Journal of Innovative Optical Health Sciences
Vol. 4, No. 4 (2011) 409−420

DOI: 10.1142/S1793545811001502

HOW DOES ANESTHESIA AFFECT VARIOUS LEVELS OF EXPERIMENTAL TRAUMATIC BRAIN INJURY?

BARBIRO-MICHAELY EFRAT, MANOR TAMAR,
ROGATSKY GENNADY and MAYEVSKY AVRAHAM*
The Mina and Everard Goodman Faculty of Life-Sciences
The Leslie and Susan Gonda Multidisciplinary Brain Research Center
Bar-Ilan University, Ramat-Gan, 52900, Israel
**mayevsa@mail.biu.ac.il*

Accepted 28 March 2011

The use of anesthetics is a well-known treatment for severely injured patients. In the present study we tested the pathophysiology of several levels of injury damage in a rat model and also tested the effect of Equithesin on brain vitality in these models. Traumatic Brain Injury (TBI) was induced using the fluid percussion injury model in four levels: mild, moderate and two levels of severe TBI. Brain real-time evaluation was performed by the multiparametric monitoring assembly (MPA) which enable cerebral blood flow (CBF) monitoring by laser Doppler flowmetry, mitochondrial NADH (Nicotinamide adenine dinucleotide) monitoring by the fluorometric technique, ionic homehostasis using special mini-electrodes, intracranial pressure (ICP) by the ICP camino device and needle electrodes for ECoG (Electrocorticogram) recording. Our results showed high correlation between the level of impact and the extent of changes in the physiological properties of the injury as indicated by the changes in all parameters monitored using the MPA device. Moreover, Equithesin improved CBF, ionic extracellular level and mitochondrial redox state following mild and moderate TBI while in severe TBI, Equithesin did not improve the metabolic state of the cerebral cortex, although it decreased the mortality rate from 66% to 20%, and following extra-severe TBI level, Equithesin did not improve survival rate. In conclusion it seems that Equithesin's protective effect exists under mild to moderate levels of injury and not in case of severe injuries.

Keywords: Cerebral blood flow; mitochondrial NADH; multiparametric monitoring.

1. Introduction

Traumatic brain injury (TBI) was recently named the "silent epidemic of the 21st century,"[1] with an incidence of 1.5 million cases per year in the United State alone. Hence it is clear that scientists' and physicians' efforts to improve the treatment and final outcome of head-trauma patients are significant.

One of these treatments include the use of anesthesia up to a comatose state, especially in severely injured patients, in order to decrease cerebral metabolism and decrease cerebral perfusion and therefore decrease intracranial pressure (ICP) and increase cerebral perfusion pressure (CPP).[2,3]

The major pathophysiological mechanisms involved with head trauma are very complicated and include the impairment of cerebral circulation, metabolism, oxygen supply and acid−base balance. This fact might explain why therapeutic attempts

that are aimed at the normalization of only one of these components have failed to improve outcome in patients with severe head injury.[4]

One of the crucial parameters being monitored in severe head injury victims is the ICP level.[5−7] This parameter is also used for the evaluation of adequate sedative-anesthetic management of these patients.[8−12] Although a diverse range of anesthetics is used in the treatment of TBI patients, isoflurane and pentobarbital are the most frequently used agents.[13] A most recent study that examined the Intensive Care management of patients with severe TBI in Austria implies that the use of barbiturates (short-term) may improve the outcome of patients after severe TBI.[6] There is also a vast experience with barbiturate application in numerous medical centers implying that barbiturates may be considered as a fine pharmacological agent usable under these conditions.[14] According to the conclusion of the Brain Trauma Foundation, high-dose barbiturate therapy decreases mortality rate of TBI patients and is efficacious in lowering ICP and in setting of uncontrollable ICP as opposed to all other conventional medical and surgical treatments used for lowering ICP.[13]

On the other hand there are scientists who claim that there is no evidence that barbiturates improve the final outcome of patients with severe head injury.[15] Moreover according to Statler *et al.*,[16] "the metabolic-suppressive properties of pentobarbital induced relatively negative effects on functional and histological parameters of the rat brain after TBI, as compared to other anesthetics." Barbiturates also have dangerous side effects, such as infection, pulmonary dysfunction, arterial hypotension and renal failure.[17] Considering the negative effects of barbiturates, in the present study the rats were anesthetized using Equithesin, which is a mixture of pentobarbital and chloral hydrate. Doing so, we decreased the dose of barbiturates being used and hence decreased the risk for negative side effects of this anesthetic in the rats that underwent TBI.

We believe that the beneficial effects of anesthesia on TBI are under disagreement, among other reasons, due to the differences in the degree of injury for which they are being used. Therefore, we wanted to apply a TBI model of several degrees of injury to be treated with Equithesine.

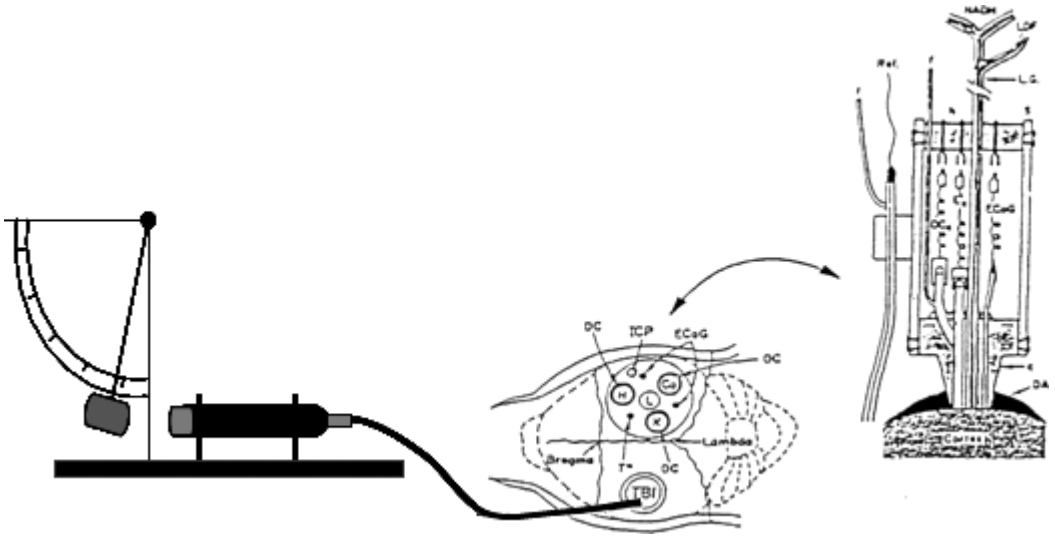
In addition, one of the limiting factors in the evaluation of anesthetics' effects on cerebral parameters is the ability of monitoring only some parameters from the brain simultaneously. It is clear that continuous multiparametric monitoring of parameters that evaluate brain blood supply and cerebral metabolism are of great benefit.[1,4,18−21] In this view, the advantage of our multiparametric monitoring probe is obvious, enabling not only the monitoring of ICP level but also real-time evaluation of cerebral hemodynamic, ionic, energetic and electrical state, under anesthesia in the experimental model of TBI.

2. Material and Methods

2.1. *The multiparametric monitoring system*

Monitoring of the cerebral cortex following TBI was performed using the multiparametric monitoring system (MPA) (Fig. 1). The MPA, 6 mm in diameter, contained optical fibers for NADH redox state fluorometry, a laser Doppler probe, selective mini-electrodes for the measurements of extracellular levels of K^+, Ca^{2+} and H^+ each surrounded with a DC potential electrode, ICP probe, brain temperature thermistor and two electrodes for electrocardiogram (ECoG) monitoring.

The principle of NADH monitoring from the surface of the tissue (1 mm depth) is based on the fact that excitation light (366 nm) passes from the fluorometer, which contains appropriate filters, to the brain via a bundle of optical fibers made of quartz. The emitted light (450 nm), together with the reflected light at the excitation wavelength (366 nm), is transferred to the fluorometer via another bundle of optical fibers.[22] Changes in the reflected light are correlated to changes in tissue blood volume and therefore serve to correct for hemodynamic artifacts in NADH monitoring.[23] The corrected fluorescence (NADH) is obtained by subtracting the reflectance from the fluorescence signal at a 1:1 ratio.[23] Real-time monitoring of CBF from the same cortical area was achieved using the laser Doppler flowmetry technique, which is based on the Doppler shift reflecting the flow of red blood cells in the microcirculation at a depth of 1−2 mm and the reflectance at 632 nm that is mainly affected by changes in tissue blood volume.[24−26] ICP was monitored by the Camino device (model V420, USA). Extracellular K^+, Ca^{2+} and H^+ ions level were monitored using selective mini-electrodes attached to Ag/AgCl holders. The DC steady potential was



(c) (b) (a)

Fig. 1. (a) A schematic presentation of the MPA and its location on the cerebral cortex. The MPA includes a central light guide made of optical fibers to transport the excitation light from the light source (a metal Halide Lamp) to the cerebral tissue, and the emission light (fluorescence and reflectance) from the tissue to the photo-detectors as well as optical fibers for CBF monitoring. (b) The tip of the probe includes optical fibers for NADH monitoring (Ex — Excitation, Em — Emission), for blood flow monitoring ($LDF_{in/out}$), mini-electrodes for the monitoring of extracellular levels of K^+, H^+ and Ca^{2+} each surrounded with a DC electrode for the monitoring of DC steady potential. (c) The fluid percussion injury device for the induction of traumatic brain injury.

measured by highly stable Ag/AgCl electrodes connected via a salt bridge located around the ion's electrodes. A reference electrode was located below the skin of the animal's neck.

All of the signals monitored during the experiment were digitized and transmitted to a multichanneled computerized data acquisition and recording system (Labview A/D software, National Instruments Co., USA) for further analysis.

2.2. *Animal preparation*

All experiments were performed in accordance to the Guidelines of the Animal Care Committee of Bar-Ilan University. Wistar male rats (250−300 g) were anesthetized with an IP injection (0.3 ml/100 gw) of Equithesin (each ml contains: pentobarbital 9.72 mg, chloral hydrate 42.51 mg, magnesium sulfate 21.25 mg, propylene glycol 44.34% w/v, alcohol 11.5% and distilled water).

The rat was fixed in a head holder and a midline incision in the skin was made exposing the parietal and frontal bones of the rat's skull. For the MPA a 6 mm hole was drilled in the parietal bone and the Dura mater was removed. Two stainless steel screws were drilled in the frontal and parietal bones to ensure better fixation of the monitoring device to the cerebral cortex. Another hole (3 mm) was drilled at the contralateral parietal bone for the connection of the fluid percussion injury device. All devices were then fixated on the rat's cortex using dental acrylic cement.

After the fixation of the monitoring device to the rat's scalp, a short session of anoxia was induced by exposing the rat to pure N_2 until respiratory arrest. This short anoxic test is routinely used in all of our experiments in order to make sure that the MPA is placed correctly on the cerebral cortex and that brain function is normal at the basal state. Our vast experience showed that this short anoxic episode has no negative effect on the cerebral tissue as well as on the whole-body physiological parameters such as blood pressure, respiratory and heart rate, body temperature etc.[27]

In the group of the non-anesthetized rats, the rats were placed in a restriction cage in which they were left to recover and to fully awaken for two hours (post anoxia), then after, TBI was induced. In the anesthetized rats two hours after anoxia, E-th was injected and the rats were anesthetized, then after TBI was induced. In the control group after the short anoxia monitoring proceeded for four hours with no other perturbation. At the end of the experiment, all rats were anesthetized and then sacrificed using pure N_2.

2.3. *Fluid percussion injury*

TBI was induced according to the model of fluid percussion injury[28–30] first applied by Sullivan (1976) using a special device (VCU Biomedical ENG. Facility, Richmond, Virginia, USA). We calibrated the device using nitrogen gas in such a manner that an angle of 15° yielded an injury of 1.15 ± 0.14 atm, $20° = 1.78 \pm 0.28$ atm, $25° = 2.13 \pm 0.34$ atm and $30° = 4.34 \pm 1.08$ atm.

In our experiments injuries at the different levels were induced as follows:

Mild TBI: a group of 9 rats were exposed to injury at strength of 1.15 ± 0.14 ATM, from an angle of 15°.

Moderate TBI: a group of 12 rats were exposed to injury at strength of 1.78 ± 0.28 ATM, from an angle of 20°.

Severe TBI: a group of 15 rats were exposed to injury at strength of 2.13 ± 0.34 ATM, from an angle of 25°.

Extra-severe TBI: a group of 10 rats were exposed to injury at strength of 4.34 ± 1.08 ATM, from an angle of 30°.

3. Statistical Analysis

Monitoring was performed at a rate of 1 sample per second, and each minute, 60 samples were averaged. The results were presented at intervals of 5 min for 4 h. Statistical analysis was performed using the SAS and SPSS software. For the evaluation of the significance of the changes in a certain parameter following TBI, the student, 2-tail, paired *t*-test was performed. The difference between anesthetized and non-anesthetized rats under a certain TBI level, was tested using unpaired t-test. For the evaluation of significant differences between the mortality rates of the various groups the Exact Fisher Test was used.

4. Results

The effects of severe TBI on the hemodynamic, metabolic and electrical activities of the brain in a typical experiment are presented in Fig. 2. As TBI was induced, ICP immediately increased to the level of 60 mmHg followed by a further increase to the level of 100 mmHg. Cerebral Blood Flow (CBF) completely decreased, and partly recovered (up to 40%) approximately 7 min post TBI. The decrease in CBF was associated with an increase of 10% in

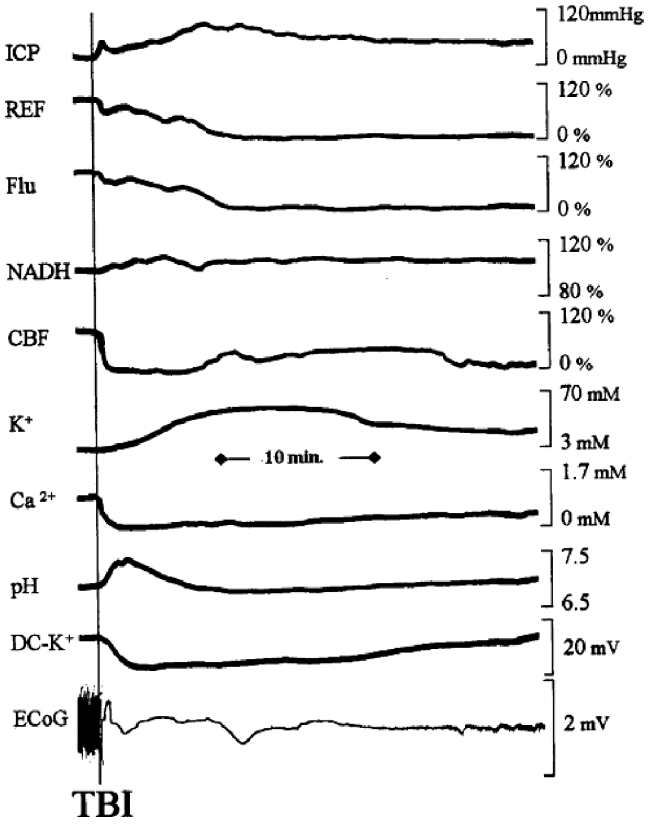


Fig. 2. Typical responses of one rat from the severe TBI group. The monitored parameters include: intracranial pressure (ICP), reflectance (REF), fluorescence (Flu), corrected NADH (NADH), cerebral blood flow (CBF), extracellular levels of K^+, H^+ and Ca^{2+}, DC-potential of the K^+ electrode and the cortical spontaneous electrical activity (ECoG).

mitochondrial NADH level, which partly recovered later on. The reflectance and fluorescence dramatically decreased by 100%. The extracellular level of K^+ significantly increased, reaching very high levels of approximately 50 mM, following with a decrease in the DC potential of K^+, which recovered within 20 min post injury. Extracellular pH level also increased immediately after TBI following with full recovery. The ECoG was significantly depressed, showing a gradual minor recovery 20 min post TBI.

In the non-anesthetized rats, the severe TBI group showed a mortality rate of 66% (6 of 9 rats). In all cases the rats died immediately after the induction of the injury and not during the additional four hours of monitoring hence the rats that died are not included in the results showed in Figs. 3–5. Administration of E-th significantly decreased the percentage of mortality in the severe TBI group to 20% ($p = 0.029$). However in the extra-severe TBI group, 70% (7 of 10 rats) of the rats died immediately

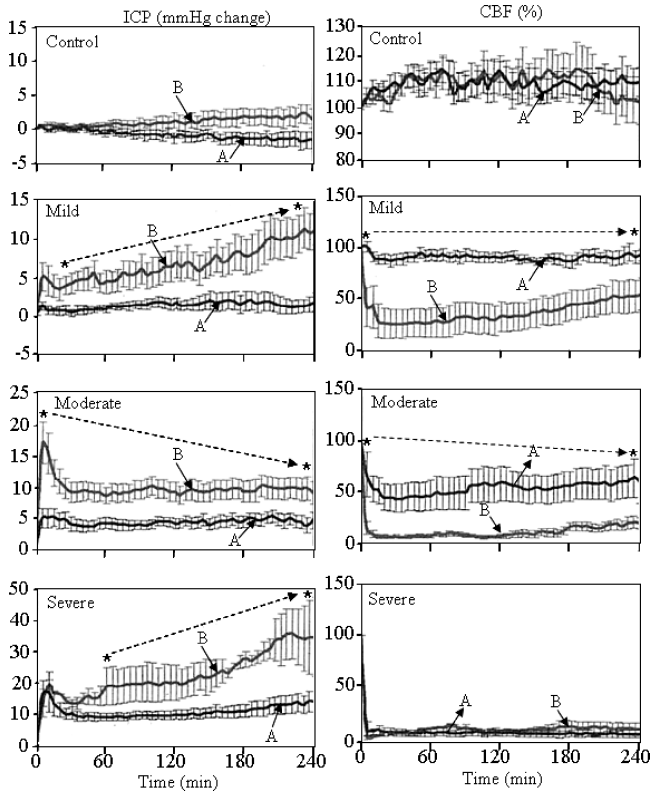


Fig. 3. The changes (mean ± S.E.) in the intracranial pressure (ICP), cerebral blood flow (CBF) following three levels of TBI (mild, moderate and severe) and in control rats, induced in anesthetized rats (A) and non-anesthetized rats (B). The dashed arrows identify periods in which a significant difference between the two groups A and B was found (*) $p < 0.05$.

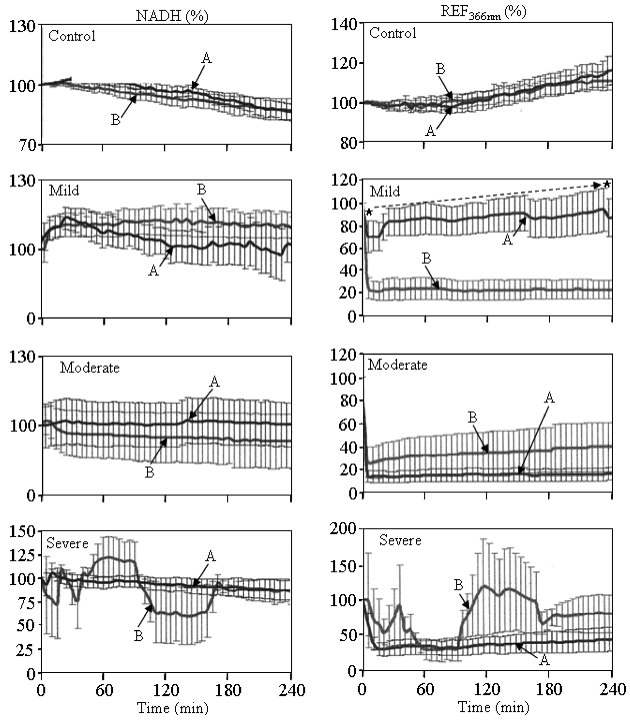


Fig. 4. The changes (mean ± S.E.) in the reflectance at 366 nm (REF_{366}) and mitochondrial function (NADH) following three levels of TBI (mild, moderate and severe) and in control rats, induced in anesthetized rats (A) and non-anesthetized rats (B). The dashed arrows identify periods in which a significant difference between the two groups A and B was found (*) $p < 0.05$.

after injury and when anesthesia was used 100% of the rats died within 15 min. Since these rats were monitored only for a very short duration (around 15 min), their results are not shown; however, it should be mentioned that this group was very similar to the severe group except for the responses of extracellular levels of K^+ and DC-potential of K^+, in which no recovery was observed, namely irreversible ischemic depolarization was developed, as opposed to full recovery of these parameters in the severe group, namely reversible ischemic depolarization was observed.

The effect of anesthesia on ICP is presented in Fig. 3. As seen in the control non-anesthetized rats, a trend of increase in ICP is seen, whereas in the anesthetized control rats ICP decreased by 3−4 mmHg 160 min after the injection of E-th.

In all non-anesthetized TBI groups a biphasic rapid response (increase/decrease) in ICP was seen following with a secondary gradual increase in ICP with time. However, at the anesthetized rats the secondary increase in ICP was prevented therefore ICP levels were significantly different during almost the entire experimental period (4 h) ($p < 0.05$ to $p < 0.01$). Moreover during the impact, the maximum increase in ICP in the moderate group was significantly higher ($p < 0.05$) in the non-anesthetized rats as compared to the anesthetized rats while in all other groups similar maximal levels of ICP were monitored. As for CBF anesthesia had no effect in the control group however while the immediate response of CBF to TBI was a significant decrease of 75%−95% in the mild and severe group respectively, under anesthesia CBF decreased only by 10% in the mild TBI, by 60% in the moderate group and by 95% in the severe group. Therefore there were no differences between CBF under anesthesia versus awakens in the severe group, whereas in both other TBI groups CBF levels were significantly different between both treatments ($p < 0.05$). The changes in NADH level (Fig. 4) showed no significant differences between the various levels of TBI as well as in the control groups.

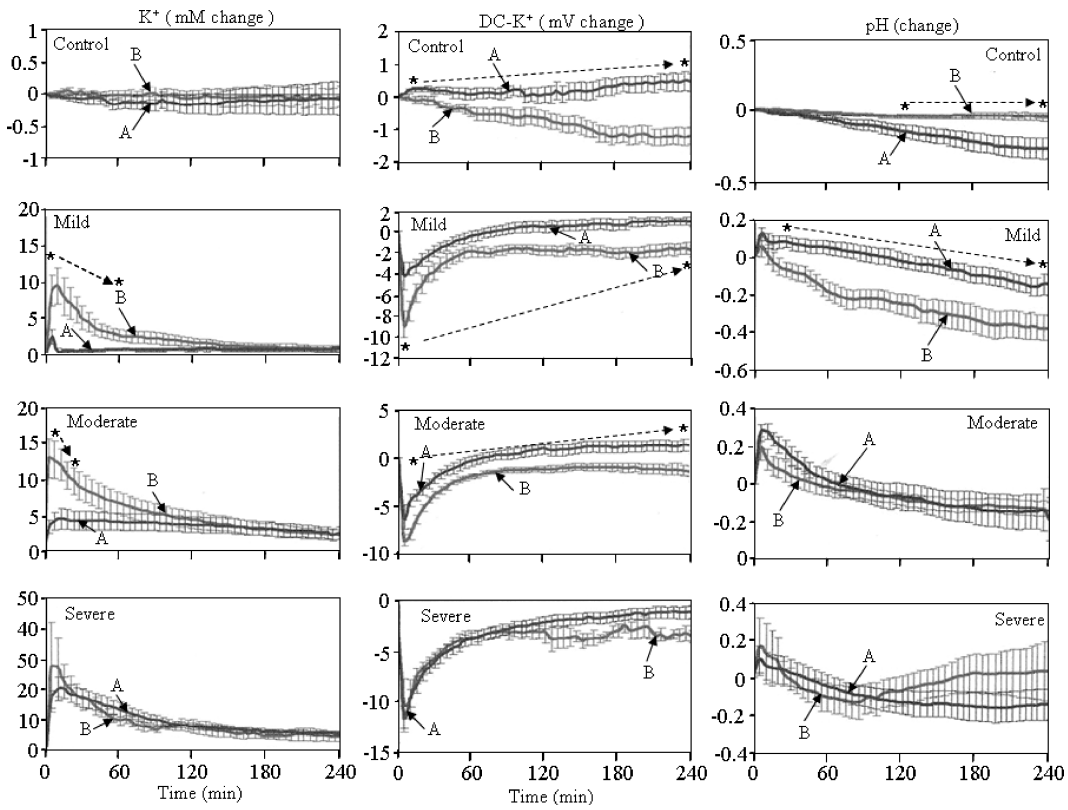


Fig. 5. The changes (mean ± S.E.) in the extracellular levels of K^+ and H^+ (pH) and the changes in DC-potential of K^+, following three levels of TBI (mild, moderate and severe) and in control rats, induced in anesthetized rats (A) and non-anesthetized rats (B). The dashed arrows identify periods in which a significant difference between the two groups A and B was found (*) $p < 0.05$.

As seen in the control group, NADH decreased with time by 13% in the anesthetized rats as well as in the non-anesthetized rats. Following mild TBI in the non-anesthetized rats NADH increased by 10% and remained so until the end of the experiment, while in the anesthetized rats NADH increased by 15% following with a decrease back to basal levels. At the moderate TBI group, there were no differences in NADH level between non-anesthetized and anesthetized rats. In the severe TBI model, anesthesia kept NADH stable while in the non-anesthetized rats NADH increased and decreased and the variability in the group was very high. This fact may be one of the reasons for lack of significant difference between the two treatments. As for the reflectance at 366 nm (Fig. 4), under control an increase of 15% was monitored in both experimental groups. When mild TBI was induced the reflectance significantly decreased in the non-anesthetized rats ($p < 0.05$) and remained low through the entire monitoring period. In the anesthetized rats a decrease of 30% in the reflectance was seen followed by an increase to the level of 90%. When these two groups were compared, a significant difference between them was seen ($p < 0.05$). However following severe TBI, both groups showed a significant initial decrease of 75%–87% in non-anesthetized and anesthetized rats, respectively; thereafter, the reflectance in the anesthetized rats remained low while in the non-anesthetized rats, reflectance was unstable with periods of increase and decrease. These changes lead to no significant differences between the two treatments at this level of TBI. While extracellular K^+ level remained stable at the control groups, following TBI reversible ischemic depolarization was seen, at all three levels of TBI ($p < 0.05$), namely K^+ level increased significantly followed by full recovery. However, in the non-anesthetized rats the pick value of extracellular K^+ was significantly higher than the increase seen at the anesthetized rats in the mild and moderate groups ($p < 0.05$). The increase in extracellular level of K^+ was associated with a negative DC shift in all experiments, although under anesthesia this decrease in DC-potential was smaller than the one seen in the non-anesthetized rats at the mild and moderate groups ($p < 0.05$ to $p < 0.01$).

Following severe injury DC potential was similar in non-anesthetized and anesthetized rats. The changes in extracellular pH level showed a significant decrease in the control anesthetized rats two hours post injury. In non-anesthetized mild TBI rats pH decreased to a greater extent than in the anesthetized mild TBI rats ($p < 0.05$ to $p < 0.01$). Following moderate injury, pH level was changed at the same way in both groups, only the peak of increase in pH was higher under anesthesia versus awakens ($p < 0.05$). At the severe group pH decreased in the anesthetized rats whereas in the non-anesthetized rats approximately 90 min post injury pH returned to its basal level. There were no differences between the non-anesthetized rats and anesthetized rats at this level of injury.

5. Discussion

In the present study we used the MPA device for real-time monitoring of the cerebral cortex following three levels of fluid percussion brain injury in anesthetized and non-anesthetized rats. The first part of this discussion will relate to our unique monitoring approach and the three levels of injury induced, whereas, the second part will discuss the involvement of anesthesia in this model.

5.1. *Tissue viability evaluated by the MPA following various levels of TBI*

The MPA probe was placed contralaterally to the injury site following four levels of TBI. It is important to clarify that although the level of injury induced in our severe group is sometimes related to as moderate injury in other studies,[31–34] our results clearly demonstrated significant differences between this group and the moderate group, therefore we decided to classify the four levels of injury as mild, moderate, severe and extra-severe. The differences that were detected in the hemodynamic, metabolic, ionic and electrical aspects indicated that the severe level of injury is stronger than the one induced in the moderate group and results with different conditions in the cerebral cortex that are not affected from anesthesia.

As for the location of the monitoring device, the MPA was placed contralaterally to the impact hemisphere, based on our preliminary results, which were recently published. This study showed that monitoring of the cerebral cortex using the MPA under moderate or severe head injury is practically feasible only in the contralateral hemisphere.[35] As indicated, the contralateral site of monitoring has the advantage of being very close to the injury site and hence clearly demonstrate the hemodynamic and metabolic changes following the injury. These changes include a decrease in CBF,[32,36] increase of NADH redox state level, disruption of ionic homeostasis and decrease of DC potential. However, this location is less affected from the direct injury that induces cell rupture, hemorrhage insults and edema,[37] which are changes that deeply affect the absorption character of the tissue, disabling the use of optical monitoring technique such as the fluorometry. The location that is close to the impact site yet is not the exact impact site can be addressed to as the "penumbra of the impact site," as also widely used in the model of focal brain ischemia, which is another model for brain injury,[38] or at the model of brain retraction which is also involved with focal ischemia and include the ischemic core, at the retractor position and the ischemic penumbra at the circumference of this position.[39] In both these models for brain injury, the evaluation of the cerebral damage is mostly performed in the penumbra zone, especially when a therapeutic perspective is being tested.

The extent of physiological changes monitored by the MPA in the cerebral tissue of both anesthetized and non-anesthetized rats was correlated with the mortality rate of rats. These results confirm previous studies[28,40,41] that also showed a decrease in survival rate as injury was more severe. The high mortality rate following severe and extra-severe TBI probably resulted from the fact that in such cases the impact is so strong, also reaching the brain stem, which is responsible for the respiratory and cardiovascular functions, as was previously indicated.[42] In rats that survived the TBI, the increase in ICP following TBI as well as the level of CBF decrease were proportional to the level of TBI applied, as previously presented in other studies as well.[18,36,40,41]

Since the skull is close and not flexible, following TBI, ICP increases gradually due to the development of hemorrhage insults and edema.[18,43] Our results showed also such an increase in the severe non-anesthetized TBI group. In addition, CBF remained low and extracellular K^+ level increased, ECoG was depressed and in many cases ischemic

depolarization was developed as previously reported in another study.[40] Whereas, in the mild non-anesthetized TBI group, ICP increased with time; however, this increase was associated with an increase in CBF, and a decrease of the reflectance (implying for increased cerebral blood volume — CBV). In this case the increase of ICP, previously reported,[44] evokes due to an increase in the metabolic demands of the injured tissue. When autoregulation mechanisms are intact and activated, vasoconstriction of small arteries will occur and CBV and ICP will decrease[45,46] whereas, when autoregulation is damaged there will be no improvement in the cerebral functional state.

Although CBF reached very low levels in the moderate and severe injuries of non-anesthetized rats, the extracellular levels of K^+ and the DC potential were fully recovered (reversible ischemic depolarization). This indicates that there is still enough energy in the tissue for ionic homeostasis even though CBF is low. Whereas in the extra-severe group irreversible ischemic depolarization was seen namely, the extracellular level of K^+ increased and remained high until the rat died. These results point out for the importance of monitoring the extracellular level of K^+ which gives indication for the energetic state of the tissue (since the activity of the Na^+/K^+ATPase's which are responsible for the maintenance of ionic homeostasis, is directly dependent on ATP level), minimizing the risk for misinterpretation of tissue state using laser Doppler flowmetry alone.

Following TBI oxygen supply to the brain is often insufficient, leading to a decrease in energy production.[47] Previous studies showed that the mitochondria, which are the main generator for ATP, are damaged during TBI due to lack of oxygen, resulting with mitochondrial dysfunction.[21,47–49] In our study, there was a problem in the evaluation of mitochondrial function via the analysis of NADH level. The main reason rises from the character of the injury which induces hemorrhage and edematous insults in the cerebral tissue and leakage of ions from the intracellular space to the extracellular space yielding significant changes in the optical character of the tissue. Our vast experience in real-time monitoring of the mitochondrial NADH using the fluorometric technique showed that the accumulation of blood beneath the monitoring probe increases tissue absorption, thus reflectance decreases, as observed in the current study too. Edema induces an increase in tissue reflectance and so does the leakage of ions and water movement between the intracellular and extracellular spaces. The overall effects of all of these changes yields unstable levels of tissue reflectance and also affect tissue fluorescence, thus inducing artifacts in the monitoring of mitochondrial NADH level. In addition, it could be that the impact induced a slight movement of the monitoring probe on the surface of the cerebral cortex in such a manner that the contact between the MPA and the tissue was affected. Since the fluorometric technique demands good contact with the monitored tissue such a movement automatically induce artifacts in the monitoring of the fluorometric parameters, namely the reflectance, fluorescence and thus corrected NADH.[50]

5.2. *The involvement of Equithesin in the fluid percussion injury model*

Analgesia and sedation are an essential part of the therapy in patients with severe head injury.[16,19,51] Pentobarbital is a barbiturate that is commonly used as an adjunct therapy in patients suffering from intracranial hypertension after severe TBI.[13] It is also used in experimental studies where it was found to reduce infract size after cerebral ischemia,[52,53] reduce CBF[54] as well as stabilize its level.[55] However, in the present study, pentobarbital alone was not used since this anesthetic is known to deeply affect cerebral metabolism[56,57] and depress cardiac and respiratory functions.[58] These effects would have forced the usage of artificial ventilation, complicating the experimental protocol, which was already very complicated due to its real-time, long-lasting monitoring format. Instead we used Equithesin, which is a mixture of pentobarbital with other anesthetics, which enables the use of lower doses of pentobarbital hence decreases the risk for complications rising from high doses of pentobarbital. As for the effects of chloral hydrate, which is also an anesthetic, here we had no problem of negative effects of high doses since the toxicokinetic model of chloral hydrate in rats is involved with oral administration of 1−12 doses of 50−200 mg/kg body weight whereas, in our model the dosage of chloral hydrate that the rats were injected to (within the Equithesine mixture) was approximately 1.4 mg/kg, which is a very low dosage.[59]

In the present study, the protective effect of anesthesia on the parameters monitored by the MPA was seen only at the mild and moderate TBI groups where the impact caused a smaller increase in ICP of the anesthetized rats as opposed to the increase in ICP at the non-anesthetized rats. Moreover, in the anesthetized rats, ICP remained significantly low for the entire experimental period while CBF remained significantly high as compared to the non-anesthetized rats. However in the severe group anesthesia did not improve CBF. As for the extracellular level of K^+, a trend of higher levels of K^+ is seen in the non-anesthetized rats (during the first 15 min post TBI). Although this level was not significantly different from the level monitored in the anesthetized rats, one must take under account that in the severe group the results presented are the mean levels of only three rats that survived the injury, thus the standard errors are huge, minimizing the difference between the two groups. To summarize, anesthesia clearly improved ICP level which decreased immediately after the impact and stayed relatively low as compared to the non-anesthetized rats and tended to decrease the peak levels of extracellular K^+. In this view it seems that by monitoring these two parameters good indication for the tissue state can be achieved and that the monitoring of only CBF by laser Doppler flowmetry is not enough, as previously indicated. Moreover, if the injury is extremely severe, yielding irreversible ischemic depolarization, as seen in the extra-severe TBI, anesthesia will have no protective effect even though ICP may recover. Whereas, if TBI induces reversible ischemic depolarization, which is characterized by full recovery of extracellular level of K^+ and DC-potential, than anesthesia can improve the final outcome, namely the survival rate.

A similar approach was also suggested in a new clinical study in which TBI patient were infused with pentobarbital. Their results showed an increase in tissue oxygen concentration, following pentobarbital infusion only in patients with moderate levels of ICP and who do not suffer from very low levels of CPP, whereas in patients with more compromised brain physiology, pentobarbital induced negative effect on brain tissue oxygen.[60]

In conclusion, our results demonstrate the importance of monitoring not only CBF and ICP but also extracellular K^+ level and DC-potential for better evaluation of the cerebral function following TBI.

As for the protective effects of anesthesia on the cerebral hemodynamic and metabolic parameters, following TBI, our study showed that this effect exists only under mild and moderate TBI, whereas the effects of anesthesia on the final outcome seem to depend on the reversibility of the ischemic depolarization in the tissue, in such a manner that if ischemic depolarization is irreversible, anesthesia will not help and the rat will probably die.

Acknowledgments

This work was supported by the Mina and Everard Goodman Faculty of Life Sciences, and the Leslie and Susan Gonda Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan, Israel.

References

1. Y. Shen, Z. Kou, C. W. Kreipke, T. Petrov, J. Hu, E. M. Haacke, "*In vivo* measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility weighted imaging," *Magn. Reson. Imaging* **25**, 219–227 (2007).
2. H. I. Chen, N. R. Malhotra, M. Oddo, G. G. Heuer, J. M. Levine, P. D. LeRoux, "Barbiturate infusion for intractable intracranial hypertension and its effect on brain oxygenation," *Neurosurgery* **63**, 880–886 (2008).
3. A. Gremmelt, U. Braun, "Analgesia and sedation in patients with head-brain trauma," *Anaesthesist* **44**, S559–S565 (1995).
4. A. Zauner, T. Clausen, O. L. Alves, A. Rice, J. Levasseur, H. F. Young, R. Bullock, "Cerebral metabolism after fluid-percussion injury and hypoxia in a feline model," *J. Neurosurg.* **97**, 643–649 (2002).
5. M. Czosnyka, P. J. Hutchinson, M. Balestreri, M. Hiler, P. Smielewski, J. D. Pickard, "Monitoring and interpretation of intracranial pressure after head injury," *Acta Neurochir. Suppl.* **96**, 114–118 (2006).
6. W. Mauritz, I. Janciak, I. Wilbacher, M. Rusnak, "Severe traumatic brain injury in Austria IV: Intensive care management," *Wien. Klin. Wochenschr.* **119**, 46–55 (2007).
7. B. Schaller, "Craniocerebral trauma — new pathophysiological and therapeutic viewpoints. 1: Management of the acute phase," *Swiss. Surg.* **8**, 123–137 (2002).
8. R. M. Chesnut, L. F. Marshall, "Management of head injury. Treatment of abnormal intracranial pressure," *Neurosurg. Clin. N. Am.* **2**, 267–284 (1991).

9. M. J. Kanter, R. K. Narayan, "Management of head injury. Intracranial pressure monitoring," *Neurosurg. Clin. N. Am.* **2**, 257−265 (1991).
10. N. Lundberg, "Continuous recording and control of ventricular fluid pressure in neurosurgical practice," *Acta Psychiatr. Scand. Suppl.* **36**, 1−193 (1960).
11. L. A. Steiner, P. J. Andrews, "Monitoring the injured brain: ICP and CBF," *Br. J. Anaesth.* **97**, 26−38 (2006).
12. G. Citerio, I. Piper, M. Cormio, D. Galli, S. Cazzaniga, P. Enblad, P. Nilsson, C. Contant, I. Chambers, "Bench test assessment of the new Raumedic Neurovent-P ICP sensor: A technical report by the BrainIT group," *Acta Neurochir.* (*Wien.*) **146**, 1221−1226 (2004).
13. "The Brain Trauma Foundation. The American Association of Neurological Surgeons. The Joint Section on Neurotrauma and Critical Care. Computed tomography scan features," *J. Neurotrauma* **17**, 597−627 (2000).
14. R. M. Chesnut, L. F. Marshall, S. B. Marshall, "Medical management of intracranial pressure," in *Head Injury*, P. R. Cooper, Ed. (London, Williams & Wilkins, 1993), pp. 225−246.
15. I. Roberts, "Barbiturates for acute traumatic brain injury," *Cochrane Database Syst. Rev.* CD000033 (2000).
16. K. D. Statler, H. Alexander, V. Vagni, C. E. Dixon, R. S. Clark, L. Jenkins, P. M. Kochanek, "Comparison of seven anesthetic agents on outcome after experimental traumatic brain injury in adult, male rats," *J. Neurotrauma* **23**, 97−108 (2006).
17. A. Gremmelt, U. Braun, "Analgesia and sedation in patients with head-brain trauma," *Anaesthesist* **44**, S559−S565 (1995).
18. E. G. Pfenninger, A. Reith, D. Breitig, A. Grunert, F. W. Ahnefeld, "Early changes of intracranial pressure, perfusion pressure, and blood flow after acute head injury. Part 1: An experimental study of the underlying pathophysiology," *J. Neurosurg.* **70**, 774−779 (1989).
19. E. Bedell, D. S. Prough, "Anesthetic management of traumatic brain injury," *Anesthesiol. Clin. North Am.* **20**, 417−439 (2002).
20. F. S. Kahveci, N. Kahveci, T. Alkan, B. Goren, E. Korfali, K. Ozluk, "Propofol versus isoflurane anesthesia under hypothermic conditions: Effects on intracranial pressure and local cerebral blood flow after diffuse traumatic brain injury in the rat," *Surg. Neurol.* **56**, 206−214 (2001).
21. A. Zauner, W. P. Daugherty, M. R. Bullock, D. S. Warner, "Brain oxygenation and energy metabolism. Part I: Biological function and pathophysiology," *Neurosurg.* **51**, 289−301 (2002).
22. A. Mayevsky, "Biochemical and physiological activities of the brain as *in vivo* markers of brain pathology," in *Cerebral, Revascularization*, E. F. Bernstein, A. D. Callow, A. N. Nicolaides, E. G. Shifrin, Eds. (Med-Orion Pub., 1993), pp. 51−69.
23. A. Mayevsky, "Brain NADH redox state monitored *in vivo* by fiber optic surface fluorometry," *Brain Res. Rev.* **319**, 49−68 (1984).
24. D. Arvidsson, H. Svensson, U. Haglund, "Laser Doppler flowmetry for estimating liver blood flow," *Am. J. Physiol.* **254**, G471−G476 (1988).
25. R. L. Haberl, M. L. Heizer, A. Marmarou, E. F. Ellis, "Laser Doppler assessment of brain microcirculation: Effect of systemic alterations," *Am. J. Physiol.* **256**, H1247−H1254 (1989).
26. U. Dirnagl, B. Kaplan, M. Jacewicz, W. Pulsinelli, "Continuous measurement of cerebral cortical blood flow by laser Doppler flowmetry with a rat stroke model," *J. Cereb. Blood Flow Metab.* **9**, 589−596 (1989).
27. A. Mayevsky, G. G. Rogatsky, "Mitochondrial function *in vivo* evaluated by NADH fluorescence: From animal models to human studies," *Am. J. Physiol. Cell Physiol.* **292**, C615−C640 (2007).
28. C. E. Dixon, B. G. Lyeth, J. T. Povlishock, R. L. Findling, R. J. Hamm, A. Marmarou, H. F. Young, R. L. Hayes, "A fluid percussion model of experimental brain injury in the rat: Neurological, physiological and histological characterizations," *J. Neurosurg.* **67**, 110−119 (1987).
29. H. G. Sullivan, J. Martinez, D. P. Becker, J. D. Miller, R. Griffith, A. O. Wist, "Fluid-percussion model of mechanical brain injury in the cat," *J. Neurosurg.* **45**, 521−534 (1976).
30. C. E. Dixon, R. L. Hayes, "Fluid percussion and cortical impact models of brain injury," in *Neurotrauma*, R. K. Narayan, J. E. Wilberger, J. T. Povlishock, Eds. (McGraw-Hill, New York, 1996), pp. 1337−1356.
31. F. Y. Sun, A. I. Faden, "N-methyl-D-aspartate receptors mediate post-traumatic increases of protein kinase C in rat brain," *Brain Res.* **661**, 63−69 (1994).
32. M. J. Ahn, E. R. Sherwood, D. S. Prough, C. Y. Lin, D. S. DeWitt, "The effects of traumatic brain injury on cerebral blood flow and brain tissue nitric oxide levels and cytokine expression," *J. Neurotrauma* **21**, 1431−1442 (2004).
33. D. S. Prough, G. C. Kramer, T. Uchida, R. T. Stephenson, H. L. Hellmich, D. S. Dewitt, "Effects of hypertonic arginine on cerebral blood flow and intracranial pressure after traumatic brain injury combined with hemorrhagic hypotension," *Shock* **26**, 290−295 (2006).

34. N. Marklund, S. Sihver, B. Langstrom, M. Bergstrom, L. Hillered, "Effect of traumatic brain injury and nitrone radical scavengers on relative changes in regional cerebral blood flow and glucose uptake in rats," *J. Neurotrauma* **19**, 1139−1153 (2002).
35. T. Manor, E. Barbiro-Michaely, G. Rogatsky, A. Mayevsky, "Real-time multi-site multi-parametric monitoring of rat brain subjected to traumatic brain injury," *Neurol. Res.* **30**, 1075−1083 (2008).
36. J. K. Muir, M. Boerschel, E. F. Ellis, "Continuous monitoring of post-traumatic cerebral blood flow using laser-Doppler flowmetry," *J. Neurotrauma* **9**, 355−362 (1992).
37. H. J. Thompson, J. Lifshitz, N. Marklund, M. S. Grady, D. I. Graham, D. A. Hovda, T. K. McIntosh, "Lateral fluid percussion brain injury: A 15-year review and evaluation," *J. Neurotrauma* **22**, 42−75 (2005).
38. P. Lipton, "Ischemic cell death in brain neurons," *Physiol. Rev.* **79**, 1431−1568 (1999).
39. E. Barbiro-Michaely, G. Bachbut, A. Mayevsky, "Effects of compression injury on brain mitochondrial and tissue viability evaluated by multiparametric monitoring system," *Proc. SPIE* **6848**, 68480M-1−68480M-8 (2008).
40. M. L. Prins, S. M. Lee, C. L. Cheng, D. P. Becker, D. A. Hovda, "Fluid percussion brain injury in the developing and adult rat: A comparative study of mortality, morphology, intracranial pressure and mean arterial blood pressure," *Brain Res. Dev. Brain Res.* **95**, 272−228 (1996).
41. I. Yamakami, T. K. McIntosh, "Effects of traumatic brain injury on regional cerebral blood flow in rats as measured with radiolabeled microspheres," *J. Cereb. Blood Flow Metab.* **9**, 117−124 (1989).
42. L. E. Thibault, D. F. Meaney, B. J. Anderson, A. Marmarou, "Biomechanical aspects of a fluid percussion model of brain injury," *J. Neurotrauma* **9**, 311−322 (1992).
43. G. Rogatsky, A. Mayevsky, N. Zarchin, A. Doron, "Continuous multiparametric monitoring of brain activities following fluid-percussion injury in rats: Preliminary results," *J. Basic Clin. Physiol. Pharmacol.* **7**, 23−43 (1996).
44. D. F. Kelly, R. K. Kordestani, N. A. Martin, T. Nguyen, D. A. Hovda, M. Bergsneider, D. L. McArthur, D. P. Becker, "Hyperemia following traumatic brain injury: Relationship to intracranial hypertension and outcome," *J. Neurosurg.* **85**, 762−771 (1996).
45. M. J. Rosner, S. D. Rosner, A. H. Johnson, "Cerebral perfusion pressure: Management protocol and clinical results," *J. Neurosurg.* **83**, 949−962 (1995).
46. M. J. Rosner, S. Daughton, "Cerebral perfusion pressure management in head injury," *J. Trauma* **30**, 933−940 (1990).
47. B. H. Verweij, J. P. Muizelaar, F. C. Vinas, P. L. Peterson, Y. Xiong, C. P. Lee, "Impaired cerebral mitochondrial function after traumatic brain injury in humans," *J. Neurosurg.* **93**, 815−820 (2000).
48. R. Vagnozzi, A. Marmarou, B. Tavazzi, S. Signoretti, P. D. Di, B. F. del, A. M. Amorini, G. Fazzina, S. Sherkat, G. Lazzarino, "Changes of cerebral energy metabolism and lipid peroxidation in rats leading to mitochondrial dysfunction after diffuse brain injury," *J. Neurotrauma* **16**, 903−913 (1999).
49. X. B. Jiang, K. Ohno, L. Qian, B. Tominaga, T. Kuroiwa, T. Nariai, K. Hirakawa, "Changes in local cerebral blood flow, glucose utilization, and mitochondrial function following traumatic brain injury in rats," *Neurol. Med. Chir.* (*Tokyo*) **40**, 16−28 (2000).
50. A. Mayevsky, G. G. Rogatsky, "Mitochondrial function *in vivo* evaluated by NADH fluorescence: From animal models to human studies," *Am. J. Physiol. Cell Physiol.* **292**, C615−C640 (2007).
51. B. J. McGrath, M. J. Matjasko, "Anesthesia and head trauma," *New Horiz.* **3**, 523−533 (1995).
52. D. S. Warner, S. Takaoka, B. Wu, P. S. Ludwig, R. D. Pearlstein, A. D. Brinkhous, F. Dexter, "Electroencephalographic burst suppression is not required to elicit maximal neuroprotection from pentobarbital in a rat model of focal cerebral ischemia," *Anesthesiology* **84**, 1475−1484 (1996).
53. G. K. Shiu, E. M. Nemoto, J. Nemmer, "Dose of thiopental, pentobarbital, and phenytoin for maximal therapeutic effects in cerebral ischemic anoxia," *Crit. Care Med.* **11**, 452−459 (1983).
54. M. M. Todd, J. Weeks, "Comparative effects of propofol, pentobarbital, and isoflurane on cerebral blood flow and blood volume," *J. Neurosurg. Anesthesiol.* **8**, 296−303 (1996).
55. K. S. Hendrich, P. M. Kochanek, J. A. Melick, J. K. Schiding, K. D. Statler, D. S. Williams, D. W. Marion, C. Ho, "Cerebral perfusion during anesthesia with fentanyl, isoflurane, or pentobarbital in normal rats studied by arterial spin-labeled MRI," *Magn. Reson. Med.* **46**, 202−206 (2001).
56. J. E. Bazin, "Effects of anesthetic agents on intracranial pressure," *Ann. Fr. Anesth. Reanim.* **16**, 445−452 (1997).
57. A. Gremmelt, U. Braun, "Analgesia and sedation in patients with head-brain trauma," *Anaesthesist* **44**, S559−S565 (1995).

58. K. H. Reid, M. Paskitti, S. Z. Guo, T. Schmelzer, V. Iyer, "Experience with ketamine and sodium pentobarbital as anesthetics in a rat model of cardiac arrest and resuscitation," *Resuscitation* **57**, 201−210 (2003).
59. F. A. Beland, T. C. Schmitt, N. F. Fullerton, J. F. Young, "Metabolism of chloral hydrate in mice and rats after single and multiple doses," *J. Toxicol. Environ. Health A* **54**, 209−226 (1998).
60. H. I. Chen, N. R. Malhotra, M. Oddo, G. G. Heuer, J. M. Levine, P. D. LeRoux, "Barbiturate infusion for intractable intracranial hypertension and its effect on brain oxygenation," *Neurosurg.* **63**, 880−886 (2008).