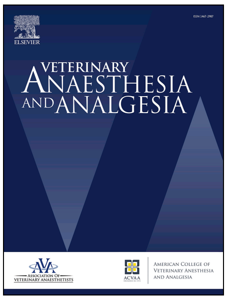

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RESEARCH STUDY

Mandibular nerve block in juvenile Nile crocodile: a cadaveric study

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Running title

Mandibular nerve block in Nile crocodiles

Authors' contribution

CB, HD, PM: study design, data collection, analysis and interpretation, manuscript preparation

CA: study design, statistical analysis and interpretation, manuscript preparation, editing

AC, DD: study design, critical appraisal of the manuscript

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Conflict of interest statement

The authors declare no conflict of interest

MAIN DOCUMENT

Abstract

Objective To develop a technique for performing the mandibular nerve block in Nile crocodiles.

Study design Experimental cadaveric study.

Animals A group of 16 juvenile Nile crocodile heads.

Methods To study the course of the mandibular nerve, one head was dissected. Computed tomography (CT) examination was performed in two heads to identify useful landmarks. Thereafter, a hypodermic needle was inserted through the external mandibular fenestra of 17 hemimandibles (13 heads) and a mixture of methylene blue and iohexol was injected. Injection volumes were 0.5 (n = 7) and 1.0 mL (n = 10) for hemimandibles < 15 and ≥ 15 cm long, respectively. Iohexol spread and nerve staining with methylene blue were assessed with CT and anatomical dissection, respectively. Data were analysed with either one sample t-test or Mann-Whitney Rank Sum test. $p <$ 0.05

Results Both anatomical dissection and imaging confirmed the external mandibular fenestra as a useful anatomical landmark for needle insertion. The CT images acquired after needle positioning confirmed that its tip was located on the medial bony mandibular surface formed by the fusion of the angular and coronoid bone in 100% of the cases. In all the hemimandibles, the rostro-caudal spread of contrast was greater than 23 mm. The length of the stained mandibular nerve in the temporal region and of the stained medial branch of the mandibular nerve, as well as the dorso-ventral and medio-lateral spread of iohexol, was greater in group 1.0 than in group 0.5 ($p < 0.001$). The caudal spread of iohexol was greater in group 1.0 than in group 0.5 ($p = 0.01$).

Conclusions and clinical relevance The technique developed in this study is feasible. Both injection volumes resulted in staining of the mandibular nerve. The spread of contrast in the anatomical region of interest may result in successful sensory block.

Keywords crocodilians, mandibular nerve, nerve block, Nile crocodile, trigeminal nerve

Introduction (

Little is known about the pharmacokinetics and pharmacodynamics of analgesic drugs in reptiles. Doses and the reported intervals of administration are often extrapolated from other species, based on the assumption that the analgesics would be effective regardless of biodiversity (Whiteside 2014). Moreover, nociception and pain are challenging to recognise in reptiles and, therefore, likely to be undertreated (Mosley 2011). In support of this hypothesis, a recent survey revealed that only 39.5% of the practitioners dealing with these animal species routinely provide analgesia to them (Read 2004).

In such a scenario, the use of local anaesthetics might produce antinociception and improve pain management in reptiles and enhance animal welfare. Local anaesthetics act by interrupting sensory and motor transmission from the peripheral to the central nervous system, thus decreasing the requirement for systemic analgesic drugs (Mosley 2011). The popularity of locoregional anaesthesia has been increasing over the last decade both in mammals and reptiles. Although, in most of the published work involving reptiles, the effectiveness of locoregional anaesthesia is anecdotally reported rather than evidence-based (d'Ovidio & Adami 2019). Despite the lack of species-specific pharmacokinetic information , the literature suggests that locoregional anaesthetic techniques may be used in reptiles to a similar extent to their use in mammals (Rivera et al. 2011; Chatigny et al. 2016; Ferreira & Mans 2019). Local anaesthetics may have onsets and durations of action in these species that are similar to those reported in other vertebrates (Perry & Nevarez 2018; Mans et al. 2019).

Regarding the mandibular nerve block, little information is published describing its clinical use in crocodilians, for either surgical or diagnostic procedures (Wellhan et al. 2006). An earlier study reported the use of the electrolocation to perform this nerve block in crocodilian species other than Nile crocodiles (Wellehan et al. 2006). The technique was considered effective, based on behavioural indicators, in American alligators (*Alligator mississippiens*), dwarf crocodiles (*Osteolaemus tetrapis*) and Yacare caimans (*Caiman yacare*); however, no clear conclusions could be drawn for Nile crocodiles. Furthermore, no cadaveric studies were conducted prior to the clinical trial in order to identify useful landmarks and potential complications of this nerve block in the living animal.

The primary aim of this study was to describe the course of the mandibular nerve in Nile crocodiles and identify useful anatomical landmarks to enable the mandibular nerve block to be performed in this species. A secondary aim was to assess macroscopic nerve staining, as well as the spread of both the dye and the contrast medium in the anatomical area of interest.

Materials and methods

The study was conducted under ethical approval of the Clinical Research Ethical Review Board (CRERB) of the Royal Veterinary College of the University of London (licence number: URN 2019 1874-3).

A total of 16 thawed juvenile Nile crocodile heads, euthanized 1 year earlier for reasons unrelated to the current study and frozen immediately thereafter, were used. All the anatomical tissues used in this study were from animals weighing between 1.75 and 6 kg euthanized and 32 months or less in age. The body length, measured from the nares to the distal end of the tail, was less than 120 cm.

The length of each mandible from the rostral to the caudal limit was measured with a ruler using a line that bisected the external mandibular fenestra. Based on this, the heads were classified as either large (mandibles ≥ 15 cm long) or small (mandibles < 15 cm long).

Phase I: anatomical and tomographic study

In this phase, three heads, and a total of six hemimandibles, were used. In order to expose the mandibular nerve, one head was dissected, and the course of the nerve was studied in addition to the anatomy of the region of interest. Dissection of the orbit and the temporal region allowed visualisation of the ophthalmic, maxillary and mandibular branches of the trigeminal nerve. Medial and lateral dissection of the mandible were performed to evaluate the mandibular nerve in relation to the external and internal mandibular fenestrae.

A computed tomography (CT) examination of two heads (one large and one small) was performed to identify landmarks that could be used to enable the mandibular nerve block to be performed. A 16 mm long 21 gauge hypodermic needle (Henry Schein Inc., NY, USA) was then inserted percutaneously by the same investigator (CB) through the rostro-dorsal portion of the external mandibular fenestra of the hemimandibles. The needle was advanced, at a 45° caudo-rostral angle, until the tip reached the medial surface formed by the fusion of the angular and coronoid bones (Fig. 1). Thereafter, a CT examination was performed to confirm the correct position of the needle tip, which was anticipated to be in close proximity to the mandibular nerve. CT scans were acquired using a 320 multi-detector row unit (Canon Aquilion One Genesis Edition; Canon Medical Systems Ltd, UK). The heads were placed in a dorsal or ventrally recumbent position and their entirety was included in the scanned field of view. Technical variables were standardized for all scans and included: helical scan mode, reconstruction slice thickness 0.5 mm, tube rotation time 1s, 120 kVp, 120 mA, 512 × 512 image matrix, high frequency spatial reconstruction algorithm. Images were reviewed using commercially available viewing software (OsiriX v.6.5.2. 64bit; Pixmeo SARL, Switzerland).

Phase II: injection and contrast-dye spread evaluation

In this phase, 13 heads (17 hemimandibles) were used, which were divided according to their size into those < 15 cm in length and ≥ 15 cm long. The injectate solution was prepared by mixing methylene blue (Ethyl-thionium Chloride Injection 1% w/v; Martindale Pharmaceuticals Ltd, UK) with iohexol (Omnipaque, 300 mg iohexol mL^{-1}; GE Healthcare AS, Norway) in a 1:1 ratio. In this study, two different injection volumes were used: 0.5 (group 0.5; $n = 7$) and 1.0 mL (group 1.0; $n = 10$) for hemimandibles measuring < 15 or ≥ 15 cm, respectively.

For each hemimandible, the same investigator (CB) inserted the needle as previously described in phase I. For each head, a first plain CT scan was performed, as in phase I, with the needle in place. Thereafter, the dye-contrast solution was injected over 30 seconds, by the same investigator (CB), and the needle removed immediately after injection. A second CT scan was performed 10 minutes after the injection.

All images were evaluated by a board-certified radiologist (HD). For the plain scan, the lateromedial angulation of the needle was measured, relative to the surface of the mandibular bone. Additional measurements performed included: maximal rostro-caudal and dorso-ventral lengths of the mandible and maximal rostro-caudal and dorso-ventral lengths of the external mandibular fenestra for each injected side. After injection, the radiologist measured the following variables: spread of contrast into the mandibular canal and calvarium; spread of contrast through the internal mandibular fenestra; medial spread into the masticatory muscles of the temporal region.

Additionally, the dorso-ventral and medio-lateral rostral and caudal spreads of contrast relative to the position of the needle tip were measured. This was done in order to characterise the spread of contrast medium into the anatomical structures of interest. Finally, the sum of the rostral and caudal spread of contrast was calculated to evaluate the overall spread of injectate along the length of the main trunk of the mandibular nerve.

After acquisition of the images, a third investigator (PM) dissected each head in order to evaluate nerve staining. The temporal region, the external mandibular fenestra and the medial and lateral aspects of each hemimandible were dissected and examined. A qualitative 0 − 2 score was used to determine the staining of the internal mandibular fenestra, staining of medial branch of the mandibular nerve and staining of the mandibular nerve into the temporal region. The score was based on a previously published scale (Portela et al. 2017) and modified for use in this species and on the anatomical structures of interest in the current study. The score was as follows: 0 = no staining; 1 = weak staining; 2 = adequate staining (Appendix A). Additionally, during dissections the length of the stained portion of the mandibular nerve, including both the branch exiting from the internal mandibular fenestra and the portion of the nerve extending into the temporal region, was measured with a ruler.

Statistical analysis

Normality of data was analysed with the Kolmogorov-Smirnov test. Numerical variables were analysed with either one-sample *t*-test or Mann-Whitney Rank Sum test, depending on data distribution. Categorical variables were analysed with the use of contingency tables and a Chi-squared test. Commercially available software was used (SigmaStat 4.0 and SigmaPlot 10; Systat Software Inc., NV, USA). The level of significance was always defined as $p < 0.05$. Data are presented as either mean ± standard deviation (SD) or median and range [minimum − maximum], depending on data distribution.

Results

Phase I: anatomical and tomographic study and development of the technique

In Nile crocodiles, the maxillary and the mandibular nerve branches emerge from the lateral part of the trigeminal ganglion and exit the trigeminal fossa through the maxillary-mandibular foramen. The maxillary nerve turns rostrally (Fig. 2a), whereas the mandibular nerve emerges into the adductor chamber, passing between the adductor mandibulae posterior muscle (m.), the adductor mandibulae internus m. and the adductor mandibulae externus m. (Fig. 2b). The mandibular nerve passes caudo-ventrally and laterally to the cartilage *transiliens* and then enters the inferior alveolar canal of the mandible, after giving off the mylohyoid nerve (Fig. 2c). Lateral dissection of the external mandibular fenestra showed that the mandibular nerve crosses the fenestra caudo-rostrally, at the level of the dorsal rim formed by the dentary and surangular bones, before entering the inferior alveolar canal. The further exposure of the internal mandibular fenestra, followed by removal of the intramandibularis m., showed the rostro-medial path of the mandibular nerve along the mandible. Along its mandibular path, the mandibular nerve gives off various large and small branches. These branches emerge from different neurovascular foramina, located on the medial aspect of the splenial bone as well as on the lateral aspect of the dentary bone. Among the secondary nerve branches, the medial branch exiting from the internal mandibular foramen is the largest (Fig. 2d).

Plain CT scans performed on the two heads were found to be useful for identification of the bony landmarks used for the nerve block. In particular, the main area of interest was identified as the external mandibular fenestra. This was palpable on the caudal third of the lateral aspect of the mandible, just caudal to a line traced tangentially to the lateral canthus of the eye and perpendicular to the long axes of the mandible. The CT scan showed that the external mandibular fenestra is delineated by the fusion of three bones: the dentary (rostral), the surangular (dorso-caudal) and the angular bone (ventro-caudal; Fig. 3). Based on the CT findings, both medial aspects of the angular and coronoid bones were identified as useful anatomical landmarks to guide needle insertion and advancement. These bones extend more caudally on the medial aspect of the mandible than the lateral dentary bone. Finally, the CT examination confirmed the presence, on the medial aspect of the mandible, of an internal mandibular fenestra formed by the splenial bone (rostral) and the angular bone (caudal).

Based on the findings of both anatomical dissection and CT examination, the mandibular nerve block technique was reproduced, as described in material and methods (Fig. 1). Thereafter, the CT examination was performed to assess the accuracy of needle positioning. CT images confirmed that the needle tip had reached the medial aspect of the mandible, at the entrance of the mandibular nerve into the inferior alveolar canal.

Phase II: injection and contrast-dye spread evaluation

In this phase, 13 juvenile Nile crocodile heads were included. The authors had to exclude nine hemimandibles as some anatomical structures were compromised owing to their use in another study. This meant that 17 intact hemimandibles were used in this study. Of these, seven hemimandibles were < 15 (9.7 ± 2.6) cm long while the remaining 10 were ≥ 15 (17.1 ± 2.8) cm long.

The CT images acquired after needle positioning confirmed the correct location of the latter in 17/17 (100%) hemimandibles. Resistance to injection was perceived in three out of 17 cases, possibly as a result of the proximity to the surface of the bone. In these cases, the needle was withdrawn by 1 − 2 mm, and the injection was reattempted and performed successfully. As shown in the CT scans, the median angle of the needle with respect to the mandibular bone was 52° (35° − 59°).

In all of the hemimandibles, iohexol spread in a caudal, rostral, dorso-ventral and medio-lateral direction from the injection site (Fig. 4). The sum of the rostral and caudal spread of contrast was greater than 23 mm in 100% of the hemimandibles included in the study. No spread into the calvarium was identified.

Staining of the mandibular nerve in the temporal region and staining of the medial branch of the mandibular nerve occurred in 100% of the hemimandibles (Fig. 4). The details of staining of the nerve with methylene blue and iohexol distribution in the anatomical area of interest, as revealed by anatomical dissection and tomography, respectively, are shown in Table 1.

Discussion

The results of this study indicate that the technique for performing a mandibular nerve block by insertion of the needle through the external mandibular fenestra, is feasible in juvenile Nile crocodiles. This technique may be particularly useful when a nerve stimulator is unavailable, and it offers some advantages in terms of personnel safety over the intraoral approach previously described in crocodilians (Wellehan et al. 2006). Both of the injection volumes investigated here consistently resulted in staining of the mandibular nerve and spread of contrast to the anatomical region of interest without spread to other anatomical areas such as the calvarium.

In order to overcome the lack of homogeneity between the sizes of the heads - and consequently of the lengths of the hemimandibles and associated nerves - two different volumes of injectate were used in the study. It was speculated that 0.5 and 1.0 mL would be comparable injection volumes for mandibles measuring < 15 and ≥ 15 cm, respectively. However, statistical analysis refuted this assumption and showed that a volume of 1.0 mL resulted in greater spread of injectate than 0.5 mL, despite it being applied to longer and larger nerves. The injection volume of 1.0 mL, as opposed to 0.5 mL, consistently resulted in staining of longer portions of the mandibular nerve and wider spread of contrast in the anatomical area of interest. Based on these observations, it can be postulated that the two volumes were not comparable and that, presumably, a volume less than 1.0 mL may have been used in hemimandibles ≥ 15 cm in length to obtain adequate nerve staining. With respect to this specific aspect, the authors aimed to achieve a minimum length of nerve staining of 23 mm, which was based on previously published work in amphibians. This nerve length was suggested as the minimal necessary for local anaesthetics to produce a reduction in the conduction of action potentials (Raymond et al. 1989).

Measuring the exact length of the stained mandibular nerve was one of the main challenges of the present study. As revealed by dissection, once the mandibular nerve enters the inferior alveolar canal, it runs within the inner portion of the mandible, where it is protected and hidden by the bone. Then, it gives rise to a number of nerve branches of different sizes that exit the medial surface of the mandible through multiple foramina. This makes some portions of the nerve inaccessible and therefore impossible to examine. For this reason, we used the sum of the lengths of the rostral and caudal spread of contrast - measured on the CT images - as a surrogate of the total length of the nerve stained. Both injection volumes resulted in a total spread of contrast that was notably longer than 23 mm. This finding is promising for the clinical effectiveness of the nerve blockade in juvenile Nile crocodiles based on the previously published work in amphibians (Raymond et al. 1989).

The staining of the portion of the mandibular nerve located in the temporal region was one of the main differences between the two groups. The larger injection volume resulted in greater nerve staining in this specific anatomical area. George & Holliday (2013) investigated the anatomy of the trigeminal nerve in *Alligator mississippiensis*. They described the motor and sensory components of the mandibular nerve in relation to its axonal density and its anatomical course. Those authors determined that the motor component of the mandibular nerve is predominantly in its proximal portion, at the level of the temporal region. Based on those findings and our results, we propose that the mandibular nerve would have a similar organisation and function in Nile crocodiles. Therefore, we suggest that using volumes smaller than 1.0 mL may produce less motor blockade and, therefore, fewer postoperative complications. This is particularly important in crocodilians, which rely on the mandibular motor function to form a seal at the back of the oral cavity during submersion (Fleming 2001).

The technique of mandibular nerve block described in this study may also be applicable to other crocodilians species. Previous studies investigating the path of the mandibular nerve in alligators found comparable results (George & Holliday 2013; Wellehan et al. 2016). Further research is warranted to support this hypothesis, and to investigate the suitability of the technique for use in adult Nile crocodiles and to evaluate its analgesic effectiveness in clinical cases.

This study has some limitations. Owing to the use of thawed crocodile heads, the diffusion of iohexol-dye solution described in this study may be different from the spread of contrast in live animals. Furthermore, a different spread and distribution of local anaesthetics from that of the iohexol-dye solution cannot be excluded.

Conclusions

The mandibular nerve block technique described in this study is feasible and easy to perform in juvenile Nile crocodiles. An injection volume ranging from 0.5 to 1.0 mL, depending on the size of the crocodile, is proposed to provide a sensory block in clinical cases. However, prospective clinical trials should be performed to evaluate the usefulness of the technique and the volumes of local anaesthetics described in the current studied in live juvenile crocodiles.

References

Chatigny F, Kamunde C, Creighton et al. (2017) Uses and doses of local anesthetics in fish, amphibians, and reptiles. J Am Assoc Lab Anim Sci 56, 244−253.

d'Ovidio D, Adami C (2019) Locoregional anesthesia in exotic pets. Vet Clin Exot Anim 22, 301−304.

Ferreira TH, Mans C (2019) Evaluation of neuroaxial anaesthesia in bearded dragon (*Pogona vitticeps*). Vet Anaesth Analg 46, 126−134.

Fleming GJ (2001) Crocodilian anesthesia. Vet Clin North Am Exot Anim Pract 4, 119−145.

George ID, Holliday CM (2013) Trigeminal nerve morphology in *Alligator mississippiensis* and its significance for crocodyliform facial sensation and evolution. Anat Rec 296, 670−680.

Mans C, Steagall P, Sladky KK (2019) Regional Anesthesia and Analgesia. In: Mader's Reptile and Amphibian Medicine and Surgery (3rd edn). Mader D, Stahl S, Divers J (eds.) Elsevier, St. Louis, USA, pp. 475−479.

Mosley C (2011) Pain and nociception in reptiles. Vet Clin Exot Anim 14, 45−60.

Perry SM, Nevarez JG (2018) Pain and its control in reptiles. Vet Clin Exot Anim 21, 1−16.

Portela DA, Campoy L, Otero PE et al. (2017) Ultrasound-guided thoracic paravertebral injection in dogs: a cadaveric study. Vet Anaesth Analg 44, 636−645.

Raymond SA, Steffensen SC, Gugino LD et al. (1989) The role of length of nerve exposed to local anesthetics in impulse blocking action. Anesth Analg 68, 563−570.

Read MR (2004) Evaluation of the use of anesthesia and analgesia in reptiles. J Am Vet Med Assoc 224, 547−552.

Rivera S, Divers SJ, Knafo SE et al. (2011) Sterilisation of hybrid Galapagos tortoises (*Geochelone nigra*) for island restoration. Part 2: phallectomy of males under intrathecal anaesthesia with lidocaine. Vet Rec 22, 78

Wellehan JFX, Gunkel CI, Robertson KSA et al. (2006) Use of nerve locator to facilitate administration of mandibular nerve blocks in crocodilians. J Zoo Wildl Med 37, 405−408.

Whiteside DP (2014) Analgesia. In: Zoo Animal and wildlife immobilization and anesthesia (2nd edn). West G, Heard D, Caulkett N (eds). Wiley Blackwell, IA, USA. pp. 83−108.

Appendix A

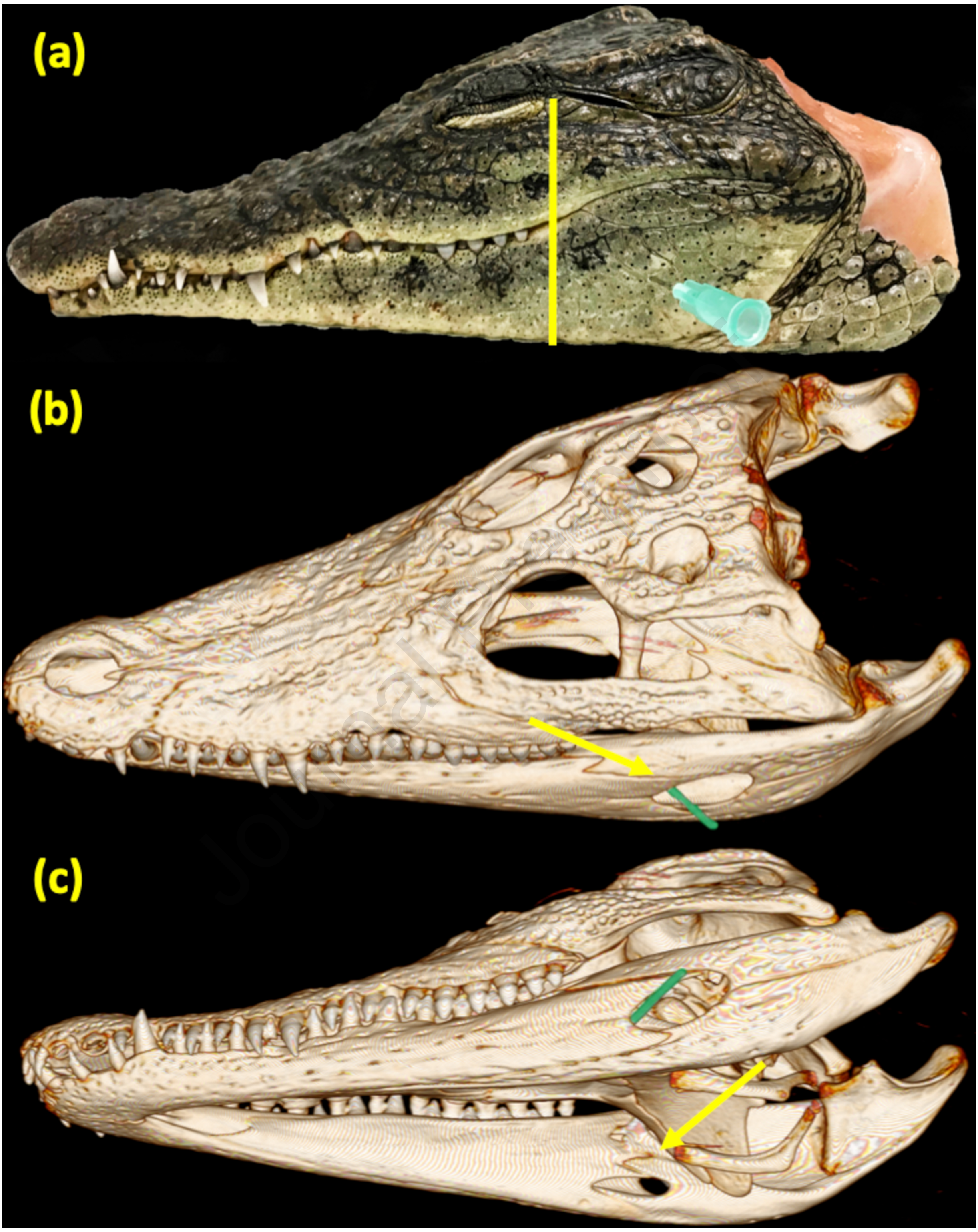
Qualitative 0 - 2 score used to evaluate the stain of the nerves (medial branch of the mandibular nerve and mandibular nerve into the temporal region) and of the internal mandibular fenestra. The score was developed based on a previously published scale (Portela et al. 2017), modified for use in this species and the anatomical structures of interest in the current study.

Qualitative score	**Description**
0	No staining: the nerve or medial mandibular fenestra not stained by the dye
1	Weak staining: the nerve or medial mandibular fenestra not completely stained by the dye and for the nerve, additionally, the dye is not diffused to the entire circumference
2	Adequate staining: the nerve or medial mandibular fenestra was completely stained by the dye and for the nerve, additionally the dye is diffused to the entire circumference

Table 1 Measurement of contrast (iohexol) and dye (methylene blue) spread, in millimetres (mm), after injection of two different volumes of a contrast and dye 1:1 mixture in the external mandibular fenestra of 17 hemimandibles of 13 euthanized juvenile Nile crocodiles. The injection volumes used were 0.5 mL (group 0.5) and 1.0 mL (group 1.0) for hemimandibles measuring < 15 and ≥ 15 cm, respectively. The level of significance was defined as $p < 0.05$. Data are presented as mean ± standard deviation and median [minimum − maximum], depending on data distribution. The stain quality evaluated during anatomical dissection was scored as follows: 0 = no staining; 1 = weak staining; 2 = adequate staining (Appendix A; Portela et al. 2017).

Variable	**Group 0.5 (*n* = 7)**	**Group 1.0 (*n* = 10)**	**Test**	***p* value**
Length of staining of the mandibular n. in the temporal region (mm)*	12 ± 4	24 ± 6	One-sample *t*-test	**< 0.001**
Length of staining of the medial branch of the mandibular n. (mm)*	11 [8 − 15]	30 [21 − 31]	Mann-Whitney U-test	**< 0.001**
Stain quality of the internal mandibular fenestra*	Weak: 40% Adequate: 60%	Weak: 14% Adequate: 86%	Fisher exact test	0.338
Stain quality of the mandibular n. in the temporal region area*	Weak: 50% Adequate: 50%	Weak: 14% Adequate: 86%	Fisher exact test	0.304
Medio-lateral spread of contrast from IS (mm)†	17 ± 2	27 ± 4	One-sample *t*-test	**< 0.001**
Rostral spread of contrast from IS (mm)†	39 ± 22	62 ± 37	One-sample *t*-test	0.149
Caudal spread of contrast from IS (mm)†	30 [21 − 41]	48 [39 − 53]	Mann-Whitney U-test	**0.011**
Rostral + Caudal spread of contrast from IS (mm)†	69 ± 20	109 ± 39	One-sample *t*-test	**0.025**
Spread quality of contrast through the internal mandibular fenestra†	Weak: 86% No spread: 14%	Weak: 90% No spread: 10%	Fisher exact test	1.000

n.: nerve; IS: injection site; *: measured during anatomical dissection †: measured on the images acquired with computerised tomography.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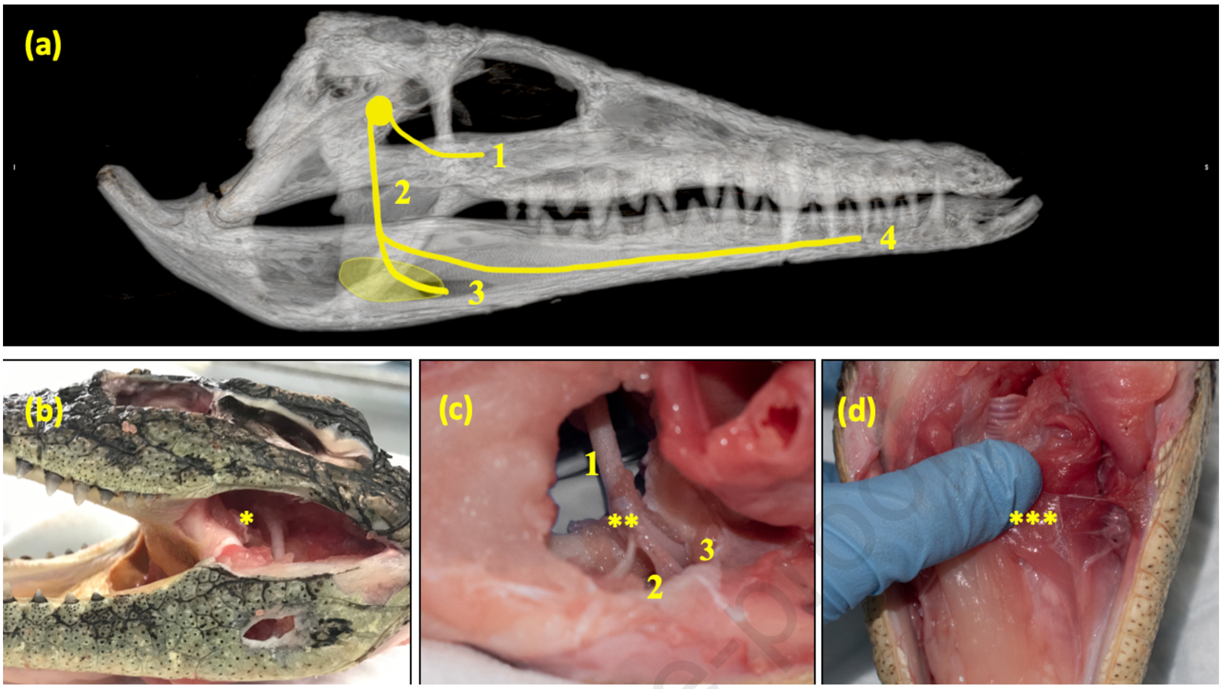
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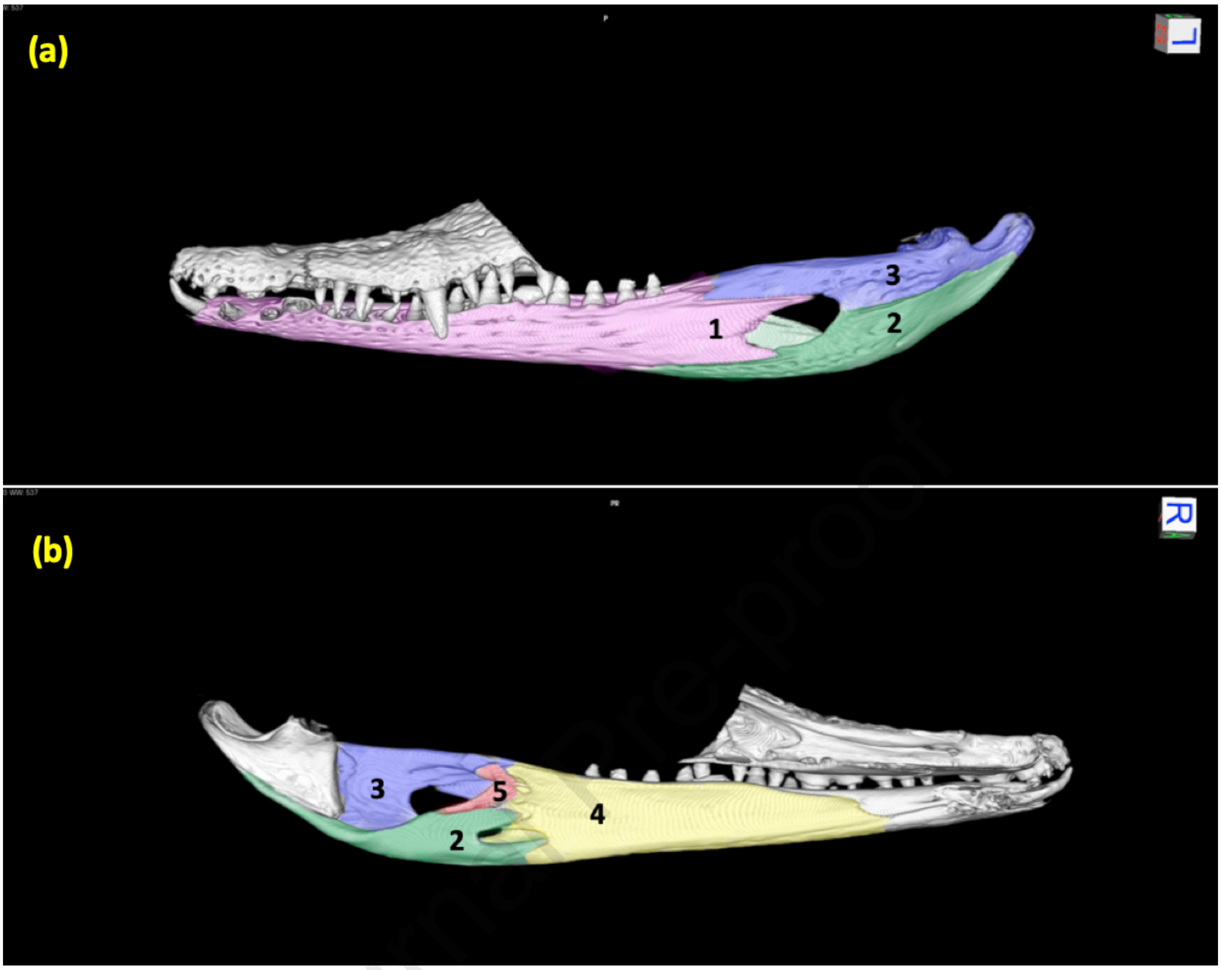
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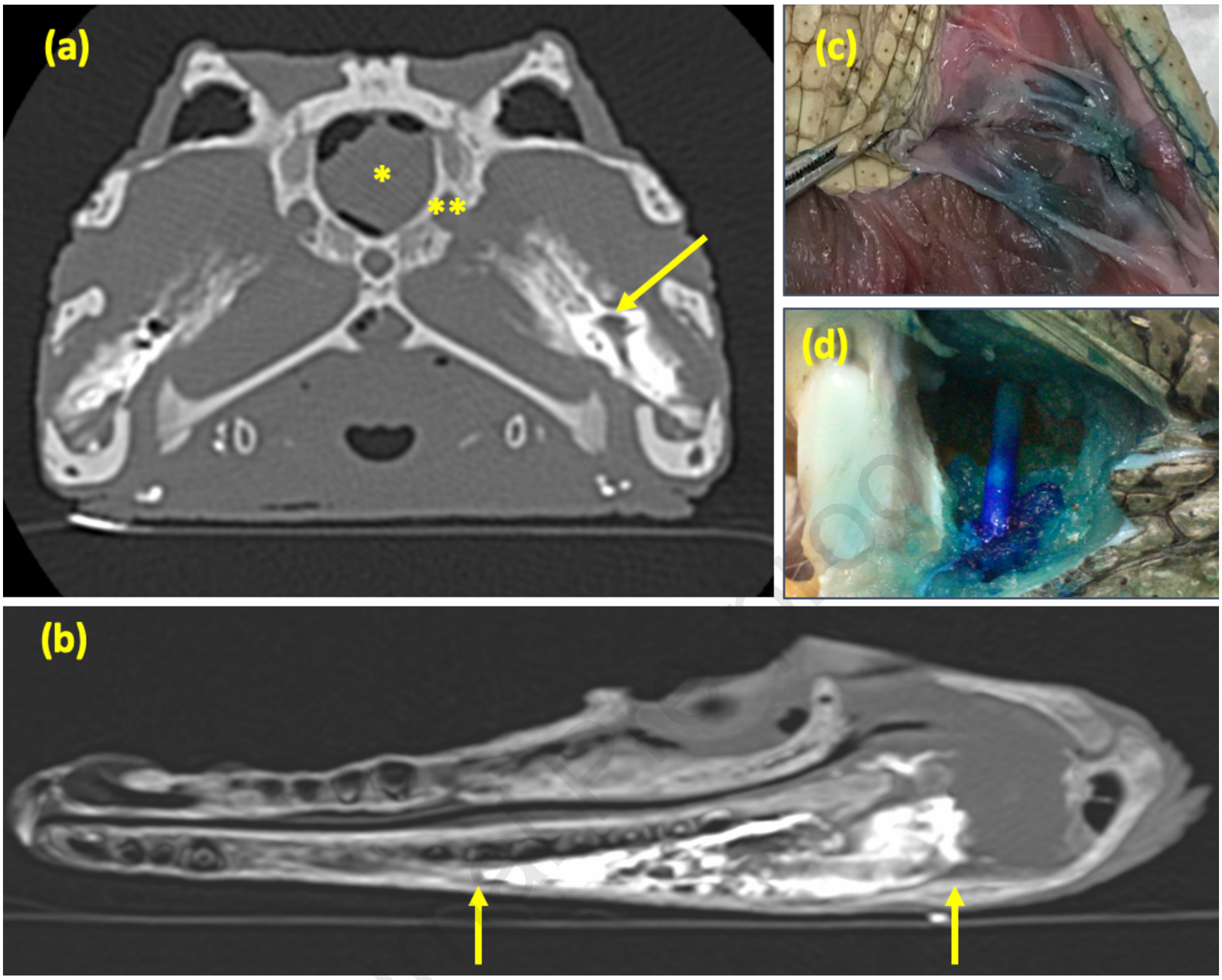
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LEGEND FOR THE FIGURES

Figure 1 Mandibular nerve block in a juvenile Nile Crocodile head cadaver (a) and the 3D tilted reconstructions from the dorsally (b) and from ventrally(c) with the use of computed tomography. The external mandibular fenestra was palpable on the caudal third of the lateral aspect of the mandible, immediately caudal to a line tangential to the lateral canthus of the eye and perpendicular to the long axes of the mandible (yellow line). The hypodermic needle (21 gauge) was inserted percutaneously, with a 45° caudo-rostral angle, through the rostro-dorsal portion of the external mandibular fenestra [yellow arrow in (b)]. The needle was then advanced until its tip reached the medial surface formed by the fusion of the angular and coronoid bones [yellow arrow in (c) pointing at the fusion of the angular and coronoid bones the contralateral side]. The green line in (b) and (c) represents the needle.

Figure 2 Course of the mandibular nerve (n.) (a) Computed tomographic examination of a juvenile Nile crocodile skull with superimposed graphic representation of the external mandibular fenestra (yellow ellipse) and of the trigeminal ganglion and the maxillary and mandibular branches of the trigeminal n. (in yellow). The maxillary n. (1) runs rostrally, whereas the mandibular n. (2) runs caudo-ventrally and then enters the inferior alveolar canal (4) of the mandible, after giving off the mylohyoid n. (3) (b) Anatomical dissection of the temporal region (*mandibular n.) (c) Anatomical dissection of the medial aspect of the mandible that reveals the mandibular n. trunk (1). The mandibular nerve enters the inferior alveolar canal of the mandible (3) after giving off the mylohyoid nerve (2) (**). (d) Anatomical dissection of the medial aspect of the mandible that shows the medial branch (***) of the mandibular n. originating from the internal mandibular fenestra.

Figure 3 Computed tomographic reconstruction of the lateral (a) and medial (b) aspects of the hemimandible. The external mandibular fenestra is delimited by the fusion of the dentary (1), the angular (2) and the surangular (3) bones. The internal mandibular fenestra is formed by the angular (2) and the splenial (4) bones. The coronoid bones (5) is also present on the medial aspect of the mandible.

Figure 4 (a) Transverse CT reconstruction of the head at the level of the external mandibular fenestra following bilateral injection. Contrast has spread into the medial musculature, and on the left (right side of the image), is outlining the trigeminal nerve and its branches (yellow arrow). The endocranial cavity (*) and trigeminal fossa (**) are also identified. (b) Sagittal oblique CT reconstruction of the head following injection, showing spread of contrast along the mandibular canal; arrows indicate rostral and caudal extent of the spread. (c) Sample image of weak staining of the medial branch of the mandibular nerve in line with the qualitative score used for the study (Appendix A; Portela et al. 2017). The image was taken during the anatomical dissection performing the technique of mandibular nerve block with dye-contrast solution. (d) Sample image of adequate staining of the mandibular nerve in the temporal region in line with the qualitative score used for the study (Appendix A; Portela et al. 2017). The image was taken during the anatomical dissection after performing the technique of mandibular nerve block with dye-contrast solution.