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Effect of Isoflurane, Atracurium, Fentanyl, and Noxious Stimulation on Bispectral Index in Pigs

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The study reported here was done to determine the relationship between anesthesia depth and bispectral index (BIS) in stimulated pigs. Isoflurane minimal alveolar concentration (MAC) was determined using the tail-clamp method in 16 Yorkshire/Landrace-cross pigs with mean ± SEM weight of 27.7 ± 1.76 kg. One week later, BIS, ECG, heart rate, arterial blood pressure, esophageal temperature, end-tidal CO_2 tension and isoflurane concentration, arterial pH, PaO_2, $PaCO_2$, plasma bicarbonate concentration, and base excess were determined at each of five isoflurane MAC-multiples: 0.8, 1.0, 1.3, 1.6, and 2.0. Six treatments were studied: isoflurane; isoflurane and atracurium; isoflurane, atracurium, and fentanyl; isoflurane with noxious stimulation; isoflurane and atracurium with noxious stimulation; and isoflurane, atracurium, and fentanyl with noxious stimulation. The noxious stimulus during BIS measurement was the same as that for MAC determination. Each pig was studied three times (n = 8), and order of MAC-multiples and treatments was randomized. Data were evaluated by use of general linear model analysis of variance and linear regression analysis, with statistical significance set at $P < 0.05$. Significant differences in BIS values were identified between MAC-multiples within each treatment and between treatment 3 compared with treatments 2 and 4. Significant differences also were observed within and between treatments for heart rate, arterial blood pressure, and PaO_2. Use of BIS appears reliable for identification of light versus deep anesthesia, but is of limited use for discrimination between isoflurane MAC-multiples of 1 and 1.6. We conclude that, compared with other treatments, atracurium and noxious stimulation had no significant effect on BIS.

Pigs are frequently used in cardiovascular research as models of human health-care problems. Cardiovascular surgical procedures are often performed in pigs under light inhalant anesthesia supplemented with opioids. Opioids have become prominent choices for analgesic adjuncts in the anesthetic management of patients and animals with cardiovascular disease because of their minimal impact on cardiovascular function.

Yet, species differences in the pharmacologic properties of analgesic agents have been recognized (17). Nitrous oxide is one example of an analgesic with marked differences in analgesic potency between humans and pigs (25, 27). Efficacy of opioid analgesia in pigs can be difficult to assess because some opioids (e.g., morphine) are known to stimulate awake pigs (22). The reduction of isoflurane minimal alveolar concentration (MAC) by morphine administered to pigs is significantly less than that in dogs or humans (4, 23). The MAC-sparing effect of the potent opioid fentanyl has been determined in pigs (13). Moon and colleagues reported that doses of fentanyl conventionally used in dogs or humans are typically too low to significantly decrease the inhalant anesthetic requirement in pigs. Inadequate analgesia during surgery has been observed when opioid anesthetic protocols used in humans or dogs are extrapolated to laboratory and client-owned pigs, and may result in poor surgical conditions or misinterpretation of experimental data (23).

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Pigs anesthetized for cardiovascular surgery may receive a neuromuscular blocking agent (e.g., atracurium) to improve muscle relaxation and to facilitate mechanical ventilation as part of a balanced anesthetic protocol. Paralysis of skeletal muscle prevents movement in response to surgical stimuli, thereby removing a valuable indicator of inadequate anesthesia depth. Cardiovascular responses during anesthesia do not necessarily provide reliable or meaningful information related to anesthesia depth (15). Thus, paralyzed pigs undergoing surgery could be over-anesthetized, with resultant increase in morbidity and mortality, or under-anesthetized, creating an inhumane situation. In either instance, data obtained will be negatively impacted. Pigs that are anesthetized for surgery by use of opioid analgesics in combination with inhaled anesthetics and neuromuscular blocking agents may be at substantial risk for intraoperative awareness.

A reliable means of monitoring anesthesia depth in paralyzed animals would reduce the chance of either anesthetic overdose or intraoperative awareness, and thereby improve the quality of data from studies involving surgery. An objective measure of anesthesia depth would also benefit institutional animal care and use committees by providing assurance that investigators use proper anesthetic protocols. Monitoring CNS depression using electroencephalography (EEG) during anesthesia has been the focus of study for decades with these goals in mind. However, direct examination of the EEG has not proven adequately reliable or time responsive for use under operative circumstances. More recently, processed EEG monitoring has evolved as a way to provide immediate feedback to the anesthetist with regard to the patient's CNS activity. Spectral edge frequency, total power, beta-to-delta frequency ratios, and other specific parameters derived

from computer-processed EEG have been evaluated with some success, but interpretation of the information can vary greatly depending on selection of anesthetic agents.

A new approach to intraoperative EEG processing with substantial improvement in reliability has been developed. The bispectral index (BIS) is a proprietary monitoring device that provides information related to anesthesia depth and is readily interpreted. In addition, BIS has proven reliable as an indicator of anesthesia depth in people given a variety of anesthetic agents and adjuncts (20, 24). Bispectral index is a unit-less number between 0 and 100 derived from the processed EEG (16). Numerous citations have correlated anesthesia depth with BIS in humans (3, 9, 10, 18, 21). People undergoing surgery are typically maintained at a depth of anesthesia that yields a BIS value between 40 and 60 and reliably prevents intraoperative awareness.

Few reports related to use of BIS as an indicator of anesthesia depth in animals are available. In our laboratory, depth of isoflurane- and sevoflurane-induced anesthesia was inversely correlated to BIS in dogs (5, 6). In goats anesthetized with isoflurane, BIS values significantly changed in response to intubation and noxious stimulation (2). Results of a study in unstimulated pigs did not indicate reliable correlation between isoflurane anesthesia depth and BIS (7), whereas those of another study in pigs indicated reliable correlation of BIS values to anesthesia depth during surgery using propofol anesthesia (19).

The goal of the study reported here was to assess a method of objectively measuring adequacy of anesthesia depth in pigs, specifically in pigs for which anesthesia is difficult to monitor such as those given a neuromuscular blocking agent during a noxious procedure. The objectives of this study were to determine the relationship of BIS values to isoflurane-induced anesthesia depth in pigs and to determine the effects of noxious stimulation on BIS values. The specific aims were: to determine the dose response of isoflurane and BIS in pigs, using pre-determined MAC-multiples for individual pigs; to determine BIS values associated with noxious stimulation at various depths of isoflurane-induced anesthesia; to determine the effects of infusions of atracurium and fentanyl on BIS during isoflurane anesthesia; and to determine the effects of infusions of atracurium and fentanyl on BIS during isoflurane-induced anesthesia combined with noxious stimulation.

Materials and Methods

Animals. Sixteen Yorkshire/Landrace-cross pigs (*Sus scrofa*; 8 females and 8 males), 80 to 110 days of age and weighing (mean ± SEM) 27.7 ± 0.73 kg, were studied over a 4-week period. Pigs were test negative for porcine respiratory and reproductive syndrome, pseudorabies, and mycoplasmosis and were farrowed by members of the University of Illinois' (Urbana) breeding herd, which is vaccinated for and determined to be free (based on serologic test results from cross-sectional blood sampling) of H1N1 and H3N2 strains of influenza. Pigs were housed indoors in groups of four per pen and were fed a standard ration, with water available ad libitum. One week was allowed for acclimatization prior to study. The study was approved by the university's institutional animal care and use committee and was conducted in compliance with local and federal guidelines governing laboratory animal care and housing at facilities approved by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Food, but not water, was withheld the evening prior to study. Each pig was anesthetized for study on four occasions at weekly intervals: week 1 for determination of isoflurane MAC; and weeks 2-4 for receiving treatments 1, 2, and 3 or treatments 4, 5, and 6. Thus, 8 pigs received three treatments involving different anesthetic protocols and 8 pigs received three treatments using those protocols while administering a noxious stimulus. This design allowed study of each pig in a short period (approx. 30 days) to minimize effects of growth so that a relatively uniform head size was used throughout the study. The CBC and serum biochemical values were found to be within reference ranges for each pig prior to study. Pigs were weighed at the beginning of each study day, and mean body weights for each treatment were calculated and compared by use of analysis of variance (ANOVA).

Procedure. Each pig's isoflurane MAC was determined using the tail clamp method. On subsequent study days, pigs were instrumented for measurement of BIS, ECG, direct arterial blood pressure, esophageal temperature, and end-tidal gas (CO_2 and isoflurane) concentrations. Pigs were assigned to one of six treatments and were anesthetized at each of five MAC-multiples (0.8, 1.0, 1.3, 1.6, and 2.0) on the basis of each animal's pre-determined MAC value for isoflurane. Treatments (1-6, respectively) were: isoflurane; isoflurane and the neuromuscular blocking agent, atracurium (atracurium besylate, Baxter Healthcare Corp., Deerfield, Ill.) administered as a loading dose (2.5 mg/kg of body weight, i.v.) followed by a constant rate of infusion (46 µg/kg/min, i.v.); isoflurane, atracurium and fentanyl (Abbott Laboratories, N. Chicago, Ill.) administered as a loading dose (75 µg/kg, i.v.) followed by a constant rate of infusion (50 µg/kg/h, iv); isoflurane and noxious stimulus; isoflurane, atracurium, and noxious stimulus; and isoflurane, atracurium, fentanyl, and noxious stimulus. The noxious stimulus was the same as that used for determination of MAC and was applied for 1 min, or until the pig responded with purposeful movement (e.g., 0.8 MAC) at the beginning of the BIS recording period. The order of study of treatments and isoflurane MAC-multiples was randomized. Twenty minutes were allowed for equilibration at each MAC-multiple prior to recording BIS. The BIS data were collected for 5 min, and the median BIS value was determined for the recording period at each isoflurane MAC-multiple.

Determination of MAC. The technique for determination of MAC used in our laboratory has been described (26). Briefly, anesthesia in each pig was induced by use of a mask, the trachea was intubated, and anesthesia was maintained for 20 min at 1.3% end-tidal isoflurane concentration. A padded sponge clamp was placed on the tail at the second palpable coccygeal vertebra distal to the base of the tail. The clamp was closed to full ratchet position and was held in place for 60 sec or until the pig responded with gross purposeful movement. Typically, gross purposeful movement was characterized by avoidance or escape behavior such as lifting and waving of the head along with paddling of all 4 limbs. Although it was unpleasant, the fully closed clamp could be tolerated for 1 min when placed on the investigator's finger. This method prevented mechanical tissue damage and preserved the tail for application of the stimulus over subsequent weeks of study. If the pig did not respond to application of the clamp, the end-tidal anesthetic concentration was reduced by 10%, a 20-min equilibration period was allowed, and the clamp was reapplied. When the pig did respond

to application of the clamp, end-tidal concentration was increased by 10%, another 20-min equilibration period was allowed, and the clamp was reapplied to further categorize the MAC. The MAC was determined by averaging the highest end-tidal concentration when the pig responded and the lowest end-tidal concentration when the pig did not respond. After MAC was identified, anesthetic administration was discontinued and the pigs were allowed to recover.

Physiologic monitoring. On each day of BIS measurement, pigs were positioned in sternal recumbency in a webbed stanchion custom-made for transporting and restraining pigs. The limbs dangled freely below the webbing, and pigs readily tolerated this simple method of restraint. Anesthesia with isoflurane was induced in pigs by use of a mask (Isoflo, Abbott Laboratories), and the trachea was intubated via direct laryngoscopy. Anesthesia was maintained with isoflurane in oxygen using a precision vaporizer (Isoflurane Vapor 19.1, North American Drager, Telford, Pa.) and a rebreathing circuit on an anesthesia machine (Narkovet 2, North American Drager, Telford, Pa.). Ventilation was controlled by use of a mechanical ventilator (Hallowell EMC, Pittsfield, Mass.) to maintain normocapnia ($PaCO_2$ = 35 to 40 mmHg).

The marginal ear vein and auricular artery were catheterized (Angiocath, 20-gauge, 48 mm, Becton-Dickinson, Sandy, Utah). The arterial catheter was connected to a mercury-calibrated blood pressure transducer and physiologic monitor (Datascope 3000A, Datascope Corp, Paramus, N.J.). Heart rate was determined by counting arterial pulse waves per minute. A three-lead ECG and esophageal temperature were continuously monitored using the physiologic monitor. Arterial blood samples were collected at the end of each MAC-multiple BIS recording period. Arterial blood gas tensions (PaO_2 and $PaCO_2$) and pH were measured within 5 min of sample collection using a calibrated blood gas machine (Ciba-Corning Blood Gas Machine, model 288, Medfield, Mass.). Bicarbonate concentration and base excess were calculated and reported by the blood gas machine. End-tidal CO_2 and isoflurane concentrations were measured from samples taken at the tracheal carina using a calibrated side-stream sampling anesthetic gas analyzer (Datascope Multinex 4100 Plus, Datascope Corp, Paramus, N.J.).

Measurement of BIS. Bispectral index was measured using the A-2000 BIS monitor (Aspect Medical Systems Inc, Natick, Mass.) with version 3.4 software. The BIS was recorded every 5 sec for 5 min after equilibration at each MAC-multiple, and data were stored on a computer. The BIS is reported as a unit-less whole number between 0 and 100. Filters were set at low cut = 2 Hz, 50/60 Hz filter at 60 Hz and high cut = 70 Hz. At startup, the monitor requires skin-electrode impedance < 7.5 kS, and thereafter provides for continuous impedance checking, with impedance < 2 kS at 16 Hz. High-frequency activity (70 to 110 Hz) is identified as electromyographic activity measured in decibels with respect to 0.0001 μV^2, and is graphed in real time with the BIS. The monitor has automatic artifact detection and displays a signal quality index as a function of good epochs and suppressed epochs in the previous 120 (61.5 sec) used for calculation of BIS. The percentage of epochs in the last 63 sec in which the EEG signal is suppressed is expressed as the suppression ratio (SR). Burst suppression was identified as isoelectric analog EEG for at least 1 sec and was detected by the monitor, as indicated by increased suppression ratio (SR > 1). Presence of burst suppression at deeper levels of isoflurane anesthesia was readily identified by spike activity followed by isoelectric EEG and increased SR. When paradoxical increases in BIS (> 60) were observed along with SR > 1, data were not recorded.

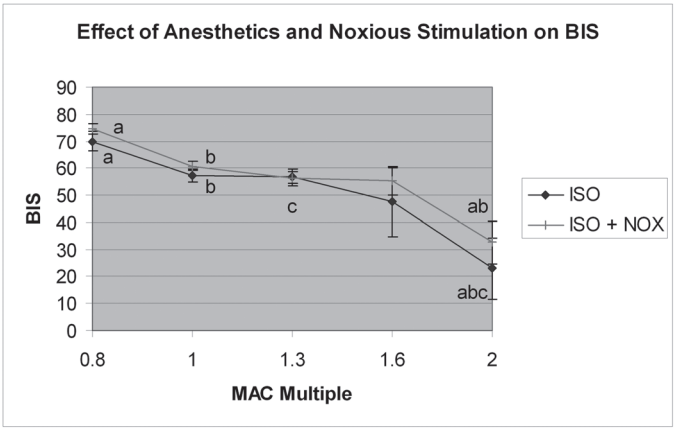

Figure 1. Effect of anesthetic and noxious stimulation on bispectral index (BIS) in 8 pigs (treatment 1, isoflurane [ISO] versus treatment 4, isoflurane with noxious stimulation [ISO+NOX]). Points represent mean values for 8 pigs; error bars represent SEM. Within treatments data points marked by the same letter are significantly ($P < 0.05$) different.

Electrodes. A modified ECG cable was connected to the BIS cable distal to the analog-to-digital converter. Three 29-gauge platinum needle electrodes (E2-31 cm, Grass Instruments, Astro-Med Inc., West Warwick, R.I.) were connected to the modified cable and placed subdermally. The primary lead was placed on the midline approximately a third of the distance from a line connecting the zygomatic processes of the frontal bone and the most caudal portion of the external frontal crest that was palpable. Ground and secondary leads were placed subdermally near the rostral base of the right ear and over the right temporal bone, respectively.

Statistical analysis. Data are reported as mean ± SEM. Data from each MAC-multiple within each treatment were compared by use of a general linear model ANOVA with commercially available software (NCSS, Kaysville, Utah). For comparison of treatment means, the Tukey-Kramer test was used. The level of significance was set at $P < 0.05$. The MAC-multiples also were converted to each pig's corresponding end-tidal isoflurane concentration, and their relationship to BIS values was analyzed by use of linear regression.

Results

Mean ± SEM body weights of pigs for each treatment were: 1) 29.4 ± 1.7; 2) 29.1 ± 1.7; 3) 29.2 ± 1.4; 4) 26.4 ± 1.9; 5) 25.0 ± 1.9; and 6) 27.1 ± 2.1 kg. Mean body weights were not significantly different among treatments. Mean ± SD isoflurane MAC for this group of 16 pigs was 1.3 ± 0.2%. The first 2 pigs receiving fentanyl recovered from anesthesia after study in an excited state (one each with and without noxious stimulation) that was characterized by frequent rolling about the recovery cage. Subsequently, at the end of each experiment, pigs treated with fentanyl were given naloxone (0.8 mg, i.v.) at the time isoflurane was discontinued. After beginning this practice, all pigs recovered from anesthesia smoothly and unremarkably. Pigs were returned to their pen when they could stand and walk without ataxia.

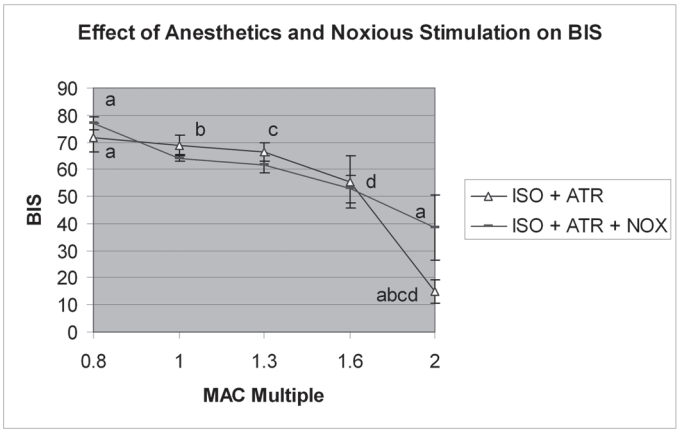

Figure 2. Effect of anesthetic and noxious stimulation on BIS in 8 pigs (treatment 2, isoflurane and atracurium [ISO+ATR] versus treatment 5, isoflurane and atracurium with noxious stimulation [ISO+ATR+NOX]).

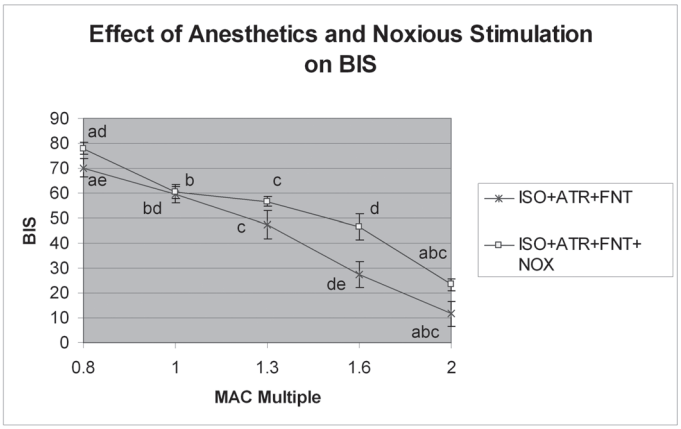

Figure 3. Effect of anesthetic and noxious stimulation on BIS in 8 pigs (treatment 3, isoflurane, atracurium, and fentanyl [ISO+ATR+FNT] versus treatment 6, isoflurane, atracurium, and fentanyl with noxious stimulation [ISO+ATR+FNT+NOX]).

Within all treatments, BIS values at 0.8 and 1.0 isoflurane MAC were significantly different from those at 2.0 MAC (Fig. 1-3). The BIS values at 1.3 and 1.6 isoflurane MAC were significantly different from those at 2.0 MAC within treatments 1, 2, 3, and 6, and treatments 2, 3, and 6, respectively. Within treatments 3 and 6, BIS values for 0.8 MAC were significantly different compared with those for 1.6 MAC. Within treatment 3, BIS values at 1.0 isoflurane MAC also were significantly different from those at 1.6 MAC. Between treatments, BIS values at 1.6 MAC were significantly higher in treatments 2 and 4 compared with treatment 3. Values at other MAC multiples were not significantly different among treatments.

All pigs that received noxious stimulation (treatment 4) during 0.8 MAC isoflurane responded with purposeful movement, whereas 3 pigs responded at 1.0 MAC. In addition, one pig each from treatment 1 (isoflurane) and treatment 4 (isoflurane with noxious stimulation) spontaneously moved during the equilibration period for 1.6 MAC.

Linear regression analysis of BIS values plotted against end-tidal isoflurane concentration was done for each treatment. Regression coefficients for each treatment are given in Table 1.

Heart rate at 0.8 isoflurane MAC was significantly lower in treatment 1 compared with treatment 3 (Table 2). Heart rate was not significantly different among other treatments or within MAC multiples.

Table 1. Regression coefficients for end-tidal isoflurane concentration versus bispectral index (BIS) values for each treatment; BIS = m(ETISO) + b, where BIS = bispectral index value, m = slope, ETISO = end-tidal isoflurane concentration, and b = y-intercept

Treatment	Intercept	Slope	R^2
1	102.4	–30.7*	0.80
2	113.3	–32.9*	0.66
3	108.3	–37.4*	0.90
4	99.4	–29.4*	0.74
5	98.5	–26.3*	0.59
6	106.0	–35.1*	0.84

*Indicates slopes that are significantly ($P < 0.05$) different from zero.
Treatments: 1 = isoflurane; 2 = isoflurane and atracurium; 3 = isoflurane, atracurium and fentanyl; 4 = isoflurane with noxious stimulation; 5 = isoflurane and atracurium with noxious stimulation; 6 = isoflurane, atracurium, and fentanyl with noxious stimulation.

Systolic arterial blood pressure at 0.8 isoflurane MAC within treatments 3 and 6 was significantly higher than that at 1.6 and 2.0 MAC (Table 2). Within treatment 3, systolic arterial blood pressure at 0.8 isoflurane MAC was significantly higher than that at 1.3 MAC. Within treatment 6, systolic arterial blood pressure at 1.0 isoflurane MAC was significantly higher than that at 2.0 MAC.

Mean arterial blood pressure for treatments 3 and 6 was significantly different between the same MAC-multiples as was systolic arterial blood pressure. In addition, mean arterial blood pressure at 0.8 isoflurane MAC was significantly higher in treatment 3 compared with either treatment 1 or 4 (Table 2).

Diastolic arterial blood pressure values were significantly different among the same treatments and MAC multiples as was mean arterial blood pressure except that, within treatment 6, the value at 1.0 isoflurane MAC was not significantly different from any other value (Table 2).

The PaO_2 at 0.8 isoflurane MAC was significantly lower than that at 1.0 MAC within treatment 5 (Table 2). Other values for PaO_2 were not significantly different within or between treatments. There were no significant differences within or between treatments for esophageal temperature, end-tidal CO_2 tension, arterial pH, $PaCO_2$, arterial bicarbonate concentration, or base excess (Table 2).

Discussion

The isoflurane MAC value determined for this group of pigs was slightly lower than that reported in previous studies (4, 11, 23). Factors that may affect the determination of the MAC value include breed or strain of pig and the type of noxious stimulus used to elicit a response. Our method for producing the noxious stimulus has been used in previous studies involving anesthetized pigs (25, 26). To minimize variability in application of the stimulus, the same clamp was used throughout the study.

As a general rule, BIS decreased for all treatments as the isoflurane MAC-multiple increased. Although the previously reported relationship of BIS with MAC-multiple was linear in the dog for the range of 0.8 to 2 MAC (5), this relationship was less clear in the pig. Although BIS values were significantly different in all treatments when comparing the light depths of anesthesia with the deepest depth, there were rarely statistically significant differences observed for isoflurane MAC-multiples between 1.0 and 1.6. It appears that, during moderate-to-deep anesthesia, pigs undergo activation of some EEG parameter in such a way

Table 2. Mean ± SEM values for physiologic variables during BIS measurement in anesthetized pigs

Variable	Treatment	MAC-multiple 0.8	1.0	1.3	1.6	2.0
HR(bpm)	1	111 ± 8.8*	112 ± 8.8	112 ± 8.8	114 ± 11.2	109 ± 11.2
	2	142 ± 8.8	131 ± 8.8	122 ± 8.8	114 ± 9.4	106 ± 10.2
	3	162 ± 8.8*	138 ± 8.8	123 ± 8.8	128 ± 9.4	122 ± 9.4
	4	127 ± 8.8	120 ± 8.8	122 ± 8.8	119 ± 9.4	111 ± 9.4
	5	146 ± 8.8	122 ± 8.8	121 ± 8.8	117 ± 9.4	111 ± 11.2
	6	153 ± 8.8	146 ± 8.8	133 ± 8.8	124 ± 8.8	125 ± 10.2
SBP(mmHg)	1	98 ± 6.3	93 ± 6.3	87 ± 6.3	90 ± 8.0	80 ± 8.0
	2	106 ± 6.3	105 ± 6.3	91 ± 6.3	90 ± 6.8	75 ± 7.3
	3	130 ± 6.3abc	106 ± 6.3	92 ± 6.3a	88 ± 6.8b	75 ± 6.8c
	4	99 ± 6.3	94 ± 6.3	90 ± 6.3	84 ± 6.8	72 ± 6.8
	5	105 ± 6.3	93 ± 6.3	90 ± 6.3	85 ± 6.8	76 ± 8.0
	6	122 ± 6.3ab	113 ± 6.3c	98 ± 6.3	83 ± 6.3a	74 ± 7.3bc
MBP(mmHg)	1	72 ± 5.6*	66 ± 5.6	62 ± 5.6	62 ± 7.0	54 ± 7.0
	2	80 ± 5.6	74 ± 5.6	64 ± 5.6	63 ± 5.9	52 ± 6.4
	3	104 ± 5.6*†abc	80 ± 5.6	68 ± 5.6a	65 ± 5.9b	52 ± 5.9c
	4	73 ± 5.6†	68 ± 5.6	67 ± 5.6	60 ± 5.9	54 ± 5.9
	5	82 ± 5.6	69 ± 5.6	63 ± 5.6	60 ± 5.9	53 ± 7.0
	6	95 ± 5.6ab	88 ± 5.6c	74 ± 5.6	61 ± 5.6a	52 ± 6.4bc
DBP(mmHg)	1	56 ± 5.2*	52 ± 5.2	47 ± 5.2	49 ± 6.6	42 ± 6.6
	2	62 ± 5.2	56 ± 5.2	48 ± 5.2	48 ± 5.6	42 ± 6.1
	3	88 ± 5.2*†abc	66 ± 5.2	55 ± 5.2a	52 ± 5.6b	42 ± 5.6c
	4	56 ± 5.2†	54 ± 5.2	52 ± 5.2	47 ± 5.6	44 ± 5.6
	5	66 ± 5.2	55 ± 5.2	50 ± 5.2	47 ± 5.6	42 ± 6.6
	6	78 ± 5.2ab	70 ± 5.2	58 ± 5.2	48 ± 5.2a	39 ± 6.1b
T (°C)	1	37.5 ± 0.3	37.4 ± 0.3	37.4 ± 0.3	37.7 ± 0.4	37.6 ± 0.4
	2	37.7 ± 0.3	37.6 ± 0.3	37.8 ± 0.3	37.8 ± 0.3	37.9 ± 0.4
	3	37.6 ± 0.3	37.3 ± 0.3	37.5 ± 0.3	37.8 ± 0.3	37.8 ± 0.3
	4	37.1 ± 0.3	37.0 ± 0.3	37.0 ± 0.3	37.1 ± 0.3	37.1 ± 0.3
	5	37.3 ± 0.3	37.2 ± 0.3	37.4 ± 0.3	37.2 ± 0.3	37.4 ± 0.4
	6	37.1 ± 0.3	37.6 ± 0.3	37.5 ± 0.3	37.2 ± 0.3	37.4 ± 0.4
$etCO_2$(mmHg)	1	37.8 ± 1.6	36.1 ± 1.6	36.1 ± 1.6	36.8 ± 2.0	37.0 ± 2.0
	2	40.3 ± 1.6	35.0 ± 1.6	35.4 ± 1.6	37.0 ± 1.7	32.2 ± 1.8
	3	38.0 ± 1.6	35.9 ± 1.6	35.1 ± 1.6	37.0 ± 1.7	35.9 ± 1.7
	4	39.9 ± 1.6	39.9 ± 1.6	38.5 ± 1.6	36.7 ± 1.7	35.9 ± 1.7
	5	36.8 ± 1.6	33.9 ± 1.6	32.1 ± 1.6	36.7 ± 1.7	34.4 ± 2.0
	6	39.0 ± 1.6	36.8 ± 1.6	35.0 ± 1.6	36.4 ± 1.6	35.8 ± 1.8
pH	1	7.505 ± 0.016	7.528 ± 0.017	7.526 ± 0.017	7.507 ± 0.021	7.500 ± 0.021
	2	7.477 ± 0.017	7.504 ± 0.017	7.518 ± 0.017	7.513 ± 0.018	7.545 ± 0.019
	3	7.502 ± 0.017	7.518 ± 0.017	7.504 ± 0.017	7.495 ± 0.018	7.494 ± 0.018
	4	7.491 ± 0.017	7.494 ± 0.017	7.505 ± 0.017	7.510 ± 0.018	7.516 ± 0.018
	5	7.509 ± 0.017	7.526 ± 0.017	7.538 ± 0.017	7.476 ± 0.019	7.514 ± 0.021
	6	7.476 ± 0.017	7.513 ± 0.017	7.512 ± 0.018	7.516 ± 0.017	7.521 ± 0.019
PaO_2(mmHg)	1	593 ± 18	590 ± 18	563 ± 18	592 ± 23	577 ± 23
	2	602 ± 18	597 ± 18	561 ± 18	563 ± 20	542 ± 23
	3	573 ± 18	583 ± 18	574 ± 18	576 ± 20	535 ± 20
	4	584 ± 18	611 ± 18	606 ± 20	604 ± 20	581 ± 20
	5	517 ± 18a	616 ± 18a	575 ± 18	593 ± 21	586 ± 23
	6	564 ± 18	617 ± 18	605 ± 20	581 ± 20	599 ± 21
$PaCO_2$(mmHg)	1	37.4 ± 1.8	35.0 ± 1.8	35.5 ± 1.8	35.1 ± 2.3	35.3 ± 2.3
	2	40.3 ± 1.8	37.4 ± 1.8	35.3 ± 1.8	36.2 ± 2.0	33.3 ± 2.3
	3	37.4 ± 1.8	36.4 ± 1.8	37.0 ± 1.8	37.1 ± 2.0	36.7 ± 2.0
	4	37.8 ± 1.8	38.4 ± 1.8	37.6 ± 1.8	36.7 ± 2.0	35.9 ± 2.0
	5	36.5 ± 1.8	34.4 ± 1.8	32.9 ± 1.8	40.2 ± 2.1	35.1 ± 2.3
	6	41.4 ± 1.8	37.6 ± 1.8	38.0 ± 2.0	38.4 ± 1.8	36.7 ± 2.1
HCO_3(g/dl)	1	29.6 ± 0.7	28.9 ± 0.7	29.4 ± 0.7	27.6 ± 0.9	27.1 ± 0.9
	2	29.0 ± 0.7	29.2 ± 0.7	28.6 ± 0.7	28.7 ± 0.7	27.4 ± 0.8
	3	28.8 ± 0.7	29.1 ± 0.7	28.7 ± 0.7	28.2 ± 0.7	27.7 ± 0.7
	4	28.8 ± 0.7	29.5 ± 0.7	29.5 ± 0.7	29.0 ± 0.7	28.6 ± 0.7
	5	28.8 ± 0.7	28.3 ± 0.7	27.8 ± 0.7	29.2 ± 0.8	28.0 ± 0.9
	6	30.2 ± 0.7	30.9 ± 0.7	29.2 ± 0.8	29.9 ± 0.7	29.7 ± 0.8
Base excess	1	6.9 ± 0.6	7.1 ± 0.6	7.2 ± 0.6	5.6 ± 0.7	5.9 ± 0.7
	2	6.1 ± 0.6	6.8 ± 0.6	6.5 ± 0.6	6.6 ± 0.6	6.2 ± 0.7
	3	6.6 ± 0.6	7.2 ± 0.6	6.6 ± 0.6	5.9 ± 0.6	5.4 ± 0.6
	4	6.1 ± 0.6	6.8 ± 0.6	7.0 ± 0.6	6.7 ± 0.6	6.5 ± 0.6
	5	6.6 ± 0.6	6.5 ± 0.6	6.3 ± 0.6	6.2 ± 0.7	6.0 ± 0.7
	6	7.2 ± 0.6	7.5 ± 0.6	6.8 ± 0.7	7.4 ± 0.6	7.7 ± 0.7

*Indicates values significantly ($P < 0.05$) different between treatments. Letters next to values that are the same for MAC-multiples within a treatment indicate significant differences.

The asterisk (*) and the dagger (†) indicate values significantly ($P < 0.05$) different between the treatments indicated within the same MAC-multiple. Like letters next to values within the same treatment indicate significant ($P < 0.05$) differences between MAC-multiples. BIS is bispectral index; MAC is minimal alveolar concentration; HR (bpm) is heart rate in beats per minute; SBP (mmHg) is systolic arterial blood pressure in millimeters of mercury; MBP (mm Hg) is mean arterial blood pressure in millimeters of mercury; DBP (mm Hg) is diastolic arterial blood pressure in millimeters of mercury; T (°C) is esophageal temperature in degrees Centrigrade; $etCO_2$ (mmHg) is end-tidal carbon dioxide tension in millimeters of mercury; pH is the negative logarithm of the hydrogen ion concentration in arterial blood; PaO_2 (mmHg) is the oxygen tension of arterial blood in millimeters of mercury; $PaCO_2$ (mmHg) is the carbon dioxide tension of arterial blood in millimeters of mercury; and HCO_3^- (g/dl) is bicarbonate ion concentration of arterial blood in grams per deciliter.

that the proprietary algorithm (designed for human EEG) overestimates the BIS value, indicating less depression of CNS function. This phenomenon was most notable around 1.6 isoflurane MAC. Pigs, in which anesthesia depth was changed from 1.3 to 1.6 isoflurane MAC, began to have decreased BIS, but after the equilibration period, this apparent EEG activation predominated, causing reversal in the BIS trend toward a higher value. Frequently this occurred at the time of onset of burst suppression. Burst suppression is frequently observed in most mammalian species given large doses of a general anesthetic, and is characterized by sudden high amplitude spikes followed by a variable period of isoelectric EEG. The BIS monitor appears to interpret burst suppression EEG activity as evidence of arousal in pigs and reports an increase in BIS during these episodes, even though anesthesia depth is at steady state. Therefore, BIS values obtained during burst suppression, or near the anesthesia depth at which burst suppression has its onset, are unreliable in pigs. We observed that this activation of EEG was transitory in nature and was diminished or abolished as anesthesia depth was increased to 2 MAC in nearly all instances.

Addition of atracurium to isoflurane anesthesia had no apparent effect on BIS. Atracurium has no analgesic or MAC-sparing properties. Only one treatment with atracurium (treatment 3) had a significantly different (lower) BIS value at the same MAC-multiple compared with treatment without atracurium (treatment 4). However, treatment 4 involved noxious stimulation whereas treatment 3 did not, and arousal due to stimulation may explain the increased BIS in this instance.

Addition of fentanyl to the combination of isoflurane and atracurium had a noticeable stabilizing effect on BIS relationship with anesthesia depth, particularly during the EEG-activation zone, around 1.6 isoflurane MAC. Compared with that for other treatments, regression analysis of data from treatment with fentanyl (treatments 3 and 6) yielded had the highest R^2 values, indicating better accounting for variation in BIS due to variation in end-tidal isoflurane concentration. The dose of fentanyl used in this study was associated with a 25% decrease in isoflurane MAC in another study of pigs (13). Thus, it is possible that the addition of fentanyl had a depressing effect on CNS activity that countered the apparent activation of EEG observed in pigs not receiving fentanyl while maintained at 1.6 isoflurane MAC.

An effect of noxious stimulation on BIS was not evident. Although treatment 3 (isoflurane, atracurium, and fentanyl) had a lower BIS value at 1.6 isoflurane MAC compared with that for treatment 4 (isoflurane with noxious stimulation), the importance of this single finding is questionable. As expected, pigs given noxious stimulation during isoflurane alone (treatment 4) responded with purposeful movement at 0.8 and 1.0 isoflurane MAC. Interestingly, spontaneous movement occurred in 2 pigs at 1.6 isoflurane MAC (one each from treatments 1 and 4). Both responses occurred at a time when stimulus was not applied. However, the spontaneous movement in these instances was qualitatively different from the purposeful movement observed in stimulated pigs and did not appear to be escape behavior. Whether the EEG activation observed at 1.6 isoflurane MAC was a cause of this spontaneous movement could not be determined in our study. Pigs maintained at the isoflurane anesthesia depth associated with burst suppression of the EEG have been documented to have motor responses to nociceptive stimuli, possibly indicating that suppression of movement during noxious stimulation involves processing in the spinal cord (8). Thus, it is possible that BIS (brain) and reflex (spinal) motor responses to noxious stimuli are not highly correlated.

For all treatments, BIS values < 60 were observed at 1.3% isoflurane, which is 1 MAC for this group of pigs. Avoidance of arousal reactions in pigs during surgical stimulation by maintaining depth of anesthesia so that BIS is 40 to 60 has been recommended by other investigators (19). It would seem prudent, when considering non-verbal species, to err on the conservative side to ensure loss of consciousness and prevention of pain perception. Thus, using the loading and maintenance doses for atracurium and fentanyl described herein, our results suggest a minimal anesthetic depth of 1 MAC isoflurane to prevent awareness in pigs undergoing noxious procedures performed during isoflurane, isoflurane and atracurium, or isoflurane, atracurium, and fentanyl anesthesia.

An important aspect of our study involved pre-determination of isoflurane MAC values for each individual pig. If we had studied BIS relationships to anesthesia depth by choosing a published isoflurane MAC value, our results would likely have been less meaningful. Lack of correlation between BIS values with depth of anesthesia in pigs may be due to increased variance associated with use of a published MAC value rather than actual individual MAC values.

The changes in heart rate and systemic arterial blood pressure were consistent with expected physiologic responses to altered inhalant anesthesia depth. In all treatments, mean arterial blood pressure at 2.0 isoflurane MAC was less than the recommended minimum during clinical anesthesia (60 to 70 mmHg). Effects of hypotension on BIS have not been recognized. During acute hypertensive episodes in anesthetized people, the BIS was unaffected (14). However, when epinephrine was administered to patients during propofol sedation, BIS increased when the patient was aroused, as indicated by sedation scores (1). We cannot rule out a possible effect that hypotension may have had on the BIS values in the pigs anesthetized with 2.0 MAC isoflurane. In sevoflurane-anesthetized pigs, there was lack of correlation between BIS and arterial blood pressure or heart rate (12).

The significant difference in PaO_2 observed between 0.8 and 1.0 isoflurane MAC in treatment 5 should not be considered clinically relevant. Both mean values for oxygen tension were above 500 mmHg, and are typical for animals maintained by use of inhaled anesthetics enriched with high concentrations of oxygen. There were no notable outlying data points for either of these observations. Lack of significant changes in other measured variables support the standardization of each pig's physiologic status during recording of BIS throughout the study.

In conclusion, BIS appears to have limited usefulness for monitoring degree of CNS depression in pigs. The apparent EEG activation between 1.6 and 2.0 MAC inhibits the ability to discriminate between mid-levels of isoflurane-induced CNS depression. The BIS was useful for prediction of the point at which suppression of motor responses to noxious stimuli will occur in pigs.

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