Review Article

# Ketamine-induced neurotoxicity in neurodevelopment: A synopsis of main pathways based on recent *in vivo* experimental findings

**Konstantina Kalopita, Athanasios Armakolas, Anastassios Philippou, Apostolos Zarros[1], Panagoula Angelogianni**

Physiology Laboratory, Medical School, National and Kapodistrian University of Athens, Athens, Greece, [1]Institute of Cancer Sciences, University of Glasgow, Glasgow, Scotland, UK

## Abstract

Ketamine, a phencyclidine derivative and *N*-methyl-D-aspartate (NMDA) receptor antagonist, is widely used as an anesthetic, analgesic, and sedative agent in daily pediatric practice. Experimental studies have suggested that early prenatal or postnatal exposure to ketamine can induce neuroapoptosis, and establish neurobehavioral deficits that are evident in adulthood. However, most of the currently available clinical evidence is derived from retrospective and observational clinical studies. We, herein, attempt a brief review of the cellular and molecular mechanisms suggested to mediate ketamine-induced developmental neurotoxicity, utilizing a selected number of recent *in vivo* experimental evidence.

**Keywords:** Ketamine, neurodevelopment, neurogenesis, NMDA receptors, oxidative stress

## Introduction

Although intravenous anesthetic agents are typically considered as safe to be administered during pediatric surgery, preclinical and clinical evidence has recently emerged regarding their potential neurotoxicity. Several studies have demonstrated that anesthetic exposure in early age may lead to long-term cognitive impairment as well as learning deficits.[1-4] The United States Food and Drug Administration has raised the concern of pediatric anesthetic neurotoxicity as a major public health issue,[5] and toward that direction, the Smart-Tots initiative has been carried out.[6,7] Moreover, a number of clinical studies have been performed in recent years,[8,9] and symposia are now assessing both the preclinical and clinical data on the potential correlation between anesthetic exposure and developmental neurocognitive impairment.[10] A central role in the ongoing debate on the potential developmental neurotoxicity of anesthetic agents is played by ketamine [Figure 1a], a *N*-methyl-D-aspartate (NMDA) receptor antagonist that is widely used in the pediatric anesthesia practice and in sub-anesthetic doses for sedation during diagnostic procedures.

Most of the currently available clinical evidence is derived from retrospective and observational clinical studies, and thus, very little can be concluded from them with regard to the mechanisms involved. We, herein, attempt a brief review of the cellular and molecular mechanisms suggested

**Address for correspondence:** Dr. Apostolos Zarros, Lab 112, L1, Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow, G61 1QH, Scotland, UK.
E-mail: apostolos.zarros@glasgow.ac.uk

**Access this article online**

Quick Response Code:

Website: www.joacp.org

DOI: 10.4103/joacp.JOACP_415_19



**How to cite this article:** Kalopita K, Armakolas A, Philippou A, Zarros A, Angelogianni P. Ketamine-induced neurotoxicity in neurodevelopment: A synopsis of main pathways based on recent *in vivo* experimental findings. J Anaesthesiol Clin Pharmacol 2021;37:37-42.

**Submitted:** 07-Dec-2019 **Accepted:** 07-Jan-2020 **Published:** 10-Apr-2021

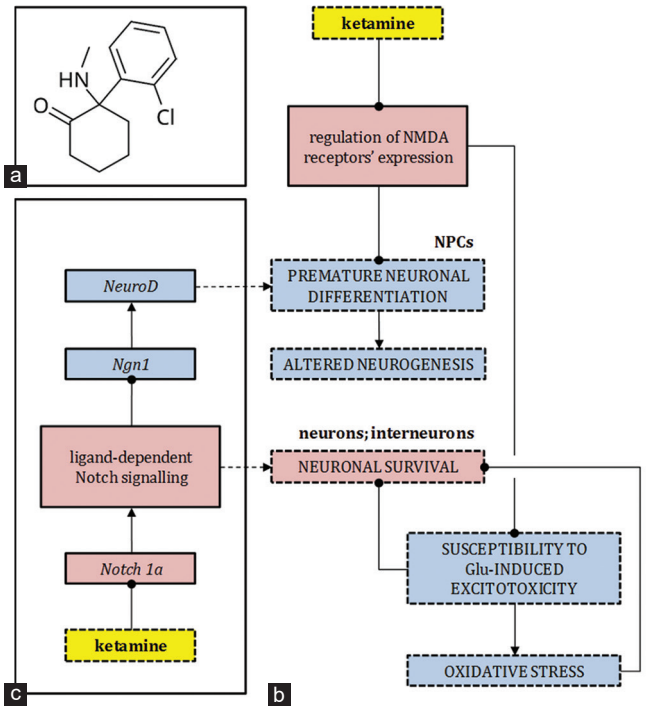


**Figure 1:** Pathways of ketamine-induced developmental neurotoxicity. (a): Chemical structure of ketamine. (b): It is well-established that ketamine deregulates the NMDA receptors' expression and as a result increases the neuronal susceptibility to the excitotoxic effects of Glu; the latter leads to a deregulation of the neuronal $Ca^{2+}$ signaling and—among other effects on the neuronal machinery—triggers the generation of oxidative stress and, in some cases, even the mitochondrial apoptotic pathway.[15,18,19,22,23] Recent experimental evidence suggests that the same deregulation of the NMDA receptors' expression leads to premature differentiation of NPCs.[26] (c): Ketamine has been reported to downregulate *Notch 1α*,[17] that in turn is expected to affect negatively the ligand-dependent Notch signaling in the proneural domain. The latter inhibition would upregulate *Ngn1* in the NPCs and decrease the possibility of neuronal survival in differentiated neurons. In the first case, the ketamine-induced upregulation of the *Ngn1* expression could lead to an upregulation of *NeuroD* expression (a critical factor for neuronal differentiation), leading to premature neuronal differentiation. The fact that Kanungo *et al.*[17] reported a downregulation of the *NeuroD* expression was suggested by the authors themselves to be a result of fewer surviving differentiated neurons as a result of the exposure to ketamine. Elements in blue color background indicate an increase, upregulation or enhancement, while elements in red color background indicate a decrease, downregulation or inhibition, as a result of the exposure to ketamine. $Ca^{2+}$: calcium; Glu: glutamate; NeuroD: neurogenic differentiation (transcription factor); Ngn1: neurogenin-1; NMDA: *N*-methyl-D-aspartate; Notch 1α: neurogenic locus notch homolog protein 1 alpha; NPCs: neural progenitor cells

to mediate ketamine-induced developmental neurotoxicity, utilizing a selected number of recent *in vivo* experimental evidence [Table 1].[11-24] Nevertheless, the translational value of the preclinical data discussed in this review is interpreted with caution.[25]

## Main pathways of ketamine-induced developmental neurotoxicity

Neuroapoptosis, a major consequence of ketamine's developmental toxicity and one of the first to be reported,[2] is now known to be not only dose-dependent[15,20] but also time-evolving[24] and dependent on the exposure time-window.[12,14,26-29] Caspase-3 protein level increase as well as the induction of neuroapoptosis seems to be "hallmarks" of ketamine-induced developmental neurotoxicity in both rodents and non-human primates.[12,15,19,22] Recent *in vivo* experimental evidence suggests that neuroapoptosis is only an aspect of a more complex pathophysiological cascade involved in ketamine-induced developmental neurotoxicity [Figure 1b]. Specifically, the deregulation of the NMDA receptors' expression (overexpression) and the induction of oxidative stress as a result of increased cellular susceptibility to glutamate (Glu) and calcium ($Ca^{2+}$) mobilization are evident and/or implied by a number of studies in rodents.[15,18-23]

### *Deregulation of NMDA receptors' expression*

As the antagonistic action on the NMDA receptor is one of the main mechanisms of the anesthetic and analgesic effect of ketamine, it comes as no surprise that a study performed on Sprague-Dawley rats has revealed a major and fulminant ketamine-induced upregulation of NMDA receptor subunit NR1 in the PND7 frontal cortex.[20] An earlier study investigating gene expression profiling in frontal cortical areas of age-matched (PND7) Sprague-Dawley rats that received ketamine, identified perturbations and confirmed an upregulation of NMDA receptors.[21]

The deregulation of the expression of the NMDA receptors contributes to the neuronal susceptibility to the excitotoxic effects of Glu after the clearance of ketamine, leading to a major deregulation of the neuronal $Ca^{2+}$-signaling, and to the generation of oxidative stress.[20] Moreover, due to the fact that Glu is an established regulator of neural progenitor cell (NPC) differentiation, and as NMDA receptors are considered to promote neuronal differentiation (through the overexpression of *NeuroD* as a result of neuronal excitation), premature neuronal differentiation becomes an additional consequence of the exposure to ketamine during neurodevelopment [Figure 1b].[26]

### *Mitochondrial dysfunction and oxidative stress/mitochondrial apoptotic pathway*

The deregulation of the neuronal $Ca^{2+}$ signaling as a result of the increased susceptibility to the excitotoxic effects of Glu has been reported to provoke mitochondrial dysfunction and the generation of oxidative stress in the hippocampi of rats exposed to ketamine during neurodevelopment.[15,18,19,22] Mitochondrial dysfunction in ketamine-exposed rat brains has been associated with a downregulation of critical components of the extracellular signal regulated kinase (ERK) signaling cascade,[15,18] implying a decreased capacity to perform critical gene transcription and translation. In the hippocampus, the latter could result to an impairment of synaptic consolidation and to a difficulty in the maintenance of long-term potentiation.[30]

**Table 1: Selected recent (2009-2019) *in vivo* experimental studies providing critical insight to the cellular and molecular mechanisms underlying ketamine-induced developmental neurotoxicity**

| Study | Species used; age at exposure | Exposure details (ED); main findings (MF); importance of the study (IMP) |
|---|---|---|
| Aligny *et al.*[11] | FVB-Tg(GadGFP)45704Swn transgenic mice; from GD15 to GD20 | ED: pregnant mice received ketamine at 50 mg/kg, daily, subcutaneously, *via* injection; MF: (i) at PND45, mice exposed to ketamine demonstrated significant loss of Gad67-GFP interneurons in cortical layers II-IV, accompanied by a significant reduction in the dendritic spine density of these same interneurons; (ii) GABA levels as well as GAT-1 and GAT-3 expression levels were found increased in PND45 ketamine-exposed male mice (but not in the female ones) as compared to controls; (iii) an exacerbated Glu-induced $Ca^{2+}$ mobilization was evident in PND45 ketamine-exposed female mice only, accompanied by higher levels of spontaneous locomotor activity; IMP: this study has targeted a time-window in which the migration of GABAergic precursors occurs in the developing murine brain, has utilized a dose of ketamine that can induce anesthesia for 3 h, and has demonstrated that although the ketamine-induced interneuronal loss is evident in both male and female mice, a sex-dependent adaptation of the GABAergic neurotransmission seems to provoke sex-specific behavioral deficits in adulthood |
| Brambrink *et al.*[12] | rhesus macaques; fetuses (GD120) and neonates (PND6) | ED: pregnant macaques (on GD120) received ketamine intravenously at a dose scheme of 10 mg/kg bolus, followed by a continuous infusion of 10-85 mg/kg of ketamine per h, for 5 h (supplemented with additional anesthetic-depth maintenance boluses); neonate macaques received ketamine intravenously at a dose scheme of 20 mg/kg bolus, followed by a continuous infusion of 20-50 mg/kg of ketamine per h, for 5 h (supplemented with additional anesthetic-depth maintenance boluses); MF: (i) both fetal and neonatal macaque brains exposed to ketamine demonstrated a significant increase of activated caspase-3 positivity as compared to controls; (ii) the pattern of the ketamine-induced neuroapoptosis was different in macaque fetuses than that in the respective neonates; IMP: this study has used a ketamine perfusion protocol of 5 h in order to match the depth and duration of anesthesia considered as "standard" in anesthetic drug testing and is among the few studies ever to demonstrate the differences in ketamine-induced neuroapoptotic injury as a result of different exposure time-windows, in nonhuman primates |
| Dong *et al.*[13] | Sprague-Dawley rats; GD17 | ED: pregnant rats on GD17 were exposed to ketamine *via* intraperitoneal injection at different doses (1, 2, 10, 20, 40, and 100 mg/kg), followed by an intraperitoneal injection of BrdU 24 h later; MF: GD19 fetuses demonstrated a dose-dependent reduction of BrdU-positive cells in the VZ and SVZ of their brain cortex, just 48 h after their exposure to ketamine; IMP: this study demonstrates that even in low doses, ketamine can inhibit cell proliferation in critical neurogenic regions of the developing rat brain |
| Huang *et al.*[14] | Sprague-Dawley rats (male); PND7 | ED: rats were exposed to 4 intraperitoneal injections of ketamine (40 mg/kg each) at 1 h intervals; MF: (i) ketamine-exposed rats demonstrated a transient disruption of their NSC proliferation and differentiation, as revealed by a series of well-devised experiments using BrdU along with a panel of other immunofluorescence stains; (ii) exposure to ketamine caused an inhibition of neuronal migration and in the granule cell layer of the hippocampal dentate gyrus of PND37 and PND44 rats, accompanied by a reduced growth of astrocytes in the hippocampal dentate gyrus; (iii) 2-month-old rats previously exposed to ketamine demonstrated a lower performance in the Morris water maze test than their age-matched controls; IMP: this is a critical study for the understanding of the role of the hippocampus in the ketamine-induced developmental neurotoxicity, with important leads regarding neuronal migration and glial growth |
| Huang *et al.*[15] | Sprague-Dawley rats; PND7 to PND9 | ED: rats were exposed to ketamine *via* intraperitoneal injections, at various doses (25, 50, and 75 mg/kg), once daily for 3 days; MF: 24 h after the last injection, ketamine-exposed rats demonstrated an increased number of apoptotic cells in the dentate gyrus and the CA1 region of their hippocampi only at the highest dose scheme (75 mg/kg), supported by decreased expression levels of p-PKCγ, p-ERK1/2, and Bcl-2; (ii) female rats of the same treatment group (75 mg/kg) demonstrated a decreased performance in the Morris water maze test at PND60; IMP: this study provides evidence of a PKCγ-ERK signaling pathway-mediated rat hippocampal neurodegeneration as a result of exposure to ketamine at a high dose (75 mg/kg) during the PND7-PND9 time-window, that is consistent with learning and memory impairment as assessed in adulthood in female only rats |

*Contd...*

**Table 1: Contd...**

| Study | Species used; age at exposure | Exposure details (ED); main findings (MF); importance of the study (IMP) |
|---|---|---|
| Jeevakumar *et al.*[16] | CB6-Tg[Gad1-EGFP] G42Zjh/J mice (male); PND7 to PND11 | ED: rats received subcutaneous injections of ketamine at 30 mg/kg on PND7, PND9, and PND11; MF: (i) rats exposed to ketamine demonstrated a significant reduction of PV-expressing interneurons in the medial prefrontal cortex, in adulthood; (ii) adult rats exposed to ketamine demonstrated schizophrenia-like behavioral performance when assessed on a battery of behavioral experiments between PND90 and PND120; IMP: the authors claim this method of utilizing the neurodevelopmental exposure to ketamine could act as a model for the experimental simulation of the "*cognitive and negative symptoms of schizophrenia*" |
| Kanungo *et al.*[17] | Transgenic *(hb9:GFP) Danio* rerio (zebrafish); embryos | ED: zebrafish embryos were exposed to 0.5 and 2 mM of ketamine for 2 or 20 h; MF: (i) when administered for 20 h, ketamine at 2 mM decreased cranial and motor neuron populations, as well as the axon length of the latter; (ii) ketamine suppressed the expression of the *Notch 1α* gene and downregulated the expression of the motor neuron-inducing *NeuroD* and *Gli2b*, while it upregulated the expression of *Ngn1*; IMP: this is a unique experimental study that describes a new pathway of ketamine-induced developmental neurotoxicity through the manipulation of differentiating and differentiated neurons (for more details, see Figure 1) |
| Li *et al.*[18] | Wistar rats; GD14 | ED: pregnant rats received an intravenous injection of ketamine (200 mg/kg) for 3h; MF: (i) PND30 offspring rats exposed to ketamine during gestation demonstrated decreased levels of ERK, p-ERK, PKA, p-PKA, p-CREB, and BDNF in their hippocampi; (ii) these same rats demonstrated an impaired performance in a battery of behavioral tests as a result of their exposure to ketamine on GD14; IMP: this is a representative study of this group of authors, demonstrating the role of the ERK-CREB signaling pathway in ketamine-induced developmental neurotoxicity |
| Li *et al.*[19] | Wistar rats; GD19 | ED: pregnant rats received an intravenous injection of ketamine (200 mg/kg) for 3 h; MF: (i) GD19 rat embryos exposed to ketamine demonstrated the induction of oxidative stress in parallel to increased levels of cleaved-caspase-3, Bax, LC3-II, and ATG5 protein levels, as well as decreased Bcl-2, ATG4, and P62 protein levels in their hippocampi; IMP: this study provides evidence of a potential ketamine-induced ROS-mediated activation of the mitochondrial apoptotic pathway in the developing hippocampus, as well evidence of autophagy as a result of the exposure to ketamine under the specific experimental conditions |
| Liu *et al.*[20] | Sprague-Dawley rats; PND7 | ED: rat pups received ketamine at different doses (5, 10, and 20 mg/kg) in 1, 3, or 6 subcutaneous injections at 2-h intervals, on PND7; MF: only the rats receiving 6 injections of 20 mg/kg of ketamine demonstrated significantly increased apoptotic death in their frontal cortex, while *in situ* hybridization revealed an overexpression of the NR1 subunit of the NMDA receptor of these same rats' frontal cortex; IMP: this simple study demonstrates a ketamine-induced "compensatory" upregulation of the NMDA receptors and apoptosis, both after repeated exposures of ketamine at the highest dose tested |
| Shi *et al.*[21] | Sprague-Dawley rats; PND7 | ED: rat pups received ketamine at 20 mg/kg in 6 subcutaneous injections at 2-h intervals, on PND7; MF: the rats receiving ketamine had their frontal cortical areas' RNA profiled and identified perturbations were further investigated with the use of other techniques, revealing an upregulation of NMDA receptors; IMP: this study is supplementary to that of Liu *et al.*[20] |
| Yan *et al.*[22] | Sprague-Dawley rats; PND7 to PND10 | ED: rat pups received ketamine at 75 mg/kg, in 3 intraperitoneal injections at 24-h intervals, starting on PND7; MF: (i) exposure to ketamine provoked an increase of HIF-1α, cleaved-caspase 3 and p53 protein levels in the PND11 rat hippocampi, in addition to a decreased Bcl-2/Bax ratio; (ii) the administration of YC-1, L-carnitine or nimodipine; IMP: this study suggests that the ROS/HIF-1α pathway is activated in ketamine-induced hippocampal neurodegeneration |
| Ye *et al.*[23] | C57BL/6 mice; PND10 to PND17 or PND30 to PND37 | ED: mice were administered ketamine at 100 mg/kg per day for 7 consecutive days *via* intraperitoneal injection; MF: the levels of pyroptosis-related proteins (caspase-1, caspase-11, NLRP3, IL-1β, and IL-18) were found to be significantly increased after multiple doses of ketamine administration; IMP: this is a unique study suggesting the caspase-1-dependent pyroptosis could be an essential component of the mitochondrial apoptotic pathway triggered by ketamine |

*Contd...*

**Table 1: Contd...**

| Study | Species used; age at exposure | Exposure details (ED); main findings (MF); importance of the study (IMP) |
|---|---|---|
| Zhao *et al.*[24] | Sprague-Dawley rats; GD14 | ED: pregnant rats were exposed to ketamine at a sedative dose totalling to approximately 144 mg/kg over 2 h; MF: (i) cell apoptosis and neuronal loss were evident in newborn (PND0) rats as a result of exposure to ketamine on GD14; (ii) PND30 rats previously exposed to ketamine demonstrated more abundant dendritic branching in their prefrontal cortical neurons; IMP: this study suggests that prenatal exposure to ketamine deregulates the neurodevelopment in the prefrontal cortex of rats in a time-evolving manner |

*In the FVB-Tg (GadGFP) 45704Swn transgenic mice, the expression of a green fluorescent protein is controlled by the Gad1 gene promoter in the GABAergic interneurons of ganglionic eminences.[11] The full-term gestation of the rhesus macaque is 165 days.[12] BrdU is an S-phase marker of the cell cycle.[13,14] YC-1 is an inhibitor of HIF-1α.[22] ATG4: autophagy-related protein 4; ATG5: autophagy-related protein 5; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; BDNF: brain-derived neurotrophic factor; BrdU: 5-bromo-2'-deoxyuridine; $Ca^{2+}$: calcium; cAMP: cyclic-AMP; ERK: extracellular signal regulated kinase; CREB: cAMP response element-binding protein; GABA: γ-aminobutyric acid; GAT-1: GABA transporter 1; GAT-3: GABA transporter 3; GD: gestational day; Gli2b: GLI family zinc finger 2b; Glu: glutamate; HIF-1α: hypoxia-inducible factor 1 alpha; IL-1β: interleukin-1 beta; IL-18: interleukin-18; LC3: microtubule-associated proteins 1A/1B light chain 3B; LC3-II: the lipid-modified form of LC3; NeuroD: neurogenic differentiation (transcription factor); Ngn1: neurogenin-1; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; NMDA: N-methyl-D-aspartate; Notch 1α: neurogenic locus notch homolog protein 1 alpha; NR1: subunit 1 of the NMDA receptor; NSC: neural stem cell; p-CREB: phosphorylated cAMP response element-binding protein; p-ERK: phosphorylated extracellular signal regulated kinase; p-ERK1/2: phosphorylated extracellular signal regulated kinase 1/2; PKA: protein kinase A; PND: postnatal day; p-PKA: phosphorylated protein kinase A; p-PKCγ: phosphorylated protein kinase C gamma; PV: parvalbumin; ROS: reactive oxygen species; SVZ: subventricular zone; VZ: ventricular zone*

Also, triggering of the mitochondrial apoptotic pathway has been reported to evolve autophagy and caspase-1-dependent pyroptosis in rodents.[19,23]

### *Deregulation of neurogenesis through premature neuronal differentiation*

An experimental study on transgenic zebrafish embryos[17] has put forward a new candidate pathway of ketamine-induced developmental neurotoxicity through the manipulation of differentiating and differentiated neurons [Figure 1c]. More specifically, zebrafish embryos were exposed to 0.5 and 2 mM of ketamine for 2 or 20 h; when administered for 20 h, ketamine at 2 mM was found not only to decrease cranial and motor neuron populations, and the axon length of the latter, but also to: (i) suppress the expression of the *Notch 1*α gene, (ii) downregulate the expression of the motor neuron-inducing *NeuroD* and *Gli2b*, and (iii) upregulate the expression of *Ngn1*.[17]

The reported ketamine-induced downregulation of *Notch 1*α[17] is expected to affect negatively the ligand-dependent Notch signaling in the proneural domain. The latter inhibition would upregulate *Ngn1* in the NPCs and decrease the possibility of neuronal survival in differentiated neurons. In the first case, the ketamine-induced upregulation of the *Ngn1* expression could lead to an upregulation of *NeuroD* expression, leading to premature neuronal differentiation.[26] A downregulation of the *NeuroD* expression has been reported[17] and it was suggested to be a result of fewer surviving differentiated neurons as a result of the exposure to ketamine [Figure 1c].

This—yet to be confirmed in mammals—mode of ketamine-induced developmental neurotoxicity could explain the findings of Aligny *et al.*[11] on FVB-Tg(GadGFP) 45704Swn transgenic mice, where both the migration and the cytomorphology of GABAergic interneurons in the cortical layers II-IV were significantly affected by maternal exposure to ketamine from GD15 to GD20. It could also account for the dose-dependent inhibition of cell proliferation in critical rat neurogenic regions such as the ventricular and the subventricular zones by a single intraperitoneal injection of ketamine on GD 17.[13]

## Other findings of interest

Interestingly, the reduction of parvalbumin-expressing interneurons in the adult murine medial prefrontal cortex as a result of exposure to ketamine during the PND7 to PND11 time-window seems to be compatible with the expression of a phenotype that could act as a model for the experimental simulation of the "*cognitive and negative symptoms of schizophrenia*".[16] Moreover, a critical and noteworthy study for the understanding of the role of the hippocampus in the ketamine-induced developmental neurotoxicity, with important leads regarding neuronal migration and glial growth, has been performed by Huang *et al.*[14] In that well-designed study on Sprague-Dawley rats, ketamine-exposed rats on PND7 demonstrated a transient disruption of their neural stem cell proliferation and differentiation, and an inhibition of neuronal migration and in the granule cell layer of the hippocampal dentate gyrus upon reaching PND37 and PND44, which were accompanied by reduced growth of astrocytes in the hippocampal dentate gyrus.[14]

## The "bigger picture" and translational perspectives

Despite the progress recorded regarding the understanding of the neurodevelopmental toxicity of ketamine, the clinical translation of the aforementioned experimental findings should be done with caution and only after

considering that: (i) in clinical pediatric or obstetric practice, ketamine is rarely used as a stand-alone anesthetic agent,[12] (ii) sex-dependent differences with regards to the developmental neurotoxicity of ketamine seem to exist,[11] and (iii) *in vivo* experimental studies involving a maternal exposure to ketamine rarely provide details of the maternal hemodynamic and respiratory stability; the latter being a critical interfering factor for the reliability of that type of study.[13] The pathways presented in this review seem to form a bigger picture in which the extent and the nature of the neuronal susceptibility to ketamine during neurodevelopment is strongly dependent on the experimental conditions employed.

## Financial support and sponsorship
Nil.

## Conflicts of interest
There are no conflicts of interest.

# References

1. Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, Olson MD, *et al.* Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. Pediatrics 2011;128:e1053-61.
2. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, *et al.* Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283:70-4.
3. Vutskits L, Davidson A. Update on developmental anesthesia neurotoxicity. Curr Opin Anaesthesiol 2017;30:337-42.
4. Walters JL, Paule MG. Review of preclinical studies on pediatric general anesthesia-induced developmental neurotoxicity. Neurotoxicol Teratol 2017;60:2-23.
5. Kuehn BM. FDA considers data on potential risks of anesthesia use in infants, children. JAMA 2011;305:1749-50, 1753.
6. Ramsay JG, Rappaport BA. SmartTots: A multidisciplinary effort to determine anesthetic safety in young children. Anesth Analg 2011;113:963-4.
7. Ramsay JG, Roizen M. SmartTots: A public-private partnership between the United States Food and Drug Administration (FDA) and the International Anesthesia Research Society (IARS). Paediatr Anaesth 2012;22:969-72.
8. Davidson AJ, Disma N, de Graaff JC, Withington DE, Dorris L, Bell G, *et al.* Neurodevelopmental outcome at 2 years of age after general anaesthesia and awake-regional anaesthesia in infancy (GAS): An international multicentre, randomised controlled trial. Lancet 2016;387:239-50.
9. Gleich SJ, Flick R, Hu D, Zaccariello MJ, Colligan RC, Katusic SK, *et al.* Neurodevelopment of children exposed to anesthesia: Design of the Mayo Anesthesia Safety in Kids (MASK) study. Contemp Clin Trials 2015;41:45-54.
10. Lee JJ, Sun LS, Levy RJ. Report on the Sixth Pediatric Anesthesia Neurodevelopmental Assessment (PANDA) Symposium, "Anesthesia and Neurodevelopment in Children". J Neurosurg Anesthesiol 2019;31:103-7.
11. Aligny C, Roux C, Dourmap N, Ramdani Y, Do-Rego JC, Jégou S, *et al.* Ketamine alters cortical integration of GABAergic interneurons and induces long-term sex-dependent impairments in transgenic Gad67-GFP mice. Cell Death Dis 2014;5:e1311.
12. Brambrink AM, Evers AS, Avidan MS, Farber NB, Smith DJ, Martin LD, *et al.* Ketamine-induced neuroapoptosis in the fetal and neonatal rhesus macaque brain. Anesthesiology 2012;116:372-84.
13. Dong C, Rovnaghi CR, Anand KJ. Ketamine exposure during embryogenesis inhibits cellular proliferation in rat fetal cortical neurogenic regions. Acta Anaesthesiol Scand 2016;60:579-87.
14. Huang H, Liu CM, Sun J, Hao T, Xu CM, Wang D, *et al.* Ketamine affects the neurogenesis of the hippocampal dentate gyrus in 7-day-old rats. Neurotox Res 2016;30:185-98.
15. Huang L, Liu Y, Jin W, Ji X, Dong Z. Ketamine potentiates hippocampal neurodegeneration and persistent learning and memory impairment through the PKCγ-ERK signaling pathway in the developing brain. Brain Res 2012;1476:164-71.
16. Jeevakumar V, Driskill C, Paine A, Sobhanian M, Vakil H, Morris B, *et al.* Ketamine administration during the second postnatal week induces enduring schizophrenia-like behavioral symptoms and reduces parvalbumin expression in the medial prefrontal cortex of adult mice. Behav Brain Res 2015;282:165-75.
17. Kanungo J, Cuevas E, Ali SF, Paule MG. Ketamine induces motor neuron toxicity and alters neurogenic and proneural gene expression in zebrafish. J Appl Toxicol 2013;33:410-7.
18. Li X, Guo C, Li Y, Li L, Wang Y, Zhang Y, *et al.* Ketamine administered pregnant rats impair learning and memory in offspring via the CREB pathway. Oncotarget 2017;8:32433-49.
19. Li Y, Li X, Zhao J, Li L, Wang Y, Zhang Y, *et al.* Midazolam attenuates autophagy and apoptosis caused by ketamine by decreasing reactive oxygen species in the hippocampus of fetal rats. Neuroscience 2018;388:460-71.
20. Liu F, Paule MG, Ali S, Wang C. Ketamine-induced neurotoxicity and changes in gene expression in the developing rat brain. Curr Neuropharmacol 2011;9:256-61.
21. Shi Q, Guo L, Patterson TA, Dial S, Li Q, Sadovova N, *et al.* Gene expression profiling in the developing rat brain exposed to ketamine. Neuroscience 2010;166:852-63.
22. Yan J, Huang Y, Lu Y, Chen J, Jiang H. Repeated administration of ketamine can induce hippocampal neurodegeneration and long-term cognitive impairment via the ROS/HIF-1α pathway in developing rats. Cell Physiol Biochem 2014;33:1715-32.
23. Ye Z, Li Q, Guo Q, Xiong Y, Guo D, Yang H, *et al.* Ketamine induces hippocampal apoptosis through a mechanism associated with the caspase-1 dependent pyroptosis. Neuropharmacology 2018;128:63-75.
24. Zhao T, Li C, Wei W, Zhang H, Ma D, Song X, *et al.* Prenatal ketamine exposure causes abnormal development of prefrontal cortex in rat. Sci Rep 2016;6:26865.
25. Disma N, Hansen TG. Pediatric anesthesia and neurotoxicity: Can findings be translated from animals to humans? Minerva Anestesiol 2016;82:791-6.
26. Dong C, Anand KJ. Developmental neurotoxicity of ketamine in pediatric clinical use. Toxicol Lett 2013;220:53-60.
27. Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol 2013;106-107:1-16.
28. Zanghi CN, Jevtovic-Todorovic V. A holistic approach to anesthesia-induced neurotoxicity and its implications for future mechanistic studies. Neurotoxicol Teratol 2017;60:24-32.
29. Cheung HM, Yew DTW. Effects of perinatal exposure to ketamine on the developing brain. Front Neurosci 2019;13:138.
30. Zarros A, Byrne AM, Boomkamp SD, Tsakiris S, Baillie GS. Lanthanum-induced neurotoxicity: Solving the riddle of its involvement in cognitive impairment? Arch Toxicol 2013;87:2031-5.