Research proposal

Performing nerve stimulator-guided inferior alveolar nerve blocks in horses and their evaluation by means of CT technology and anatomical dissection.

Introduction

When equine exodontial procedures are performed it is common to use a regional nerve block in the standing sedated horse. These regional nerve blocks provide preemptive analgesia, reduce pain perception and therefore lower the sedation dose requirements. Furthermore it provides superior analgesia in comparison with that following systemic sedation with for instance alpha2-agonists, opioids and nonsteroidal anti-inflammatory drugs, which results in a more compliant patient and a safer work environment. This combined makes for faster recovery. Therefore using regional nerve blocks improve the horses' welfare.[6,16]

To perform a mandibular foramen block, also known as an inferior alveolar nerve block, the mandibular nerve has to be desensitized prior to entering the mandibular foramen. This foramen is situated on the medial aspect of each mandibular ramus. The mandibular nerve innervates the ipsilateral lower dental arcade. To prevent impulse transmission along the nerve, it is required to reduce voltage-gated sodium conductance to nearly 0. This can be achieved through blocking with local anesthetics, covering a distance of at least three nodes of Ranvier or 6mm of exposed nerve. This blocks the intra-cellular sodium channels and therefore prevents depolarization.[15]

Before entering the mandibular foramen and continuing through the mandibular channel, the mandibular nerve branches, giving off the lingual branch, which provides the tongue with sensory and motor innervation. The mandibular nerve is accompanied by the mandibular alveolar artery, which can lead to complications, such as haematoma formation, when performing an inferior alveolar nerve block.[4,6,19] Because of the close anatomical relationship between the inferior alveolar and lingual nerve, depositing large volumes of local anesthetics can lead to inadvertent anesthesia of the lingual branch, which may lead to self-inflicted tongue trauma.[2,19] Excessive volumes may also cause neuropraxia and temporary lip paralysis.[19]

When delivering peripheral local anesthesia to the horse's head, the most commonly used method is a blind injection technique with volumes up to 10-15mL of local anesthetics.[6,19] Various authors have described different approaches when performing the inferior alveolar nerve block.[3,5,6,19] In 2011, Coomer stated that clinically, the success of achieving local anesthesia via the described techniques is variable.[3] Researchers have tried to invent other ways to use more delicate techniques. In humans, ultrasound-guided injection has become a routine technique for regional anesthetic procedures.[13] The comparison between an ultrasound-guided injection technique and a blind technique when performing a maxillary nerve block in the horse was described by O'Neill in 2014.[14] In 2006, Wellehan described the use of the nerve stimulator-guided technique in order to locate and block the inferior alveolar nerve in crocodilians.[20]

Research goals

This research will consist of two parts. Part I will be a pilot study to determine which blind injection technique (vertical vs caudal approach) is the most accurate for localizing the mandibular foramen and nerve, in order to obtain the best results in Part II. During Part II the most accurate blind injection technique from Part I will be compared to a nerve stimulator-guided injection technique, while both being administered from the same approach.

The purpose of this research is to develop a nerve stimulator-guided injection technique of the equine mandibular nerve. The technique should be reliable, accurate and with a lower risk of complications than the commonly used blind technique. Because of the anatomy of the horse's head and individual differences in anatomy, the area in which a block can be performed anesthetizing the inferior alveolar nerve only, without desensitizing the lingual branch, is relatively small. Hopefully, in the future, this new technique can be applied in daily practice.

Hypothesis

Part I: *"The use of the blind ventral injection technique, will not lead to a significantly higher amount of inferior alveolar nerve staining from dye deposited in the area surrounding the nerve, when comparing this technique to the blind caudal injection technique."*

Part II: *"The use of a nerve stimulator-guided technique for performing the inferior alveolar nerve block leads to improved localization of the mandibular nerve with more accuracy and a significantly smaller distance between the blocking site and the foramen, in comparison to using the common blind technique."*

If hypothesis Part II is true and a significant difference from the null hypothesis is found, the currently recommended large volumes (10-15mL) for performing such a block will not be necessary, therefore lowering the risks of complications.

Materials and methods

Part I: Cadaver study

Before performing the nerve stimulator-guided technique on anesthetized Shetland ponies (Part II), Part I of the study was conducted on 8 adult Shetland cadaver heads in order to determine which commonly used blind injection approach (ventral versus caudal) is most accurate to obtain an effective block. These heads were obtained from a slaughterhouse and had been separated from the body at the level of the atlanto-occipital joint. They were stored at -20°C and thawed in cold water 36 h prior to use.

Prior to Part I, two cadaver heads were used in a pilot study in order to gain proper information about the dosage use of Methylene blue coloring dye and CT-contrast medium (Xenetix® 350, lobitridol 768 mg/mL). They were administered in a 1:1 ratio. Different volumes and techniques were applied on each hemi-mandible. The first cadaver head was injected with 2mL and 4mL solution, followed by 0,5mL of air to pass the solution along the needle. The second cadaver head was injected with 0,5mL and 1mL solution, followed by 0,5mL of air. After injection, the stylet was replaced within the needle and one of the needles was left in place. CT examination of the two heads revealed that the injected solution of 0,5mL, containing 0,25mL of contrast volume, was insufficient to measure foramen coverage. Also, addition of air resulted in too many artifacts; therefore air was no longer administered. Volumes of 2mL and 4mL solution spread along a large area, which led to difficulties trying to measure distance between needle tip and foramen. The larger volumes also resulted in large stained, blue areas, which made it impossible to determine the impact of nerve staining as a result of difference in volume administered.

Therefore, during Part I a volume of 1mL (1:1 ratio of contrast medium and dye) was selected and administered with an 18G spinal needle. To ensure the administered volume would remain equal, the needle volume of 0,1mL contrast medium was added to the solution. After injection the stylet was reinserted and both needles were left in place. Because of this, the heads were positioned in dorsal recumbency, after injection and during CT.

The two blind approaches were randomly applied to each side of the cadaver heads; one technique per hemi-mandible and all nerve blocks were performed by the same researcher. The mandibular foramen was located via topographical landmarks. These landmarks were found by drawing two imaginary lines. The first line was drawn along the occlusal surface in horizontal direction to the caudal aspect of the mandible, and the second line from the lateral canthus ventrally. The second line intersected the first line at a 90-degree angle. To obtain a more likely desensitization of the nerve the investigator aimed for a site 1cm dorsal and caudal to the foramen (figure 1). With the ventral approach, the spinal needle was inserted on the ventral aspect of the rami immediately ventral to the topographical landmarks. With the caudal approach, the needle was inserted at the intersection of the body and the ramus of the mandible. The insertion ran along the medial aspect of the mandible, in rostral-dorsal direction towards the site 1cm dorsal and caudal to the foramen.

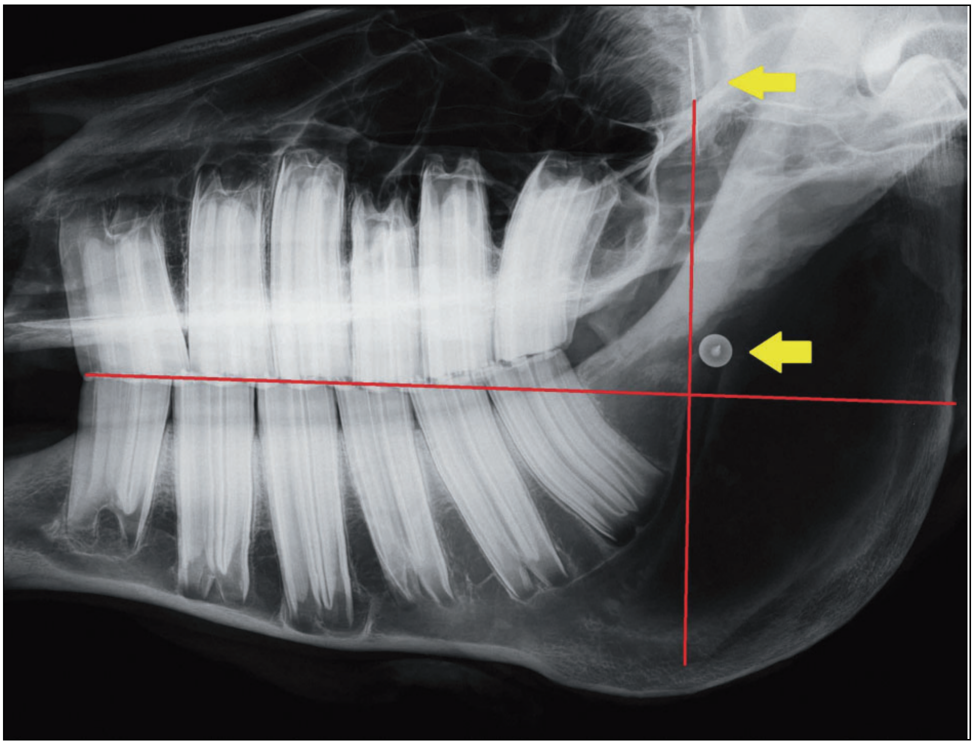


Figure 1: Radiograph of a hemi-mandible showing the 2 imaginary lines pointing out the location of the mandibular foramen. Lower yellow arrow shows aiming target area[6].

CT technology was used to determine the accuracy of the injections. The CT scans were acquired with the cadaver heads in dorsal recumbency with a 64 slice CT scanner. The protocol utilised was 140 KVp, 328 mAs and 1 mm slice thickness. Bone (W 3000 L600) and soft tissue (W 300 L 50) algorithms were applied and a standard matrix of 512 x 512 pixels used. The same ECVDI (European collage of

Veterinary Diagnostic Imaging) resident, who was not aware of the patients' history at the time of evaluation, examined the CT-scans. Using the PACS system, 3D reconstructions were used to determine whether the contrast medium was in contact with the region of the mandibular foramen (yes/no) and percentage of the foramen covered in contrast medium (0-25%, 25-50%, 50-75%, 75-100%). 2D, multiplane reconstructions were made, in order to measure the distance from the mandibular foramen to the tip of the needle in sagittal plane and record the distance with caudal and dorsal being positive vectors.

Anatomical dissection was conducted within 2h after the first injection. The heads were measured for anatomical properties: distance between the medial canthi, nose circumference rostral to the facial crest and distance from commissure to the temporomandibular joint (TMJ) were determined. The inferior alveolar nerve and the lingual nerve were approached ventrally, by dissecting on the medial side of the mandible. An independent observer, who was blinded for the technique and approach used for application of the block, classified staining of the nerve as a hit, partial hit or a miss. These hits will later be combined into positive- or negative blocks. A positive block is referred to when the inferior alveolar nerve is classified as a hit and the lingual nerve as a miss or a partial hit. A partial hit will not completely desensitize the nerve and therefore will not prevent depolarization. Figure 2 shows an example of a positive block. A negative block is referred to when the inferior alveolar nerve is missed or classified as a partial hit. These hits are combined into a positive- or negative block because it is undesirable to desensitize the lingual nerve. Desensitizing of the lingual nerve has been seen to lead to self-inflicted tongue trauma and this is why it is of the utmost importance to locate the most accurate blocking area.[2] In order to determine which of the two blind approaches is the most accurate, the amount of positive hits and the needle tip closest to the foramen are decisive.

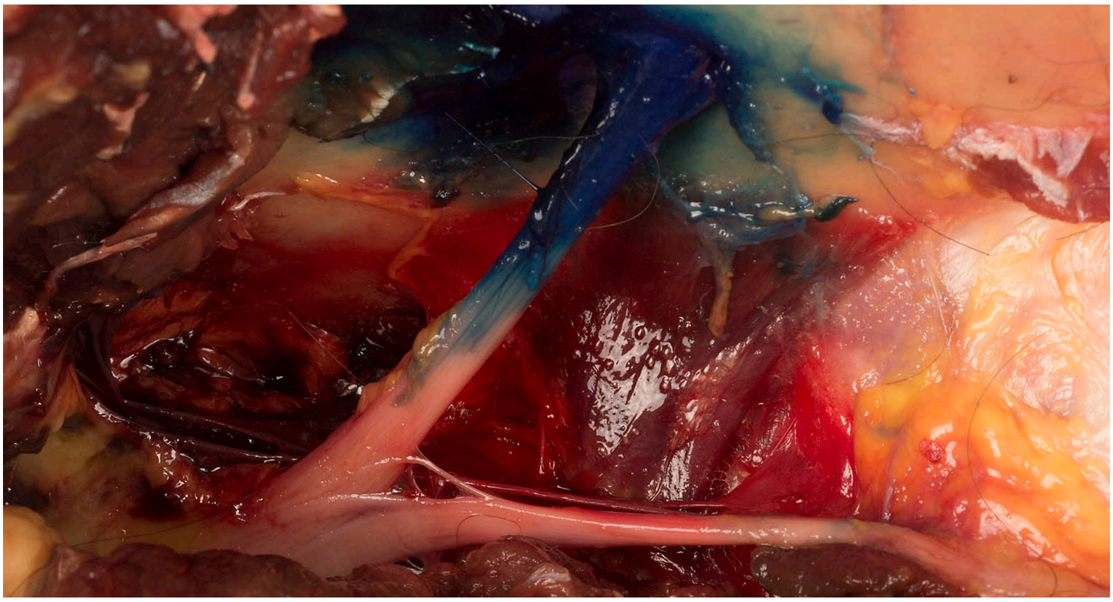


Figure 2: A positive block with a stained inferior alveolar nerve and a clear lingual nerve. This is a picture of the medial side of the right mandible. The head is in dorsal recumbency with the caudal side on the left and the rostral side on the right.

All data were analyzed with IBM SPSS Statistic version 24. The binominal data were compared with the Fisher's exact test and the continuous data, if not normally distributed, were compared with the Mann Whitney U test. When normal distribution did occur the data were compared with the T-test.

Part II: Clinical cases

During Part II, 7 anesthetized Shetland ponies were used for performing the nerve stimulator-guided technique of blocking the inferior alveolar nerve. These animals were used in another study, unrelated to this research question, and euthanasia was included at the end of protocol due to further lab work on joints. Ethical permission was obtained, by national and local authorities (CCD, DEC, IvD), for the use of these animals during these studies. Before euthanasia, the ponies were anesthetized and the nerve stimulator-guided injection technique was performed on both hemi-mandibles. It was decided to use the ventral approach during Part II, because no significant difference was found during Part I (see results) between the caudal and ventral blind approach. Another reason was the researcher being more experienced with applying the ventral approach. TIVA (total intravenous anesthesia) was used to induce and maintain anesthesia. First, adequate sedation was achieved by sedating with detomidine hydrochloride© (10-20µg/kg bodyweight i.v. to desired effect). After sedation the skin at the level of the jugular vein was clipped and an i.v. catheter was placed in the jugular vein. Induction was performed with a combination of midazolam (0,011- 0,044 mg/kg bodyweight) followed by ketamine (2,2 mg/kg bodyweight rapidly i.v.). Once the ponies were fully induced, anesthesia was maintained with triple drip, containing 10mg of detomidine and 1000mg of ketamine to 500mL of 10% guaifenesin, with an infusion speed of 1 mL/kg bodyweight/ h).[12] The nerve stimulator (Stimuplex® DIG, B. Braun) was set on an initial current of 1.3 mA and a frequency of 2 Hz. The ground electrode was placed on the ventral border of the ipsilateral masseter, close to the entry point of the Stimuplex® A, B. Braun needle. When a motor response from the temporalis and masseter muscle was achieved, resulting in a jaw jerk, the current was gradually reduced to a threshold of 0.6- 0.4 mA.[10] When the jaw jerk was still discernible at this threshold, the same volume (1mL in a 1:1 ratio of contrast medium and dye) was administered. To ensure the administered volume would remain equal, the needle volume of 0.4mL NaCl was used to pass the dye along the tubing of the needle. The needles were left in place. After euthanasia the cadaver heads were studied with the same protocol and parameters as performed in Part I of the study.

Because the first seven attempts showed such different results in comparison to the second seven attempts, the possibility of a learning effect when using the flexible Braun needle was kept in mind. This is why it was decided to divide the results of the nerve stimulator-guided group into two separate groups. They were each compared with the ventral blind approach and with each other.

Results

Staining of nerves

The average measurements of the heads were calculated and are shown in the table 1.

Table 1: Several measurements of the heads		
	Average (cm):	**SD (cm):**
Medial canthi[1]	13.75	1.04
Nose circumference[2]	26.75	1.75
Commissure to TMJ[3]	47.63	4.17

[1] The distance between the medial canthi.
[2] The circumference of the nose, rostral to the facial crest.
[3] The distance between the commissure and the temporomandibular joint.

With the caudal approach a hit was achieved 6 out of 8 times, a partial hit and a miss were both achieved 1 out of 8 times. With the ventral approach a hit was achieved 3 out of 8 times, a partial hit was achieved 1 out of 8 times and a miss was achieved 4 out of 8 times. These results are shown in table 2. Staining of the nerve and average distance form needle tip to mandibular foramen was compared between both blind approaches, but no significant difference was found ($p = .234$, $Z = -1.260$).

Table 2: Staining of the inferior alveolar nerve							
	Positive	**%**	**Partial**	**%**	**Negative**	**%**	**Total**
Blind caudal approach	6	75.0	1	12.5	1	12.5	8
Blind ventral approach	3	37.5	1	12.5	4	50.0	8
Neurostim ventral[1]	6	42.9	2	14.3	6	42.9	14

[1] The nerve stimulator-guided ventrally approached technique.

The nerve stimulator-guided technique was applied 14 times, by means of the ventral approach. Positive hits of the inferior alveolar nerve were achieved 6 out of 14 times, partial hits were achieved 2 out of 14 times and 6 out of 14 times the nerve was missed. These results are also show in table 2.

Table 3: Positive and negative blocks[1]					
	Positive	**%**	**Negative**	**%**	**Total**
Blind ventral approach	1	12.5	7	87.5	8
Neurostim ventral[2]	2	14.3	12	85.7	14

[1] A positive block is referred to when a positive staining of the inferior alveolar never in combination with a negative or partial staining of the lingual nerve was achieved. Because partial staining of the lingual nerve would still ensure feeling to the tongue. A negative block is referred to when the inferior alveolar nerve is not or only partially stained.
[2] The nerve stimulator-guided ventrally approached technique.

When using the blind ventral technique, a positive block was achieved in 1 out of 8 times as compared to 2 out of 14 times when using the nerve stimulator-guided technique. Both techniques gave similar results for the negative block. These results are shown in table 3.

CT assessment of contrast medium

When the ventral blind technique was used, the CT-scans showed that in 5 out of 8 attempts, the contrast medium was in close contact with the region surrounding the mandibular foramen. Table 4 shows that the use of the nerve stimulator-guided technique results in a higher amount of placements of contrast medium near the mandibular foramen. A positive result was achieved 10 out of 14 times when the nerve stimulator-guided technique was used.

Table 4: Contrast medium in close contact to the mandibular foramen					
	Positive	**%**	**Negative**	**%**	**Total**
Blind ventral approach	5	62.5	3	37.5	8
Neurostim ventral[1]	10	71.4	4	28.6	14

[1] The nerve stimulator-guided ventrally approached technique.

Looking at the percentage of foramen coverage with contrast medium, results show that the nerve stimulator-guided technique had 75-100 % coverage in 4 out of 14 attempts. Table 5 shows the comparison to the ventral blind technique, which did not result in 75-100% coverage of the foramen.

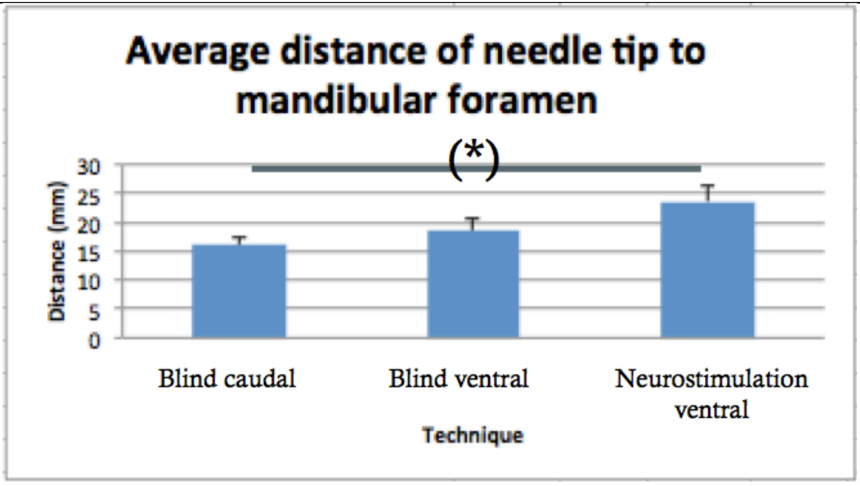
Table 5: Percentage of foramen covered with contrast medium

	0 – 25%	**%**	**25 – 50%**	**%**	**50 – 75%**	**%**	**75 – 100%**	**%**	**Total**
Blind ventral approach	4	50.0	3	37.5	1	12.5	0	0.0	8
Neurostim ventral[1]	4	28.6	5	35.7	1	7.1	4	28.6	14

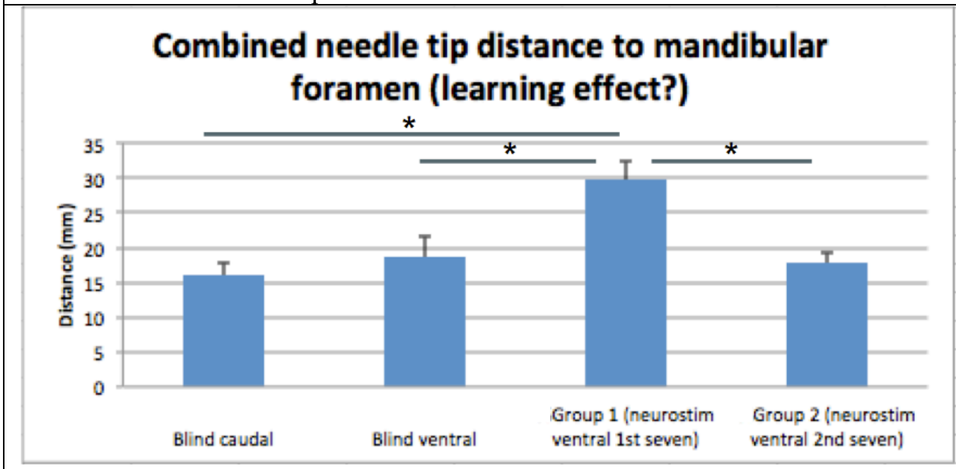
[1] The nerve stimulator-guided ventrally approached technique.

The mean and SD of the different techniques, shown in graph 1, resulted in the following: caudal blind (16.00 mm ± 1.47 mm), ventral blind (18.26 mm ± 1.90 mm) and the total nerve stimulator-guided group (23.72 mm ± 2.62 mm) (mean ± SD). This shows the caudal blind technique to insert the needle tip the closest to the mandibular foramen. It was decided to divide the nerve stimulator-guided group into two groups, resulting in a mean ± SD of: first seven (29.68 mm ± 3.13 mm) and second seven (17.76 mm ± 2.86 mm). These results show that the second group of the nerve stimulator-guided technique also has a short average distance of the needle tip tot the mandibular foramen (graph 2).

Several measurements were compared. Graph 1 compares the distance between needle tip and mandibular foramen related to the different techniques from Part I and Part II. No significant difference was found when the blind caudal and the blind ventral technique were compared ($p = .208$, $Z = -1.260$). As was the case when the blind ventral technique and the nerve stimulator-guide technique were compared ($p = .453$, $Z = -.751$). A trend was found however when de blind caudal technique was compared to the nerve stimulator-guided group ($p = .088$, $Z = -1.707$). Dividing the nerve stimulator-guided group into two groups (first seven attempts and second seven attempts) resulted in several significant results (graph 2). The first significant difference was found comparing the ventral blind results and the first seven results of the nerve stimulator-guided group ($p = .008$, $Z = -2.662$). The second significant difference was found when comparing the caudal blind results and the first seven results of the nerve stimulator-guided group ($p = .001$, $Z = -3.240$). The third significant difference was found when the first seven results and the second seven results from the nerve stimulator-guided group were compared ($p = .006$, $Z = -2.747$). A comparison was also made between both blind techniques and the second half of the nerve stimulator-guided group, which showed no significant difference (ventral blind $p = .908$, $Z = -.116$; caudal blind $p = .203$, $Z = -1.273$).



Graph 1: Comparison of average distance of needle tip to mandibular foramen between different techniques from Part I and Part II.



Graph 2: Comparison of the average distance of needle tip to mandibular foramen when using the divided groups of the nerve stimulator-guided technique.

Figure 3 shows the exact needle tip placement and its relationship to the mandibular foramen for the ventral blind and the nerve stimulator-guided technique. These exact coordinates were calculated using measurements obtained from CT-imaging. The most desirable needle placement was thought to be in the dorsal-caudal area, close to the mandibular foramen, because this is where the alveolar inferior nerve runs, just before entering the mandibular foramen. This result was only obtained in 2 out of 22 (9.09%) times. By far the most needle tips, 12 out of 22 attempts, were placed in the ventral-caudal area (54.55%) and almost all needle tips were placed ventrally of the mandibular foramen. When this is compared to the combined staining results, referred to as either positive or negative blocks, it shows that both needle tip placements in the dorso-caudal area gave a negative block due to staining of the lingual nerve. The three positive blocks that were obtained by correct combined staining were all placed in the ventro-caudal area. Needle tips placed in the ventro-cranial area almost never lead to staining of the inferior alveolar nerve and in some cases staining of the lingual nerve.

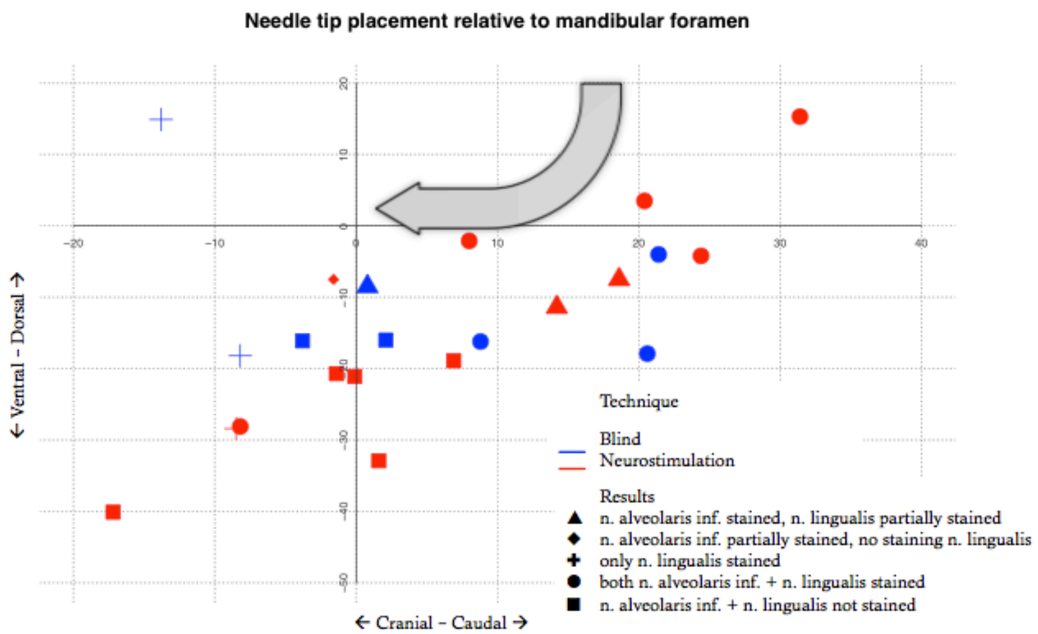


Figure 3: This figure shows the exact needle tip placements relative to the mandibular foramen. The cross-section between x-axis and y-axis being the foramen and the grey arrow being the alveolar inferior nerve.

Out of the 22 attempts in performing an inferior alveolar nerve block 12 attempts led to (partially) staining of the inferior alveolar nerve (54.55%). However in 11 out of 12 (91.67%) attempts the lingual nerve was also (partially) stained, leading to a rejection of what could have been a positive block.

During this study all needles were left in place, making a more accurate detection during CT-imaging possible because the needle tip could easily be detected on the CT-scan. Measurements were made from the needle tip to the foramen, but because the needles were left in place during transport, it may have occurred that the needles suffered some displacement. Therefore, this could have created bias when measuring the distances on CT-imaging. Also when measuring distances between needle tip and foramen 2D- imaging was used instead of 3D, so error in the z-axis (medial vs lateral) was not account for due to the assumption that the needle tip was always adjacent to bone. This was not always the case with the nerve stimulator-guided technique.

Discussion

The inferior alveolar nerve block is used when equine exodontial procedures are performed in the standing sedated horse. It provides preemptive analgesia and reduces pain perception. In order to gain more clarity in this complex anatomic area and to avoid damaging or anesthetizing other structures, the nerve stimulator-guided technique was introduced in this study as a tool that might form a possible addition in equine exodontial procedures.

Nerve stimulation in humans

According to our knowledge no research attempting nerve stimulation in horses has yet been described, so it was unclear which exact nerve response could be expected. Nerve stimulation in order to specifically locate nerves is a method that has been used in humans for quite some time now. [1,8,10,11,17]

Kumar describes the use of a peripheral nerve stimulator for the mandibular nerve block in humans. He also used a Stimuplex® DIG, B Braun stimulator and a Stimuplex® A, B. Braun needle in both studies. A ground electrode was placed on the anterior border of the ipsilateral masseter and the needle was inserted via the lateral extra-oral approach, with a initial current at 1.3 mA and a frequency of 2 Hz. Once the desired motor response from the temporalis and masseter muscle was achieved, resulting in a jaw jerk, the current was gradually reduced to 0.6 – 0.4 mA while maintaining the muscle contraction seen as a visible jaw jerk. Kumar uses the peripheral nerve stimulator in patients with distorted anatomy and describes it as a sure, safe and easy option when performing a mandibular nerve block, especially in cases with distorted anatomy.[10,11]

In 1997 C.N. Barthram executed a performance study comparing six different nerve stimulators. On of these stimulators was the Braun Stimuplex® DIG, the same stimulator as used in this study. He described this stimulator as being the most accurate of the test group.[1] Because of this description and because the Stimuplex® DIG was the nerve stimulator available at the time, values used by Kumar have been extrapolated to the horse for the use in this study.

Jochum however was not as fond of the Braun Stimuplex® DIG. In his objective assessment from 2006, he describes this nerve stimulator to have a inaccurate impulse duration of 0.1 ms, a >50% overshoot of the stimulating signal and under experimental conditions the current intensities <0.2 mA could not be measured. He warns anesthetists to be aware of the limitations of the stimulator being used.
The article of Jochum being more recent then the 1997 study, suggests that different results could have been obtained when using a different type of nerve stimulator.[8]

A note should be made. Because of anatomical alveolar nerve differences and because it was unclear which response was expected from using the nerve stimulator in horses, it might be possible that some false positive muscle contractions occurred.

Anesthesia of lingual nerve

Harding discusses that due to the close relationship between the lingual- and the inferior alveolar nerve using larger volumes may lead to unwanted blockage of the lingual nerve, which could result in self-inflicted tongue trauma.[2,6,18] This is one of the reasons why in general practice a mandibular nerve block is not often performed on both sides during the same procedure. This study shows that even a small volume can lead to staining of the lingual nerve either by itself or in combination with the inferior alveolar nerve. Using lager volumes will obviously lead to more staining of this particular nerve.

The positive blocks in this study were mainly located in the ventro-caudal area. These blocks however all had a partially stained lingual nerve, therefore being classified as a positive block, as defined when constructing the protocols of this study. This implies that with larger volumes all the positive blocks in this study would be classified as negative ones, due to complete positive staining of the lingual nerve. This shows the difficulty of only staining the inferior alveolar nerve, even with small volumes. Therefore it is questionable if it is even possible to find the exact anatomical location for only blocking the inferior alveolar nerve.

The most desirable needle placement was thought to be in the dorsal-caudal area, close to the mandibular foramen, because this is where the alveolar inferior nerve runs just before entering the mandibular foramen. Because almost all needle tips were placed ventrally of the mandibular foramen and the three positive blocks that were obtained were in the ventral-caudal area, it might be suggested that the aiming site for performing an inferior alveolar nerve block should be 1cm ventral and caudal to the foramen instead of the now used area being 1cm dorsal and caudal to the foramen. When using smaller volumes the aiming site becomes more important. This doesn't mean, although it is common use to apply lager volumes at a less accurate aiming side, this is best veterinary practice.

During anatomical dissection some anatomical nerve differences were found (figure 4). Due to the small number of horses used during this study, this suggests that anatomical differences in between horses may occur more often then we might think. Therefore having the exact anatomical location for only blocking the inferior alveolar nerve would not ensure an effective analgesia. In general practice this would lead to the use of lager amounts of local anesthetics, which will inevitably lead to the unwanted anesthesia of the lingual nerve.

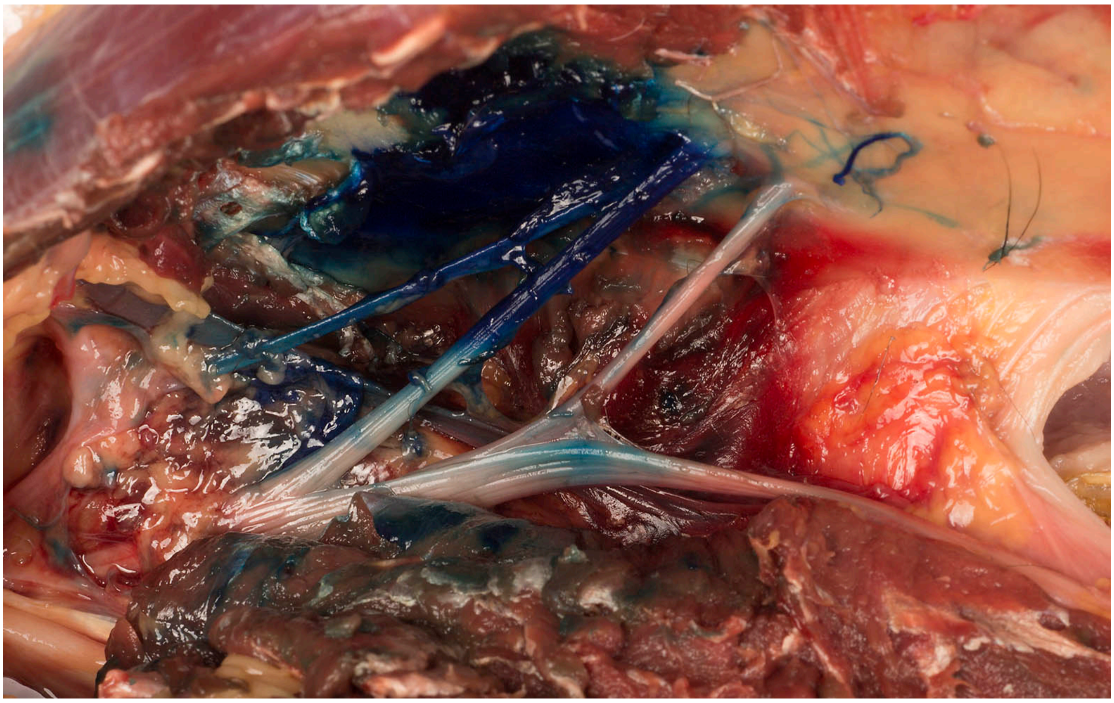


Figure 4: Anatomical differences in between horses. Note the two positively stained mandibular branches and the third unstained mandibular branch entering a second mandibular foramen. This would probably make for ineffective analgesia.

Little is known about the incidence of self-inflicted oral trauma after using the inferior alveolar nerve block. A recent published retrospective study by Tanner & Hubbell reports the incidence of complications associated with regional nerve block. 270 regional nerve blocks to the head were performed in 162 horses in a second opinion referral hospital. The inferior alveolar nerve block was set 51 times in 38 patients using the conventional method. 21 patients received a bilateral mandibular nerve block. Self-inflicted lingual trauma was seen in two horses the day after the procedure and one of these horses received a bilateral nerve block, although lingual maceration was only seen on one side. These horses were observed eating normally 24 hours post surgery.[18] This suggests that self-inflicted lingual trauma can easily be overlooked.

Perhaps the focus should lie on using the most fitting local anesthetic with the most suitable anesthetic duration regarding the specific procedure. Limiting the amount of oral movement and ensuring the local anesthetic effect has worn off before feeding the horse will help to prevent unwanted self-inflicted tongue trauma. Tanner & Hubbell confirmed this. After they found the first two cases developing self-inflicted tongue trauma, all subsequent horses receiving an inferior alveolar nerve block had feed withheld and a muzzle placed for a minimum of four hours. No cases of self-inflicted lingual trauma occurred after this management change.[18] These measures will lower the risk of self-inflicted tongue trauma and should be applied after performing an inferior alveolar nerve block, because avoiding lingual anesthesia using the current techniques seems unavoidable.

Small volumes

Reviewing the results from table 2, a clear difference is seen in positive staining of the alveolar nerve between the caudal blind approach versus the ventral blind approach and the nerve stimulator-guided technique. However no statistical significant differences were detected. Only a small number of heads was used during this study especially in Part I, which might be a reason for not finding a significant difference when comparing the different techniques. A possible explanation for this higher outcome with the caudal blind approach could be that the cadaver heads that were used in the Part I had been cut off at the atlanto-occipital joint, thereby missing a large part of the neck making it easier to gain the correct position for applying the block. This approach may prove to be more difficult in vivo. For this reason the ventral approach was chosen during Part II.

During this study the volume that was used had to be kept very low, because assessing CT-imaging and dissection would otherwise have been impossible due to overly staining and inaccuracy in CT measurements. Therefore only 1 mL of solution was used instead of the currently recommended volumes of anesthesia (10-15mL).[6,19] Because of the large number of staining's and partial staining's already obtained with this relatively small volume, it is more than likely that when volumes are enlarged a larger number of positive inferior alveolar nerve hits will occur. Therefore the partial staining in table 2 could be combined with the full staining leading to a clear difference between the caudal blind approach and the other two techniques. These results are as followed: caudal blind approach respectively 87,5% (7/8), followed by the nerve stimulator-guided technique with 57,14% (8/14) and the ventral blind approach resulted in 50% (4/8). Unfortunately the difference between the caudal blind approach and the nerve stimulator-guided technique is also not significant ($p = .167$), but it does suggest that a more accurate localization of the nerve could be obtained while using the nerve stimulator-guided technique via the caudal approach.

The significant difference between the currently used volumes and the 1 mL that was used in this study suggests that the currently recommended volumes may not be necessary. Harding also recommended this assumption.[6] Question remains though if these small volumes will give sufficient analgesia in living animals. Also the blockage of three nodes of Ranvier (6 mm) required to form a proper nerve block in other studies was impossible to check because of euthanasia at end of our protocol.[15]

When reviewing the results from CT-imaging and dissection two trends can be found. While both techniques from Part II have a relatively low amount of positive staining of the inferior alveolar nerve when looking at the dissection results, both techniques score higher results when looking at the contrast medium being in close contact to the mandibular foramen. In both cases the nerve stimulator-guided technique scores slightly better. The question arises which parameter is the most valid for predicting a positive block in a clinical scenario. Both substances have different physical characteristics and could therefore have different diffusion distances. This however requires further research. While methylene blue shows a low success rate when using a small volume, the contrast medium seems to have a higher success rate with the same volume. Meaning: if staining of the nerve is the most valid parameter a smaller volume will not be very successful in generating a desensitized alveolar nerve. If however, contrast medium and CT-imaging are the most valid parameter a smaller volume will establish better results. Because CT assessment is generally accepted as the golden standard, this would be the most likely valid parameter. Therefore using small volumes would suggest a higher outcome when given in a clinical scenario.

Learning effect

This study resulted in several significant differences. Because the first and the second seven attempts of the nerve stimulator-guided technique showed such big differences, it was thought this might be due to a learning effect. The Stimuplex® A, B. Braun needle is much more flexible than a normal spinal needle. Therefore the researcher might have had to adjust the technique that was used during needle placement. This may explain why the distance of the needle tip to the mandibular foramen in the first seven attempts was significantly larger when compared to both blind techniques and the second seven attempts (graph 2). Another explanation suggesting a learning effect was involved, was obtained by studying the results. The contrast medium was in close contact to the mandibular foramen during all seven attempts in the second group of the nerve stimulator-guided technique. This only occurred 3 out of 7 times in the first group. Furthermore staining of the inferior alveolar nerve occurred 6 out of 7 times in the second group when compared to 2 out of 7 times in the first group. Taken these results together with the percentage of foramen coverage being far more accurate in de second group, this strongly suggests that the researcher needed a few attempts to refine the nerve stimulator-guided technique.

Other guided techniques

Ultrasound-guided local anesthetic techniques have been used in humans for quiet some time now and meta-analysis comparing blind techniques versus ultrasound-guided techniques have shown that ultrasound guidance reduces the incidence of complications and improves quality of the block. When using this technique for the mandibular nerve block in humans, the ultrasound probe has to be placed intraorally medial to the mandibular ramus. Introduction of this technique has improved the outcome of inferior alveolar nerve block substantially in comparison to the common blind technique. Ultrasound-guidance may also improve the inferior alveolar nerve block in horses, but seems rather impractical due to the anatomy of the mouth. An alternative for the intraoral placement is to place the ultrasound probe to the ventromedial aspect of the mandibular ramus.[7]

Johnson performed this approach in 2019. This study was conducted on 8 cadaver heads with neck. Injection volumes of 2.5 mL and 5 mL were randomly administered, 1 of 2 volumes per hemi-mandible. Accuracy of the block was visualized with CT contrast medium and methylene blue to show nerve staining on dissection. Assessment by CT imaging resulted in a positive inferior alveolar nerve block in 81.3% (13/16) and the dissection success rate was 68.8% (11/16). The lager volume deemed more successful on CT-imaging than on dissection 87.5% (7/8) and 62.5% (5/8), compared to the smaller volume 75% (6/8) and 75% (6/8). 2 out of 8 larger injections resulted in only staining the lingual nerve but not the inferior alveolar nerve. Johnson concluded that although the ultrasound-guided approach improved visualization of the anatomical landmarks and thus provides a more precise localization of the injection side, this technique is challenging and no more accurate than the common blind technique. Johnson also describes the significant risk of desensitizing the lingual nerve, even while using the ultrasound-guided technique and a small volume of 2.5 mL.[9]

Conclusion

Although the nerve simulator-guided technique seems to localize the nerve with more accuracy, this study shows no beneficial effect when compared to the ventral blind approach in localizing the inferior alveolar nerve. The differences however are small and due to the limited number of horses used, no significant difference could be detected. Therefore the hypothesis Part II has to be rejected. Further research, repeated with a lager number of horses, will have to show if the hypothesis will then be approved. Because no significant difference from the null hypothesis was found, it cannot be said for certain that the currently recommended large volumes are not necessary.

This study shows the difficulty in only staining the inferior alveolar nerve, despite the technique used. Over 50% of the attempts during Part II led to staining or partial staining of the inferior alveolar nerve, despite of the relatively small volume used. Unfortunately in over 90% of these attempts the lingual nerve was also affected. This shows us that when using larger volumes the lingual nerve will inevitably be anesthetized. Practitioners should therefore be well informed about the local anesthetic they are applying and adjust the anesthetic to the specific duration of the procedure. Limiting the amount of oral movement and ensuring the local anesthetic effect has worn off before feeding the horse should also be applied in preventing unwanted self-inflicted oral trauma.

The in this study desired positive blocks, being the alveolar nerve stained and the lingual nerve not or partially stained, were only achieved three times and were all located in the ventro-caudal area close to the mandibular foramen (< 20 mm caudally and < 13 mm ventrally). This implicates that this is the area in which the most accurate blocking can be performed. Therefore the recommended aiming site used in this study might have been to far dorsally, which should be taken in consideration during future research.

In this study several significant differences were found when comparing all techniques and the distance of their needle tip placement to the mandibular foramen. These significant differences all contribute to the impression of a certain learning effect when first using the Stimuplex® A, B. Braun needle. These significant effects were no longer present when both blind techniques were compared to the second seven attempts of the nerve simulator-guided technique and therefore it can be concluded that this is caused by a learning effect.

The hypothesis Part I is approved, because no significantly higher amount of inferior alveolar nerve staining occurred when the ventral blind and the caudal blind technique were compared. However further research is recommended to determine if better results will be gained with the nerve stimulator-guided technique when using the caudal approach in vivo. Although not significant, Part I showed more positive staining of the inferior alveolar nerve with the caudal approach when compared to the ventral approach. More research is also recommended to reveal if well placed small volume nerve blocks, set with guided techniques will give a sufficient analgesia in living horses.

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