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Alpha-chloralose is a Suitable Anesthetic for Chronic Focal Cerebral Ischemia Studies in the Rat: A comparative study

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Abstract

α -chloralose is widely used as an anesthetic in studies of the cerebrovasculature because it provides robust metabolic and hemodynamic responses to functional stimulation. However, there have been no controlled studies of focal ischemia in the rat under α -chloralose anesthesia. Artificially ventilated rats were prepared using 1.2–1.5 % isoflurane anesthesia for filament occlusion of the right middle cerebral artery (MCA), and anesthesia was either switched to α -chloralose (60 mg/kg bolus, 30 mg/kg/hr; n=10) or was maintained on 1% isoflurane (n=10). Following temporary MCA occlusion EEG was monitored from a screw electrode and changes in cerebral blood flow (rCBF) measured with a laser Doppler probe placed over the ischemic cortex. This study shows that α -chloralose is a safe anesthetic for ischemia studies and provides excellent survival. Compared with isoflurane, the cortical and total infarct volumes are larger in the α -chloralose anesthetized animals, while the functional outcome at 72 hours is similar. The total duration of peri-infarct flow transients (PIFTs) is also significantly longer in α -chloralose anesthetized animals. The average amplitude of the flow transients showed a good correlation with the extent of edema in all animals as did the total duration of non-convulsive seizures (NCS) in the α -chloralose anesthetized animals.

Keywords

α -chloralose; edema; focal ischemia; isoflurane; non-convulsive seizures; peri-infarct flow transients

1. Introduction

Long term stable anesthesia is the aim of any regimen employed in stroke research. Studies of cerebrovascular or electrophysiological mechanisms in neuroscience require that normal physiological reflexes be preserved as much as possible. Since the introduction of functional magnetic resonance imaging (fMRI) and optical imaging techniques in the early 1990s for the investigation of brain function, there has been increasing interest in neuronal-hemodynamic coupling and associated metabolic changes. Although surgical preparation is commonly performed in these studies under a gaseous anesthetic such as isoflurane or halothane, transfer to α -chloralose for the coupling measurement has become standard. In anesthetized animals α -chloralose gives the most robust functional response (Ueki et al., 1992), but its use as an anesthetic is still controversial because of its questionable analgesic properties, and its tendency to produce convulsions. Because of its long lasting action and a recovery characterized by

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myoclonic and seizure like motor activity, α -chloralose has been considered as a “non-recovery” anesthetic in stroke research. The purpose of this study was to place into perspective the strengths and weaknesses of α -chloralose (AC) in the rat model of middle cerebral artery (MCA) occlusion at a dose commonly used in functional studies in order to assess the possible use of α -chloralose in chronic stroke studies. The secondary aims of this study were to compare α -chloralose at this dose with a commonly used gaseous anesthetic, isoflurane (ISO), on blood flow, EEG background activity, non-convulsive seizures, peri-infarct flow transients (PIFT), and infarct volume.

2. Results

2.1 Physiological variables

All of the physiological parameters were within normal limits both during the surgical preparation and during MCA occlusion (MCAO). There were no differences between the two groups in arterial blood gases, but there were a few differences in arterial blood pressures, glucose, and body temperature. Both the preischemic blood pressures (ISO: 108 ± 8 mmHg vs AC: 118 ± 9 mmHg) ($p=0.027$) and the postischemic blood pressures (ISO: 110 ± 8 mmHg vs AC: 119 ± 10 mmHg) ($p=0.029$) were significantly higher in the α -chloralose group; however during ischemia the blood pressures were almost identical (table 1). The animals in the α -chloralose group showed a statistically significant lower blood glucose concentration (102 ± 9 mg/dl) than did animals in the isoflurane group (115 ± 17 mg/dl) ($p=0.038$) prior to occlusion. Although body temperature in the isoflurane group (37.7 ± 0.2 °C) was significantly higher than in the α -chloralose group (37.3 ± 0.3 °C) ($p=0.005$) during ischemia, this difference was quite small.

2.2 Laser Doppler Flowmetry

In animals of both groups, MCAO resulted in an immediate reduction of cerebral blood flow (CBF) to 18–32% of baseline in the territory supplied by the ipsilateral MCAO. The flow dropped to $25 \pm 5\%$ of baseline in the ISO group, and to $21 \pm 4\%$ in the AC group. The blood flow showed a slight, continuous increase with time from $36 \pm 8\%$ of baseline (averaged over the first 30. min.) to $39 \pm 8\%$ of baseline (averaged between 60–90. min) in the ISO group. The residual CBF in the AC group was almost identical in the early ($35 \pm 10\%$) and late phase ($36 \pm 8\%$) after MCAO. The CBF changes showed significant differences only in the reperfusion phase with the average flow in the α -chloralose group ($161 \pm 40\%$ of baseline) being higher than in the isoflurane group ($117 \pm 36\%$ of baseline) ($p=0.018$).

2.3 Peri-infarct flow transients

Peri-infarct flow transients occurred in all the rats subjected to MCAO over the 90 minutes of ischemia (figure 1). The flow transient occurring within 3–4 minutes of occlusion with a characteristic biphasic signature (Hossmann, 1996; Shin et al., 2006) was considered a flow manifestation of anoxic depolarization. The following flow transients were mostly monophasic and hyperemic, with only a small fraction being biphasic (ISO: 17.3%, AC: 10.7%) and were considered to be flow manifestations of peri-infarct depolarizations. The number, 12 ± 3 , ($p=0.002$) and the total duration, 1550 ± 177 sec ($p<0.001$) of the PIFT episodes were significantly higher in the AC group than in the ISO group (8 ± 3 and 956 ± 318 sec respectively) (table 2). The average amplitudes (ISO: $12 \pm 8\%$ vs AC: $13 \pm 6\%$ of baseline) of the flow transients were similar in the two groups. The mean amplitude of the PIFT showed a positive correlation with the extent of edema in both the α -chloralose ($r=0.653$, $p=0.040$) and the isoflurane groups ($r=0.654$, $p=0.040$), with a relationship so similar that the data could be combined ($r=0.629$, $p=0.003$) (figure 2).

2.4 EEG

In animals of both groups, MCA occlusion resulted in a reduction in the power of the EEG signal over time. Figures 3A and 3B show the time course of loss of EEG power as a function of post occlusion time for the isoflurane and the α -chloralose treated animals. In the 10 minute period before occlusion (baseline, -10 to 0 in 3A and /B) the total power in the AC treated animals was 2.8 times as great as that in the ISO animals. The ratio was relatively constant over the frequencies 4–25Hz (range 2.5–3.7, figure 3C). Power rapidly dropped in the first ten minutes following occlusion (note the scale change in power from figure 3C to 3D), falling to approximately the same levels in both groups by 40 minutes post occlusion (figure 3A and 3B). Averaged over the period of 40–80 minutes post occlusion, total ISO power dropped to 23.8% of baseline activity, and total AC power dropped to 9.6% of baseline. We examined the delay of EEG power drop off with respect to flow by cross-correlating flow and EEG power (as described in methods), and found no statistical difference, with a delay of 2.3 ± 1.6 seconds for the ISO group and 3.4 ± 3.0 for the AC group. Recovery of EEG power following reperfusion was slower than was the loss of power during occlusion. Following 10 minutes reperfusion, the ISO group had returned to 26.8% of baseline activity and the AC group to 12.0% of baseline; after 20 minutes, the recovery was 29.4% and 14.5% respectively. The incidence of non-convulsive seizures (NCS) (percentage of animals with electric seizure) was similar in the two groups-isoflurane (7/10) and α -chloralose (6/10), and no significant differences in number of events (ISO: 6 ± 3 vs AC: 6 ± 5) and total duration of electric seizure (ISO: 409 ± 292 sec vs AC: 375 ± 271 sec) were measured (table 2). Non-convulsive status epilepticus did not occur in any of the animals. There was no significant correlation between NCS and infarct volume or neurological score/outcome. However a significant correlation was found between the extent of edema and the total duration of NCS in animals of the chloralose group ($r=0.877$, $p=0.003$) (figure 4) but not the isoflurane group ($r=0.15$, $p=0.68$).

2.5 Histology

Temporary MCA occlusion produced histological damage in the cortex and striatum in both groups with animals under chloralose anesthesia having a significantly larger cortical infarct. In the cortex the infarct volume in the AC group was 169 ± 28 mm³ compared to 137 ± 18 mm³ in the isoflurane group ($p=0.007$), while in the striatum the infarct volumes were similar (AC: 66 ± 10 ; ISO: 62 ± 9 mm³ (NS) (figure 5). The volume of total infarct was also significantly different in the two groups (ISO: 198 ± 17 mm³ vs AC: 234 ± 33 mm³) ($p=0.006$), while the extent of edema was similar (table 2).

2.6 Neurological score

All of the animals in both groups survived the ischemia and reperfusion period (zero mortality). The neurobehavioral score for the isoflurane group had a median of 9 at 24 hours and 9.5 at 72 hours not significantly different than the scores of the chloralose group (9.7 at 24 hours and 10.2 at 72 hours).

3. Discussion

α -chloralose is widely used as an anesthetic in studies of the cerebrovasculature because of its presumed minimal depression of autonomic function. However, there have been no controlled studies of focal ischemia in the rat under α -chloralose anesthesia because of its potential convulsant properties and its long lasting action. In the current study we demonstrated that α -chloralose provides excellent survival following focal cerebral ischemia. Compared with isoflurane, the cortical and total infarct volume is larger in the α -chloralose anesthetized animals, while the functional outcome at 72 hours is similar. The total duration of PIFTs is also significantly longer in α -chloralose anesthetized animals. The average amplitude of the flow transients related to ischemic depolarisations showed a good correlation with the extent

of edema in all animals as did the total duration of NCS in the α -chloralose anesthetized animals.

General anesthetics have been in use for more than 150 years, yet only within the past 40 years have we begun to understand the mechanism of action of these compounds. The vast majority of research has been primarily focused on the anesthetics used in the clinic with little work been done with nonvolatile drugs used in the laboratory and by veterinarians. Limited studies suggest that α -chloralose effects GABA-A receptor activity (Kumamoto and Murata, 1996). It does not bind to either the benzodiazepine or the barbiturate sites but potentiates the GABA-induced current by increasing the affinity for GABA (Garrett and Gan, 1998). A generalized inhibition can lead to synchronization of the cortex, a documented feature of this anesthetic (Winters and Spooner, 1966). Unlike isoflurane, α -chloralose produces an electroencephalogram characterized by intermittent high amplitude transients.

α -chloralose anesthetized animals exhibit myoclonic movements, jerks, and tonic extensions of the limbs, and even generalized convulsions can occur in the peri-anesthetic period. Cariani (2000) hypothesized that α -chloralose can lead to increased cortical activity despite “anesthesia”, making it attractive for use in cerebral activation studies.

Although it is widely believed that α -chloralose anesthesia has no remarkable, negative impact on the cardio-or cerebrovasculature, its precise effect on cardiovascular reflexes, cerebral circulation and the autonomic nervous system is strongly debated in the literature. In the present study we found higher arterial blood pressures (10 mmHg) prior to and following the ischemic period in the α -chloralose anesthetized animals. It is well known that isoflurane causes both respiratory and cardiovascular depression, the latter occurring predominantly due to a fall in systemic vascular resistance (Eger, 1981). On the other hand nitrous oxide, which remained at the 70% level, stimulates the sympathetic nervous system (Maze and Fujinaga, 2000). The arterial blood pressure was determined by the interaction of these two anesthetics during “isoflurane” anesthesia. In the animals anesthetized with α -chloralose, the vasodilative effect of isoflurane is not present and a slightly higher blood pressure may be expected. CBF and cerebral blood volume (CBV) are not affected by concentrations of isoflurane between 0.6 and 1.1 MAC (0.7–1.3%), but at higher concentrations (1.6 MAC in the human), CBF doubles (Eger, 1981). Similarly it is only at higher concentrations that an increase in ICP is observed (Grosslight et al., 1985) thereby decreasing perfusion pressure. An MRI study in rat showed that the isoflurane/ awake CBF ratio is 1.46, but the response to CO₂ was lower with isoflurane, likely due to isoflurane's “ceiling “effect“ of higher basal CBF values (Sicard et al., 2003). Unfortunately the cerebrovascular effects of α -chloralose have not been extensively studied. The cerebral blood flow under α -chloralose anesthesia in the rat is between 0.69–0.85 ml/g/min (Duong et al., 2000; Tsekos et al., 1998; Ueki et al., 1988), so the calculated ISO/AC ratio is 1.7–1.9. In the present study the mean initial drop in flow after MCAO was slightly larger in the α -chloralose group ($p=0.053$), and hyperemia during reperfusion was greater, due in part to the vasodilative features of isoflurane (Lorenz et al., 2001). Our subjective experience with α -chloralose indicates that physiological variables can be easily maintained, and although α -chloralose anesthetized rats require approximately 2 hours more postoperative care, we did not lose any animals because of a potential adverse effect of α -chloralose on survival.

One important aim of this study was to make comparisons between the two anesthetics in cerebral ischemia. It was therefore important to find doses of the two drugs similar with respect to “anesthetic depth”. To define the right doses is difficult since α -chloralose produces a unique pattern of anesthesia (both excitant and depressant) and EEG, has no significant analgesic effect at doses frequently used (Dudley et al., 1982), and it is not a volatile agent. Our choice of anesthetic dose was based on cerebral metabolism studies. Dudley and colleagues (Dudley et al., 1982) reported that a 60mg/kg single dose of α -chloralose reduces the cerebral

glucose utilization rate to 35–47% of that in the awake preparation in the cortex, and Nakao et al. (2001) found that a 50 mg/kg bolus followed by a 40 mg/kg/h maintenance dose reduced glucose utilization to 36% in the sensory motor cortex. These reductions in cerebral glucose metabolism in the cortex are very similar to the reductions observed with isoflurane at the concentrations used in the present study (Krafft et al., 2000; Ori et al., 1986). In addition, the α -chloralose dosing used (60 mg/kg bolus; 30 mg/kg/hr maintenance) is in line with the average dosing used in a variety of functional activation studies (64 ± 15 mg/kg; 34 ± 10 mg/kg/hr).

Both the AC and ISO groups also received nitrous oxide (N_2O) so as to minimize painful stimuli, most important for the α -chloralose animals, and so as to minimize required doses of both α -chloralose and isoflurane. In humans, nitrous oxide acting synergistically with the volatile agents, can cause a significant increase in cerebral blood flow (Sakabe et al., 1976), and in a study in goats (Pelligrino et al., 1984), one of the few to examine the effects of N_2O in isolation from other anesthetics, both CBF and cortical CMR_{O_2} increase to 165%–170% of control during 70% inhalation. Although N_2O alters both cerebral blood flow and metabolism, the use of this anesthetic agent during functional studies in ischemia is justified. Besides the pain due to the surgery, the stereotaxic frame, the ventilator, and the forepaw electrodes all are noxious stimuli which can induce surrounding inhibition (Luo et al., 2005) and increases in blood flow (Erdos et al., 2003) in rat somatosensory cortex potentially interfering with the results in stimulation studies. About two-thirds of the functional studies reported in the literature have been done in combination with N_2O .

A hyperglycemic response has been observed in several studies using isoflurane (Lattermann et al., 2001; Saha et al., 2005) potentially due to impaired glucose clearance and increased glucose production. It is important to stress that although there was a significant difference in blood glucose level between the two groups, all animals remained normoglycemic; therefore an effect of glucose on infarct size or outcome is unlikely.

The effect of volatile agents in ischemic insults has been intensively investigated in different stroke models, but a comparison with α -chloralose is lacking in rodents. In the cat following permanent MCA occlusion, halothane anesthetized animals had a smaller cortical infarct volume than did α -chloralose anesthetized animals possibly due to halothane's ability to reduce perifocal spreading depression-like depolarization (Saito et al., 1997), although other studies a slightly lower halothane dose and a shorter survival period (6 hrs instead of 16 hrs) found the damage to be the same (Browning et al., 1997). Anesthetics, similar to other agents, can mitigate ischemic damage by a variety of mechanisms including reduced energy depletion from suppression of electrical activity, enhanced oxygen and glucose supply provided by increased intras ischemic CBF, and an interference with pathophysiological processes on the molecular level. Halothane and isoflurane have both been found to be neuroprotective possibly through a reduction of ischemia induced glutamate accumulation (Patel et al., 1995), gap junction blockade inhibiting peri-ischemic depolarization (PID) (Mantz et al., 1993), an increase in basal CBF, and a reduction in cerebral metabolic rate. There is some evidence, however, that isoflurane delays, by activating alternative cell death mechanisms, but does not prevent cerebral infarction in rats subject to focal ischemia (Kawaguchi et al., 2000). A recent study in filament MCA occlusion suggests that isoflurane (1.8%) improves long term neurologic and histologic outcome in comparison to the awake state (Sakai et al., 2007). In the present study the histological outcome was favorable in the isoflurane anesthetized group compared to the α -chloralose group at 72 hours, while the functional outcomes were similar. The difference between the functional and histological outcome may be related to the site of damage. Our neurological test battery consisted of two tests: a postural reflex test first described by Bederson et al. (1986) and sensorimotor placing tests developed by De Ryck et al. (1989) and used to describe neurological changes following photothrombosis where the cortex only is affected. Filament occlusion of the middle cerebral artery causes damage in both the cortex and the

striatum, which contribute non-proportionally to the test battery. In the present study, the damage in the striatum was similar in the isoflurane and the α -chloralose groups, with the observed histological difference originating in the cortex. The better correlation between the damage in the striatum and the neurological score than between the total damage and neurological score is consistent with data from other laboratories using a similar ischemic model and neurological test battery (Belayev et al., 1996). A potential disconnect between histological and functional data is the main reason that many investigators measure both outcome parameters in experimental studies.

In various animal models of cerebrovascular disease, peri-infarct depolarization, a spreading depression like phenomenon, has been found to contribute to ischemic injury through its high metabolic and excitotoxic impact (Back et al., 1996; Nedergaard and Astrup, 1986). The expansion of the depolarized core coincides with the occurrence of repetitive and spontaneous PIDs (Hossmann, 1996). A wave of PID induces an increase in blood flow only in the penumbra where perfusion is not severely reduced. Arteriolar vasodilatation in the penumbra may result in further perfusion deficits in the surrounding already hypoperfused areas (steal phenomenon) (Nallet et al., 2000; Pinard et al., 2002). Although no data exists for isoflurane, under halothane anesthesia, the number of PIDs in the rat is approximately 2–8 events/hour (Hartings et al., 2003a; Pinard et al., 2002; Schuler et al., 2001) with an average duration of 2.0 ± 1.0 min (Pinard et al., 2002). The propagation of PID is multi-directional; the most common pattern is frontocaudal with a velocity of 3–8 mm/min., but rostrocaudal and lateromedial propagation have also been observed. In transient MCAO, PIDs occur in a biphasic pattern with the onset time and total duration of delayed PIDs (8–24 hours post MCAO) correlating with infarct volume (Hartings et al., 2003a). Our findings, recorded with one laser probe, are similar to those reported from electrophysiological recordings. In our series the mean number of PIFTs was $8 \pm 3/90$ min in the isoflurane group and $12 \pm 3/90$ min in the α -chloralose group, with an average duration of 130 ± 15 sec and 134 ± 24 sec respectively. In twelve animals anesthetized with α -chloralose and in which two laser probes were placed on the thinned skull 3 mm apart; the mean velocity of PIFT was 3–6 mm/min in 7 animals and 12–18 mm/min in 5 animals (unpublished observations). The majority of flow transients propagated rostrocaudal, consistent with previous observations (Hartings et al., 2003a). The measurement of velocity is often complicated and limited because the wave morphology of the flow transients is usually not identical beneath the two probes. Unfortunately there are other limitations to detecting flow transients with laser Doppler. Especially during isoflurane anesthesia, the “wave” morphology is sometimes “obscured”. In our studies, however, we excluded only one animal from the twenty because of artifacts and a poor laser Doppler signal. It is worthy of note, based on our previous experience, that the amplitude of the flow transients could be even less than 5% in those cases where CBF after occlusion is less than 20% of baseline.

The total duration of PIFTs during occlusion and the cortical infarct volume were significantly higher in the α -chloralose group. Consistent with the literature we did not find any correlation between the infarct size and the parameters of “early” PID (0–90 min. post MCAO) in either group. If all animals are taken together, however, the mean amplitude of the PIFT showed a positive correlation with the extent of edema.

Cytotoxic edema is the earliest form of edema that develops in acute ischemic stroke usually preceding the formation of vasogenic edema that reflects damage to the blood brain barrier. Edema starts to develop after 2–3 h of ischemia (Young et al., 1987) and reaches its maximum at 24–48h, slightly earlier than when our histological measurements were made (72 hours). PID/PIFT could promote both types of edema by both increasing the metabolic burden and energy deficit, and by causing a deterioration of the microcirculation through endothelial swelling, plugging of the microvasculature, and by increasing arteriolar diameter (del Zoppo and Hallenbeck, 2000; Pinard et al., 2002) Although we recorded PIFTs only during the 90

minutes of MCAO, ischemic depolarization can continue over 24h even in transient ischemia, hereby facilitating the edema formation over several hours. Parameters other than the number of PIDs/PFTs such as the amplitudes of these hyperemic events could be important in the pathophysiology of stroke and could bear prognostic importance as well.

Non-convulsive seizures have received relatively limited attention both clinically and experimentally. The rat MCAO model is widely used in stroke studies and importantly, mimics the EEG abnormalities observed in brain-injured patients, including seizures, spreading depolarizations, polymorphic delta activity and periodic lateralized epileptiform discharges (Hartings et al., 2003b; Lu et al., 2001). The incidence of NCS in this model is 81%, the mean number of NCS/animal is 10.6 over a 2 hour observation period, and the mean duration of each episode is 60 sec (Hartings et al., 2003b). The occurrence and duration of NCS in brain injured patients are critical determinants of outcome and mortality (Jordan, 1995; Litt et al., 1998; Young et al., 1996). Although electric seizures and acute brain injury work synergistically to worsen outcome, the underlying pathomechanism is still unclear. In a comprehensive study testing antiepileptic drugs in the rat MCAO model Williams and colleagues found that there was no significant correlation between infarct volume and the number of NCS events for vehicle-treated animals (Williams et al., 2004). However, when data from all treatment groups were considered together, there was a low (but positive) correlation. In our study, the parameters of NCS were similar, although the shorter recording time and the one-lead EEG may account for a slightly lower incidence in our animals. We did not manage to find any correlation between NCS and infarct volume or neurological score/outcome. However a significant correlation was found between the extent of edema and the total duration of NCS in animals of the chloralose group. Several papers, both in humans and animals, report that (non)convulsive status epilepticus causes changes in the redistribution of intracellular and extracellular water presumably due to cytotoxic edema (Chu et al., 2001; Wall et al., 2000; Wiesmann et al., 1997). A possible link between edema and electric seizure is supported by the observation that the incidence of NCS in this study (12/13) was significantly higher in those animals of both groups where the extent of edema was higher than 10 %.

The result of the EEG power analysis can be summarized as follows: α -chloralose treated animals have higher levels of baseline EEG power than isoflurane anesthetized animals. Power falls in both the AC and ISO animals with about a 2–3 second delay to a common, low, level. This drop is much larger percentage-wise for the AC group than the ISO group due to the much greater activity in the AC group prior to occlusion. There appears to be no frequency effect of the anesthetic, as the fall off in activity across frequencies is similar for both anesthetics. For both AC and ISO the recovery following perfusion was slow and not fully characterized by the experiments performed. If we take into consideration the higher baseline activity under AC anesthesia, there appears to be no difference in the two conditions.

In summary this study demonstrates that chronic cerebral ischemia studies can be undertaken in the rodent anesthetized with α -chloralose with no mortality. Since α -chloralose is the preferred anesthetic for neuronal-hemodynamic coupling studies, this facilitates functional activation protocols under conditions of cerebral ischemia. Doses of α -chloralose normally used for functional activation studies increases infarct size compared to isoflurane. This study demonstrates the use of laser Doppler as a detector/monitor of peri-infarct flow transients, and shows the excellent correlation between edema and the parameters of PFTs and NCS which potentially has prognostic and therapeutic implications in the clinic.

4. Experimental procedure

4.1 Surgical preparation

All procedures performed were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Adult male Sprague-Dawley rats (290–340g) were anesthetized with 4% isoflurane for induction in a mixture of 50% nitrous oxide and 50% oxygen. The animals were intubated endotracheally, then mechanically ventilated and maintained on 1.2–1.5% isoflurane (1.0–1.25 MAC) (Sarraf-Yazdi et al., 1998) in 70% nitrous oxide and 30% oxygen during surgery. Exhaled CO₂ levels were monitored by a capnograph (MicroCapnograph 240; Columbus Instruments, Ohio, USA), and the ventilator was adjusted to keep the levels within the normal range. Temperature probes were inserted into the rectum and the right temporalis muscle and the temperature was kept at 37° C with a separate heating lamp and blanket regulated by a homeothermic control unit. The tail artery was cannulated with a polyethylene catheter (PE-50) for the measurement of arterial blood pressure and arterial blood gasses. Mean arterial blood pressure was monitored continuously throughout the animal preparation using the PowerLab (ADInstruments, Colorado Springs, CO, USA) data acquisition system.

4.2 Laser Doppler flowmetry and EEG recording

The head of the animal was placed in a stereotaxic frame, a midline scalp incision was made and the skull was exposed. A laser Doppler flow (LDF) probe (tip diameter 1mm, fiber separation 0.25 mm) attached to a flowmeter (PeriFlux 4001; Perimed, Stockholm, Sweden) was affixed over a 1 mm-diameter circular area of thinned skull (5 mm lateral to midline, 2mm posterior to Bregma). Cortical EEG was monitored using a stainless steel electrode (5 mm lateral to midline, 4mm posterior to Bregma) overlying the territory of the right middle cerebral artery. The EEG activity was amplified 200 times and sampled at 100 Hz with a high pass filter set at 2 Hz (PowerLab). The reference electrode was placed 2–3 mm anterior to Bregma. Both blood flow and the unipolar electrical activity were recorded continuously during the ischemia and the reperfusion phase using PowerLab. The initial drop and the average flow during ischemia and reperfusion were calculated as a percentage of the baseline prior to MCA occlusion. Criteria for identifying peri-infarct flow transients (PIFTs) were the following: (a) flow transient with amplitude greater than 5% of preischemic baseline; (b) a duration of blood flow changes longer than 60 sec.; (c) stable blood pressure during the event. The criteria for PIFTs were based on our experience with simultaneous laser Doppler and laser speckle monitoring during filament MCA occlusion in rat. The duration of the PIFT was determined by computing a mean and standard deviation of the LDF signal both prior to and following a PIFT. The start of the PIFT was determined by the time at which the LDF signal changed by more than two standard deviations of the mean prior to the PIFT, and the end of the PIFT defined when the LDF signal fell to within two standard deviations of the mean following the PIFT (figure 6). The amplitude was obtained as the percent change in CBF from the lowest mean (either prior to or following the PIFT) to the peak.

EEG activity was analyzed for changes in total power, and spectral power content throughout the course of the experiment. The raw EEG signal was also examined for electrical seizure activity (described below). We used the *pwelch* routine in MATLAB (The MathWorks, Natick, MA, USA) to determine power in the following manner: The EEG signal was aligned on the time of occlusion, and spectral power analyzed in 5 second bins after removing bins which contained seizure activity or large noise events. Remaining bins were averaged over ten minute periods. The delay in the reduction of EEG power in relation to changes in blood flow was determined by cross correlating blood flow (averaged over one second bins) with EEG power (also averaged over one second bins) across three minutes of data, starting one minute before

the occlusion event (MATLAB *xcorr*). The peak of the cross-correlogram was used to determine the delay between decreasing flow and decreasing EEG power.

All seizure events were verified at a digital recording speed of 25 mm/s for scoring of non-convulsive seizure (NCS) episodes. Criteria for identifying NCS events were as follows (Williams et al., 2004): (a) the occurrence of repetitive spikes or spike and wave discharges recurring at frequencies > 1 Hz, or continuous polyspiking; (b) spike amplitude greater than background activity; (c) duration of continuous seizure activity (defined by (a) and (b)) longer than 10 s. Consecutive seizure episodes were considered a single event if not separated by more than 10 s.

4.3 Transient MCA Occlusion

The animal was placed supine on a plastic holder and prepared for MCA occlusion using the intraluminal filament model (Shimazu et al., 2005). Briefly, the right common carotid artery (CCA) was isolated from the surrounding connective tissue. The right external carotid artery was ligated and a 0.37–0.39 mm silicone coated nylon filament (Doccol Corporation, Redlands, CA, USA) was inserted through the CCA into the internal carotid artery (ICA). The filament was gently advanced until LDF indicated adequate MCA occlusion by a sharp decrease in ipsilateral blood flow to 20–30% of baseline. After 90 minutes of occlusion the filament was gently removed. Prompt and proper reperfusion was monitored by LDF for 20 minutes.

4.4 Experimental regimen

Two groups of rats randomized to anesthetic condition were studied (n=10 each). In the first group (AC) after the surgical preparation α -chloralose was administered intraperitoneally (60 mg/kg). Five minutes later the isoflurane (but not the N₂O) was discontinued and anesthesia maintained on α -chloralose (30 mg/kg/hr). Sixty minutes after the isoflurane was discontinued, the MCAO was occluded. Following the ischemia, animals were weaned off of the ventilator starting 60 min after the start of reperfusion. First the ability of the animal to breathe spontaneously was checked. If it was necessary, the respiratory rate and the tidal volume were incrementally decreased based on the monitored end-tidal CO₂ level. After weaning from the respirator the animals continued to receive oxygen rich air (FiO₂ 30%) via the endotracheal tube, care being taken to maintain normal levels of exhaled CO₂ and the other physiological variables in the postischemic phase. The depth of anesthesia was monitored constantly by pinching the tail and extremities. Approximately 120 minutes after the MCAO was reopened, the animals were extubated. A laryngeal toilette was performed and the intact state of vocal chords was confirmed. The extubated animals continued to receive oxygen via a nose cone and the core temperature was kept at 37.0–38.0 C with a heating blanket for an additional hour. Once the animals were capable of moving about they were put back in their cages. The elapsed time between start of reperfusion and the start of animal ambulation was about 180–210 minutes.

A second group of animals was kept on isoflurane (ISO) (and N₂O) both during the surgical and ischemic phase of the experiment with timing of the start of ischemia matched to the AC group. Post surgery the isoflurane was maintained at 1.0 %. After reopening the MCAO a protocol similar to the AC group was followed. Since isoflurane is a volatile, short lasting anesthetic the process of weaning the animal from the respirator was much shorter and easier. Approximately 90 minutes after reopening the MCAO, the animals were extubated. The animals in this group recovered quickly after discontinuing the isoflurane and they did not require special observation after the extubation, except a laryngeal toilette. The time span between the start of reperfusion and the recovery of the animals in this group was 90–100 minutes.

4.5 Neurological Evaluation

A neurological evaluation was performed at 24 and at 72 hours post-injury according to the protocol of Belayev (Belayev et al., 1996). Postural reflex, visual placing in forward and sideways directions, tactile placing of the dorsal and lateral paw surfaces and proprioceptive placing were tested by an investigator who was blinded to the experimental group. These tests were each scored from 0 to 2, and the neurological deficit was calculated as the sum of the scores of the individual tests ranging from 0 (no deficit) to 12 (maximum deficit). Mortality rate was compared between animals of two groups at 72h post MCAO. Animals with subarachnoidal hemorrhage were excluded from the analysis.

4.6 Infarct volume measurement

Rats were sacrificed 72 hours after MCAO and the brain was removed from the skull and cooled in ice cold saline for 20 minutes. The brain was sectioned in the coronal plane at 2-mm intervals using a rodent brain matrix and the brain slices were incubated in PBS containing 2% 2,3,5-triphenyltetrazolium chloride (TTC) at 37.0° C for 10 minutes. The TTC-stained sections were photographed with a digital camera and the damaged area determined using a computer based image analyzer (AIS 6.0; Imaging Research Inc., St. Catharines, ON, Canada). To avoid artifacts attributable to edema, the damaged area was calculated by subtracting the area of the normal tissue in the hemisphere ipsilateral to the stroke from the area of the hemisphere contralateral to the stroke. Total lesion volumes in cortex and striatum were calculated by summation of the infarct areas of 6 brain slices integrated by the thickness. An edema index was calculated from the total volume of the hemisphere ipsilateral to the MCA occlusion and the total volume of the contralateral hemisphere (Yanamoto et al., 1996), a technique validated by a combined MRI imaging / water content study (Gerriets et al., 2004). The investigator was blinded to anesthetic condition when analyzing the data.

4.7 Statistical analysis

Results are expressed as mean±SD. Significant differences between groups were determined with an ANOVA with repeated measures followed by a Tukey test as appropriate. Correlations between parameters was tested with Pearson Product Moment Correlation. A p value less than 0.05 was considered significant.

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Reference List

- Back T, Ginsberg MD, Dietrich WD, Watson BD. Induction of spreading depression in the ischemic hemisphere following experimental middle cerebral artery occlusion: effect on infarct morphology. *J. Cereb. Blood Flow Metab* 1996;16:202–213. [PubMed: 8594051]
- Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. *Stroke* 1986;17:472–476. [PubMed: 3715945]
- Belayev L, Alonso OF, Busto R, Zhao W, Ginsberg MD. Middle cerebral artery occlusion in the rat by intraluminal suture. Neurological and pathological evaluation of an improved model. *Stroke* 1996;27:1616–1622. [PubMed: 8784138]
- Browning JL, Heizer ML, Widmayer MA, Baskin DS. Effects of halothane, alpha-chloralose, and pCO₂ on injury volume and CSF beta-endorphin levels in focal cerebral ischemia. *Mol. Chem. Neuropathol* 1997;31:29–42. [PubMed: 9271003]
- Cariani P. Anesthesia, neural information processing, and conscious awareness. *Conscious. Cogn* 2000;9:387–395. [PubMed: 10993666]

- Chu K, Kang DW, Kim JY, Chang KH, Lee SK. Diffusion-weighted magnetic resonance imaging in nonconvulsive status epilepticus. *Arch. Neurol* 2001;58:993–998. [PubMed: 11405815]
- De Ryck M, Van RJ, Borgers M, Wauquier A, Janssen PA. Photochemical stroke model: flunarizine prevents sensorimotor deficits after neocortical infarcts in rats. *Stroke* 1989;20:1383–1390. [PubMed: 2799870]
- del Zoppo GJ, Hallenbeck JM. Advances in the vascular pathophysiology of ischemic stroke. *Thromb. Res* 2000;98:73–81. [PubMed: 10812160]
- Dudley RE, Nelson SR, Samson F. Influence of chloralose on brain regional glucose utilization. *Brain Res* 1982;233:173–180. [PubMed: 7059799]
- Duong TQ, Silva AC, Lee SP, Kim SG. Functional MRI of calcium-dependent synaptic activity: cross correlation with CBF and BOLD measurements. *Magn Reson. Med* 2000;43:383–392. [PubMed: 10725881]
- Eger EI. Isoflurane: a review. *Anesthesiology* 1981;55:559–576. [PubMed: 7027831]
- Erdos B, Lacza Z, Toth IE, Szelke E, Mersich T, Komjati K, Palkovits M, Sandor P. Mechanisms of pain-induced local cerebral blood flow changes in the rat sensory cortex and thalamus. *Brain Res* 2003;960:219–227. [PubMed: 12505675]
- Garrett KM, Gan J. Enhancement of gamma-aminobutyric acidA receptor activity by alpha-chloralose. *J. Pharmacol. Exp. Ther* 1998;285:680–686. [PubMed: 9580613]
- Gerriets T, Stolz E, Walberer M, Muller C, Kluge A, Bachmann A, Fisher M, Kaps M, Bachmann G. Noninvasive quantification of brain edema and the space-occupying effect in rat stroke models using magnetic resonance imaging. *Stroke* 2004;35:566–571. [PubMed: 14739415]
- Grosslight K, Foster R, Colohan AR, Bedford RF. Isoflurane for neuroanesthesia: risk factors for increases in intracranial pressure. *Anesthesiology* 1985;63:533–536. [PubMed: 4051214]
- Hartings JA, Rolli ML, Lu XC, Tortella FC. Delayed secondary phase of peri-infarct depolarizations after focal cerebral ischemia: relation to infarct growth and neuroprotection. *J. Neurosci* 2003a; 23:11602–11610. [PubMed: 14684862]
- Hartings JA, Williams AJ, Tortella FC. Occurrence of nonconvulsive seizures, periodic epileptiform discharges, and intermittent rhythmic delta activity in rat focal ischemia. *Exp. Neurol* 2003b; 179:139–149. [PubMed: 12618120]
- Hossmann KA. Perinfarct depolarizations. *Cerebrovasc. Brain Metab Rev* 1996;8:195–208. [PubMed: 8870974]
- Jordan KG. Neurophysiologic monitoring in the neuroscience intensive care unit. *Neurol. Clin* 1995;13:579–626. [PubMed: 7476820]
- Kawaguchi M, Kimbro JR, Drummond JC, Cole DJ, Kelly PJ, Patel PM. Isoflurane delays but does not prevent cerebral infarction in rats subjected to focal ischemia. *Anesthesiology* 2000;92:1335–1342. [PubMed: 10781279]
- Krafft P, Frietsch T, Lenz C, Piegras A, Kuschinsky W, Waschke KF. Mild and moderate hypothermia (alpha-stat) do not impair the coupling between local cerebral blood flow and metabolism in rats. *Stroke* 2000;31:1393–1400. [PubMed: 10835462]
- Kumamoto E, Murata Y. Enhancement by lanthanide of general anesthetic-induced GABAA-receptor current in rat septal cholinergic neurons in culture. *J. Neurophysiol* 1996;75:2294–2299. [PubMed: 8793742]
- Lattermann R, Schricker T, Wachter U, Georgieff M, Goertz A. Understanding the mechanisms by which isoflurane modifies the hyperglycemic response to surgery. *Anesth. Analg* 2001;93:121–127. [PubMed: 11429352]
- Litt B, Wityk RJ, Hertz SH, Mullen PD, Weiss H, Ryan DD, Henry TR. Nonconvulsive status epilepticus in the critically ill elderly. *Epilepsia* 1998;39:1194–1202. [PubMed: 9821984]
- Lorenz IH, Kolbitsch C, Hormann C, Luger TJ, Schocke M, Felber S, Zschiegner F, Hinteregger M, Kremser C, Benzer A. Influence of equianaesthetic concentrations of nitrous oxide and isoflurane on regional cerebral blood flow, regional cerebral blood volume, and regional mean transit time in human volunteers. *Br. J. Anaesth* 2001;87:691–698. [PubMed: 11878518]
- Lu XC, Williams AJ, Tortella FC. Quantitative electroencephalography spectral analysis and topographic mapping in a rat model of middle cerebral artery occlusion. *Neuropathol. Appl. Neurobiol* 2001;27:481–495. [PubMed: 11903931]

- Luo W, Li P, Chen S, Luo Q. Surrounding inhibition in rat somatosensory cortex during noxious electrical stimulation of the sciatic nerve. *Conf. Proc. IEEE Eng Med. Biol. Soc* 2005;2:1777–1779. [PubMed: 17282560]
- Mantz J, Cordier J, Giaume C. Effects of general anesthetics on intercellular communications mediated by gap junctions between astrocytes in primary culture. *Anesthesiology* 1993;78:892–901. [PubMed: 7683851]
- Maze M, Fujinaga M. Recent advances in understanding the actions and toxicity of nitrous oxide. *Anaesthesia* 2000;55:311–314. [PubMed: 10781114]
- Nakao Y, Itoh Y, Kuang TY, Cook M, Jehle J, Sokoloff L. Effects of anesthesia on functional activation of cerebral blood flow and metabolism. *Proc. Natl. Acad. Sci. U. S. A* 2001;98:7593–7598. [PubMed: 11390971]
- Nallet H, MacKenzie ET, Roussel S. Haemodynamic correlates of penumbral depolarization following focal cerebral ischaemia. *Brain Res* 2000;879:122–129. [PubMed: 11011013]
- Nedergaard M, Astrup J. Infarct rim: effect of hyperglycemia on direct current potential and [14C]-deoxyglucose phosphorylation. *J. Cereb. Blood Flow Metab* 1986;6:607–615. [PubMed: 3760045]
- Ori C, Dam M, Pizzolato G, Battistin L, Giron G. Effects of isoflurane anesthesia on local cerebral glucose utilization in the rat. *Anesthesiology* 1986;65:152–156. [PubMed: 3740504]
- Patel PM, Drummond JC, Cole DJ, Goskowitz RL. Isoflurane reduces ischemia-induced glutamate release in rats subjected to forebrain ischemia. *Anesthesiology* 1995;82:996–1003. [PubMed: 7717573]
- Pelligrino DA, Miletich DJ, Hoffman WE, Albrecht RF. Nitrous oxide markedly increases cerebral cortical metabolic rate and blood flow in the goat. *Anesthesiology* 1984;60:405–412. [PubMed: 6424512]
- Pinar E, Nallet H, MacKenzie ET, Seylaz J, Roussel S. Penumbral microcirculatory changes associated with peri-infarct depolarizations in the rat. *Stroke* 2002;33:606–612. [PubMed: 11823677]
- Saha JK, Xia J, Grondin JM, Engle SK, Jakubowski JA. Acute hyperglycemia induced by ketamine/xylazine anesthesia in rats: mechanisms and implications for preclinical models. *Exp. Biol. Med.* (Maywood.) 2005;230:777–784. [PubMed: 16246906]
- Saito R, Graf R, Hubel K, Fujita T, Rosner G, Heiss WD. Reduction of infarct volume by halothane: effect on cerebral blood flow or perifocal spreading depression-like depolarizations. *J. Cereb. Blood Flow Metab* 1997;17:857–864. [PubMed: 9290583]
- Sakabe T, Kuramoto T, Kumagai S, Takeshita H. Cerebral responses to the addition of nitrous oxide to halothane in man. *Br. J. Anaesth* 1976;48:957–962. [PubMed: 791308]
- Sakai H, Sheng H, Yates RB, Ishida K, Pearlstein RD, Warner DS. Isoflurane provides long-term protection against focal cerebral ischemia in the rat. *Anesthesiology* 2007;106:92–99. [PubMed: 17197850]
- Sarraf-Yazdi S, Sheng H, Miura Y, McFarlane C, Dexter F, Pearlstein R, Warner DS. Relative neuroprotective effects of dizocilpine and isoflurane during focal cerebral ischemia in the rat. *Anesth. Analg* 1998;87:72–78. [PubMed: 9661549]
- Schuler OG, Plesnila N, Otto D, Baethmann AJ, Back T. Early thrombolysis inhibits peri-infarct depolarizations in embolic MCA occlusion. *Neuroreport* 2001;12:3943–3946. [PubMed: 11742216]
- Shimazu T, Inoue I, Araki N, Asano Y, Sawada M, Furuya D, Nagoya H, Greenberg JH. A peroxisome proliferator-activated receptor-gamma agonist reduces infarct size in transient but not in permanent ischemia. *Stroke* 2005;36:353–359. [PubMed: 15618443]
- Shin HK, Dunn AK, Jones PB, Boas DA, Moskowitz MA, Ayata C. Vasoconstrictive neurovascular coupling during focal ischemic depolarizations. *J. Cereb. Blood Flow Metab* 2006;26:1018–1030. [PubMed: 16340958]
- Sicard K, Shen Q, Brevard ME, Sullivan R, Ferris CF, King JA, Duong TQ. Regional cerebral blood flow and BOLD responses in conscious and anesthetized rats under basal and hypercapnic conditions: implications for functional MRI studies. *J. Cereb. Blood Flow Metab* 2003;23:472–481. [PubMed: 12679724]
- Tsekos NV, Zhang F, Merkle H, Nagayama M, Iadecola C, Kim SG. Quantitative measurements of cerebral blood flow in rats using the FAIR technique: correlation with previous iodoantipyrine autoradiographic studies. *Magn Reson. Med* 1998;39:564–573. [PubMed: 9543418]

- Ueki M, Linn F, Hossmann KA. Functional activation of cerebral blood flow and metabolism before and after global ischemia of rat brain. *J. Cereb. Blood Flow Metab* 1988;8:486–494. [PubMed: 3392113]
- Ueki M, Mies G, Hossmann KA. Effect of alpha-chloralose, halothane, pentobarbital and nitrous oxide anesthesia on metabolic coupling in somatosensory cortex of rat. *Acta Anaesthesiol. Scand* 1992;36:318–322. [PubMed: 1595336]
- Wall CJ, Kendall EJ, Obenaus A. Rapid alterations in diffusion-weighted images with anatomic correlates in a rodent model of status epilepticus. *AJNR Am. J. Neuroradiol* 2000;21:1841–1852. [PubMed: 11110536]
- Wiesmann UC, Symms MR, Shorvon SD. Diffusion changes in status epilepticus. *Lancet* 1997;350:493–494. [PubMed: 9274593]
- Williams AJ, Tortella FC, Lu XM, Moreton JE, Hartings JA. Antiepileptic drug treatment of nonconvulsive seizures induced by experimental focal brain ischemia. *J. Pharmacol. Exp. Ther* 2004;311:220–227. [PubMed: 15140918]
- Winters WD, Spooner CE. A neurophysiological comparison of alpha-chloralose with gamma-hydroxybutyrate in cats. *Electroencephalogr. Clin. Neurophysiol* 1966;20:83–90. [PubMed: 4161321]
- Yanamoto H, Hong SC, Soleau S, Kassell NF, Lee KS. Mild postischemic hypothermia limits cerebral injury following transient focal ischemia in rat neocortex. *Brain Res* 1996;718:207–211. [PubMed: 8773789]
- Young GB, Jordan KG, Doig GS. An assessment of nonconvulsive seizures in the intensive care unit using continuous EEG monitoring: an investigation of variables associated with mortality. *Neurology* 1996;47:83–89. [PubMed: 8710130]
- Young W, Rappaport ZH, Chalif DJ, Flamm ES. Regional brain sodium, potassium, and water changes in the rat middle cerebral artery occlusion model of ischemia. *Stroke* 1987;18:751–759. [PubMed: 3603602]

Figure 1.

Dynamic changes in LDF (laser Doppler flow) during 90 min. of middle cerebral artery occlusion in a chloralose and an isoflurane anesthetized rat. According to our criteria (see text) thirteen events of peri-infarct flow transients (PIFTs) in the chloralose and eleven flow transients in the isoflurane anesthetized animal can be seen. The reperfusion is also remarkably greater in the chloralose anesthetized animal.

Figure 2.

The relationship between the extent of edema and mean amplitude of PIFTs (peri-infarct flow transients) in the isoflurane (open circles) and chloralose (closed circles) animals. The linear regression (solid line) and 95% confidence limits (dotted lines) was performed on the combined groups ($r=0.629$, $p=0.003$).

Figure 3.

Change in EEG power over time. Power is shown in a common color scale for isoflurane (ISO) (A) and α -chloralose (AC) (B) treated. Red indicates larger power values, blue indicates smaller values. Power in single Hz frequency bins is plotted versus time with zero (0) being the time of occlusion (C). Power in individual frequency bins averaged from 10 minutes before the occlusion to the time of the occlusion. Error bars indicate the standard error across animals. The values shown are those represented in the -10 to 0 minute columns in panels A and B. Blue lines indicate data from ISO treated animals, red lines indicate data from AC treated animals. (D) to (F); consecutive 10 minute periods following occlusion, otherwise same as (C).

Figure 4.

Relationship between extent of edema and total duration of non-convulsive seizures (NCS) in the chloralose anesthetized group ($r=0.877$, $p=0.003$). The linear regression (solid line) and 95% confidence limits (dotted lines) are shown. There was no correlation between extent of edema and NCS duration in the isoflurane group.

Figure 5.

Bar graphs show measurements of TTC-determined cortical (A) and striatal (B) infarct size at six (1–6: rostral to caudal) stereotaxic levels in α -chloralose (AC) and isoflurane (ISO) groups.

Figure 6.

Schematic showing both an anoxic depolarization (left of figure) and a peri-ischemic flow transient (PIFT) (right of figure). The duration (Dur) of the PIFT was defined as the time period when blood flow deviated by more than two standard deviations (shaded area) from the mean prior to and following the PIFT. The amplitude (Amp) was obtained as the percent change in CBF from the lowest mean (either prior to or following the PIFT) to the peak.

Table 1

Physiological variables measured in separate groups at baseline, during ischemia, and during early reperfusion

SAP (mmHg)	ISO	AC
Baseline	108±8	118±9 [*]
Ischemia	120±10	121±7
Reperfusion	110±8	119±10 [*]
Core temperature (°C)		
Baseline	37.2±0.1	37.2±0.1
Ischemia	37.7±0.2 [*]	37.3±0.3
Reperfusion	37.4±0.2	37.3±0.2
72 h post injury	37.5±0.5	37.1±0.5
Pericranial temperature (°C)		
Baseline	36.9±0.2	36.9±0.2
Ischemia	37.2±0.2	37.0±0.3
Reperfusion	36.9±0.2	36.8±0.3
Blood gas (baseline)		
PCO ₂ (mmHg)	37.8±2.6	36.9±2.6
PO ₂ (mmHg)	117±15	125±16
pH	7.46±0.02	7.47±0.03
Blood gas (ischemia)		
PCO ₂ (mmHg)	37.8±3.2	38.8±3.1
PO ₂ (mmHg)	114±17	122±14
pH	7.45±0.04	7.43±0.03
Glucose (baseline) (mg/dl)	115±17	102±9 [*]
Loss of body weight at 72h	21±3%	19±3%

SAP: systemic arterial blood pressure; ISO: isoflurane group; AC: α -chloralose group. Baseline: 15 minutes prior to middle cerebral artery occlusion (MCAO); Ischemia: 20–30 minutes post MCAO; Reperfusion: 30 minutes after start of reperfusion. All data are expressed as mean±SD.

* Significant differences between groups ($p<0.05$).

Table 2
Mean of PIFT, NCS, edema and infarct size in separate groups

	ISO	AC
Peri-infarct flow transients (PFIT)		
Number of events	8±3	12±3**
Total duration of PIFTs (sec)	956±318	1550±177**
Amplitude of PIFTs (% of baseline)	12±8	13±6
Non-convulsive Seizures (NCS)		
Incidence	7/10	6/10
Number of NCS	6±3	6±5
Total duration of CS (sec)	409±292	375±271
Edema	13±5%	11±5%
Infarct size (mm³)		
Striatum	62±9	66±10
Cortex	137±18	169±28*
Total	198±17	234±33*

ISO: isoflurane group; AC: α -chloralose group. All data are expressed as mean±SD.

Significant difference between groups:

*
p<0.05

**
p<0.01