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Paradoxical Increase in the Bispectral Index during Deep Anesthesia in New Zealand White Rabbits

Alexander Romanov,[1,*] Rajinder-Singh Moon,[2] Mei Wang,[2] and Shailendra Joshi[2]

Objective monitoring of the level of anesthesia is crucial in carefully controlled translational neuroscience studies. The usefulness of bispectral index (BIS) in monitoring human anesthesia is well established. However, the validity of its application remains unproven in laboratory animals. We assessed whether BIS could be used reliably in monitoring the depth of deep anesthesia in 8 New Zealand white rabbits. Experimental baseline anesthesia was maintained with continuous infusion of propofol and administration of isoflurane, both of which were titrated to EEG activity. The rabbits were allocated randomly to receive 3 increasing concentrations of common anesthetic drugs (etomidate, propofol, and isoflurane) aimed to produce burst suppression of EEG activity yielding at least 10 s of sustained EEG silence. Rabbits had a 20-min recovery interval between challenges. Transient cerebral hypoperfusion to produce reversible EEG silence due to ischemia was induced as a fourth challenge, followed by terminal arrest, in each animal. BIS, EEG, and physiologic data were analyzed for each rabbit. We observed stable BIS values in the range of 40 to 60 during the administration of baseline anesthesia. However, as the depth of anesthesia deepened with the anesthetic drug challenges, the BIS value paradoxically increased with increasing doses. The BIS signal quality index declined while the total power decreased. In contrast to these unexpected results, BIS values decreased rapidly to near 0 during terminal arrest, as expected. Therefore, we do not consider BIS to be a useful method for monitoring deep levels of anesthesia in laboratory rabbits.

Abbreviation: BIS, bispectral index.

Precise monitoring of anesthesia can be challenging in both human and veterinary medicine. Depth of anesthesia may affect outcome of experimental results directly or indirectly through hemodynamic effects on a body. The usefulness of the bispectral index (BIS) in monitoring anesthesia in human patients has been well established.[5,6,19] BIS is an algorithm derived from a complex statistical analysis of the EEG to monitor and record the electrical activity of the brain. The BIS variable is dimensionless and ranges from 0, which indicates complete cortical silence, to 100 (that is, the subject is awake and alert), reflecting the levels of the subject's loss of sensation during anesthesia. During human surgery, a BIS value of 40 to 60 correlates with a surgical plane of anesthesia.[3,21,23] In addition, BIS monitoring has been used with various degrees of success in diverse animal species, both in laboratory research and veterinary practice (Table 1). No single parameter is an effective measure of all aspects of anesthesia, including analgesia, amnesia, hypnosis or narcosis, suppression of reflexes, and immobility. Furthermore, not all anesthetic drugs or drug combinations affect all of these components of anesthesia equally.[13] Our current interest in using BIS for rabbit experiments arose because of the inconsistent disruption of the blood–brain barrier that we encountered with intracarotid injections of mannitol that were critical to our drug-delivery protocols in previous experiments.[12] Because anesthetic agents can affect the permeability of the blood–brain barrier to mannitol, we hypothesized that depth of anesthesia might have a similar effect.[9,20] If this effect does occur, then standardizing the depth of anesthesia by using BIS values could help us achieve more consistent results in our experiments. The aim of the current study was to assess the usefulness and reliability of BIS monitoring during increasing depth of anesthesia in rabbits.

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[1]Department of Institute of Comparative Medicine and [2]Department of Anesthesiology, Columbia University Medical Center, New York, New York.
[*]Corresponding author. Email: ar2772@columbia.edu

Materials and Methods

All procedures in this study were performed under an approved IACUC protocol in a USDA-inspected and AAALAC-accredited animal research facility (Columbia University). The rabbits were obtained from an approved commercial vendor (Harlan Laboratories, Oxford, MI) and were negative for 26 common rabbit viral, bacterial, fungal, and parasitic pathogens, according to the health monitoring program established at the vendor's animal facility. Each rabbit underwent physical examination prior to shipment and on receipt at our animal facility and was found to be healthy. During the acclimation period (2 d or more after the delivery date), rabbits were group-and pair-housed in standard (60 × 30 × 17 in.) stainless steel rabbit cages (Allentown Caging, Allentown, NJ) and provided free access to commercial feed (LabDiet 5326 pellets, PMI Nutrition International, Brentwood, MO) and water. Daily environmental enrichment was provided to each rabbit in the form of elevated cage shelves, food treats, toys, and human interaction with husbandry and veterinary technicians.

Anesthetic and surgical preparations. Female juvenile (n = 8; age, 8 to 9 wk; mean weight, 1.6 kg) New Zealand white rabbits were enrolled in this experiment. Animals were anesthetized initially with intramuscular ketamine (50 mg/kg; Ketathesia, Abbott Laboratories, North Chicago, IL) supplemented with intradermal bupivacaine (0.5 to 1.0 mL per site; Bupivacaine

Table 1. Outcomes of BIS studies across animal species

Publication	Reference no.	Animal	Device	Lead placement	Anesthesia or drug	Outcome
Morgaz and colleagues, 2009	18	Dog	BIS EEG monitor(model A-2000,Aspect Medical Systems)	2 cm lateral and1 cm caudal over right temple	Sevoflurane	BIS could be used to estimate anesthetic depth in dogs (puppies)
Martin-Jurado and colleagues, 2008	16	Chicken	BIS EEG monitor (model A-2000, Aspect Medical Systems)	Not described	Isoflurane	BIS could be used to monitor the degree of unconsciousness in chickens during isoflurane anesthesia
Antognini and colleagues, 2000	1	Goat	BIS monitor (model A-1050, Aspect Medical Systems)	Platinum needles (3-4 cm lateral to midline; 3-4 cm caudal to the eyes)	Isoflurane	BIS could be used as a monitoring parameter
Howard and colleagues, 2006	11	Dolphin	2 BIS EEG monitors (Quatro A-2000, Aspect Medical Systems)	Vertically on the head, above and behind the eyes	Propofol–atropine–diazepam	Different BIS values were found in each side of the dolphin brain
Haga and colleagues, 1999	10	Pig	BIS EEG monitor (model A-1050TM, Aspect Medical Systems)	1 cm caudal to the lateral angle of the eye and 1 cm medial to the temporal line	Isoflurane	BIS value did not accurately reflect depth of isoflurane anesthesia at surgical levels in pigs
Martın-Cancho and colleagues, 2006	15	Rabbit	BIS EEG monitor (model A-1050TM, Aspect Medical Systems)	1 cm caudal to the lateral angle of the eye, on midline frontal bone 3 cm from each electrode	Propofol–sevoflurane	BIS value was not useful for predicting speed of recovery from anesthesia in rabbits
Masamune and colleagues, 2009	17	Rabbit	BIS monitor (model A-2000, Aspect Medical Systems)	As for reference 13	JM-1232(–)	BIS had no clinical or statistical significance in JM-1232(–)– infused rabbits
Belda and colleagues, 2010	4	Horse	BIS EEG monitor (Quatro, Aspect Medical Systems)	8–10 cm caudal to lateral angle of the eye; electrodes laterally away from midline	Halothane–sevoflurane	BIS did not predict intraoperative movement in anesthetized horses
Schmidt and colleagues, 2001	22	Pig	BIS EEG monitor (Aspect Medical Systems)	Not described	Xenon gas–pentobarbital-buprenorphine-pancuronium	BIS value was found to be inaccurate during recovery from xenon anesthesia

0.25%, Hospira, Lake Forrest, IL) along incision sites and intravenous bolus injections of propofol (3 to 5 mg/kg; Propofol 1%, APP Pharmaceuticals, Schaumburg, IL,) into the left marginal ear vein as needed to achieve a surgical plane of anesthesia. Surgical preparation consisted of cannulation of the left marginal ear vein for injections and of the left femoral artery for direct blood pressure measurements (20 gauge, 1.88 in., BD Insyte Autoguard IV catheter, Becton Dickenson, Franklin Lakes, NJ), tracheostomy for mechanical ventilation, placement of silastic loops around the carotid arteries for occlusion, skull exposure through a dorsal midline incision for the placement of laser Doppler leads (1.5-m fiber-optic laser Doppler lead wire with 6 mm stainless steel tip for plastic adapter) over the frontal cortex area, and placement of stainless steel skull screws (2 mm threaded length; diameter, 1 mm) to secure EEG and BIS leads. In addition, a 6-mm burr hole craniotomy was performed on the right side of the skull just lateral to midline to enable subdural placement of reduced NADH fluorescent probe in the parietal region of the cortex to assess tissue ischemia (Figure 1). The rabbit's head was placed in a custom stereotaxic apparatus for craniotomy and electrode placement.

The key to our experimental protocol was to ensure sufficient depth of surgical anesthesia prior to anesthetic challenges. During the recording phase, baseline anesthesia was maintained by using an infusion pump (Colleague CX, Baxter Healthcare, Deerfield, IL) to provide continuous intravenous infusion of propofol (20 mg/kg/h) and by vaporizer (Vapor 19.1 vaporizer, Draeger Medical, Telford, PA) to provide 0.5% end-tidal isoflurane and 100% oxygen. The depth of anesthesia was monitored by pinching a hindpaw every 5 min to ensure a lack of response to surgical stimuli. In our experience, this level of anesthesia corresponds to a mixed EEG pattern of high amplitude–low frequency and low amplitude–high frequency activity on the density spectral array display region of the BIS monitor screen. Rabbits were mechanically ventilated continuously by using a small-animal ventilator (Harvard Apparatus, Holliston, MA) at a tidal volume of 10 mL/kg and a rate of 45 to 55 breaths per minute to maintain end-tidal CO_2 at 35 to 40 mm Hg.

Experimental protocol. Our protocol incorporated 4 challenges prior to terminal cardiac arrest. The 3 anesthetic drugs (propofol, etomidate [Etomidate 2 mg/mL solution, Bedford Laboratories, Bedford, OH], and isoflurane [Forane, Baxter Healthcare]) were randomized by using a random sequence generator. The fourth challenge, transient cerebral hypoperfusion, was followed by terminal arrest. We had determined in our preliminary studies that intravenous anesthetic drugs had to be injected as small boluses within 20 s to have a cumulative effect. Given the level of baseline anesthesia, with incremental dosing, the total intravenous dose of propofol required to produce a sustained EEG silence of at least 10 s in duration was 25 to 35 mg; etomidate required 3 to 4 mg. Propofol was injected in 5-mg boluses, whereas etomidate was injected in 0.4-mg boluses. Isoflurane concentrations were increased in 0.5% intervals. We allowed stabilization of end-tidal isoflurane concentrations for 30 s at each interval. Each challenge lasted approximately 5 min for etomidate and propofol but longer (5 to 10 min) for isoflurane, due to the prolonged time required to stabilize end-tidal concentrations. There was a 20-min recovery interval between challenges. In addition to measuring BIS scores during pharmacologically induced EEG silence, we wanted to investigate whether EEG silence induced by transient cerebral hypoperfusion affected BIS in a manner similar to terminal cardiac arrest after administration of intravenous KCl. Therefore, prior to euthanasia with intravenous KCl (8 mEq; KCl 2 mEq/mL solution, Hospira), we induced cerebral hypoperfusion by occluding the carotid arteries and inducing hypotension with single bolus injections of esmolol (15 to 30 mg; Esmolol 10 mg/mL solution, APP Pharmaceuticals) and adenosine (15 to 30 mg; Adenosine 3 mg/mL solution, Bedford laboratories) to decrease mean arterial blood pressure to 10 to 20 mm Hg for 1 to 2 min.

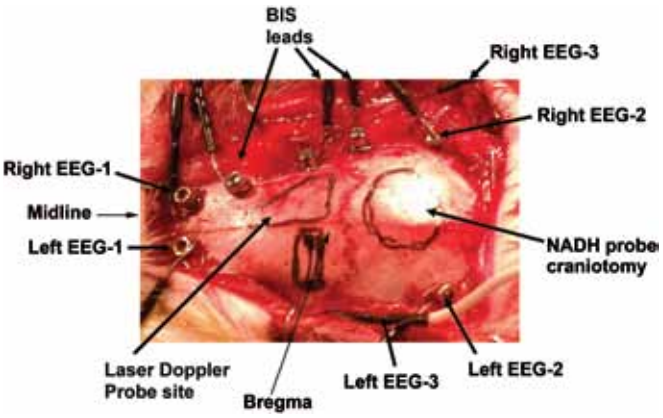

Figure 1. Placement of the BIS and EEG leads and NADH and laser Doppler probes on a rabbit's skull. The dorsal view is shown, with the rostral skull on the left; midline and bregma indicated by arrows.

Data collection and analysis. EEG and physiologic data (including heart rate, mean arterial blood pressure, esophageal body temperature, blood oxygen saturation, and ECG) were monitored and recorded on a designated data collection system (Power Lab 8/35, AD Instruments, Boston, MA) for analysis. BIS data were obtained by using a commercially available monitor (BIS 2000 System, Aspect Medical Systems, Norwood, MA). The standard human BIS leads were modified by using conductive epoxy to attach stainless steel subdermal electrodes (catalog no. RL-SND110-2.5, Rhythmlink International, Columbia, SC) to the ends of the standard BIS leads. We used the 3-lead BIS setup configuration that was compatible with our BIS system. Although methods of placing more than 3 leads have been developed by other investigators (Table 1), we opted to use fewer electrodes given the space constraints in small animals like rabbits. Furthermore, BIS leads were placed in positions analogous to those of the human skull by using the orbit and coronal sutures as a guide. The BIS leads were positioned between the EEG leads on the side of placement (Figure 2). Independent of BIS, EEG monitoring was performed on the ipsilateral and contralateral sides by using bioamplifiers whose recording parameters were optimized to obtain similar EEG tracing bilaterally and similar to the raw EEG displayed on the BIS monitor (Figures 1 and 2). During the recording, the BIS system continuously tested itself for electrode impedance (set to less than 7.5 kΩ), which appeared to be not affected by the lead modifications, and raw EEG filters were turned on (2 to 70 Hz with notch). The notch filter included filters for 50- and 60-Hz frequencies. The data from the BIS monitor were downloaded to an independent computer every 5 s through the USB cable and company-provided software (Figure 2). We monitored and recorded six independent BIS-associated parameters: total power, BIS, suppression ratio, spectral edge frequency, signal quality index, and EMG activity. Raw EEG data displayed on the BIS monitor (Figure 3) were compared with the EEG recorded by the designated EEG data collection system.

Among BIS parameters,[2] total power indicates the strength of the EEG signal based on a decibel scale. Decreases in total power generally correlate with deeper levels of anesthesia. The suppression ratio is a calculated parameter that indicates the

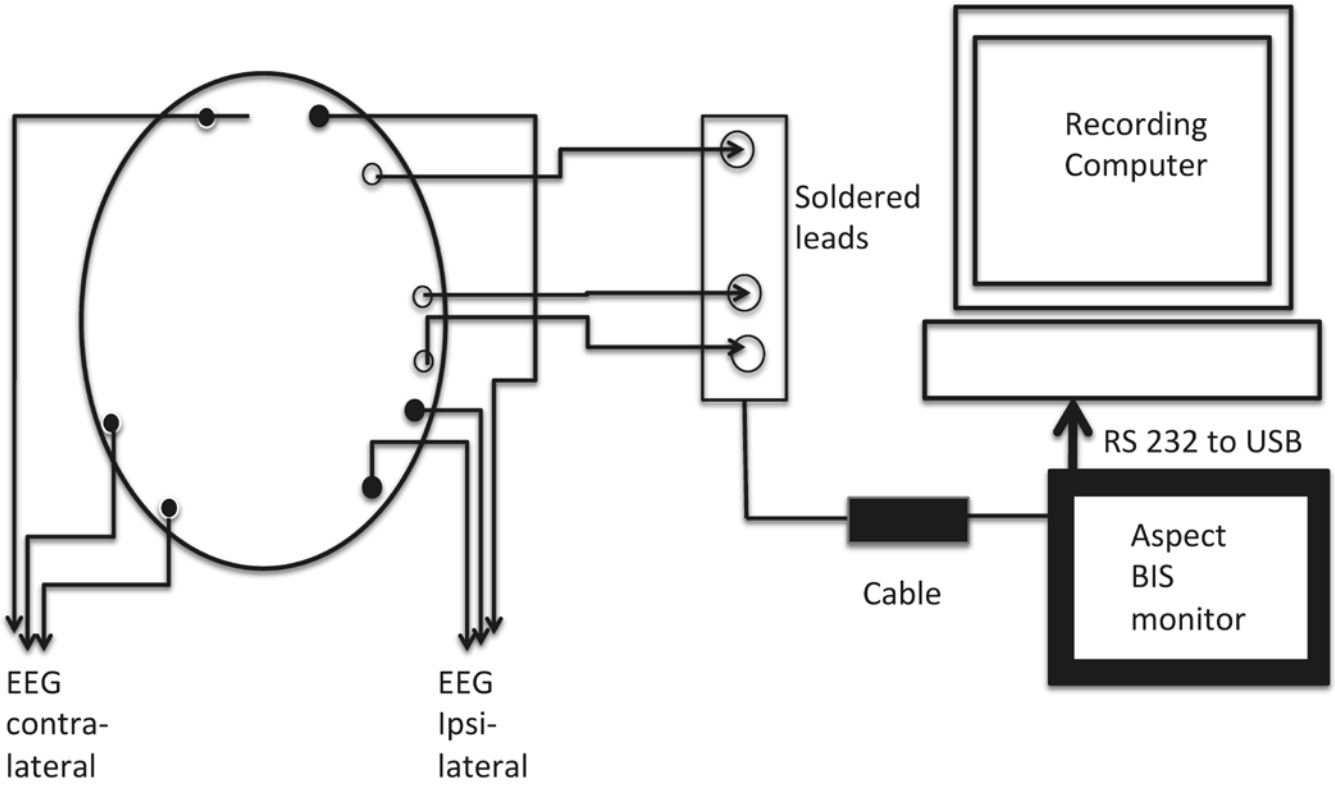

Figure 2. Diagram of the experimental monitoring and recording setup. The oval represents the rabbit's skull, with top of the oval corresponding with the rostral skull.

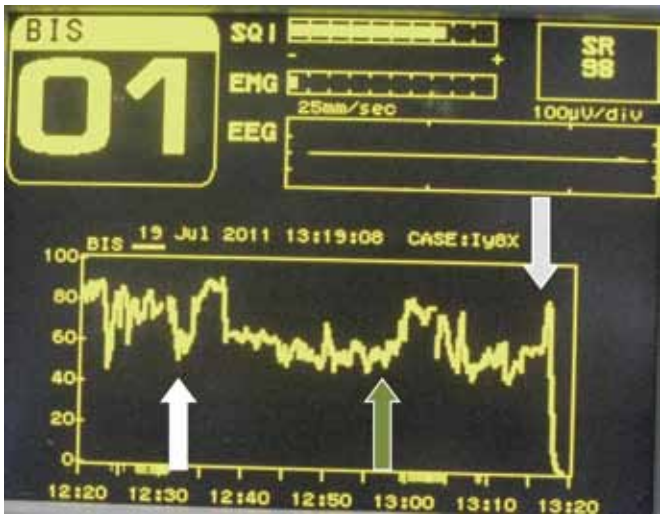

Figure 3. BIS monitor screenshot showing changes in BIS during 2 challenges and terminal cardiac arrest. Injection of propofol boluses (white arrow) yielded a sustained increase in the BIS values. Transient interruption of cerebral blood flow (light green arrow) increased BIS values also. Euthanasia with KCl during propofol and isoflurane anesthesia (gray arrow) resulted in a decrease of BIS values to near 0.

existence of an isoelectric ('flat line') condition. The suppression ratio is the percentage of time over the last 63-s period that the signal is considered to be in the suppressed state. For example, a suppression ration of 50 indicates an isoelectric condition over 50% of the last 63-s review. Increases in the suppression ratio indicate deeper levels of anesthesia. The spectral edge frequency is a line that is superimposed over the density spectral array (power distribution) display where 95% of the total power lies on one side of the line and 5% lies on the other. The spectral edge frequency value is a point on the line at any given time and ranges from 0 to 30 Hz. A decrease in the spectral edge frequency value generally correlates with a deeper anesthetic state. The signal quality index is a measure of the quality of the EEG channel signal and is calculated based on impedance data, artifact, and other variables. The signal quality index graph represents a scale of 0 to 100, marked in the intervals of 10. Optimal signal quality (that is, low interference) of the EEG channel source is indicated when the bar approaches the 100 mark on the graph. The signal quality index is not affected by the suppression ratio. EMG activity is power (in dB) from muscle activity, as well as other high-frequency artifacts, in the frequency range of 70 to 110 Hz. The EMG graph represents a scale of 0 to 100, marked in intervals of 10. During anesthesia, EMG activity is optimal when the bar on the graph is empty.

The data for each challenge were recorded continuously (Figure 4) and analyzed at only 2 time points—at baseline and at the burst-suppression pattern (complete EEG silence for at least 10 s). In addition, laser Doppler cerebral blood flow (PeriFlux System 5000, Perimed AB, Stockholm, Sweden) and relative changes in NADH fluorescence (custom NADH tissue spectroscope designed by our lab) were recorded in real time. Cerebrovascular resistance was calculated by dividing mean arterial pressure by laser Doppler cerebral blood flow.

To calculate the sample size for this study, we used an online calculator (http://www.statisticalsolutions.net). We assumed a known mean BIS value of 50, a 20% change in the value for significance, with a standard deviation (σ) for this population of 10% in the 2-sided test, an α value of 0.05, and power of 0.8, to arrive at a sample size of 8. Mean and standard deviations were analyzed by using factorial and repeated-measures ANOVA with posthoc Bonferroni–Dunn tests (StatView, SAS

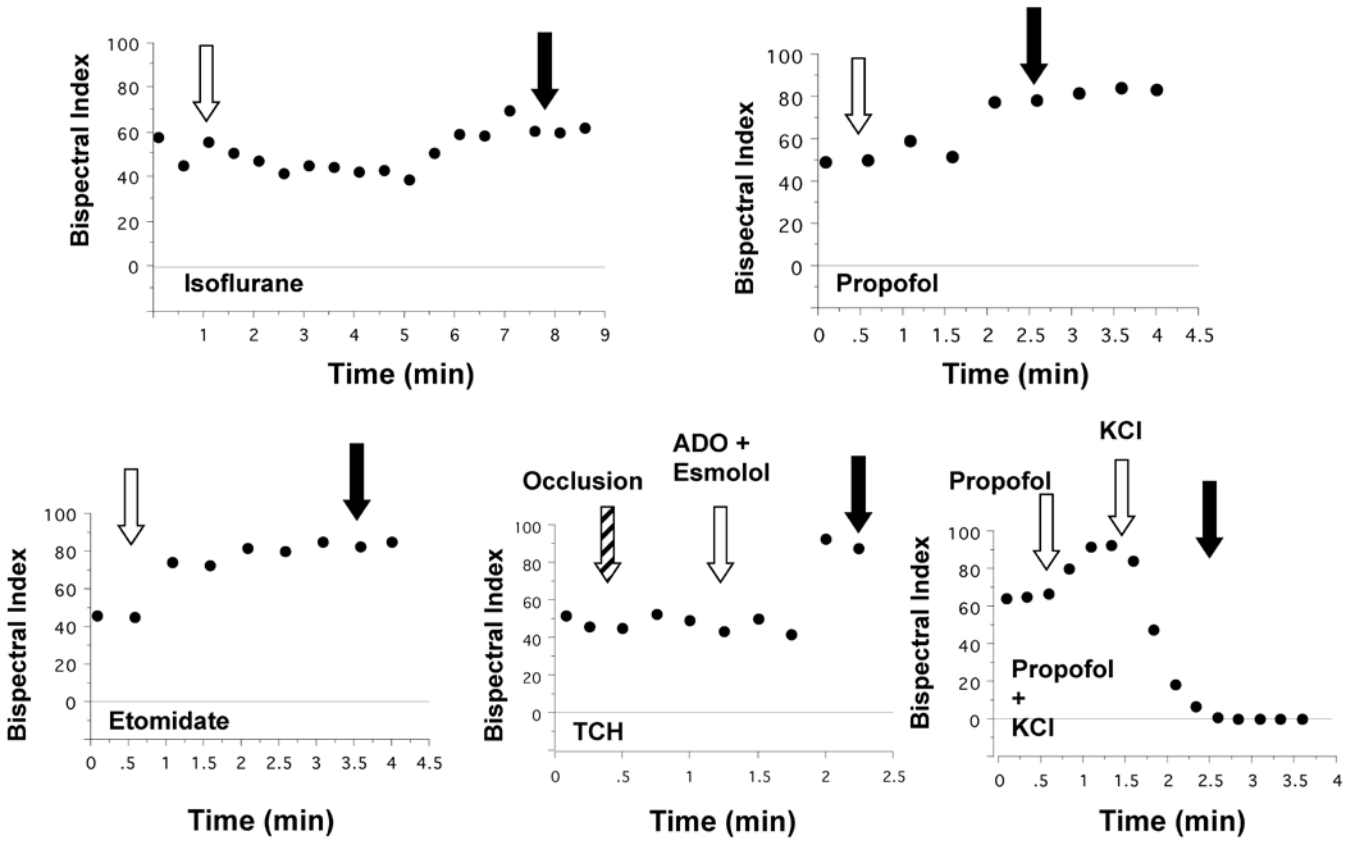

Figure 4. Changes in BIS during EEG silence after 4 challenges and terminal cardiac arrest. White arrows indicate the onset of a challenge; black arrows mark the timing of EEG silence. The crosshatched arrow indicates arterial occlusion during transient cerebral hypoperfusion (TCH). ADO, adenosine.

Institute, Cary, NC). A P value less than 0.05 was considered statistically significant.

Results

The BIS data analysis yielded some inexplicable results. Although the total power of the EEG decreased with all anesthetic drug and transient cerebral hypoperfusion challenges, the BIS values showed paradoxical increases. In addition, BIS values remained high during EEG recovery from silence. In contrast to the 4 earlier challenges, euthanasia with KCl resulted in a significant decrease in BIS to the expected near-0 values (Figure 4). The signal quality index decreased with increasing doses of anesthetic drugs but increased after terminal cardiac arrest. The suppression ratio was unaffected by anesthetics and increased only after euthanasia, whereas EMG values decreased during EEG silence with all challenges (Table 2).

Hemodynamic and blood flow responses at baseline and during EEG silence are shown in Table 3. Prearrest hemodynamic parameters were comparable across all challenges. A significant ($P < 0.05$) decrease in body temperature was seen during terminal cardiac arrest. Heart rate decreased during all challenges except for isoflurane. Mean arterial pressure decreased across all challenges but the decline was significantly ($P < 0.05$) greater (relative to baseline) during cerebral hypoperfusion and terminal cardiac arrest. Cerebral blood flow decreased with EEG silence across all challenges, but cerebrovascular resistance increased. The NADH probe did not reveal any brain ischemia during EEG silence due to anesthetic drugs but showed a significant ($P < 0.05$) increase in ischemia during cerebral hypoperfusion and euthanasia.

Discussion

Several reports suggest that BIS can be used successfully to monitor deep levels of anesthesia in dogs, pigs, chickens, and goats.[1,7,8,16,18] Other investigators were able to record BIS values in dolphins;[11] however, multiple studies found BIS to be unreliable as an anesthesia-monitoring tool in pigs, rabbits, cats, and horses.[4,10,14,15,22] Pertinent aspects of several studies, including differences in BIS lead placement, are outlined in Table 1. There are no standardized lead placement criteria for optimal BIS monitoring in different animal models, because investigators develop their own approaches to the locations and types of leads (implanted, subdermal, or patch), doses and combinations of anesthesia agents, and evaluation of the depth of the anesthetic state. We used small stainless-steel screws to avoid the noise typically generated by scalp muscle in an attempt to bring the lead system close to the brain and thereby decrease impedance. We also tried to mimic the lead positioning and to choose anesthetics commonly used in human medicine.[6,23] All of these variables may explain why we did not observe the anticipated attenuation of BIS values with increasing depths of anesthesia at burst suppression, even though BIS has provided a reliable measure of the depth of anesthesia in several other investigations.[1,7,8,16,18] For many years, we successfully used EEG to monitor depth of anesthesia and to ensure hemodynamic stability and lack of movements in anesthetized rabbits. This consistent pattern encouraged us to evaluate BIS monitoring in this species, so that we could derive an easily quantifiable, reproducible, and reliable level of anesthetic depth. Yet we observed some unexpected results: although the total power of the EEG and EMG values decreased with all 3 anesthetic-induced

Table 2. Changes in BIS parameters (arbitrary units) during EEG silence

	Isoflurane		Propofol		Etomidate		Cerebral hypoperfusion		Terminal arrest	
	Baseline	Silence	Baseline	Silence	Baseline	Silence	Baseline	Silence	Baseline	Silence
Total power	79 ± 1	64 ± 11[a]	78 ± 2	63 ± 11[a]	79 ± 2	68 ± 9[a]	77 ± 4	59 ± 15[a]	78 ± 2	42 ± 4[a,b]
Bispectral index	49 ± 9	78 ± 6[a]	52 ± 8	86 ± 4[a]	50 ± 10	83 ± 5[a]	50 ± 10	75 ± 21[a]	50 ± 11	3 ± 2[a,b]
Spectral edge frequency	11 ± 2	12 ± 2	10 ± 2	13 ± 3[a]	10 ± 3	14 ± 3[a]	11 ± 4	15 ± 5	11 ± 2	15 ± 9
Silence ratio	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 4	0 ± 0	95 ± 5[a,b]
Signal quality index	83 ± 16	41 ± 24[a]	64 ± 17	59 ± 30[a]	67 ± 30	44 ± 26[a]	70 ± 21	76 ± 23	75 ± 20	100 ± 0[b]
Electromyogram	45 ± 2	41 ± 4[a]	45 ± 2	42 ± 4[a]	46 ± 2	43 ± 4[a]	45 ± 2	39 ± 6[a]	44 ± 4	31 ± 1[a,b]

[a]Significant ($P < 0.05$) difference between baseline value and EEG silence.
[b]Significant posthoc difference between challenges ($P < 0.005$).

Table 3. Hemodynamic parameters during EEG silence

	Isoflurane		Propofol		Etomidate		Cerebral hypoperfusion		Terminal arrest	
	Baseline	Silence	Baseline	Silence	Baseline	Silence	Baseline	Silence	Baseline	Silence
Esophageal body temperature (°C)	37.3 ± 0.9	37.3 ± 0.9	37.4 ± 0.7	37.3 ± 0.7	37.1 ± 0.9	37.3 ± 0.9	37.1 ± 1.0	37.1 ± 0.9	36.4 ± 1.4	35.6 ± 1.0[a,b]
O_2 pulse saturation (%)	99 ± 3	96 ± 11	99 ± 2	100 ± 2	100 ± 3	100 ± 1	100 ± 2	90 ± 12	92 ± 15	82 ± 15[a]
Heart rate (bpm)	279 ± 22	277 ± 34	280 ± 18	265 ± 23[b]	281 ± 24	265 ± 24[b]	280 ± 23	220 ± 35[a,b]	274 ± 36	0 ± 0[a,b]
Mean arterial pressure (mm Hg)	69 ± 11	38 ± 14[b]	67 ± 9	34 ± 8§[c]	68 ± 7	53 ± 17[b]	67 ± 14	18 ± 12[a,b]	55 ± 13	1 ± 0[a,b]
Laser Doppler blood flow (arbitrary units)	175 ± 70	138 ± 61[b]	183 ± 65	139 ± 48[b]	180 ± 66	122 ± 28[b]	176 ± 75	70 ± 77[b]	159 ± 30	8 ± 3[a,b]
Cebrovascular resistance (arbitrary units)	2.6 ± 1.0	3.7 ± 0.9[b]	2.8 ± 1.0	4.3 ± 1.7[b]	2.7 ± 1.1	2.7 ± 1.9	2.8 ± 1.3	4.4 ± 3.1	3.0 ± 0.7	6.9 ± 3.4[b,c]
NADH (arbitrary units)	0 ± 0	0.01 ± 0.01	0 ± 0	0.02 ± 0.03	0 ± 0	0.01 ± 0.01	0 ± 0	0.23 ± 0.23[a,b]	0 ± 0	0.41 ± 0.09[a,b]

[a]Significantly ($P < 0.005$) different (posthoc) from values for other challenges.
[b]Significant ($P < 0.05$) difference between baseline value and that during EEG silence.
[c]Significantly ($P < 0.005$) different from value for etomidate.

challenges and with cerebral hypoperfusion (indicating a deeper anesthetic state), paradoxical increases in BIS values and spectral edge frequency (correlating with a 'lighter' anesthetic state) and decreases in the signal quality index (more EEG interference) occurred. However, as expected, after euthanasia, BIS values declined to near 0, and the signal quality index improved.

Before discussing the results further, we have to address certain technical issues. EEG recording in the laboratory environment can be suboptimal due to the presence of metal frames, electrical hardware, suboptimal grounding, and so forth. These issues become more important as the technology is scaled to smaller animals. In the current study, the possible generation of electrical artifacts by electrical motors, infusion pumps, ventilators, scavenging devices, and other monitoring equipment cannot be ruled out. However, external electrical artifacts did not seem to be a major factor in our study, because the EEG silence during terminal cardiac arrest was associated with a sharp decrease in BIS values and an improvement in the signal quality index, suggesting minimal external interference.

The biggest challenge we faced was to find a method for placing EEG leads in a position analogous to human subjects. We achieved this goal by soldering the lead wires to the BIS electrodes and then using stainless-steel screws to fix them on the skull of the rabbit. We used the coronal sutures to guide the placement of the BIS leads. The BIS monitor automatically checked electrode impedance, and the modification of the leads did not affect the impedance of the device. The raw EEG pattern shown on the BIS monitor was similar to that obtained by conventional EEG method. Throughout the experiment, EMG values remained stable and did not vary notably with any challenge. Therefore, we believe that the modification of the BIS leads and the potential sources of electrical interference in the lab did not affect the quality of the EEG signal.

In the present study, the EEG silence due to anesthetic drugs and transient cerebral hypoperfusion led to a decrease in signal quality index and paradoxical increase in BIS. EEG silence occurred during cerebral hypoperfusion despite a fairly modest reduction in cerebral blood flow (Table 3) and may have been due to both a reduction of cerebral blood flow and a greater effect of background anesthesia by way of redistribution of blood flow preferentially to the brain during hypotension. Therefore the increase in BIS values may reflect an effect of increasing anesthesia rather than cerebral ischemia (Figure 4). Figures 3 and 4 also show that even during terminal cardiac arrest, the injection of propofol prior to euthanasia resulted in a transient increase in BIS values before the final decline. Whereas signal quality index tended to decline with increasing anesthesia, euthanasia led to a dramatic improvement in the signal quality index and an equally dramatic decrease in BIS values. This pattern suggests that the degrading of signal quality with increasing depth of anesthesia was due to a factor internal to the rabbit. Our interpretation is that with increasing depth of anesthesia, as the total

EEG power decreased, intrinsic signals from the heart (electrical potentials generated due to cardiac electrical activity) affected the computed BIS values. Furthermore, the signal quality index declined during EEG silence, whereas spectral edge frequency increased. This result may be consistent with interference from a higher-frequency source, such as the rabbit's heart. Electrical interference from the heart also would explain the improvement in the signal quality index after terminal cardiac arrest. After euthanasia, as expected, immediately with the onset of loss of ECG activity, we saw EEG silence that was concurrent with the decreases in total power and BIS values and an improvement in the signal quality index.

Therefore, we suspect that in small animals such as rabbits, in which the EEG signal is weak to begin with and worsens with drug-induced EEG silence, interference from other sources of electrical signals, such as the heart, become significant and generate misleading BIS values. Even though we encountered normal base BIS recordings in anesthetized rabbits, when the anesthesia was deepened to the point of EEG silence, we observed a paradoxical increase in BIS values after all 4 experimental challenges. In conclusion, despite the various limitations of the study, including the relatively small group size, and in view of the various factors and findings discussed, we are hesitant to use BIS to monitor deep levels of anesthesia in laboratory rabbits.

Acknowledgments

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