Isoflurane suppresses early cortical activity

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Abstract

Objective: Isoflurane and other volatile anesthetics are widely used in children to induce deep and reversible coma, but they may also exert neurotoxic actions. The effects of volatile anesthetics on the immature brain activity remain elusive, however. Methods: The effects of isoflurane on spontaneous and sensory-evoked activity were explored using intracortical extracellular field potential and multiple unit recordings in the rat barrel cortex from birth to adulthood. Results: During the first postnatal week, isoflurane suppressed cortical activity in a concentration-dependent manner. At surgical anesthesia levels (1.5–2%), isoflurane completely suppressed the electroencephalogram and silenced cortical neurons. Although sensory potentials evoked by the principal whisker deflection persisted, sensory-evoked early gamma and spindle-burst oscillations were completely suppressed by isoflurane. Isoflurane-induced burst-suppression pattern emerged during the second postnatal week and matured through the first postnatal month. Bursts in adolescent and adult rats were characterized by activation of entire cortical columns with a leading firing of infragranular neurons, and were triggered by principal and adjacent whiskers stimulation, and by auditory and visual stimuli, indicating an involvement of horizontal connections in their generation and horizontal spread. Interpretation: The effects of isoflurane on cortical activity shift from total suppression of activity to burst-suppression pattern at the end of the first postnatal week. Developmental emergence of bursts likely involves a development of the intracortical short- and long-range connections. We hypothesize that complete suppression of cortical activity under isoflurane anesthesia during the first postnatal week may explain neuronal apoptosis stimulated by volatile anesthetics in the neonatal rats.

Introduction

General inhalation anesthetics such as isoflurane reversibly induce deep coma and are widely used in clinical practice. In the adult brain, their effects are associated with the so-called burst-suppression pattern of electroencephalogram (EEG), which is characterized by hyperexcitability of the cortex manifested by intermittent, highly synchronized neuronal discharges (bursts) separated by silence periods (suppression).\textsuperscript{1–3} Volatile anesthetics are also widely used in pediatric patients that require anesthesia for surgery. However, recent studies raised concern about the safety of general anesthetics in neonates and infants.\textsuperscript{4–6} Prolonged (2–6 h) exposure to general anesthetics including isoflurane at minimal alveolar concentration (MAC) (1.9–2.3% isoflurane in P\textsubscript{2}–P\textsubscript{9} rats\textsuperscript{7}) and even at sub-MAC (1.5%) concentrations causes widespread apoptosis and neurodegeneration in the developing brain, and results in long-lasting neurological and behavioral deficits in rodents and nonhuman primates.\textsuperscript{8–13} It has
been hypothesized that apoptosis is triggered by suppression of neuronal activity by these agents.\textsuperscript{14,15} While this hypothesis is compatible with neuroapoptotic effects of the drugs that suppress the activity of glutamate receptors, sodium channels or enhance GABAergic inhibition, its consistence with the effects of isoflurane and other volatile anesthetics on brain activity is less clear. Indeed, if volatile anesthetics induce a hyperexcitable state and burst-suppression pattern in the neonates as they do in adults, the neurotoxic effects of these agents would not support this hypothesis. Hence it is important to determine the effects of volatile anesthetics on the brain activity in immature animals.

The immature brain displays unique activity patterns at early developmental stages.\textsuperscript{16–18} During the first postnatal week in rodents (that corresponds to a period from midgestation to near term in human\textsuperscript{19–21}), cortical activity is characterized by intermittent bursts organized in oscillations in alpha-beta (spindle bursts) and gamma oscillations, EGOs) frequency ranges.\textsuperscript{20,22–25} These early oscillatory bursts are local events generated in thalamocortical circuits, but they can also be triggered by sensory inputs. In somatosensory cortex, they are driven by sensory feedback resulting from myoclonic twitches.\textsuperscript{22}

In the visual system, before the onset of vision, they are driven by spontaneous retinal waves.\textsuperscript{26,27} Similar activity patterns are also expressed in the human brain during the second half of gestation.\textsuperscript{20,28–35} Early oscillatory patterns activate NMDA receptors, provide conditions for synaptic plasticity, and participate in the activity-dependent formation of thalamocortical circuits.\textsuperscript{23,36} Considerable evidence indicates that in addition to the roles in synaptic plasticity, the ongoing neuronal activity also promotes neuronal survival by preventing developmental apoptosis.\textsuperscript{11,15,37–39} While several previous studies described the suppressive actions of inhalation anesthetics on the brain activity during development\textsuperscript{27,40–42} it remains largely unknown how isoflurane affects the early cortical activity during the first postnatal week at the levels of surgical anesthesia inducing neuroapoptosis.

In this study, we used intracortical recordings of electrical activity from rat barrel cortex in the rats aged from postnatal day P2 to P69 to characterize the developmental changes in the effects of isoflurane on spontaneous and sensory-evoked cortical activity. Our main finding is that isoflurane evokes burst-suppression pattern only from the second postnatal week onward, whereas during the first postnatal week, isoflurane almost completely silences cortex eliminating both spontaneous and sensory-evoked EGOs and spindle bursts. These findings provide strong support to the hypothesis that the neurotoxic effects of isoflurane in the immature brain are secondary to the suppression of neuronal activity.

### Materials and Methods

#### Surgery

This study followed Institut National de la Sante et Recherche Medicale and Kazan Federal University guidelines on animal care. Twenty Wistar rats of both sexes from the second day after birth [P2] (P0 = day of birth) till postnatal day 69 were used. Surgery was performed under isoflurane anesthesia. In brief, the skull of the animal was cleaned of skin and periosteum. Two plastic or metal bars were fixed to the nasal and occipital bones of the rats head by dental cement. The skull was covered by dental cement except for a 4–9 mm window above the barrel cortex. After surgery, animals were warmed, and left for an hour for recovery from anesthesia. During recordings, the head was fixed to the frame of the stereotactic apparatus by the attached bars; animals were surrounded by a cotton nest and heated via a thermal pad (35–37°C). A chlorided silver wire, placed in the cerebellum or visual cortex, served as a ground electrode. Isoflurane (0.5–2%, 0.2–0.4 L/min) was administered via mask adapted to the animal head leaving an access to the whiskers at the contralateral whisker pad. The level of surgical anesthesia was assessed with an alligator clip applied to the tail. The data presented for each isoflurane concentration were acquired >15 min after a change in isoflurane concentration.

#### Extracellular recordings

Extracellular recordings where performed either without any anesthesia or under slight sedation with urethane (0.2–0.4 g/kg) and/or buprenorphine analgesia (0.03 mg/kg). Extracellular local field potentials (LFP) and multiple unit activity (MUA) were recorded using 16-site linear silicon probes (100 μm separation distance between recording sites, Neuronexus Technologies, Ann Arbor, MI) placed vertical into the barrel cortex to the depth of 0–1.5 mm from the cortical surface to trace the columnar activity from all cortical layers. The principal whisker (PW) was identified by the shortest latency MUA responses in layer 4. Single whiskers were stimulated by piezo actuators (2–10 msec pulse duration, backward direction of whisker deflection, from 2 to 10 sec interstimulus intervals depending on the animal’s age to avoid depression). Auditory stimuli (10 msec white noise pulses) were provided by a piezoelectric disk beeper positioned at 5 cm from the contralateral ear. Visual stimuli (10 msec green light flash) were provided by light emitting diode positioned in front of the contralateral eye. Values for L4 and L2/3 before P4 (when Gr layer differentiates from the cortical plate) correspond to the depths
with the short latency responses and 200–300 μm above, respectively. The signals were amplified and filtered (10,000X; bandpass 0.5 Hz–10 kHz) using a custom-built 16-channel amplifier (A. Alexeev, Troitsk, Russia) or Neuralynx amplifier (Neuralynx, Bozeman, MO), digitized at 10 kHz and saved on the PC for post hoc analysis.

Data analysis

Raw data were preprocessed using a custom-developed suite of programs in Matlab analysis environment. Wide-band signal was downsampled to 1000 Hz and used as LFP signal. Positive polarity is up throughout all manuscript. For spike detection, wide-band signal was highpass filtered (>400 Hz) and negative events exceeding three standard deviations in amplitude were considered as spikes. Sensory-evoked oscillations (SEOs) were detected as the first troughs of sensory-evoked responses in L4.

For the analysis of sensory-evoked oscillations, 500 msec periods following SEP were used. For baseline activity assessment periods 200 msec prior to stimulus were used. LFPs, extracellular units, and intracellular data were analyzed by custom-written, MATLAB-based programs. Spectral analysis was carried out using Chronux toolbox procedures (http://chronux.org/). Spectral power and coherence was estimated using direct multitrape estimators (10 Hz bandwidth, 3 tapers, 200 msec spectral window padded to double its length with zeros) or continuous wavelet transformation with mother wavelet of order 6. To remove low frequencies the LFP envelope was filtered (10–100 Hz). Between groups/conditions comparison of LFP oscillation power and coherence was done based on a nonparametric jackknife-based resampling procedure. Gamma and spindle-burst oscillation peaks were detected using the following steps: (1) LFP signal was bandpass filtered (30–80 Hz for gamma and 8–30 Hz for spindle bursts), (2) times of negative troughs with amplitude greater than 10 μV were detected from filtered signal, (3) times of the detected troughs having amplitude more than 100 μV in 10 Hz high-pass LFP signal were eliminated, (4) gamma and spindle-burst oscillations were considered as minimum three cycles with periods less than 30 msec and 120 msec each, respectively, both associated with spikes. Sharp potentials were defined as single LFP deflections exceeding 10 standard deviations in amplitude with more than four spikes inside. Current source density (CSD) analysis across the cortical depth was used to eliminate volume conduction and to localize synaptic currents. CSD was computed for each recording site according to a differential scheme for second derivative and smoothed with a triangular kernel of length 3. Matlab code for EEG analysis is freely available on demand from Dr. Andrei Zakharov at mphiszav@rambler.ru.

Results

In this study we explored the effects of isoflurane on cortical activity using multisite silicone probe recordings of the LFP and MUA from the cortical barrel columns in P2–69 (n = 20) head-restrained rats.

Isoflurane completely suppresses gamma- and spindle bursts during the first postnatal week

During the first postnatal week, temporal organization of activity in barrel cortex was highly discontinuous with intermittent bursts occurring at frequency 5.6 ± 1.6 bursts/min (n = 6; P2–7; Fig. 1). Bursts were associated with intermittent LFP oscillations at spindle- and gamma frequency that were maximal in the L4 and were evident during LFP wavelet analysis and MUA spectral analysis (Fig. 1A, B, and D). Isoflurane suppressed spontaneous gamma and spindle bursts in a concentration-dependent manner (Fig. 1A and C). While at 0.5% of isoflurane, the frequency of bursts and MUA were not significantly modified, at surgical anesthesia levels (1.5–2%) isoflurane completely suppressed spontaneous spindle bursts and EGO, that were associated with a reduction in alpha-beta (8–30 Hz) and gamma (30–80 Hz) LFP power from 21 ± 4 μV²/Hz to 3 ± 1 μV²/Hz and 2.6 ± 0.7 μV²/Hz to 0.6 ± 0.1 μV²/Hz, respectively (P < 0.05; n = 6; Fig. 2B and C). Suppression of LFP activity was accompanied by almost complete suppression of neuronal firing with a drop in MUA frequency from 5.5 ± 2.3 s⁻¹ to 0.1 ± 0.1 s⁻¹ (Fig. 2A). While EEG was completely flattened in P2–5 animals, by the end of the first postnatal week rare (0.7 ± 0.3 min⁻¹) spontaneous sharp activity (amplitude of 226 ± 123 μV; half-duration of 20 ± 10 msec) associated with occasional unit firing were apparent under 1.5–2% isoflurane anesthesia (n = 2 rats). Main sinks and MUA associated with these sharp potentials were located in L4 (Fig. 3A and B).

In addition to a suppression of spontaneous activity, isoflurane also completely blocked sensory-evoked oscillatory responses during the first postnatal week. In control conditions, brief deflection of the PW evoked a characteristic oscillatory response consisting of EGO followed by spindle-burst in the corresponding cortical barrel column in P2–7 rats (Fig. 4). In keeping with previous studies, the activity during EGO and spindle bursts was essentially restricted to the granular and infragranular layers of the corresponding barrel column without any significant activation of the supragranular layers. Major sinks of the troughs of the evoked oscillatory patterns were located in L4, where the majority on neuronal firing during
sensory-evoked responses occurred (Fig. 4). Induction of isoflurane anesthesia was associated with a reduction in the power of sensory-evoked oscillations (both EGO and spindle bursts) in a concentration-dependent manner and these oscillatory responses were completely blocked at 1.5–2% of isoflurane (Fig. 4A and B). Fourier analysis of the 500 msec time window following the SEP revealed that isoflurane (1.5–2%) causes a reduction in the power of the evoked gamma and alpha-beta oscillations from 46 ± 17 μV2/Hz to 0.8 ± 0.2 μV2/Hz and 237 ± 96 μV2/Hz to 8 ± 3 μV2/Hz, respectively (Fig. 5B and C); this was accompanied by a reduction in spikes count from 35.6 ± 13.8 units to 0.9 ± 0.5 units (n = 6, P2–7 rats, Fig. 5A). While gamma and spindle-burst oscillations were completely suppressed, SEPs persisted in the presence of isoflurane without any change in SEP amplitude: SEP amplitudes in control conditions and in the presence of isoflurane were of 601 ± 158 μV and 700 ± 154 μV, respectively (Fig. S1). Spike count during SEP was also unchanged (control values, 3.4 ± 1.8 units and isoflurane, 4.0 ± 1.4 units ([n = 6] Fig. S1). In the presence of isoflurane, SEPs were often followed by a second small amplitude trough at 75 ± 10 msec after the stimulus. Stimulation of adjacent whiskers, as well as auditory or visual stimuli failed to evoke responses in both control conditions and in the presence of isoflurane in P2–7 rats (data not shown). Thus, although SEPs persisted in the presence of isoflurane, there was complete suppression of sensory-evoked and spontaneous gamma and spindle bursts during the first postnatal week.

**Developmental emergence of the burst-suppression pattern**

During the second postnatal week, spontaneous and sensory-evoked EGO and spindle bursts disappeared in counterpoint to an emergence of continuity of spontaneous activity in agreement with the results of previous studies. In the presence of isoflurane (1.5–2%) the activity became discontinuous with intermittent sharp potentials of 797 ± 219 μV occurring at 6.6 ± 2.6 min−1 and synchronizing most of MUA (n = 5 P8–15 rats; Fig. 3). These sharp potentials increased with age in frequency and amplitude, with some events attaining

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**Figure 1.** Effects of isoflurane on spontaneous activity in a cortical barrel column of neonatal rats. (A) Example traces of spontaneous electrical activity in L4 of a cortical barrel column of a P5 rat (LFP-black traces; MUA-red bars above) in control conditions and after inhalation of 0.5% and 1.5% isoflurane. Below are shown corresponding wavelet spectrograms and MUA frequency plots. Note that isoflurane (1.5%) completely suppresses ongoing LFP and MUA activity. (B) Individual oscillatory burst outlined by dashed box on panel (A) is shown on expanded time scale. (C) Dependence of spontaneous bursts frequency and MUA frequency on isoflurane concentration. (D) Power spectrum of LFP in control conditions (blue) and in the presence of isoflurane (1.5%) (red). (C–D): pooled data from six P2–7 rats.
10 mV, together with an increase in MUA recruitment (Fig. 3A and D). Resembling epileptiform population spikes, sharp potentials started to organize in complex bursts with an occurrence of 23.5 ± 6.6 bursts per minute and intraburst frequency of 12.5 ± 3.0 Hz starting from the third postnatal week (n = 9 P16–69 rats). From this developmental time point onward, adult-like burst-suppression pattern became apparent (Figs. 2, 3).

Depth analysis revealed that sharp potentials in adolescent and adult animals originate in infragranular layers 5/6, spread to the cortical surface within 10 msec attaining maximal amplitude in supragranular layers 2/3 was also located their major sink (Fig. 3B). Activation of neurons during sharp potentials also showed depth-to-surface gradient with an initial activation of L5/6 units and sequential recruitment of the L4 and L2/3 units (Fig. 3B–C, Fig. S2). This intracolumnar propagation pattern was similar to that of the cortical-driven epileptic population spikes⁴⁷ and also resembled up-states activation spread within a cortical column during slow-wave sleep.⁴⁸ Unlike in neonatal rats, where isoflurane virtually completely suppressed EEG activity and neuronal firing, sharp potentials and bursts maintained neuronal firing at significant levels in older animals (2.0 ± 0.7 s⁻¹ at P8–15 and 8.4 ± 3.2 s⁻¹ at P16–69; Fig. 2).

Isoflurane-induced sharp potentials and bursts could also be evoked by PW stimulation, as well as by stimulation of adjacent whiskers and sensory stimulations at different modalities including auditory (click) and visual (light flash) stimuli (Fig. 6). The probability of evoking sharp potentials and bursts by sensory stimuli increased with age and was more prominent at higher isoflurane concentrations. Bursts evoked by nontopographic sensory stimuli were delayed from the stimulus by 100–300 msec, where initiated in the infragranular layers and displayed CSD profile and depth-to-surface MUA spread pattern similar to that of spontaneous bursts (Fig. 6C and D and Fig. S2). The amplitude of PW-evoked SEPs amplitude was unchanged in P8–P69 rats. While spike density during SEP was not affected in P8–15 rats, it showed twofold reduction in P16–69 rats (Fig. S1).

### Discussion

The main finding of this study is that the effects of isoflurane on cortical activity differ qualitatively during the first postnatal week and in older animals. During the first postnatal week isoflurane completely suppressed spontaneous cortical activity and eliminated sensory-evoked early oscillatory patterns (EGO and spindle bursts) while in older animals isoflurane evoked burst-suppression pattern which acquired adult-like features during the third postnatal week.

During the first postnatal week isoflurane at levels of surgical anesthesia (1.5–2%) completely suppressed cortical activity patterns (EGO and spindle bursts) and silenced cortical neurons. Generation of the early activity patterns involves several mechanisms that are potential targets in the suppressive effects of isoflurane. These likely
Figure 3. Developmental emergence of the burst-suppression pattern. (A) Example traces of spontaneous activity in L4 of a cortical barrel column in control conditions (top traces) and under 1.5% isoflurane (bottom traces) in P6, P15, and P30 rats. Sharp potentials are marked by asterisks and bursts are shown by brackets. (B–C) Under isoflurane (1.5%): (B) L4 trough triggered PSTH for MUA across all layers (top) and LFP (black traces) overlaid on color coded CSD (below); (C) Color coded cross correlation for L2/3 and L5/6 units versus L4 units. (D) Age dependence of 1.5–2% isoflurane-induced bursts frequency, half-duration, amplitude, and MUA recruitment. (D) Data from twenty P2–69 rats; Each point corresponds to an individual rat; circles with error bars correspond to the group averages at P6–7, P8–15, and P16–69. Data from P2–7 rats which did not display bursts under isoflurane anesthesia (Inf = infinite) were excluded form burst analysis.
involve antagonism of isoflurane with NMDA receptors, agonistic actions on GABA(A) receptors and activation of the two-pore potassium channels. EGO and spindle bursts are generated in the thalamocortical networks and under physiological conditions they are triggered by sensory feedback resulting from myoclonic twitches. These oscillations are driven by glutamatergic synapses with AMPA and NMDA receptors contributing to their generation, whereas GABAergic interneurons support these oscillations in a developmental manner and

Figure 4. Effects of isoflurane on sensory responses in barrel cortex of neonatal rats. (A) Sensory responses evoked by principal whisker (PW) stimulation at different depth of the cortical barrel column (LFP-black traces; MUA-red bars overlaid on color coded current source density plot (CSD) in control conditions (left) and under isoflurane (1.5%). (B) Corresponding stimulus-triggered average (n = 100) for L4 wavelet spectrogram and peristimulus time histogram (PSTH) for MUA across all layers (below). (C) L4 MUA PSTH aligned to the sensory-evoked potential (SEP) onset (top) and power spectral density of L4 LFP during 500 msec time window following SEP (indicated by gray horizontal bars on panel (A)) (bottom). Shading shows jackknife deviation. (A–B): data from a P6 rat. (C): pooled data from six P2–7 rats.

Figure 5. Age dependence in the effects of isoflurane on the principal whisker evoked oscillations. (A) L4 MUA frequency in a 500 msec time window following SEP. (B–C) Power of PW evoked alpha-beta (B) and gamma (C) oscillations. Each pair corresponds to an individual rat; circles with error bars correspond to the group averages at P2–7, P8–15, and P16–69. Probability value maps for the comparisons between the activity in control conditions and under isoflurane in three age groups. Within each age group P-values were obtained from paired data comparisons.
compartmentalize the activated areas via surround inhibition. Blockade of cortical NMDA receptors reduces by half the excitatory currents during spindle bursts whereas the positive allosteric GABA(A) receptor modulator diazepam reduces their frequency. Isoflurane exerts both antagonistic actions on NMDA receptors and enhances GABA(A) receptor mediated functions and these actions are likely involved in the suppressive effects of isoflurane on early oscillatory patterns. Isoflurane also reduces transmitter release by acting on presynaptic terminals. In addition, isoflurane hyperpolarizes neurons and decreases membrane resistance via activation of the two-

pore leak potassium channels that directly decreases neuronal excitability and also likely contributes to the inhibitory actions of isoflurane at the network level. In addition to its central actions, isoflurane and other volatile anesthetics also cause immobility via hyperpolarization of motor neurons. Because spontaneous myoclonic twitches provide, via sensory feedback, the main drive for the early oscillatory bursts in somatosensory cortex, their suppression also contributes to the elimination of cortical activity by isoflurane in the neonatal rats. However, this mechanism is not too central to the suppressive effect of isoflurane on the early activity patterns in

Figure 6. Bursts under isoflurane anesthesia in adult rat barrel cortex are triggered by various sensory stimuli. (A) Example traces of responses evoked in L4 of a barrel column by brief deflections of the principal whisker (PW), second order adjacent whisker (AW2), auditory clicks, and light flashes. Stimuli onsets are indicated by vertical red lines. Note that stimuli in different modalities reliably evoke bursts in barrel cortex. (B) Example responses in different sensory modalities as above shown on expanded time scale. LFP trace (black) and MUA (red bars) are overlaid on color coded CSD. (C) Cytochromoxydase stained slice (left) and stimulus (PW-evoked response) and first population spike after stimulus (AW2, auditory and visual stimuli) triggered LFPs overlaid on CSD plots (left) and MUA PETHs across layers (right). (D) Cross correlation for L2/3 and L5/6 versus L4 units; cross correlation coefficients are color coded. (A–D) Data from a P30 rat under 2% isoflurane.
somatosensory cortex since the oscillatory activity bursts evoked by direct sensory stimulation were also completely blocked by isoflurane. Our observation of suppression of the early activity patterns by isoflurane is not limited to somatosensory cortex. Indeed, in visual cortex, where spontaneous retinal waves drive spindle bursts in visual cortex under physiological conditions, activity was virtually absent during the first postnatal week even under relatively light (0.9–1.1%) isoflurane anesthesia. During the second postnatal week, isoflurane at 0.6% specifically eliminated retinal waves-driven slow activity transients leaving short burst events—likely representing an emerging burst-suppression pattern (see also).

We found that isoflurane-induced burst-suppression pattern starts to emerge at the end of the first postnatal week in a form of recurrent, low amplitude sharp potentials, and that it progressively evolves to complex epileptic-like bursts of population spikes with age to reach adult-like burst-suppression pattern during the third postnatal week. Isoflurane-induced bursts in adolescent and adult animals could be evoked in barrel cortex not only by PW stimulation, but also by stimulation of the non-PWs as well as by stimuli in other sensory modalities (visual and auditory) that is in keeping with findings made in adult animals and patients. The laminar profile of spontaneous and sensory-evoked bursts indicates that they are initiated in and spread horizontally via the infragranular layers. Indeed, it has been shown that under isoflurane anesthesia specific optogenetic stimulation of L5 (but not L2/3) neurons is as efficient in triggering bursts as sensory stimuli in adult animals. These features of bursts appear to be similar to the network up states during slow-wave oscillations. During development, cortical-driven up-state bursts of activity can be recorded in the rats in vivo starting from the end of the first postnatal week and they evolve in amplitude and frequency to a slow-wave oscillation pattern during the second-third postnatal week. Also, driven by infragranular layers cortical giant depolarizing potentials (cGDPs) are observed in cortical slices in vitro from the end of the first postnatal week. Thus, there is a remarkable similarity in the developmental profiles of cortical up states and isoflurane-induced bursts. Because generation and spread of these both types of activity depends on intracortical connectivity, development of the short- and long-range horizontal connections between pyramidal cells in the infragranular layers seems to be a critical factor determining the development of the up states and isoflurane-induced burst-suppression pattern.

Our results provide compelling evidence to the hypothesis that the neuroapoptotic effects of isoflurane in the immature brain are caused by suppression of neuronal activity. Indeed, survival of neurons during development has been suggested to rely on physiological electrical activity, as evidenced by apoptotic and neurodegenerative effects of blocking activity in vivo and in vitro. We found that isoflurane profoundly inhibits neuronal activity in the rat cortex specifically during the first postnatal week at the anesthesia levels that have been shown to induce massive neuronal apoptosis. Developmental apoptosis is also stimulated by other anesthetic agents that inhibit early activity patterns, notably those antagonizing NMDA receptors and stimulating GABA(A) receptors. Therefore, we propose that age-specific suppression of the early cortical activity by isoflurane and other anesthetic agents, which likely reflects unique and highly sensitive to these anesthetics generative mechanisms of the early activity patterns underlies the neuroapoptotic effects of these drugs in the immature brain.

Clinical relevance of the present findings requires further confirmation of the suppressive effects of isoflurane on the early activity patterns in human. Comparative developmental studies indicate that discontinuous temporal EEG organization and the early activity patterns (delta brushes) homologous to spindle bursts in the neonatal rats are observed in human preterm neonates during the third trimester of gestation. On the basis of the results of our findings obtained in the neonatal rats one may predict that isoflurane will suppress these early cortical activity patterns and exert adverse neurodegenerative actions in the premature neonates and human fetuses in utero.

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Conflict of Interest
None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Age dependence in the effects of isoflurane (1.5–2%) on SEP amplitude (top) and L4 MUA density during SEP (bottom). Each pair corresponds to an individual rat; circles with error bars correspond to the group averages at P2–7, P8–15, and P16–69. On the right are shown probability value maps for the comparisons between the activity in control conditions and under isoflurane in three age groups. Within each age group P-values were obtained from paired data comparisons.

Figure S2. Characteristics of the responses evoked by stimuli in different sensory modalities in an adult rat under isoflurane anesthesia. Delays of the responses evoked by sensory stimuli in different modalities in an adult rat (P30) under 2% isoflurane anesthesia. Below, P-value map for the comparisons of MUA times relative to the population spikes as illustrated in main Figure 6C.