DOI 10.1007/s10517-016-3334-1
*Bulletin of Experimental Biology and Medicine, Vol. 161, No. 1, May, 2016 GENERAL PATHOLOGY AND PATHOPHYSIOLOGY* 

# Ketamine-Midazolam Anesthesia Induces Total Inhibition of Cortical Activity in the Brain of Newborn Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 161, No. 1, pp. 23-27, January, 2016
Original article submitted May 6, 2015

The effects of general anesthetics ketamine and midazolam, the drugs that cause neuroapoptosis at the early stages of CNS development, on electrical activity of the somatosensory cortex in newborn rats were studied using extracellular recording of local field potentials and action potentials of cortical neurons. Combined administration of ketamine (40 mg/kg) and midazolam (9 mg/kg) induced surgical coma and almost completely suppressed early oscillatory patterns and neuronal firing. These effects persisted over 3 h after injection of the anesthetics. We concluded that general anesthesia induced by combined administration of ketamine and midazolam profoundly suppressed cortical activity in newborn rats, which can trigger neuroapoptosis in the developing brain.

**Key Words:** *EEG; newborn; anesthesia; somatosensory cortex*

Ketamine is widely used in medical practice or short-term analgesia and anesthesia (often in combination with benzodiazepines). The effects of ketamine and benzodiazepines are related to blockage of NMDA glutamate receptors and potentiation of $GABA_A$ receptors, respectively, which is followed by sedative effects and in high doses induces surgical coma characterized by suppression of high-frequency EEG-patterns and induction of low-wave δ-oscillations [13]. Ketamine and benzodiazepines are considered relatively safe anesthetics with mild side effects in adults. However, recent studies showed that these substances can produce neurotoxic effects in immature brain during the period of active synaptogenesis (late gestation period in primates and first week after birth in rodents) [1-3,15]. It was found that neurotoxic effects of ketamine, benzodiazepines, other blockers of NMDA receptors and stimulators of $GABA_A$ receptors including ethanol and general inhalation anesthetics on the developing brain is stipulated by stimulation of neuroapoptosis, the process of programmed neuronal death [12]. In addition, long-term neurological and behavioral disturbances were observed in humans and animals exposed to these agents at the early developmental stages [3]. The main hypothesis explaining stimulation of neuroapoptosis in the developing brain is suppression of neuronal activity [9]. Electrical activity in the developing somatosensory cortex is characterized by unique activity patterns organized in oscillation bursts in α-β and γ frequency ranges [6,7]. These patterns correspond to typical activity patterns (δ-brushes) in preterm infants at the last trimester of gestation [5]. We have previously demonstrated that general inhalation anesthetic isoflurane inducing massive apoptosis in newborn rats completely suppressed spontaneous activity in the brain cortex of rat pups during the first week after birth [14]. These data fully correspond to the hypothesis on neuroapoptosis stimulation by neuronal activity blockage. However, the effects of ketamine and benzodiazepines on electrical activity of the brain in newborn rats during the critical period of high sensitivity to neuroapoptotic effects of these agents remain little studied.

Here we analyzed the effects of combined treatment with ketamine and a benzodiazepine midazolam

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on electrical activity of the somatosensory cortex in 5-7-days-old rat pups (this age corresponds to the peak of neuroapoptotic effects of these substances in the brain cortex [15]) in clinically relevant doses inducing analgesia and surgical coma.

## MATERIALS AND METHODS

Experiments were performed on newborn (postnatal days 5-7; P0 is the day of birth) Wistar rats in accordance to ethical standards of Directive 2010/63/EC on animal experiments and recommendations of the Ethical Committee of Kazan State Medical University (protocol No. 9-2013) and the French National Institute of Health and Medical Research (INSERM; protocol No. 007.08.01). Inhalation anesthetic isoflurane (0.5-1.5% in oxygen) was used for surgical manipulations. The rat was fixed using a stereotaxic adapter fixed to rat head with dental cement. Animal body was wrapped in cotton wool and placed on a thermostatic pad (35-37°C). Chlorinated silver wire was inserted into the area of visual cortex and served as combined ground and reference electrode. Local field potentials (LFP) and multiply unit potentials (MUP) were recorded without anesthesia using multichannel silicon probes (100 μ separation distance between the recording sites; NeuroNexus Technologies). The probe was inserted into the somatosensory cortex, in the representation of a forepaw, perpendicular to the surface to a depth of 1400 μ. This method allowed recording of spontaneous and induced activity in all layers of the cortical column. Sensory stimulation was provided by short-term (2-10 msec) applications of a metal stick to somatotropic areas on the skin of the contralateral paw. Spontaneous movements were registered using a piezodetector located on the back and under a forepaw. Electrical signals were amplified (10,000×; 0.5 Hz-10 kHz) using an amplifier (Neuralynx), digitized at 25 kHz, and saved on a computer for further analysis. The data were analyzed using MATLAB software. For detection of spikes (action potentials, AP), the initial signal was filtered at >400 Hz and negative events with amplitudes exceeding three standard deviations were considered as AP. Sensory-evoked potentials (SEP) were estimated as the first potential deflection in the granular layer following the stimulus. For the analysis of sensory-evoked oscillations, 500-msec interval after SEP was analyzed. LFP and MUP were detected and analyzed using a custom-developed application within MATLAB environment. Spectral analysis was performed using Chronux software.

Statistical analysis was also performed using MATLAB software. The differences between the parameters under control and experimental conditions were estimated using two-sided Wilcoxon's test. The data are presented as the mean±standard error of the mean.

## RESULTS

Under control conditions, activity in the somatosensory cortex of newborn rats was characterized by short-term bursts of collective neuronal activity (5.2±1.2 bursts/min; duration 2155±416 msec) including LFP in γ- and α-β frequency ranges (early γ oscillations and spindle bursts) associated with neuronal AP. The bursts were separated by periods of suppression that lasted 2.2±0.6 sec ($n$=6; Fig. 1). Short-term mechanical stimulation of the somatotopic part of the limb induced complex responses in the somatosensory cortex including SEP (mean amplitude 1630±220 μV, delay 34±2 msec) followed by γ- and spindle oscillations. SEP amplitude, oscillation intensity, and frequency of associated AP were maximum in the granular layer. MUP frequency during the oscillatory component of the response was 7.4±2.2 spikes over 500 msec.

Combined intraperitoneal administration of ketamine (40 mg/kg) and midazolam (9 mg/kg) significantly suppressed spontaneous electrical activity during the first minutes postinjection (Figs. 1, 2) until its disappearance in the majority of the experiments. The mean frequency of spikes decreased from 2.5±0.7 to 0.2±0.1 $sec^{-1}$ (to 11±5%, $n$=6, $p$<0.01) and the frequency of bursts decreased from 5.2±1.2 to 0.2±0.1 bursts/min (to 6±4%, $n$=6, $p$<0.01). Analysis of spontaneous motor activity showed that the frequency of myoclonic movements after the injection of anesthetics decreased to 10±3% (to 1.0±0.4 $min^{-1}$ *vs.* 10.5±1.3 $min^{-1}$ in the control; $n$=6, $p$<0.01). Deep suppression of cortical activity lasted for 3 h after anesthetic administration (Fig. 2).

The effects of combined administration of anesthetics on sensory-induced activity were less pronounced. The decrease in SEP amplitude was insignificant (to 96±12%, $n$=6, $p$>0.2). SEP delay also did not change (101±4%, $n$=6, $p$>0.2). However, the oscillatory part of the sensory response significantly decreased: the power of sensory-induced neuronal activity decreased to 19±4% ($n$=6, $p$<0.01) for γ frequency range and to 14±4% ($n$=6, $p$<0.01) for α-β frequency range. MUP frequency decreased to 50±10% from the control levels during SEP ($n$=6, $p$<0.01) and to 5±3% ($n$=6, $p$<0.01) during sensory-induced bursts.

These findings suggest that combined administration of ketamine and midazolam in clinically relevant doses used for surgical anesthesia almost completely suppressed spontaneous electrical activity and significantly reduced the oscillation part of sensory-induced response in the brain cortex of newborn rats. In adult animals, these agents induce slow wave activity on

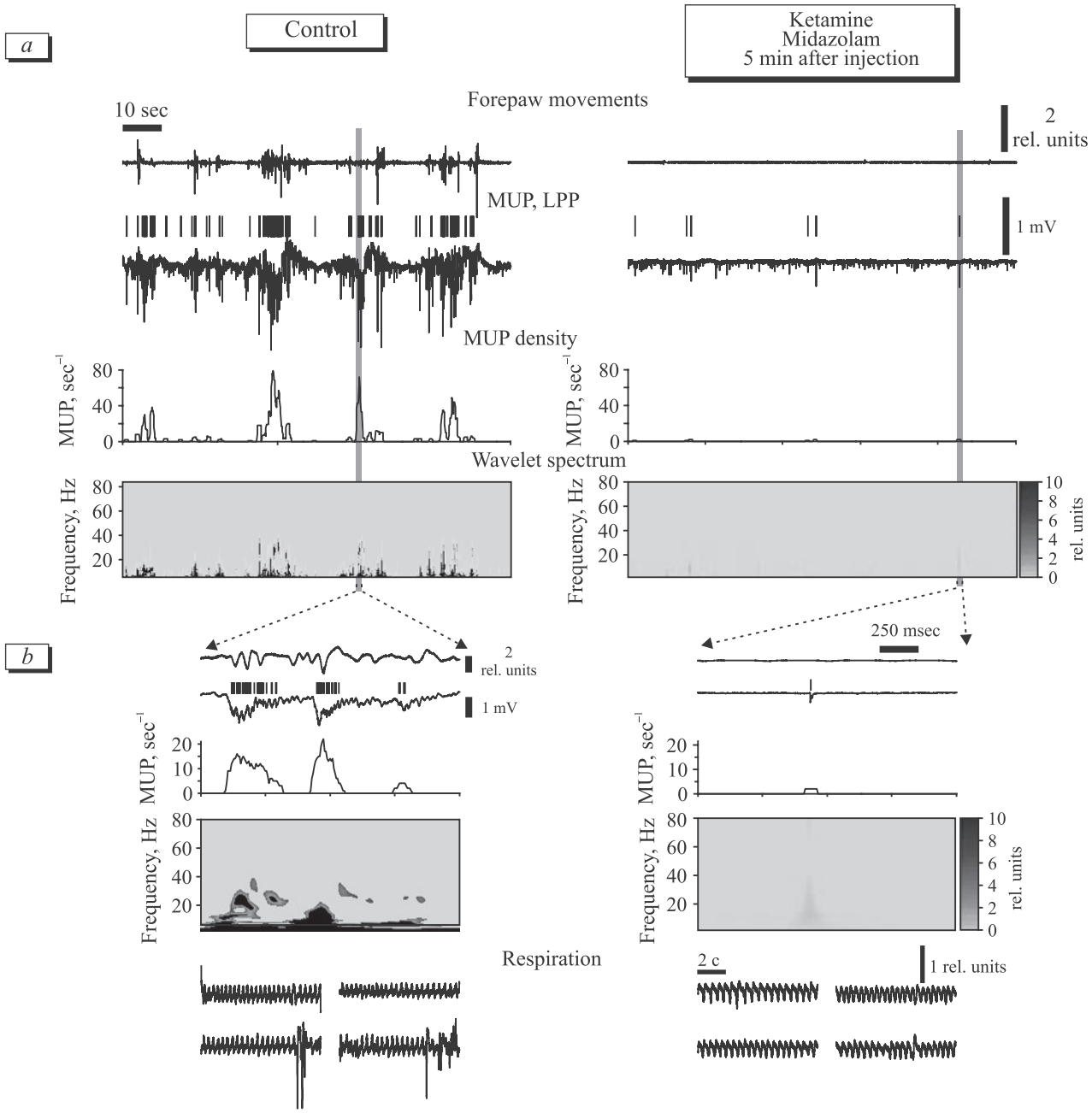


**Fig. 1.** Effects of combined treatment with ketamine (40 mg/kg) and midazolam (9 mg/kg) on spontaneous activity in layer IV of the primary somatosensory cortex of a newborn rat (P5). *a*) Mechanogram of motor activity, electrical activity in layer IV of representation of forepaws in somatosensory cortex, MUP density, and wavelet analysis; *b*) fragments of records *a* within grey bands in expanded time scale and record of respiratory movements.

EEG [13]. High sensitivity of brain activity of newborns to ketamine and midazolam can be stipulated by specific mechanisms of generation of early activity patterns. For instance, NMDA receptors, the main target of ketamine, significantly contribute to glutamatergic conduction in developing synapses, and also takes part in the generation of early network patterns [10,11]. These data can explain pronounced inhibitory effects of ketamine in newborn animals. The inhibitory effects of midazolam in newborns are less pronounced, as GABA is considered to produce depolarizing effects on immature neurons, and benzodiazepines can stimulate neuronal activity [4]. However, the stimulating effects of benzodiazepines were primarily demonstrated in *in vitro* experiments, while recent studies showed inhibitory effects of benzodiazepines on generation of early activity patterns *in vivo*, *e.g.*, enhancement of lateral inhibition mediated by GABA receptors [8,11].

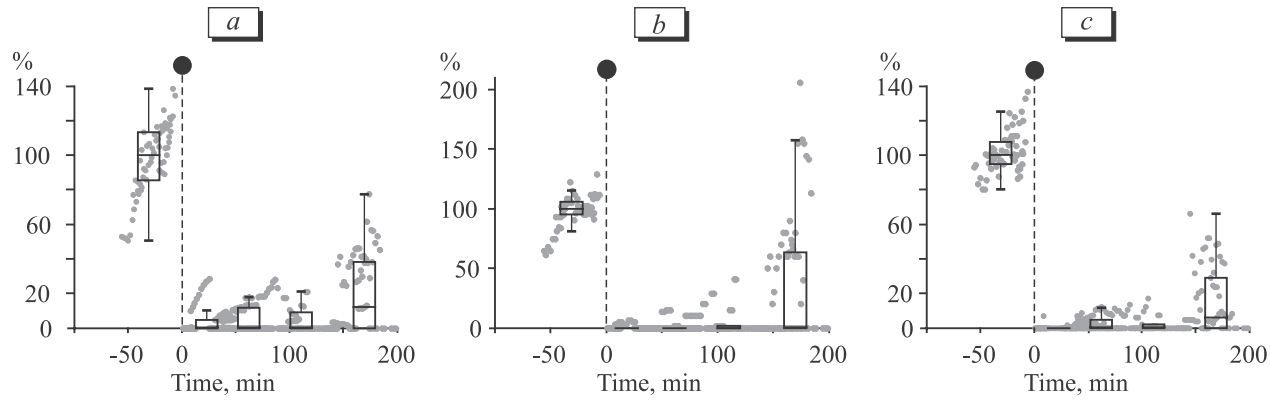


**Fig. 2.** Effects of combined intraperitoneal administration of ketamine (40 mg/kg) and midazolam (9 mg/kg) on the frequency of multiple action potentials (*a*), frequency of spike activity (*b*) in the somatosensory cortex (layer IV) in newborn rats, and the frequency of spontaneous movements (*c*). Each dark grey point represents instant average value of the parameter. Combined data of 6 experiments (P5-P7) normalized to control values are presented. Dotted line: injection of anesthetics. Points before t=0 correspond to control conditions.

In addition to these inhibitory effects induced by ketamine and midazolam in the brain cortex, suppression of motor activity (that serves as a physiological trigger of actions bursts in the somatosensory cortex of newborns [7]) can also contribute to deep suppression of activity in the brain cortex.

General anesthetics including ketamine, midazolam, ethanol, and other agents blocking NMDA receptors and stimulating $GABA_A$ receptors induce apoptosis in the developing brain, which can be the cause of behavioral impairments [1-3,15]. Our findings suggest that the combination of anesthetics ketamine and midazolam practically completely suppresses spontaneous and sensor-induced oscillatory bursts and neuron excitation in the somatosensory cortex of newborn rats. As neuronal activity contributes to survival of neurons in the developing brain [9], our results allow concluding that complete suppression of brain activity induced by general anesthetics during the first week after birth is a possible mechanism underlying their neurotoxic effects on immature brain.

Experiments were supported by the Government of the Russian Federation (grant No. 11.G34.31.0075), Ministry of Education and Science of the Russian Federation, and Program of Competitive Growth of Kazan (Volga Region) Federal University. The study was performed as a part of State Task in Research Activity of Kazan (Volga Region) Federal University.

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