Evaluation of four methods for inducing death during slaughter of American alligators (*Alligator mississippiensis*)

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Objective—To evaluate physical methods for inducing death during the slaughter of American alligators (*Alligator mississippiensis*).

Animals—24 captive hatched-and-reared American alligators.

Procedures—Baseline electroencephalograms (EEGs) were obtained for awake and anesthetized alligators. Corneal reflex, spontaneous blinking, and EEGs were evaluated after severance of the spinal cord, severance of the spinal cord followed by pithing of the brain, application of a penetrating captive bolt, or application of a nonpenetrating captive bolt (6 alligators/group).

Results—Overall, alligators subjected to spinal cord severance alone differed from those subjected to the other techniques. Spinal cord severance alone resulted in postprocedure EEG power values greater than those in anesthetized alligators, whereas the postprocedure EEG power values were isoelectric for the other 3 techniques. Corneal reflex and spontaneous blinking were absent in all alligators immediately after application of a penetrating or nonpenetrating captive bolt. One of 6 alligators had a corneal reflex up to 1 minute after pithing, but all others within that group had immediate cessation of reflexes after pithing. Mean time to loss of spontaneous blinking and corneal reflex for alligators subjected to spinal cord severance alone was 18 minutes (range, 2 to 37 minutes) and 54 minutes (range, 34 to 99 minutes), respectively.

Conclusions and Clinical Relevance—Spinal cord severance followed by pithing of the brain and application of a penetrating or nonpenetrating captive bolt appeared to be humane and effective techniques for inducing death in American alligators, whereas spinal cord severance alone was not found to be an appropriate method. (*Am J Vet Res* 2014;75:536–543)

Louisiana is home to a substantial alligator industry that relies on the responsible use of alligators as a natural resource from which hide and meat products are obtained. Annual revenue for the Louisiana alligator industry from the sale of alligator hides and meat is $60 million to $70 million.[1] The development of this industry in the early 1980s is an example of the successful balance that can be achieved between commercial use of reptiles and their conservation. In Louisiana, the population of free-range alligators has recovered from an estimate of < 200,000 animals in the early 1970s to approximately 2 million currently.[2,3] An increase in the veterinary involvement with this industry has led to a better understanding of diseases affecting captive animals and the development of research projects to improve husbandry, nutrition, and well-being of alligators.

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Abbreviation

EEG	Electroencephalogram

A critical component of animal welfare is the humane termination of life, whether for euthanasia, slaughter, depopulation, or other reasons. Methods of euthanasia must cause rapid loss of consciousness followed by cardiac or respiratory arrest and death and minimize pain, distress, and anxiety prior to loss of consciousness.[4] Slaughter is killing animals for the purpose of harvesting commodities such as meat or hides.[4] Although both terms refer to the termination of an animal's life, slaughter has a more specific and directed application.

Methods for terminating an animal's life can be classified as physical or nonphysical. Physical methods include decapitation, cervical dislocation, blunt force trauma to the head (cranial concussion), exsanguination, gunshot, penetrating captive bolt, and nonpenetrating captive bolt. Nonphysical methods include inhalation agents and injectable solutions. Some of these may be used as primary methods for terminating an animal's life, whereas others are only recommended as an adjunct or ancillary method.

Few scientific studies have been conducted to evaluate euthanasia or death during slaughter of reptiles. Most of the information available on this topic is in lay publications, textbooks, or institutional manuals but not in peer-reviewed publications. Techniques used in domestic species are commonly extrapolated and applied to reptiles maintained as pets or research animals, but such extrapolations are less applicable when dealing with reptiles in commercial operations.

Humane termination of reptiles is extremely challenging because of anatomic and physiologic differences between reptiles and mammals. Physical methods of inducing death are often not acceptable for euthanasia of pet reptiles. It is often not possible to place a catheter in a vein for administration of injectable euthanasia agents to reptiles. Even after the use of what is considered a proper euthanasia method, it is often difficult to confirm when death has occurred. The *AVMA Guidelines for the Euthanasia of Animals*[4] indicates it is often difficult to ascertain that an amphibian or reptile is, in fact, dead. Furthermore, those guidelines also indicate that although amphibians and reptiles respond to noxious stimuli and are presumed to feel pain, understanding of their nociception and response to stimuli is incomplete.[4] Some turtle species have a high tolerance to brain anoxia, which makes it difficult to accurately determine the point at which a reptile's brain has ceased to function.[5] Although this anoxia tolerance has been reported in only a few species, it has been postulated that all reptiles share this characteristic. Reflexes such as the corneal reflex are commonly used to monitor reptiles during anesthesia and could be used in the assessment of death, but they are not always consistent in reptiles and cannot be easily assessed in snakes and some gecko species because of the absence of eyelids. Given these challenges for the euthanasia of pet reptiles and those under the supervision of veterinarians, appropriate slaughter and determination of death in reptiles in commercial operations is difficult.

Physical methods to kill alligators have historically consisted of spinal cord severance alone or spinal cord severance followed by pithing of the brain. The *AVMA Guidelines for the Euthanasia of Animals*[4] recognize that although the physiologic response to a given method does not vary, the appropriateness of a method may differ in the context of euthanasia, slaughter, or depopulation. Depopulation and humane slaughter should specifically be addressed in separate AVMA documents that are being developed.[4]

The slaughter of reptiles in commercial operations is also being addressed in Europe, where the Swiss Federal Veterinary Office has provided a report[6] on the analysis of humane methods for killing reptiles used for their skins. The authors of that report[6] took into consideration all the aspects related to reptiles maintained in commercial operations and destined for slaughter. The subject of reptilian slaughter is of global interest because crocodilian hides and meat are harvested in Central and South America, Mexico, the United States, South Africa, Australasia, and Southeast Asia, and the end products of this harvest are manufactured primarily in Europe and Asia.

Crocodilian-specific information about proper methods for slaughter and euthanasia is limited. The use of a captive bolt has been described for the slaughter of caimans in Brazil.[7,8] These reports[7,8] were not clear with regard to whether the captive bolt was penetrating or nonpenetrating. According to the manufacturer of the captive bolt described in those reports,[7,8] the unit operates via a percussive method with or without the use of air injection, and shooting a penetrator against the cortex of an animal causes an injury to its nervous system.[9] The use of a captive bolt for termination of reptiles has been proposed by other sources,[4,10–14] but none have made a distinction between penetrating and nonpenetrating captive bolts.

Other recommended methods for humanely terminating the life of reptiles include concussion of the brain by striking the cranium with a blunt instrument or striking the cranium against a solid object, which is then followed by an adjunct method to ensure the brain is destroyed, if such destruction was not accomplished by the concussion.[11–17] Use of a firearm to shoot into the brain has also been recommended for reptiles.[4,6,10,12–14,16] Decapitation, exsanguination, pithing, and cervical dislocation are often cited as adjunct methods for terminating life after an animal has been rendered unconscious by other methods.[4,6,11–18] Images and diagrams of the correct location for physical destruction of the brain in reptiles and amphibians have been published.[10,19] However, the authors are not aware of any scientific studies in which the physiologic effects of various methods of terminating the life of crocodilians was examined.

Because of the limited information on methods for terminating the life of reptiles and the need to better understand methods for inducing death during slaughter of reptiles at commercial operations, the purpose of the study reported here was to evaluate physical methods to induce death in American alligators (*Alligator mississippiensis*) to determine which methods were most humane and effective. It was believed that the results would be relevant to slaughter methods for reptiles in commercial operations and would supplement information in euthanasia guidelines, which have limited information about appropriate methods for terminating the lives of reptiles.

Materials and Methods

Animals—Twenty-four captive hatched-and-reared American alligators were used in the study. There were 4 males and 20 females. Mean ± SD body length was 146.29 ± 19.98 cm, and mean body weight was 9.72 ± 4.68 kg. Animals at a local alligator ranch were selected and assigned to 4 groups by personnel from the Louisiana Department of Wildlife and Fisheries who did not have prior knowledge of the procedure that would be used on each group of alligators. The only influence of the investigators on the selection of animals was the request that each alligator have a minimum body length of 122 cm. The Louisiana State University Institutional Animal Care and Use Committee approved the study.

Electrode implantation—Twenty-four hours after alligators arrived at our laboratory, they were anesthetized for implantation of EEG recording electrodes. Hydromorphone[a] (0.25 mg/kg; 2 mg/mL of solution), ketamine hydrochloride[b] (2.5 mg/kg), and dexmedetomidine[c] (0.005 mg/kg) were administered IV in the lateral occipital sinus. Following anesthetic induction, a mouth gag that consisted of a polyvinyl chloride pipe (10 cm in length and 5 cm in diameter) was placed and secured with electrical tape. The lingual process of the basihyoid bone was displaced, and a silicone Murphy endotracheal tube (outside diameter, 7 to 10 mm) was inserted with the aid of a metal stylet. The tube was secured with porous tape and connected to an anesthesia machine for provision of supplemental oxygen and ventilation by use of a circular breathing system. Oxygen (100%) was administered, and ventilation was manually performed (8 to 11 breaths/min) to maintain the end-tidal P_{CO_2} between 35 and 45 mm Hg. Transcutaneous needle electrodes were placed to monitor the ECG. An esophageal temperature probe was inserted with the sensor tip positioned at the level of the heart; mean esophageal temperature was maintained at 28.9°C. An electrical patient warming system[d] was used to provide supplemental heat throughout the anesthetic period. Heart rate, end-tidal P_{CO_2}, esophageal temperature, respiratory rate, lead II ECG, and inspiratory fraction of oxygen were monitored with a multifunction monitor.[e]

Holes were drilled through the skin and skull with a motorized hobby drill and 2-mm-diameter bit to a depth (approx depth, 4 mm) sufficient for EEG electrode screws to be securely anchored. The skin is tightly adhered to the skull in alligators, so no effort was made to retract it prior to drilling. Electrodes consisted of self-tapping stainless steel screws[f] (diameter, 2.8 mm; length, 6.4 mm) that had previously been soldered to a short length of wire that terminated in a female electrical connector for subsequent connection to an EEG machine. Four screws were placed in positions overlying the brain (left rostral, F_3; right rostral, F_4; left caudal, C_3; and right caudal, C_4), with a ground electrode positioned between the orbital ridges (**Figure 1**). International 10-20 system electrode designations were used for convenience, and cortical locations were only approximations. After the screws were implanted, an EEG recording was obtained. At the end of the EEG recording, dexmedetomidine was reversed with atipamezole[g] (0.025 mg/kg, IM). The alligators were extubated and allowed to recover from anesthesia; they then were returned to a holding enclosure. Alligators received meloxicam[h] (0.5 mg/kg, IM) daily for postoperative analgesia after electrode implantation.

Procedures—Alligators were killed ≥ 2 days after implantation of the electrodes to ensure there were no lasting effects of the anesthetic drugs that would affect EEG recordings. Six animals were assigned by use of a randomization procedure (block randomization) to undergo 1 of 4 procedures to induce death: severance of the spinal cord and blood vessels supplying the head, severance of the spinal cord and blood vessels supplying the head followed by pithing of the brain, application of a penetrating captive bolt, and application of a nonpenetrating captive bolt. An investigator (JGN) who had 10 years of experience working with alligators, was trained in the use of the equipment, and had prior experience in euthanasia of alligators performed all procedures to ensure consistency. Alligators were not sedated or tranquilized prior to procedures. The alligators were manually restrained in ventral recumbency by assistants who placed a hand on the caudal aspect of the neck and a second hand on the pelvic area; manual restraint was necessary to ensure correct application of each method.

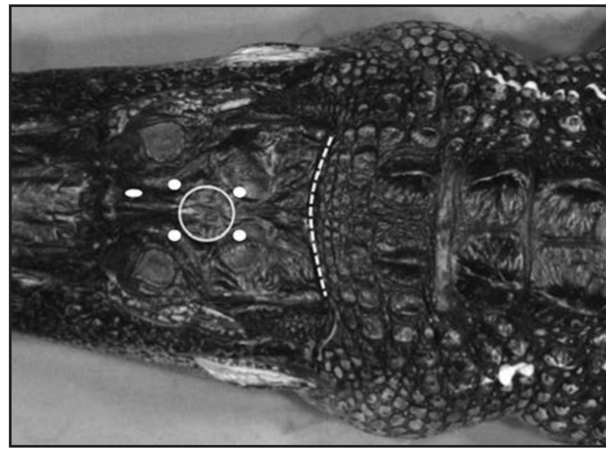

Figure 1—Photograph of the head of an American alligator (*Alligator mississippiensis*) indicating the location for spinal cord severance (dotted line) or application of a captive bolt stunner or gunshot (circle); location for the captive bolt stunner or gunshot was intended to ensure destruction of the brain. The location for EEG electrodes (white circles) and a ground electrode (white oval) used in the study are also indicated.

A knife[i] (4-inch drop point blade) was used to sever the spinal cord. The knife was inserted just caudal to the head at the base of the skull so that the cord was severed at the atlanto-occipital joint (Figure 1). The knife was inserted in a single, swift motion that severed the spinal cord and lateral occipital sinuses, which supply blood to the head. Severance of the spinal cord was verified immediately after the cutting motion was completed. For the severance-pithing group, the spinal cord and blood vessels were severed as described, which was followed immediately (within 3 seconds) by pithing of the brain by use of a 6-mm-diameter metal rod with a sharp point. The rod was inserted through the foramen magnum to the most rostral point of the brain cavity. Immediately after insertion, the rod was rotated 360° along the edges of the foramen magnum 5 times to destroy the brain tissue. Severance of the cord in this group was confirmed after pithing. A commercial stunner[j] fitted with a penetrating rod (1.1 cm in diameter and 12 cm in length) was used as the penetrating captive bolt. The same stunner[j] was used for the nonpenetrating captive bolt, but the rod had a larger (3.81 cm in diameter) nonpenetrating concussion head. The stunner was powered by a gunpowder charge (0.25 caliber and 1.25 grain). The stunner was a new unit that was stored, cleaned, and maintained as per the manufacturer's recommendations and used in accordance with directions provided by a US distributor to ensure consistency in methods. For both captive bolt groups, the stunner was placed directly over the brain at a point located on the midline immediately behind the caudal aspect of the eyes.

EEG recordings—Recordings were made at 3 time points: during anesthesia at the end of electrode implantation, immediately before the procedure, and immediately after the procedure. Recordings immediately before the procedure were obtained from awake unsedated alligators unrestrained in a tub enclosure. Recordings were continued after the procedure until the EEG became an isoelectric line. Recordings were acquired with a digital EEG system[k] and stored for later analysis. Recordings were collected for pairs of electrodes (F_3F_4, F_4C_4, C_3C_4, and F_3C_3) that circled the area above the brain.

In addition to EEG recordings, corneal reflexes and spontaneous blinking were monitored during the postprocedure period until EEG recordings were isoelectric and reflexes had ceased. The alligators were declared dead when the EEG recordings were isoelectric, spontaneous blinking had ceased, and the corneal reflex was absent. Reflexes were reassessed 1 hour after the procedure to ensure the alligators had not recovered.

Data analysis—Descriptive data were calculated for the time to loss of spontaneous blinking and corneal reflex. Frequency domain power spectrum analyses were performed on recordings from the electrode pair for the left hemisphere (F_3C_3) unless there was excess artifact, in which case recordings for the electrode pair for the right hemisphere (F_4C_4) were used. Three 10-second artifact-free epochs were analyzed for each alligator at each time period (during anesthesia at the end of electrode implantation, awake and unrestrained before the procedure, and after the procedure) and for alligators that had an EEG that did not become isoelectric immediately after the procedure (these data were arbitrarily chosen to be recordings obtained 15 minutes after the procedure).

Power spectrum analysis was performed with commercial software[l] widely used in human neurology. Binned analysis was used with Hamming windows, with 256 points/window and 50% overlapping windows. Frequency bins were grouped into traditional EEG frequency bands of δ (1 to 4 Hz), θ (4 to 8 Hz), α (8 to 13 Hz), and β (13 to 30 Hz). The EEG power was expressed in microvolts, and the analysis was analyzed over the range of 0 to 32 Hz. Results were analyzed by use of the absolute total power in each frequency band. Results were analyzed by use of linear mixed models with absolute power in each wave frequency band as the outcome variable, alligators as a random effect, and time period and method as fixed effects. The interaction between time period and method was also included as a fixed effect. Various covariance structures were incorporated into the models, which were compared with the simpler models by use of standardized residual plots and Akaike information criterion. Residual plots were used to assess linearity, homogeneity of variances, normality, and outliers. Shapiro-Wilk tests and analysis of quantile plots were conducted on the residuals of each group. Residuals were not normally distributed for standard models, but models with logarithmically transformed outcome variables had normally distributed residuals. A type III ANOVA was performed on the fixed effects. When interaction terms were not significant, main effects were reported. Post hoc comparisons were performed with a Tukey adjustment. Numeric results were back-transformed for reporting. Analyses were performed with a statistical program[m] for linear mixed modeling and contrasts. Values of $P < 0.05$ were considered significant.

Results

Intermittent seizure activity was evident in the EEG of alligators subjected to severance of the spinal cord for up to 30 minutes after the procedure. In the other groups, the EEG was isoelectric immediately after the procedure. Corneal reflex and spontaneous blinking were absent in all alligators immediately after the procedure with a penetrating captive bolt or nonpenetrating captive bolt. One of 6 alligators subjected to severance of the spinal cord followed by pithing of the brain had a corneal reflex until 1 minute after pithing, but all other alligators in that group had immediate cessation of reflexes.

Mean time until loss of spontaneous blinking in the group subjected to severance of the spinal cord was 18 minutes (range, 2 to 37 minutes). In the same group, the mean time until loss of corneal reflex after severance of the spinal cord was 54 minutes (range, 34 to 99 minutes). Time to death based on an isoelectric EEG coincided with loss of corneal reflex in this group.

In the EEG analysis, a single data point (for an awake and unsedated alligator prior to application of a penetrating captive bolt) was removed as an outlier because the standardized residual was > 6. There was a significant effect of the interaction between method and time period for all EEG frequency bands (all $P < 0.001$), except for the δ frequency band ($P = 0.18$).

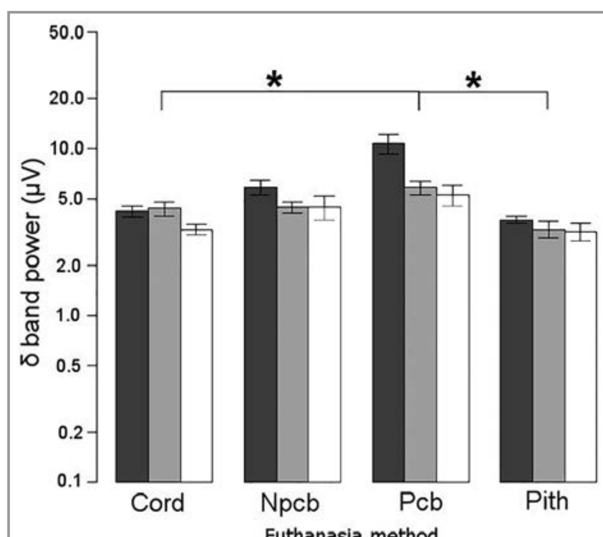

Figure 2—Power measurements for the δ frequency band obtained during each of 3 time periods (during anesthesia at the end of implantation of EEG electrodes [light gray bars], while awake and unsedated before a procedure to induce death [dark gray bars], and immediately after the procedure [white bars]) from alligators killed by each of 4 methods (6 alligators/method). There was an interval of ≥ 2 days between implantation of electrodes and procedures. The 4 methods of inducing death were as follows: severance of the spinal cord and blood vessels supplying the head (Cord), severance of the spinal cord and blood vessels supplying the head followed by pithing of the brain (Pith), application of a penetrating captive bolt (Pcb), and application of a nonpenetrating captive bolt (Npcb). *Values differ significantly ($P < 0.05$) between methods within a time period.

A positive interaction effect indicated that the power differed significantly between time periods within each group, whereas a nonsignificant interaction effect indicated that the power was similar between time periods within each group.

Power for the δ frequency band for alligators subjected to application of a penetrating captive bolt was significantly higher than that for alligators subjected to severance of the spinal cord alone (geometric mean of the difference, 1.60 μV; $P = 0.03$) or severance of the spinal cord followed by pithing of the brain (geometric mean of the difference, 1.89 μV; $P = 0.001$; **Figure 2**). There also was a significant ($P = 0.002$) effect of time period on results for the δ frequency band.

Power for the θ frequency band differed significantly among all time periods within each method, except for severance of the spinal cord followed by pithing of the brain, for which there was not a significant ($P = 0.99$) difference between the value obtained during anesthesia at the end of electrode implantation and after the procedure (**Figure 3**). The same pattern was evident among methods, with the EEG power for the θ frequency band highest for awake unsedated alligators before the procedure, next highest during anesthesia at the end of electrode implantation, and lowest after the procedure, except for alligators undergoing severance of the spinal cord alone, for which the power after the procedure was higher than during anesthesia at the end of electrode implantation.

Power for the α frequency band differed significantly among all time points within each method, except for severance of the spinal cord followed by pithing of the brain, for which there was not a significant ($P = 0.91$) difference between the values obtained during anesthesia at the end of electrode implantation and after the procedure (**Figure 4**). The same pattern was evident among methods, with the EEG power for the α frequency band highest for awake unsedated alligators before the procedure, next highest during anesthesia at the end of electrode implantation, and lowest after the procedure, except for alligators undergoing severance of the spinal cord alone, for which the power after the procedure was higher than during anesthesia at the end of electrode implantation.

Power for the β frequency band differed significantly among all time periods within each method, except for severance of the spinal cord alone, for which there was not a significant ($P = 0.52$) difference between the values obtained for awake unsedated alligators and after the procedure (**Figure 5**). Overall, values for alligators undergoing severance of the spinal cord alone differed from values for the other methods in that power values for the β frequency band were greater af-

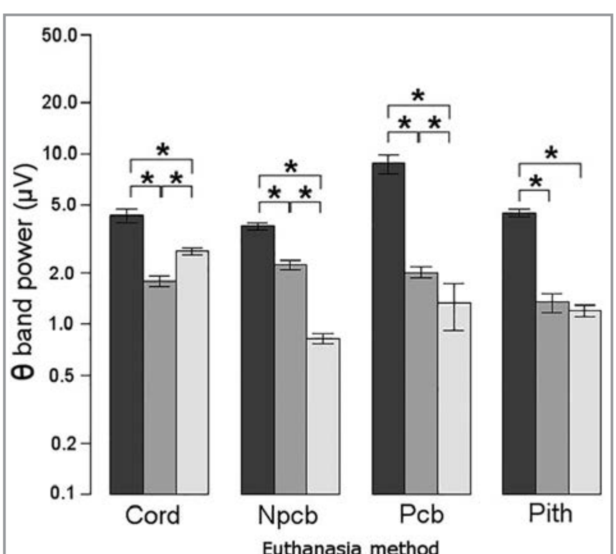

Figure 3—Power measurements for the θ frequency band obtained during each of 3 time periods from alligators killed by each of 4 methods (6 alligators/method). *Values differ significantly ($P < 0.05$) between time periods within each method. *See* Figure 2 for remainder key.

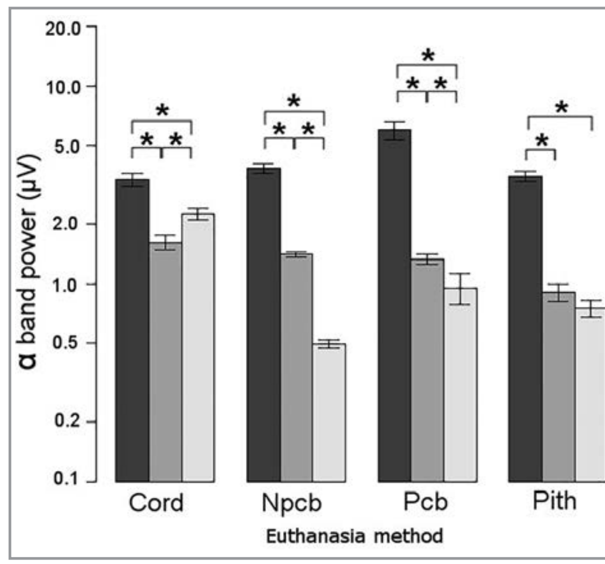

Figure 4—Power measurements for the α frequency band obtained during each of 3 time periods from alligators killed by each of 4 methods (6 alligators/method). *See* Figures 2 and 3 for remainder of key.

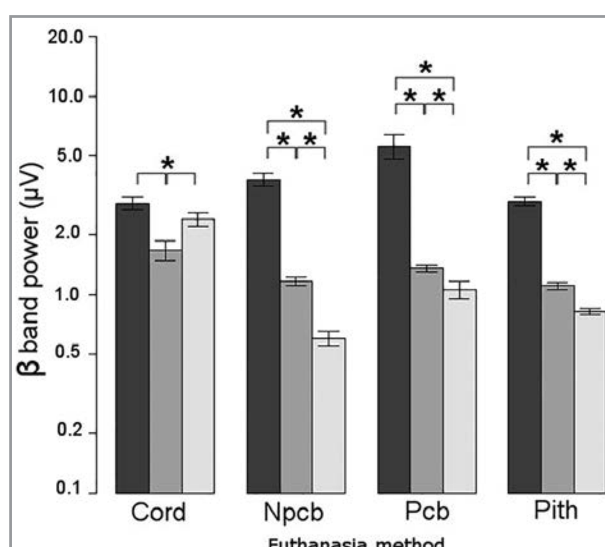

Figure 5—Power measurements for the β frequency band obtained during each of 3 time periods from alligators killed by each of 4 methods (6 alligators/method). *See* Figures 2 and 3 for remainder of key.

ter the procedure than during anesthesia at the end of electrode implantation.

Discussion

Termination of an animal's life should be performed as humanely as possible. This can be a challenge to evaluate in some animals, particularly reptile species, because of the difficulties in the ability to measure awareness or consciousness. Although consciousness in a broader sense is difficult to assess or even define in precise terms,[20] in medicine, loss of consciousness usually refers to loss of awareness of surroundings and alertness to events,[21] and anesthesia involves loss of awareness and insensitivity to pain.[22]

Little information is available for defining consciousness in reptiles. Investigators have evaluated brain activity in various reptile species in a number of studies,[23–26] but most evaluations have been of a qualitative rather than quantitative nature. The clinical usefulness of the EEG in mammals relies in part on the fact that specific frequency bands are associated with various behavioral states, and this is also presumed to be relevant in reptiles. A predominant β frequency EEG pattern (ie, high frequency and low amplitude) is associated with an alert awake state. The EEG pattern for the α frequency band (ie, lower frequency and higher amplitude) is associated with an awake relaxed state, especially when the eyes are closed. Patterns for the δ and θ frequency band have increasingly lower frequencies and higher amplitudes and are associated with deeper stages of sleep, anesthesia, or coma. When an animal goes from an awake state to a deep sleep state, the EEG changes from low amplitude and high frequency (pattern for β frequency ban) to high amplitude and low frequency (patterns for δ and θ frequency bands). As a result, there are shifts in the relative power in the 4 frequency bands with these changes in the state of consciousness. In brain-dead animals, the EEG is essentially flat (isoelectric) with little if any power in any of the frequency bands[27]; thus, the absolute EEG power is substantially reduced in comparison to that of live animals in awake, asleep, or anesthetized states. An EEG recording may be used as an index of consciousness, but interpretation of EEG recordings, especially those of reptiles, can be challenging and should be performed only by qualified individuals.

In the present study, baseline EEGs were obtained for each alligator during an awake unsedated state and during anesthesia at the end of electrode implantation, and measured values were then compared with values on postprocedure EEGs. By definition, sedation is a state of depression of the CNS and drowsiness with the patient unaware of its surroundings but responsive to painful stimuli.[28] General anesthesia is defined as induced unconsciousness characterized by controlled reversible depression of the CNS and analgesia. Anesthetized patients cannot be aroused, and the reflex functions are attenuated.[28] Therefore, we relied on EEG recordings of anesthetized alligators as an indirect measure for being unaware or unconscious. We acknowledge that this may have been a simplistic approach to a complicated process, but we believed it was the most objective measure available. We were confident with the assertion that the alligators were unaware of their surroundings during anesthesia on the basis of decreased or absent reflexes (corneal reflex and righting reflex) and results for the physiologic variables monitored (heart rate, ECG, spontaneous blinking, and responses to superficial and deep painful stimuli). None of the alligators reacted during the drilling procedure for placement of the electrodes. There was a clear visual and significant difference between the EEG recordings of the alligators when awake and unsedated and when anesthetized, which provided further credibility to our assessment that the anesthetized alligators had a depressed CNS.

Assessing the humaneness of a procedure is problematic. In the present study, specific criteria were used to classify a procedure to end life as humane. The first was depression of CNS activity immediately after the procedure to a value below that of an anesthetized state. The second was the absence of any reflexes. In other words, we defined a method of inducing death to be humane if the EEG waves after the procedure were depressed more than those during anesthesia and if the corneal reflex and spontaneous blinking were absent. Quantification of the EEG recordings allowed us to provide an objective measure of CNS depression.

Severance of the spinal cord was selected for evaluation as a means of inducing death during slaughter because it has been used at commercial crocodilian operations. The efficacy and humaneness of spinal cord severance and subsequent exsanguination have been questioned. Spinal cord severance and exsanguination are generally accepted as an adjunctive method for euthanasia but not as the sole method.[4]

Severance of the spinal cord followed by pithing of the brain was also selected for evaluation as a means of inducing death during slaughter because it has been used at commercial crocodilian operations. The efficacy and humaneness for spinal cord severance followed by pithing of the brain and subsequent exsanguination have not been reported. Pithing is considered acceptable as an adjunctive method for euthanasia.[4]

Use of captive bolts as a means of inducing death during slaughter of crocodilians has been described.[6,7] However, those reports[6,7] do not distinguish between penetrating and nonpenetrating captive bolts. Therefore, we elected to use both penetrating and nonpenetrating captive bolts to better evaluate their use, efficacy, and humaneness for inducing death in alligators. Use of a nonpenetrating captive bolt is recommended as an adjunctive method rather than the sole method for inducing death during slaughter of domestic livestock,[4] but we were interested in determining whether this technique could be used as the sole method for humanely killing alligators. For all methods of euthanasia and death during slaughter, human safety, reproducibility, consistency, and ease of application need to be considered. Alligators can inflict serious injuries to humans; therefore, restraint of the alligators is always necessary to ensure safety of the personnel. In addition, restraint allows for more consistent application, which in turn improves reproducibility and consistency. Finally, we selected methods that may have practical applications in commercial crocodilian operations and that operators would be willing and able to incorporate into current management practices.

Analysis of results for the present study revealed that severance of the spinal cord alone was not able to totally depress EEG waves. For severance of the spinal cord alone, EEG power in the α, β, and θ frequency bands was higher after the procedure than during anesthesia. The alligators retained reflexes for an extended period and had repeated periods of electrical seizure activity that could not be expressed by motor activity because of the severed spinal cord. Because this method failed to meet our definition for a humane procedure, it cannot be recommended and should not be used in alligators.

In 1 alligator killed by severance of the spinal cord followed by pithing of the brain, a corneal reflex was detected for up to 1 minute after pithing, despite the fact the EEG was isoelectric. We believe inconsistency or operator error in application of the method for this particular alligator contributed to this outcome. Pithing was delayed for 8 seconds after severance of the spinal cord because of an abnormal cutting of the cord, which delayed the pithing process. It is also possible that pithing was not performed appropriately or in the same manner as for other alligators in this group, which would have led to this unusual observation. As with any method of euthanasia or death during slaughter, consideration should be given to appropriate selection of equipment and training of operators to ensure repeatability and consistency of the method. Equipment should be serviced and maintained as per manufacturer's recommendations to ensure proper functioning. Equipment such as knives and metal rods should be maintained (eg, sharp knife blades and tips of metal rods), inspected for structural integrity, and evaluated to ensure that they are adequate and appropriate for performance of the procedures.

Investigators in another study[29] concluded that severance of the spinal cord of alligators did not cause rapid loss of consciousness, which is consistent with the results of the present study. However, the method for spinal cord severance in that previous study[29] involved the use of a chisel and a steel hammer that required 5 to 8 strikes to sever the cord. This differs notably from the method used in the study reported here. Furthermore, investigators in that previous study[29] relied solely on the assessment of reflexes, including those observed caudal to the severed cord, which do not accurately reflect information about awareness, consciousness, or humaneness of the procedure.

Decapitation, spinal cord severance, and procedures that do not involve destroying brain tissue are reported to be inhumane in reptiles because of the high tolerance of reptiles for hypoxia.[15,29] Fresh water turtles and Crucian carp (*Carassius carassius*) have the highest anoxia tolerance of vertebrates.[5,30] Most studies[31–34] on hypoxia or anoxia tolerance in reptiles have focused on fresh water turtles, specifically western painted turtles (*Chrysemys picta bellii*) and red-eared slider turtles (*Trachemys scripta*). However, investigators in 1 study[35] detected complete recovery of brainstem auditory evoked potentials in American alligators after sustained periods of hypoxia; hypoxia was induced by inverting the alligators, which substantially reduces blood flow through the carotid artery and causes an isoelectric response. Although it has probably been appropriate to assume that all reptiles have an anoxia tolerance similar to that of turtles, this has led to a lack of scientific data to support or reject such an assumption. Researchers and clinicians must be careful when extrapolating information across species and conducting studies that can prove or disprove current theories. The results of the present study provide further evidence to support the long-standing belief that severance of the spinal cord alone is not appropriate in reptiles, specifically alligators. These results clearly indicated the presence of brain activity for > 1 hour after spinal cord severance. Although it was not possible to ascertain what amount of this brain activity represented awareness or consciousness, it clearly was not within the boundaries that would be considered humane.

Results of the present study suggested that application of a penetrating or nonpenetrating captive bolt or severance of the spinal cord followed by pithing of the brain are humane, effective, and acceptable methods of inducing death that can be used for the slaughter and potentially euthanasia of alligators. However, severance of the spinal cord without destruction of the brain cannot be considered a humane method of inducing death in alligators. Humane methods must achieve nonreversible damage or destruction of the brain tissue to ensure the death of an animal. Furthermore, commercial operators should work closely with veterinarians or other qualified personnel to design appropriate slaughter plans for each facility. This plan should include training of personnel and proper use and maintenance of equipment to ensure appropriate application of the method chosen for inducing death during slaughter. This is especially important for the pithing method, given it is a 2-step procedure, to minimize operator error. The information obtained from the present study has also been used to revise the best management practices for Louisiana alligator farming[36] to ensure that procedures are up to date and conform to the most currently available scientific information.

a. West-Ward Pharmaceutical Corp, Eatontown, NJ.
b. Veta Ket, 100 mg/mL, Lloyd Laboratories, Shenandoah, Iowa.
c. Dexdomitor, 0.5 mg/mL, Pfizer Animal Health, New York, NY.
d. Hot Dog warming system, Augustine Temperature Management, Eden Prairie, Minn.
e. Datascope Passport2, Datascope Corp, Mahwah, NJ.
f. Small Parts Inc, Logansport, Ind.
g. Antisedan, 5 mg/mL, Pfizer Animal Health, New York, NY.
h. Metacam, 1.5 mg/mL, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
i. Omni Hunter 12 pt, Buck, Post Falls, Idaho.
j. Cash Special, Accles & Shelvoke Ltd, Sutton Coldfield, West Midlands, England.
k. Cadwell Easy II EEG system, Cadwell Laboratories Inc, Kennewick, Wash.
l. Insight II EEG review software, Persyst Development Corp, Prescott, Ariz.
m. Comprehensive R archive network, version 2.12.1, The R Foundation for Statistical Computing, Vienna, Austria. Available at: cran.r-project.org/. Accessed Aug 1, 2012.

References

1. Louisiana Department of Wildlife and Fisheries. Louisiana's Alligator Management Program 2012–2013 Annual Report. Available at: www.wlf.louisiana.gov/sites/default/files/pdf/page_wildlife/32693-Alligator%20Program%20Annual%20Reports/ldwf_-_la.s_alligator_mgmt_program_-_dec_2013.pdf. Accessed Jan 3, 2014.
2. Joanen T, McNease L. The management of alligators in Louisiana, USA. In: Manolis SC, Webb GJW, Whitehead PJP, eds. *Wildlife management: crocodiles and alligators*. Chipping Norton, NSW, Australia: Surrey Beatty & Sons, 1987;33–42.
3. Louisiana Department of Wildlife and Fisheries. Alligator program: general alligator information. Available at: www.wlf.louisiana.gov/general-alligator-information. Accessed Aug 13, 2013.
4. AVMA. AVMA guidelines for the euthanasia of animals; 2013 revision. Available at: www.avma.org/KB/Policies/Documents/euthanasia.pdf. Accessed Jul 29, 2013.
5. Overgaard J, Gesser H, Wang T. Tribute to P. L. Lutz: cardiac performance and cardiovascular regulation during anoxia/hypoxia in freshwater turtles. *J Exp Biol* 2007;210:1687–1699.
6. Swiss Confederation Federal Veterinary Office. Analysis on humane killing methods for reptiles in the skin trade. Available at: www.bvet.admin.ch/themen/tierschutz/04013/index.html?lang=en. Accessed Jul 29, 2013.
7. Aleixo VM. Use of captive bolt pistol for humane slaughter of crocodilians. *Crocodile Specialist Group Newsletter* 2008;27(4):24–25.
8. Campos Z. The "Zilka", a new device for humane killing of crocodilians. *Crocodile Specialist Group Newsletter* 2000;19(1):20–21.
9. GIL Equipamentos. Stunner ZILKA product information. Available at: www.gil.com.br/eng/produtos_mostra.php?id=3. Accessed Jul 29, 2013.
10. Mader DR. Euthanasia. In: Mader DR, ed. *Reptile medicine and surgery*. 2nd ed. St Louis: Saunders Elsevier, 2006;564–568.
11. Baier J. Reptiles. In: Baer CK, ed. *American Association of Zoo Veterinarians guidelines on euthanasia of non-domestic animals*. Yulee, Fla: American Association of Zoo Veterinarians, 2006;40–43.
12. Baines FM, Davies R. Euthanasia of reptiles. *Vet Times* 2004;29(2):60–72.
13. Close B, Banister K, Baumans V, et al. Recommendations for euthanasia of experimental animals: part 1. *Lab Anim* 1996;30:293–316.
14. Close B, Banister K, Baumans V, et al. Recommendations for euthanasia of experimental animals: part 2. *Lab Anim* 1997;31:1–32.
15. Cooper JE. Humane euthanasia and post-mortem examination. In: Raiti P, Girling SJ, eds. *BSAVA manual of reptiles*. 2nd ed. Gloucester, England: British Small Animal Veterinary Association, 2004;168–183.
16. Sharp T, Saunders G. Methods of euthanasia: GEN001. Available at: nrmonline.nrm.gov.au/catalog/mql:1599. Accessed Jul 29, 2013.
17. United Kingdom House of Commons. The humane killing of animals under schedule 1 to the Animals (Scientific Procedures) Act 1986; code of practice. Available at: www.official-documents.gov.uk/document/hc9697/hc01/0193/0193.asp. Accessed Jul 29, 2013.
18. McArthur S. Anesthesia, analgesia, and euthanasia. In: McArthur S, Wilkinson R, Meyer J, eds. *Medicine and surgery of tortoises and turtles*. Oxford, England: Blackwell Publishing, 2004;398–400.
19. Universities Federation for Animal Welfare and the World Society for the Protection of Animals. *Euthanasia of amphibians and reptiles: report of a joint UFAW/WSPA working party*. London: World Society for the Protection of Animals, 1989;19–35.
20. Sutherland NS. Consciousness. In: Sutherland SN, ed. *International dictionary of psychology*. 2nd ed. New York: Crossroad, 1996;95.
21. Kearse LA Jr, Rosow C, Zaslavsky A, et al. Bispectral analysis of the electroencephalogram predicts conscious processing of information during propofol sedation and hypnosis. *Anesthesiology* 1998; 88:25–34.
22. American Society of Anesthesiologists Task Force on Intraoperative Awareness. Practice advisory for intraoperative awareness and brain function monitoring: a report by the American Society of Anesthesiologists Task Force on Intraoperative Awareness. *Anesthesiology* 2006;104:847–864.
23. Huggins SE, Parsons LC, Peña RV. Further study of the spontaneous electrical activity of the brain of *Caiman sclerops*: olfactory lobes. *Physiol Zool* 1968;41:371–383.
24. Bartol SM, Musick JA, Lenhardt ML. Auditory evoked potentials of the loggerhead sea turtle. *Copeia* 1999;3:836–840.
25. Hunsaker D, Lansing RW. Electroencephalographic studies of reptiles. *J Exp Zool* 1962;149:21–32.
26. González J, Gamundi A, Rial R, et al. Nonlinear, fractal, and spectral analysis of the EEG of lizard, *Gallotia galloti*. *Am J Physiol Regul Integr Comp Physiol* 1999;277:R86–R93.
27. Wijdicks EFM. Neurology of brain death. In: Wijdicks EFM, ed. *Brain death*. 2nd ed. New York: Oxford University Press, 2011;27–68.
28. Thurmon JC, Short CE. History and overview of veterinary anesthesia and analgesia. In: Tranquilli WJ, Thurmon JC, Grimm KA, eds. *Lumb and Jones' veterinary anesthesia and analgesia*. 4th ed. Ames, Iowa: Blackwell Publishing, 2007;5.
29. Warwick C. Crocodilian slaughter methods with special reference to spinal cord severance. *Tex J Sci* 1990;42:191–198.
30. Lutz PL, Nilsson GE. Contrasting strategies for anoxic brain survival—glycolysis up or down. *J Exp Biol* 1997;200:411–419.
31. Ultsch GR, Jackson DC. Long-term submergence at 3°C of the turtle, *Chrysemys picta bellii*, in normoxic and severely hypoxic water: I. Survival, gas exchange and acid-base status. *J Exp Biol* 1982;96:11–28.
32. Herbert CV, Jackson DC. Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta bellii*. I. Blood acid-base and ionic changes during and following anoxic submergence. *Physiol Zool* 1985;58:655–669.
33. Bickler PE, Buck LT. Hypoxia tolerance on reptiles, amphibians, and fishes: life with variable oxygen availability. *Ann Rev Physiol* 2007;69:145–170.
34. Jackson DC. Hibernating without oxygen: physiological adaptations of the painted turtle. *J Physiol* 2002;543:731–737.
35. Strain GM, Tucker TA, Graham MC, et al. Brain-stem auditory evoked potentials in the alligator. Effects of temperature and hypoxia. *Electroenceph Clin Neurophysiol* 1987;67:68–76.
36. Louisiana Department of Wildlife and Fisheries and the Louisiana State University School of Veterinary Medicine. Best management practices for Louisiana alligator farming. Available at: filestogeaux.lsu.edu/public/download.php?FILE=jnevare/315265HJNtF. Accessed Jan 22, 2014.