Developmental Neurotoxicity of Ketamine: Morphometric Confirmation, Exposure Parameters, and Multiple Fluorescent Labeling of Apoptotic Neurons

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Ketamine is a widely used pediatric anesthetic recently reported (C. Ikonomidou et al., 1999, Science 283, 70–74) to enhance neuronal death in neonatal rats. To confirm and extend these results, we treated four groups of PND 7 rats with seven sc doses, one every 90 min, of either saline, 10 mg/kg ketamine, 20 mg/kg ketamine, or a single dose of 20 mg/kg ketamine. The repeated doses of 20 mg/kg ketamine increased the number of silver-positive (degenerating) neurons in the dorsolateral thalamus, where ketamine-treated animals had a death rate of neuronal apoptosis. The repeated doses of 20 mg/kg ketamine increased the number of silver-positive (degenerating) neurons in the dorsolateral thalamus to a degree comparable to ketamine increased the number of silver-positive (degenerating) neurons in the dorsolateral thalamus, where ketamine-treated animals had a death rate of neuronal apoptosis. However, blood levels of ketamine immediately after the repeated 20 mg/kg doses were about 14 μg/ml, about seven-fold greater than anesthetic blood levels in humans (J. M. Malinovsky et al., 1996, Br. J. Anaesth. 77, 203–207; R. A. Mueller and R. Hunt, 1998, Pharmacol. Biochem. Behav. 60, 15–22). Levels of ketamine in blood following exposure to the multi-10 mg/kg doses of ketamine or to a single 20 mg/kg dose ranged around 2–5 μg/ml; although these blood levels are close to an anesthetic level in humans, they failed to produce neurodegeneration. To investigate the mode of ketamine-induced neuronal death, coronal sections were stained with both Fluoro-Jade B (a green fluorescent stain selective for neurodegeneration) and DAPI (a blue DNA stain), as well as for caspase-3 (using an antisera labeled red with rhodamine). These histochemical results confirmed the developmental neurotoxicity of ketamine, demonstrated that Fluoro-Jade B (FJ-B), like silver methods, successfully stained degenerating neurons in neonatal rats, and indicated that ketamine acts by increasing the rate of neuronal apoptosis.

Key Words: neurodegeneration; apoptosis; blood levels; anesthesia; NMDA; caspase-3.

Ketamine ((±)-2-(2-chlorophenyl)-2-(methylamino)cyclohexanone) is a noncompetitive blocker of N-methyl-D-aspartate (NMDA) receptor ion channels (Anonymous, 2004). It received FDA approval in 1970 for use as an anesthetic. Ketamine is frequently used in infants and toddlers for elective surgeries, as well as for emergency room procedures (Bergman, 1999; Green and Johnson, 1990). It is short acting and provides rapid dissociative anesthesia followed by rapid recovery. In subsequent studies, it was concluded that ketamine could be safely used for anesthesia in infants and children in emergency room settings (Green et al., 2000, 2001a,b; McCarty et al., 1999). However, adverse events associated with ketamine use have been reported, including emesis, agitation during recovery, apnea, respiratory depression, and laryngospasm (Green et al., 1996, 1998a,b, 1999; Ririe et al., 1997). Sometimes, ketamine is used for postoperative analgesia or sedation after cardiothoracic procedures in one-week-old infants (Hartvig et al., 1993), and exposure for up to a month has been reported in a 14-month-old burn patient (Cederholm et al., 1990).

Recently, NMDA antagonists, whether given alone or in combination with GABA (gamma amino butyric acid) agonists, markedly increased neurodegeneration throughout the developing brain of seven-day-old rat pups (Ikonomidou et al., 1999, 2001; Ishimaru et al., 1999; Olney et al., 2000, 2002a,b,c). As observed for other NMDA antagonists such as MK-801 ((dizocilpine), (±)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine), ketamine produced elevated numbers of apoptotic neurons throughout the seven-day-old rat brain (Ikonomidou et al., 1999). However, while MK-801 neurotoxicity occurred after a single exposure, ketamine neurotoxicity was reported only after a regimen of multiple injections given during a period of about 9 h (Ikonomidou et al., 1999). The brain region most affected by ketamine was the dorsolateral thalamus, where ketamine-treated animals had a 31-fold increase in neurons stained selectively for neurodegeneration with the De Olmos silver method. These results suggested that NMDA receptor stimulation was a critical factor for neuronal survival during development and that suppression of this activity resulted in an increase in apoptotic cell death (Haberny et al., 2002; Olney, 2002; Olney et al., 2002c).
The present study sought to confirm the finding that multiple doses of ketamine administered over a short period of time to the early postnatal rat pup would result in widespread increases in neurodegeneration, using the same procedures (Ikonomidou et al., 1999). We also wished to include an independent measure of neurodegeneration, the green fluorescent stain Fluoro-Jade B (Schmued and Hopkins, 2000; Ye et al., 2001), which, unlike silver methods, can be used in multiple staining procedures for cellular apoptotic markers. Finally, we wanted to include lower doses and briefer exposures to ketamine, so that we could begin to characterize the blood levels of ketamine associated with its neurotoxicity in perinatal rat pups.

MATERIALS AND METHODS

Animals and dosing. Sprague-Dawley rat pups (Charles River, Wilmington, MA) were sexed, randomized, and returned to their dams in sets of four females and four males on PND 2. On PND 7, rats were randomly assigned to one of the four dose groups and to either the histology or blood level experiment. Animals were dosed subcutaneously with racemic ketamine hydrochloride (Ketaset, Wyeth Pharmaceuticals, Madison, NJ) or saline using a 0.3 ml syringe with a 30 gauge needle. Animals were returned to their dam between injections. Control rats (n = 10) received seven doses of saline, low dose rats (n = 14) received seven doses of 10 mg/kg ketamine, and high dose rats (n = 15) received seven doses of 20 mg/kg ketamine. The interval between repeated injections was 90 min for each of these groups. An additional group (n = 8), termed “high single,” received six doses of saline followed by a single dose of 20 mg/kg ketamine as the final injection in the series.

Blood collection. Rat pups from each group (n = 3–4) were sacrificed by decapitation 2 min after their last injection and their blood was collected for analysis of ketamine levels by high performance liquid chromatography (HPLC) using modified literature methods (Adams et al., 1992; Gross et al., 1999). The plasma was isolated from whole blood by centrifugation at 4°C. The plasma supernate was transferred to plastic cryovials, placed on dry ice until frozen, and then stored in a −80°C freezer until analysis. Gender was determined at the time of sacrifice.

Sample preparation. Extracts were analyzed from rat plasma after application to silica based solid phase extraction (SPE) C-2 cartridges using a modification of a literature SPE method (Mistry et al., 1998). Ketamine and bupivacaine, the internal standard (IS), were added to the plasma samples. Plasma samples and standards (500 ul) were diluted 1:1 with water and vortexed for 30 s. The 200 mg C-2 SPE columns (Varian, Harbor City, CA) were conditioned with (1) 3 ml of methanol (MeOH) and (2) 3 ml of water. Plasma samples were loaded onto the SPE column then rinsed with (1) 3 ml of water and (2) 3 ml of 50:50 MeOH/water. Samples were eluted with 50:50 acetonitrile/0.2 N hydrochloric acid (HCl). Samples were dried under vacuum in a spin evaporator (Savant, Farmingdale, NY) and reconstituted in 150 μl of the mobile phase.

Plasma analysis. All reconstituted standards and samples were analyzed on a Hewlett-Packard 1090 (Wilmington, DE) HPLC system equipped with a multi-channel pump, auto-injector, solvent degasser, and diode array detector (DAD). Separation was achieved on a Phenomenex C-18 Luna (2), 5 micron (250 × 4.6 mm) reverse phase HPLC column (Torrance, CA) with a Phenomenex C-18 guard cartridge (4.0 × 3.0 mm). The mobile phase was acetonitrile/10 mM phosphate, pH = 3.0 (11:89, v/v) delivered isocratically for 22 min. The flow rate was 1.0 ml/min. The injection volume for samples and standards was 50 μl. The UV detection wavelength was 205 nm. The observed retention times for ketamine and the internal standard bupivacaine were 9.9 and 15.9 min respectively.

Chemicals and reagents. Ketamine and bupivacaine were purchased from Sigma (St. Louis, MO). Acetonitrile and methanol HPLC grade were purchased from Burdick & Jackson (Muskegon, MI). HPLC grade monobasic potassium phosphate and ACS grade hydrochloric acid were purchased from Fisher Scientific (Pittsburgh, PA). Filtered 18 meg-ohm water was supplied in-house by a Millipore Milli-Q System (Bedford, MA). Blank rat plasma was purchased from Hilltop Laboratories (Scottsdale, PA).

Perfusion. The remaining rat pups (those not sacrificed for blood levels of ketamine) were deeply anesthetized with sodium pentobarbital 24 h after the initial dose (i.e., on PND 8). They were then perfused transcervically, with a saline flush (about 10 ml) followed by about 50 ml of 4% paraformaldehyde fixative in neutral cacodylate buffer. Their brains were then removed and stored in fixative until further processing.

Histological processing. The rat pup brains were then equilibrated with sucrose and embedded in gelatin as batches of 16 brains with all of the groups represented in each batch. They were then frozen prior to serial sectioning on a sledge microtome. Sections of 30 μm thickness, 16 of each of the 16 brains, and from approximately the same anteroposterior plane of section were then mounted on 2 × 3 inch glass slides. A one in five series of these slides were then stained with a cupric silver method selective for neurodegeneration (Beltraminio et al., 1993).

Certain of the remaining sections were then used for triple fluorescent staining of (1) immunoreactive caspase-3, (using a rabbit polyclonal antisera directed against the active 18 kDa form of caspase-3, Trevigen, Gaithersburg, MD) (Scallet, 1995; Scallet et al., 1988), (2) degenerating neurons using Fluoro-Jade B (Schmued and Hopkins, 2000; Ye et al., 2001), and (3) nuclear DNA using DAPI (4',6-diamidino-2-phenylindole) (Spackova et al., 2003). Briefly, the procedure involved rinsing the sections three times for 5 min each in 0.1 M phosphate buffer (pH 7.4), followed by an overnight incubation in anti-caspase-3 antisera at 5°C. The primary rabbit anti-caspase-3 antisera was diluted 1:500 in an antibody diluent prepared with 2% normal goat serum and 0.3% Triton X-100 in 0.1 M phosphate buffer. The next day, following three more 5 min rinses in buffer, sections were incubated for an hour in a 1:100 dilution in antibody diluent of the secondary antibody, a goat anti-rabbit antisera conjugated with rhodamine (Chemicon, Temecula, CA). Following three more rinses in buffer, the sections were viewed wet under green excitation for red rhodamine epifluorescence to confirm the labeling of caspase-3 immunopositive apoptotic neurons in the laterodorsal thalamus. Then the sections were rinsed in water for about 2 min and in potassium permanganate (0.06%) for 10 min with mild agitation. Following another 2 min rinse in water, the sections were incubated in 0.1% acetic acid containing a mixture of 0.0002% DAPI (Sigma Chemical, St. Louis, MO) and 0.0004% Fluoro-Jade B (Histo-Chem, Jefferson, AR). The resulting sections were then viewed individually and photographed with epifluorescence using green incident light for caspase-3, ultraviolet light for DAPI, and blue light for Fluoro-Jade B.

Morphometric methods. The dorsolateral thalamus was previously reported to have sustained the largest fold-increase in degenerating neurons following ketamine exposure (Ikonomidou et al., 1999). Thus the dorsolateral thalamus, with the addition of the medial amygdala, were selected for the purpose of morphometric confirmation of the previous findings (Ikonomidou et al., 1999). Slides were selected that contained the Lateral Dorsal thalamus, VentoLateral division (LDVL according to the rat brain atlas) at an anteroposterior level of about –2.5 mm from bregma (Paxinos and Watson, 1986). Because the rat brains were embedded in register, usually a single slide contained sections taken from the same anteroposterior level from about 12 of the 16 brains represented on each slide. If a given brain’s sections were a little more anterior or posterior than the others, it was a simple matter to select the preceding or succeeding slide in order to sample the LDVL and the medial amygdala at a comparable anteroposterior level for each brain.

The silver degeneration procedure produced high-contrast staining of dark positive neurons against a light background (see Fig. 1B). Using an image analysis system (MCID5 – Imaging Research, Inc., St. Catherine’s, Ontario, Canada), two investigators unaware of the treatment conditions agreed on an outline of the LDVL and medial amygdala from each brain. The area of the
outlined brain nucleus was then provided by the image analysis system. The
greyscale thresholds were set midway between the mean signal intensity of a
positive neuron and the mean background level for each brain section in order to
systematically segment positive neurons from background and provide a total
count as previously described (Scallet et al., 2000). Data were then expressed as
the mean frequency of degenerating, silver-positive cells per mm² of the brain
region and as fold-increases for purposes of comparison with the original report
on ketamine neurotoxicity (Ikonomidou et al., 1999).

**Statistical analysis.** Statistical analysis was by one-way ANOVA (Prism
Version 3.02, GraphPad Software, San Diego, CA) with four levels of dose: control,
low, high, and high single. Because there were no differences between the male and
female rat pups, their data were combined for analysis. Post-hoc individual means
comparisons were conducted by Tukey’s Multiple Comparisons Tests.

**RESULTS**

**Frequency of Degenerating Neurons after Exposure to Ketamine**

Compared to controls, ketamine exposure increased the
number of LDVL thalamic neurons that were positively stained

![Figure 1](image-url)

**FIG. 1.** An illustration of the appearance, distribution, and numerical density of silver-labeled degenerating neurons of the hippocampus, thalamus, and
amygdala in control and treated animals. (a) (x 2) From a control seven-day-old rat pup sacrificed 24 h after the first of a series of seven injections of 0.1 ml/kg of
saline, sc. Although the fornix (Fx), hippocampus (Hipp), and thalamus are clearly visible, only a few, sparse silver-labeled cells can be seen scattered through
these regions and in the cortex. (b) (x 2) From the comparable areas of a rat that received seven injections of 20 mg/kg ketamine. Note the dense grouping so f
silver-positive degenerating neurons in the laterodorsal thalamus, ventrolateral division (LDVL), in the ventromedial thalamus (VM), and the smaller patches of
degenerating cells in the striatum. (c) (x 10) From the region of the medial amygdalar nucleus (Med Amyg) of a control rat, with only a few cells stained
positively for degeneration. (d) (x 10) Illustration of the comparable region of a “high dose” (7 × 20 mg/kg) rat; note that considerably more medial amygdalar
neurons are silver-positive for degeneration. (e) Graph of the density of degenerating LDVL cells, identified as apoptotic (see Fig. 2), as a function of ketamine
exposure. Note that the repeated 20 mg/kg dose has a significantly (*p < 0.01) higher density of apoptotic cells than any of the other groups, which do not differ
from each other. The elevation of apoptotic cells in the LDVL represents a 28-fold increase, compared to the 31-fold increase in this same region reported by
Ikonomidou et al. (1999). (f) Indicates a parallel observation for the medial amygdala, with the repeated 20 mg/kg dose group having a significantly (*p < 0.05)
higher density of apoptotic cells than any of the other groups, which again do not differ from each other.
using the De Olmos silver method for detection of neurodegeneration (F(3,32) = 7.2, p < 0.001; see Figure 1e for quantitation and Figure 1b vs. 1a for appearance). Tukey Multiple Comparisons indicated that the “high dose” group (seven repeated doses of 20 mg/kg ketamine) was significantly greater (p values < 0.01) than each of the other three groups (control, low, and high single), which were not significantly different from each other. This elevation of the “high dose” group represented a 28-fold increase over control levels of degenerating neurons.

Ketamine exposure also increased the number of medial amygdalar neurons positively stained for neurodegeneration (F(3,32) = 5.9, p < 0.01; see Figure 1f for quantitative comparisons between groups and see Figures 1d vs. 1c for appearance). Tukey Multiple Comparisons indicated that the “high dose” group (seven repeated doses of 20 mg/kg ketamine) was significantly greater (p values < 0.05) than each of the other three groups (control, low, and high single), which were not significantly different from each other. The elevation of the “high dose” group, while statistically significant, represents only about a three-fold increase over control levels of degenerating neurons. Apparently, the numerically smaller fold-increase is because the control levels of degenerating neurons in the medial amygdala were higher than in the LDVL despite comparable levels between the two regions in the “high dose” group (see Figure 1e vs. 1f).

Fluoro-Jade B vs. DeOlmos Silver Neurodegeneration Staining in the LDVL

To compare Silver and Fluoro-Jade staining results, examine Figures 2a, 2b, 2c, and 2d. Figure 2a is a silver-stained control, easily distinguished from Figure 2b, a silver-stained section from a ketamine-treated rat pup. Figure 2c is a Fluoro-Jade-stained control of comparable appearance to the silver-stained control of Figure 2a, while the Fluoro-Jade-stained degenerating “treated” neurons in Figure 2d are quite comparable both in number and pattern to the silver-stained neurons of Figure 2b. Figure 2f is a silver-stained section from the hippocampus of a ketamine-treated rat, while Figure 2e is a nearly adjacent section from the same animal, but stained with Fluoro-Jade-B.

Multiple Fluorescent Labeling of Neurons in “High Dose” Ketamine-Exposed Rat Pups

To further investigate the mode of death exhibited by these neurons, coronal sections were stained with Fluoro-Jade B (FJ-B), a green fluorescent marker of neurodegeneration, as well as DAPI (4’, 6-diamidino-2-phenylindole dihydrochloride, a blue fluorescent DNA stain) and an antibody to the apoptotic marker, caspase-3 (CASP, labeled red with tetramethylrhodamine fluorescence).

The results from the high dose subjects (see Fig. 2) reveal that many cells in the LDVL thalamus contained one or more foci of compact, bright DAPI-stained chromatin (Fig. 2 g), an indication that they were undergoing apoptosis. Most such neurons also stained positively with both FJ-B (Fig. 2 h) and with the anti-CASP antisera (Fig. 2i), although some neurons positive for both CASP and FJ-B were not DAPI-positive for apoptosis.

Blood Levels of Ketamine

Table 1 indicates that the blood levels achieved by the repeated 20 mg/kg dosing protocol are considerably higher (around 14 mg/ml) than the blood levels in pups receiving either the multiple 10 mg/kg doses or a single dose of 20 mg/kg (each around 2–5 mg/ml).

DISCUSSION

The results indicate that the silver degeneration-selective method was successful at identifying dying cells throughout the brains of seven-day-old rat pups. While a considerable number of silver-positive cells were present even in control brains, treatment with ketamine greatly increased their numbers. The Fluoro-Jade B degeneration-selective method stained populations of cells, on adjacent sections, that were quite comparable to the silver method in distribution and numbers. The silver degeneration methods are useful for quantitative comparisons between animals or between adjacent sections. However, they are not compatible with approaches designed to combine multiple stains applied to the same section, perhaps due to the harsh extremes of pH required (Scallet, 1995). In contrast, Fluoro-Jade and Fluoro-Jade B (Schmued and Hopkins, 2000) have proven useful when combined with immunohistochemical or other fluorescent stains applied to the same tissue, as we did here for apoptotic markers.

The seven repeated doses of 20 mg/kg ketamine resulted in a 28-fold increase in the number of degenerating neurons within the laterodorsal thalamic nucleus of the rat pups. The measured increase in the present study compares very favorably with the 31-fold increase of degenerating neurons in the same region of the thalamus as reported by Ikonomidou et al. (1999). While that original report is clearly replicated by the present research, we also observed that seven repeated smaller (10 mg/kg) doses were without measurable effect. Also, a single dose of 20 mg/kg was ineffective at producing neurodegeneration.

Blood was drawn from groups of rats sacrificed on PND 7, 2 min after their last injection. The blood levels of ketamine associated with ineffective exposures were 2–5 μg/ml while the level associated with a neurotoxic exposure was about 14 μg/ml. An anesthetic blood level in humans is about 2 μg/ml (Malynovskiy et al., 1996; Mueller and Hunt, 1998), suggesting the possibility that a prolonged supra-anesthetic dose may be needed for neurotoxicity. Our animals at the repeated 10 mg/kg and single 20 mg/kg dose levels appeared anesthetized, but no actual tests of their level of analgesia or anesthesia were conducted. The fact that seven multiple 20 mg/kg doses produced higher blood levels of ketamine than a single 20 mg/kg dose suggests that repeating the injections every 90 min allowed the ketamine to
FIG. 2. An illustration of comparisons between the appearance, distribution, and approximate density of degenerating neurons as obtained using a cupric silver method (De Olmos) versus the Fluoro-Jade B approach. It also illustrates the results of multiple-staining of a single section through the laterodorsal thalamus (LDVL) of a ketamine-treated pup with anti-caspase-3, DAPI, and Fluoro Jade B. (a) (x 10) Shows only a few positive silver-labeled cells in the VLDL of a control seven-day-old rat pup, while (b) (x 10) shows many more such cells in the comparable region of a “high dose” (7 × 20 mg/kg) ketamine rat. (c) (x 10) Shows a section adjacent to the control pup in Figure 2a, but stained with Fluoro-Jade B. Note the similar numbers and localization of Fluoro-Jade B positive neurons (Fig. 2c) as compared to the silver stain (Fig. 1a). (d) (x 10) is similar in terms of the number and localization of degenerating neurons stained with Fluoro-Jade B to Figure 2b, stained with the De Olmos silver stain. (e) (x 10) Illustrates the hippocampus of a Fluoro Jade B-treated “high dose” (7 × 20 mg/kg) ketamine rat while (f) shows an adjacent section from the same animal stained for degeneration with silver. Ca1 = cornu ammonis 1, sl = stratum lacunosum, dg = dentate gyrus. (g) (x 100) is a VLDL thalamic section from a “high dose” animal in which the DNA has been stained with DAPI. The arrows mark five cells where the DNA is divided into several “packets” characteristic of apoptosis, while the circle surrounds several neurons with normal-appearing DNA. (h) (x100) is stained with Fluoro Jade B, indicating that the same five apoptotic cells from Figure 2g are also stained with Fluoro Jade B. A few cells (see arrow inside circle) are stained for Fluoro Jade B, but their nuclei do not appear apoptotic with the DAPI stain. (i) (x 100) indicates that there is substantial overlap of staining between anti-caspase-3 (red rhodamine stain in 2i) and the green Fluoro-Jade B stain (2 h).
accumulate in blood and/or tissue. The elimination half-life of ketamine in humans is around 2.5 h (1–2 h in a pediatric population) (Domino et al., 1997; Hartvig et al., 1993), so accumulation in tissue of ketamine from doses separated by only 90 min seems possible. Future work focused on the precise ketamine exposure parameters required to produce analgesia, anesthesia, and neurotoxicity will prove helpful.

Another goal of this research was to use a multiple staining approach to more reliably identify the dying cells as undergoing apoptosis (Dikranian et al., 2001). Caspase-3 is a cytoplasmic protein which can be activated by cleavage, initiated either by the stimulation of TNF (tumor necrosis factor) receptors on the surface of the membrane or by the release of cytochrome c from mitochondria undergoing calcium-activated pore transition. Following cleavage into an active 18 KDa fragment, caspase-3 is involved as an effector protein degrading downstream targets including structural proteins, inhibitors of apoptosis, DNA repair enzymes, cell cycle proteins, and signal transduction molecules which can then result in the occurrence of apoptosis (Ananth et al., 2001; McLaughlin, 2004; Vyas et al., 2002; Wang, 2000; Yuan et al., 2003). The subsequent research on NMDA antagonist-induced neurotoxicity has identified the presence of apoptotic neurons by electron microscopy and by the use of specific antisera directed against caspase-3 (Olney et al., 2002a). However, the various types of labeled cells noted to respond to NMDA antagonist exposure had to be evaluated on, at best, adjacent brain sections taken from similar regions.

Here, we showed that Fluoro-Jade B could identify apoptotic cells produced after exposure to the NMDA antagonist ketamine. Then, we used Fluoro-Jade B to stain degenerating cells in sections that were also stained for chromatin and caspase-3. We were able to show that in many cases the degenerating cells were triple-labeled; the same neuron revealed bright clumps of DAPI-positive chromatin characteristic of apoptotic nuclei, as well as positive green staining for Fluoro-Jade B and red for caspase-3. However, there were some DAPI-negative neurons that nevertheless stained positively for both Fluoro-Jade B and caspase-3 and a few that appeared to be positive for Fluoro-Jade B but not for caspase-3. Probably, the stains are differentially sensitive to the various stages that apoptotic cells undergo; further studies should be undertaken with cultured cells, where the occurrence of apoptosis can be synchronized following a toxicant exposure.

The present study confirms previous research indicating the neurotoxicity of ketamine, demonstrates multiple staining approaches for a single section that may be used to identify different stages of apoptosis, and suggests that neurotoxic blood levels of ketamine may be somewhat higher than levels required for anesthesia. Further research will be necessary to resolve concerns regarding the doses and durations of ketamine exposure that are safe for pediatric populations.

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REFERENCES


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