded in the presence of CNQX (10 μ M) at a holding potential of 0 mV. Again, TTX (1 μ M) was bath-applied for the recording of mIPSCs. Series resistance was in the range of 20–30 M Ω . Dates were discarded if the resistance changed >30% during recording or the resting membrane potential of neuron was more depolarized than –50 mV. The frequency and amplitude of mEPSCs and mIPSCs were measured using MiniAnalysis (Synaptosoft, Decatur, GA).

Drugs

Ketamine hydrochloride, the GABA_A receptor antagonist bicuculline, TTX, the AMPAR antagonist CNQX, and NMDAR inhibitor D-(–)-2-amino-5-phosphonopentanoic acid D-APV were purchased from Sigma Aldrich (St. Louis, MO, USA). The doses and injection route of antagonists were chosen based on preliminary experiments and previous reports (Zou et al., 2009a,b; Gong et al., 2010).

Statistical Analysis

The mean and SE for the control and ketamine-administered rats were compared using independent samples t tests for comparisons with control values. Significance was set at *P* values less than 0.05.

RESULTS

The LTP induction in the ACC was suppressed in neonatally ketamine treated rats

To evaluate the effect of neonatal ketamine exposure on LTP induction in the ACC, we used the TBS induction protocol as described previously (Zhao et al., 2005b; Toyoda et al., 2007). As show in Fig. 1a, LTP was induced at 5–10 min after TBS and persisted for at least 35 min in the ACC of control rats. However, the normalized amplitude of EPSCs was significantly reduced in the ACC from ketamine-treated rats (control vs. ketamine: 141.3 $\pm$ 2.4% vs. 115.1 $\pm$ 8.9% of baseline at 30 min after induction, *n*=6 cells/6 rats for both groups, * *p*<0.05, Fig.1b). These results show that LTP is dramatically suppressed in the ACC of rats that were pre-treated with ketamine neonatally.

AMPA receptor-mediated synaptic transmission in the ACC was enhanced in neonatally ketamine treated rats

It has been suggested in a previous study that the blockade of excitatory postsynaptic transmission in the hippocampus during the neonatal period interrupts the proper development of the excitatory synapses (Bellinger et al., 2002). Thus, we explored whether there was a change in excitatory synaptic transmission within the ACC after neonatal administration of ketamine by using electrical stimulation to evoke and then record AMPAR-mediated eEPSCs in ACC neurons. Input-output (I/O) relationships were obtained by measuring amplitudes of EPSCs evoked by stimulus intensities at ascending intensities of 0.25–0.5 mA with a 0.05 mA stepwise increase. As shown in Fig. 2, the I/O function of eEPSCs increased significantly in the ketamine-treated group (*n*=8 cells/7 rats) as evidenced by the greater responses to increasing intensities of stimuli when compared with control

(n=10 cells/8 rats). These results suggest that AMPAR-mediated excitatory synaptic transmission is enhanced in the ACC of rats that have been neonatally treated with ketamine.

To further determine whether presynaptic or postsynaptic mechanisms contribute to the enhanced synaptic transmission, we recorded AMPAR-mediated mEPSCs in the ACC (Fig. 3). AMPAR-mediated mEPSCs were recorded in the presence of TTX, bicuculline and D-APV. The frequency of mEPSCs in neurons located in layers II/III increased significantly in ketamine-administered group (4.2 ± 0.3 Hz, n=7 cells/5 rats) compared with the control group (2.2 ± 0.1 Hz, n=10 cells/6 rats, $p < 0.01$, Fig. 3d). However, the amplitude of mEPSCs was not affected (control vs. ketamine: 65.6 ± 3.2 pA vs. 66.6 ± 1.8 pA, Fig. 3e). These data were confirmed by the cumulative distribution plots of mEPSCs frequency (Fig. 3b) and amplitude (Fig. 3c). Specifically, the cumulative distribution plot of the mEPSC inter-event interval was shifted to the left in the ketamine-administered group compared to the control, while that of the mEPSC amplitude overlapped with the control group. These results indicate that the enhanced excitatory synaptic transmission in the ACC after neonatal ketamine administration is attributable to an increase in probability of presynaptic transmitter release.

NMDA receptor-mediated currents are not altered in neonatally ketamine-administered rats

In addition to AMPAR-mediated synaptic transmission, we also tested if there was a change in NMDAR-mediated synaptic responses following neonatal ketamine exposure. NMDAR-mediated mEPSCs were recorded at a holding potential of +40 mV with the presence of TTX, bicuculline and CNQX. Unlike the AMPAR-mediated currents, neither the frequency (control vs. ketamine: 1.3 ± 0.1 Hz vs. 1.3 ± 0.1 Hz, n=11 cells/7 rats for both groups, Fig. 4d) nor the amplitude (control vs. ketamine: 120.5 ± 18.7 pA vs. 127.7 ± 8.5 pA, n=11 cells/7 rats for both groups, Fig. 4e) of NMDAR-mediated mEPSCs was altered due to neonatal ketamine treatment. Consistently, accumulated histograms revealed no differences in the distribution of either the inter-event interval or the amplitude of mEPSCs for NMDAR-mediated synaptic transmission in the ACC (Fig. 4b and c).

Inhibitory synaptic transmission in the ACC was reduced in neonatally ketamine treated rats

Our previous work has demonstrated the importance of the excitation-inhibition imbalance in maintaining pathological pain-evoked behavioral and synaptic dysfunctions (Yan et al., 2009; Gong et al., 2010). In the present study, we hypothesized that neonatal ketamine treatment may result in a similar imbalance between excitatory and inhibitory synaptic transmission in the ACC. To this end, we examined the changes in mIPSCs in the groups of ketamine-administered and saline-administered rats. mIPSCs were recorded at a holding potential of 0 mV in the presence of TTX, CNQX and D-APV. Results show that the frequency of mIPSCs was significantly decreased in neonatal ketamine-administered group (1.1 ± 0.1 Hz, n=11 cells/7 rats) compared to the control group (3.3 ± 0.3 Hz, n=15 cells/8 rats, $p < 0.01$, Fig. 5d). However, the amplitude of mIPSCs in the ACC did not differ between the two groups (control vs. ketamine: 119.1 ± 10.6 pA vs. 113.9 ± 7.7 pA, Fig. 5e). The cumulative probability distribution of the mIPSC inter-event interval was shifted to the right in ketamine-administered group, without any alteration in the distribution of mIPSC

amplitude (Fig. 5b and c). These findings suggest that neonatal ketamine administration can also produce a suppression of the inhibitory synaptic transmission in the ACC, which is likely due to a decrease in the probability of inhibitory neurotransmitter release at presynaptic terminals.

DISCUSSION

In this study, we show that the induction of LTP in the ACC was profoundly suppressed in rats that were exposed to ketamine neonatally. Such an impairment of LTP was accompanied by an increased excitatory synaptic transmission and a decreased inhibitory synaptic transmission, strongly indicating a causal link of unbalanced excitatory/inhibitory transmissions to the impaired LTP.

LTP is believed to be the key cellular basis for information processing in the mammalian brain. As a widely used synaptic model for studying learning and memory, LTP has been reported in many brain areas including the cortex, hippocampus and spinal cord. LTP formed in the ACC may relate to chronic pain and pain-related cognitive emotional disorders (Zhuo, 2007,2008). Studies by our and other groups demonstrate that neonatal exposure to ketamine impaired spatial learning and memory in the adult animals (Huang et al., 2012; Womack et al., 2013). Also, the chronic treatment of neonatal rats with an NMDA antagonist MK-801 led to deficits in spatial task performance in the adult life (Gorter and de Bruin, 1992). Ketamine exposure causes over expression of important proteins that are involved in the normal maturation of the brain, which has been implicated in permanent changes in brain function (Viberg et al., 2008). In line with these pieces of evidence, the findings by our current study provide evidence to strongly suggest that ketamine exposure-induced neurodegeneration in the neonatal brain can cause long-lasting impairment in induction of LTP in the ACC, which is associated with learning and memory impairment later life.

LTP of excitatory synaptic transmission in the mammalian brain has been thought to be important for learning, memory, and neural development. At most excitatory synapses that exhibit LTP, synaptic responses are mediated by two distinct subtypes of ionotropic glutamate receptors, termed AMPA and NMDA receptors (APMARs and NMDARs). Most excitatory synaptic responses are solely or primarily mediated by AMPARs (Malenka and Bear, 2004). By recording of AMPAR-mediated eEPSCs, we found that excitatory synaptic transmission in the ACC was enhanced in rats pretreated with ketamine neonatally. This was evident by the fact that stimulations with the same strength produced larger responses in ketamine treatment group than in control groups. When AMPAR-mediated mEPSCs were recorded, the frequency of mEPSCs was increased without significant changes in the amplitude in ketamine administered rats. This indicates an increase in the pool of vesicles immediately available for the release or increase in the probability of neurotransmitter release. No difference in the amplitude suggests that there is no change in the efficacy of the glutamate receptors (Abekawa et al., 2011). However, there were no significant differences for amplitude and frequency in the NMDAR-mediated mEPSCs between the groups. Thus, suppressed LTP in ketamine-administered group is likely to be due to an enhancement of the AMPAR-, not NMDAR-, mediated component of excitatory transmission. Taken together,

these data imply that the excitatory synaptic transmission mediated mainly by pre-synaptic mechanism becomes enhanced in the ACC that has suffered from neurotoxic injury caused by ketamine exposure during the period of the development.

Studies have revealed that long-term synaptic plasticity is regulated by GABAergic system (Matsuyama et al., 2008). In the ACC slices of the rats that were treated with ketamine neonatally, we found that the frequency of mIPSCs was reduced but the amplitude was not significantly changed. Since reduction in frequency of miniature events could occur because of a temporal failure in release of neurotransmitter, this result suggests that the presynaptic release of GABA was reduced in the ACC that had been exposed to ketamine neonatally. This could be due to that ketamine exposure leads to the apoptotic neurodegeneration of immature GABAergic neurons seen obviously in the forebrain, like the ACC, in neonatal brains. Evidence has suggested that a remarkable increase in apoptotic neurodegeneration was observed in the frontal cortex and several major brain regions in the rats following prolonged exposure to ketamine on postnatal day 7 (Zou et al., 2009b). Desfeux et al. (2010) found that an NMDAR antagonist MK801 induced an increase of the caspase-3 activities in layers II-IV of immature neurons affecting the function of GABAergic interneurons. Ketamine when administered at a low dose to neonatal brains has been shown to interfere with dendritic arbor development of immature GABAergic neurons (Vutskits et al., 2006). In addition, prolonged exposure to other anesthetics in neonates can induce neuroapoptotic cell death in 7-day-old mice. Some of these neuronal deaths were seen in GABAergic interneurons, which contribute to a reduction in the inhibitory synaptic transmission (Istaphanous et al., 2013).

How are the enhanced excitatory and reduced inhibitory synaptic transmissions linked pathologically to the impairment of the long-term synaptic plasticity? As we know, GABA is the major inhibitory neurotransmitter in the CNS; GABAergic inhibition is also an important regulatory factor of LTP (Davies et al., 1991; Gonzalez Burgos et al., 1994). A reduction in the GABAergic inhibition resulting from loss or dysfunction of inhibitory GABAergic interneurons can indirectly increase the excitability of neurons in many circuits profoundly (Sadrian et al., 2013). GABAergic inhibition and synaptic plasticity were significantly impaired in mice with the collybistin-deficient dentate gyrus where GABA_A receptor clusters were decreased, which leads to an increased network excitability (Jedlicka et al., 2009). In the rat model of schizophrenia, deficiency in GABAergic inhibition accounts for the increase in excitability and produces hyper-excitability and a subtle deficit in synaptic plasticity, leading to alterations that may be of relevance to the behavioral and cognitive deficits (Sanderson et al., 2012). A possible explanation is that GABAergic interneurons provide an inhibitory input to the axon initial segment soma and proximal dendrites of glutamatergic pyramidal neurons, thereby regulating the output activity of these glutamatergic neurons. In the absence of GABAergic inhibition, hyperexcitability of pyramidal neurons would thus lead to impairment of LTP due to their occlusion or saturation (Tachibana et al., 2011). Thus, we propose that neurotoxic injury of GABAergic neurons resulting from neonatal ketamine exposure would directly lead to disruption of cortical inhibition and indirectly cause disinhibition of glutamatergic signaling, both contributing to excessive cortical excitability that in turn impairs the induction of LTP. Future experiments are clearly needed to explore this possibility.

Finally, it is true that the most dosing paradigms of anesthetics used in animal studies typically do not reflect doses used for pediatric patients and that there is no direct evidence from human data to either support or refute the clinical applicability of these findings in animal models (Mellon et al., 2007; Wang and Slikker, 2008). The fact is that anesthetic neurotoxicity studies in humans are not feasible because of the difficulty in obtaining neurons and performing developmental toxicity experiments in pediatric and fetal populations. With advances in pediatric and obstetric surgery that result in an increase in the duration and complexity of anesthetic procedure, the risk of neurotoxic injury in pediatric patient's brains increases as a result of anesthetic exposure. Moreover, several retrospective epidemiological studies indeed suggest that anesthetic administration early in life is associated with learning and behavioral abnormalities later in life (DiMaggio et al., 2009,2011; Wilder et al., 2009; Wilder, 2010). Therefore, studies using animal models provide a feasible way to investigate the mechanistic processes of anesthetic-induced neurotoxicity in the developing brain, which would help link to the long-term impairment of cognitive functions. Our current study provides an important cellular mechanism that the impairment of long-term synaptic plasticity following neonatal ketamine exposure contributes to cognitive sequelae.

In conclusion, our present study indicates that neonatal ketamine exposure results in increased excitatory synaptic transmission and decreased inhibitory synaptic transmission of pyramidal cells in the ACC that persist into adolescence. These alterations are likely contributing to the mechanism of abnormalities in the ACC's LTP.

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Abbreviations

ACC	anterior cingulate cortex
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
eEPSCs	evoked excitatory postsynaptic currents
mEPSCs	miniature excitatory post-synaptic current
mIPSCs	miniature inhibitory post-synaptic currents
NMDA	N-methyl-D-aspartate
LTP	long-term potentiation
TBS	theta-burst stimulation

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Highlights

The LTP induction in the ACC is suppressed in neonatally ketamine treated rats

AMPA-mediated EPSCs in the ACC are enhanced in neonatally ketamine treated rats

GABA_A-R-mediated IPSCs in the ACC are reduced in neonatally ketamine treated rats

The impaired LTP is due to unbalanced excitatory/inhibitory transmissions in the ACC

Fig. 1.

LTP induction in the ACC was suppressed in the rats treated with ketamine neonatally. a: Summarized data for LTP in the ACC slices of ketamine administered rats (filled circles) and the control group (open circles). Sample traces show averages of 7–8 eEPSCs 5 min before (1) and 30 min after (2) the theta-burst stimulation. b: Pooled data of the normalized eEPSC amplitude 30 min after LTP induction in the ACC slices. $n=6$ cells from 6 rats for both groups, * $p < 0.05$, compared with control group. Data are presented as mean $\pm$ s.e.m.

Fig. 2.

AMPA-mediated excitatory synaptic transmission was significantly increased in the ACC slices obtained from neonatally ketamine-treated rats. a: Representative traces show AMPAR-mediated EPSCs evoked by stimulus intensities of 0.3, 0.4 and 0.5 mA from control (1, n=10 cells/8 rats) and ketamine-treated groups (2, n=8 cells/7 rats). Each trace was the average from 7 to 8 responses. b: Input-output (I/O) function of eEPSC was enhanced significantly in neurons of ketamine-treated rats, ** $p < 0.01$, compared with control group. Data are presented as mean $\pm$ s.e.m.

Fig. 3.

The frequency, but not amplitude, of AMPAR mediated mEPSCs recorded from the ACC was increased in the rats pre-treated with ketamine neonatally. *a*: Representative mEPSC traces from the control (*top*) and the ketamine-treated rats (*lower*) obtained by whole-cell patch-clamp recordings treated with TTX (1 μ M), bicuculline (20 μ M) and D-APV (50 μ M). Accumulated histograms of inter-event intervals (*b*) and amplitude (*c*) of mEPSCs, and averaged frequency (*d*) and amplitude (*e*) of mEPSCs are presented for both control ($n=10$ cells/6 rats) and ketamine-exposed ($n=7$ cells/5 rats) groups. ** $p < 0.01$, compared with control group. Data are presented as mean $\pm$ s.e.m.

Fig. 4. NMDAR-mediated synaptic transmission was not altered by neonatal ketamine treatment. *a*: Representative mEPSC traces from the control (*top*) and the ketamine-treated rats (*lower*) obtained by whole-cell patch-clamp recordings treated with TTX (1 μ M), bicuculline (20 μ M) and CNQX (10 μ M). Accumulated histograms of inter-event intervals (*b*) and amplitude (*c*) of mEPSCs, and averaged frequency (*d*) and amplitude (*e*) of mEPSCs are presented for both control ($n=11$ cells/7 rats) and ketamine-exposed ($n=11$ cells/7 rats) groups. Data are presented as mean $\pm$ s.e.m.

Fig. 5.

The frequency, but not amplitude, of mIPSCs recorded from the ACC was decreased in rats pre-treated with ketamine neonatally. *a*: Representative mIPSC traces from the control (*top*) and the ketamine-treated rats (*lower*) obtained by whole-cell patch-clamp recordings with TTX (1 μ M) and CNQX (10 μ M). Accumulated histograms of inter-event intervals (*b*) and amplitude (*c*) of mIPSCs, and averaged frequency (*d*) and amplitude (*e*) of mIPSCs are presented for both control (*n*=15 cells/ 8 rats) and ketamine-exposed (*n*=11 cells/7 rats) groups. ** *p* < 0.01, compared with control group. Data are presented as mean $\pm$ s.e.m.