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RESEARCH PAPER

Peripheral nerve stimulation under ultrasonographic control to determine the needle-to-nerve relationship

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Abstract

Objective To determine the needle-to-nerve distances during electrical nerve location in dogs at different currents and pulse duration using a peripheral nerve stimulator (PNS) under ultrasound control (US), and the minimal electrical thresholds (MET) necessary to obtain a motor response (MR) after achieving needle-to-nerve contact.

Study design Prospective *in vivo* experimental trial in a clinical setting

Animals Thirty dogs, scheduled for locoregional anaesthesia of the sciatic nerve.

Methods Needle-to-nerve distance was measured ultrasonographically after obtaining the MR of sciatic nerve with 2, 1 and 0.5 mA and pulse duration 0.1 ms (NS0.1). Thereafter the needle was placed in contact with the nerve and MET was determined. The procedure was repeated with 0.3 ms (NS0.3). Finally the needle was reintroduced to contact the sciatic nerve guided only by US, thus MET-US was determined. Data were analysed using Kruskal–Wallis or Mann–Whitney tests.

Results Needle-to-nerve distances were greater when MR was obtained with 2 mA than with 1 and 0.5 mA at 0.1 and 0.3 ms. No significant differences were observed between the needle-to-nerve distances using 0.1 or 0.3 ms. The MET [median (range)] was 0.4 (0.18–1.3) mA in NS0.1, 0.32 (0.12–0.8) mA in NS0.3; while MET-US was 0.7 (0.32–1.5) mA. When the needle contacted the nerve, the MR achieved with currents below 0.3 mA was obtained in 17.2, 40 and 0% of cases using NS0.1, NS0.3 and US respectively.

Conclusions and clinical relevance The electrical current necessary to obtain a MR decreased as the needle moved towards the nerve. However when the needle tip contacted the nerve, a MR with low current intensity could not be obtained. Thus the absence of motor response at currents below 0.3 mA cannot rule out needle-epineurium contact. When ultrasound is combined with PNS, it is more important to assess the correct needle position than searching for an MR at low currents.

Keywords dog, needle to nerve distance, nerve stimulation, regional anaesthesia, sciatic, ultrasound.

Introduction

Since 1962 when Greenbaltt & Denson introduced the use of insulated needles and electrical stimulation for peripheral nerve location in clinical practice, several studies have been conducted in order to

understand and define the guidelines for peripheral nerve blocks (Greebblatt & Denson 1962; Pither et al. 1985; Urmey 2006; Dillane & Tsui 2012). The introduction of electrical nerve location in people enabled the performance of regional nerve blocks without asking the patient if paraesthesia was perceived: particularly useful in non-collaborative or sedated patients (Baranowski & Pither 1990). The method also enabled a reduction in the amount of local anaesthetics necessary to obtain an effective nerve block (De Andrés & Sala-Blanch 2001; Wenger et al. 2005).

Using the standard method for peripheral nerve stimulation, as the needle moves closer to the target nerve, less electrical current is needed to evoke a motor response of the effector muscle (Ford et al. 1984). This principle is an application of the Coulomb's law, which implies that the electrical current necessary to depolarize a nerve and thus elicit a muscular response exponentially decreases as the tip of an insulated needle advances towards the target nerve. For these reasons it was proposed that injections of local anaesthetics when a muscular response is elicited with electrical currents below 0.2 mA could be associated with intraneural injection and potential risk of nerve injury (De Andrés & Sala-Blanch 2001; Voelckel et al. 2005), while injections performed when a muscular response is elicited with 0.3–0.5 mA should be associated with correct needle placement and thus high percentage of successful blocks (Urmey 2006).

In the last decade, some controversies emerged about the safety of nerve location through electrical stimulation. Several studies have shown a dissociation between the elicitation of paraesthesia after achieving needle-to-nerve contact and the motor response, emphasizing that in 23–70% of cases the muscular twitch could be absent even delivering with high stimulating currents (Choyce et al. 2001; Urmey & Stanton 2002; Bollini et al. 2003). Moreover, after needle-to-nerve contact confirmed by ultrasound, motor response was only present in 9.8% of cases when the stimulating current was below 0.3 mA (Perlas et al. 2006), or could only be obtained with currents higher than 0.6 mA (Sauter et al. 2007). These findings highlight the discussion whether the electrical nerve location is a reliable technique to perform regional nerve blocks.

The objectives of our study were to measure, in dogs, the needle-to-nerve distances by ultrasound (US) during peripheral nerve stimulation (PNS) when a muscular response (MR) was obtained at different intensities of currents and pulse duration; and to determine the minimal electrical thresholds (MET) necessary to obtain a motor response after achieving needle-to-nerve contact. Our hypotheses were that the intensity of electrical current necessary to obtain a muscular response (MR) decreases as the needle tip moves toward the target nerve; and when the tip of the needle is contacting the nerve, a low current intensity is necessary to obtain the effector muscle response.

Materials and methods

The present prospective *in vivo* experimental trial in clinical setting was approved by the Institutional Animal Care and Use Committee of the University of Pisa (Prot. N. 2A-13372, 10/2009).

Animals

Thirty client-owned dogs, submitted to the Veterinary Teaching Hospital *"Mario Modenato"*, University of Pisa to undergo pelvic limb surgeries in which loco-regional anaesthesia of the sciatic nerve was scheduled as part of the analgesic protocol, were enrolled after obtaining owner's written consent. Patients weighting more than 40 or <15 kg, classified with the American Society of Anesthesiologists physical status ≥3, suffering from neurologic or neuromuscular diseases, clotting disorders or skin infection on puncture site were excluded from the study.

Animal instrumentation

After a clinical, haematological and biochemical evaluation to assess the good health status, dogs received 1 μg kg^{-1} of dexmedetomidine (Dexdomitor, Pfizer, Italy) and 0.1 mg kg^{-1} of methadone (Eptadone, Molteni Farmaceutici, Italy) intramuscularly. After 20 minutes, an intravenous (IV) catheter was placed in a cephalic vein. Induction and maintenance of general anaesthesia were achieved by IV administration of 3–6 mg kg^{-1} and continuous rate infusion of 15–25 mg kg^{-1} $hour^{-1}$ of propofol (Propofol Kabi 20 mg mL^{-1}, Fresenius Kabi S.r.l., Italy) respectively. After induction of anaesthesia and endotracheal intubation, dogs were administered an inspired concentration of oxygen of approximately 60% via a circle rebreathing system. Warm (38 °C) lactated Ringer's solution was administered during the entire procedure at a rate

of 2.5–5 mL kg^{-1} $hour^{-1}$. Body temperature was maintained above 37 °C using electrically heated and thermal foil blankets. During the entire procedure, heart and respiratory rates, mean non-invasive arterial pressure, end-tidal CO_2 concentration, and oesophageal temperature were monitored continuously. Dogs were carefully monitored in order to promptly detect nociceptive reactions (increases in heart rate, respiratory and/or mean arterial pressure) during nerve location. A multiparametric monitor (Mindray Beneview T5, China) was used to measure the variables described above.

Needle-to-nerve relationship evaluation

Dogs were positioned in lateral recumbency with the limb to be blocked uppermost. The hair of the lateral and caudal surface of the proximal thigh was clipped and the skin was aseptically prepared.

The needle-to-nerve relationship was evaluated using three different methods (phases) of nerve location:

- Phase-$NS_{(0.1\ ms)}$: Electrical nerve location using a pulse duration of 0.1 milliseconds (ms)
- Phase-$NS_{(0.3\ ms)}$: Electrical nerve location using a pulse duration of 0.3 milliseconds (ms)
- Phase-$US_{(0.1\ ms)}$: Ultrasound-guided nerve location

In the three phases, 100 mm 21G insulated needles with 30 short bevel (Locoplex, Laboratories Pharmaceutiques Vygon, France) were employed and connected to the negative 'black' pole (cathode) of a nerve stimulator (Plexygon, Vygon, Italy). The insulated needles were pre-filled with 0.9% saline. The positive 'red' pole (anode) of the nerve stimulator was always connected to the skin of the abdominal flank. A portable ultrasound machine with a 12 MHz linear array transducer (Venue 40, GE Medical Systems, China) was used for the US assessment of the needle-to-nerve relationship.

For the phase-$NS_{(0.1\ ms)}$, the nerve stimulator was set at 2 Hz, 0.1 ms and initially with a stimulating current of 2 mA. The insulated needle was introduced perpendicularly to the limb axis, through the caudal aspect of the pelvic limb, 1–4 cm distally to the greater trochanter of the femur depending on the dog size. The insulated needle was advanced through the semimembranosus muscle toward the sciatic nerve until either the gastrocnemious (extension of the tarsus joint) or the fibular longus (flexion of the tarsus joint) muscular response due to the stimulation of the tibial and/or peroneal component of the sciatic nerve respectively were evoked. Only brisk and vigorous MR was considered as correct end-points. As the biceps femoris, semimenbranosus and semitendinosus muscles are innervated by the caudal muscular branch of the sciatic nerve, twitches of these muscles were not considered correct end-points.

Once the proper MR was evoked, the needle was fixed in that position and in order to obtain a short axis image of the sciatic nerve and a longitudinal view of the needle (in-plane technique), the ultrasound transducer was positioned distally to the greater trochanter of the femur (Fig. 1) (Campoy et al. 2010). The nerve stimulator was turned-off and the distance between the needle tip and the outermost part of the sciatic nerve (epineurium) was recorded (Fig. 2).

Once these measurements had been completed, the ultrasound transducer was removed, the nerve stimulator was turned-on again and after reconfirming

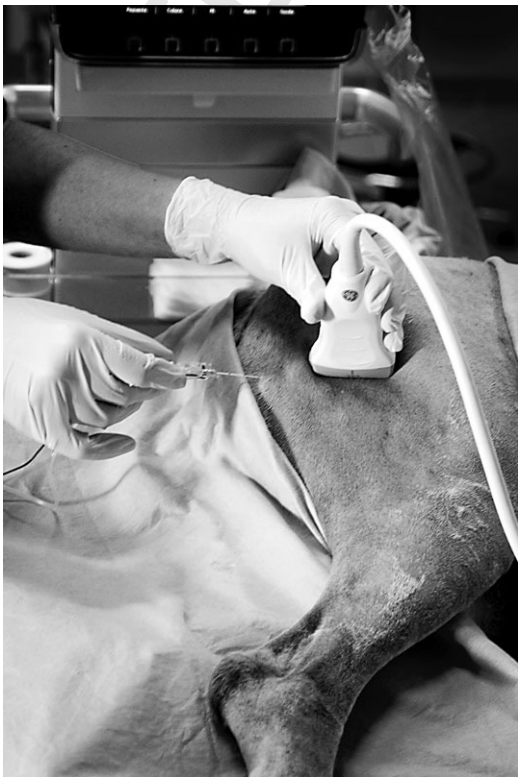

Figure 1 Lateral view of the right pelvic limb in a dog. The stimulating needle is introduced through the caudal aspect of the limb, perpendicular to the sciatic nerve. The ultrasound transducer is positioned distally to the greater trochanter of the femur in order to obtain a short axis view of the sciatic nerve and an in-plane view of the stimulating needle (Campoy et al. 2010).

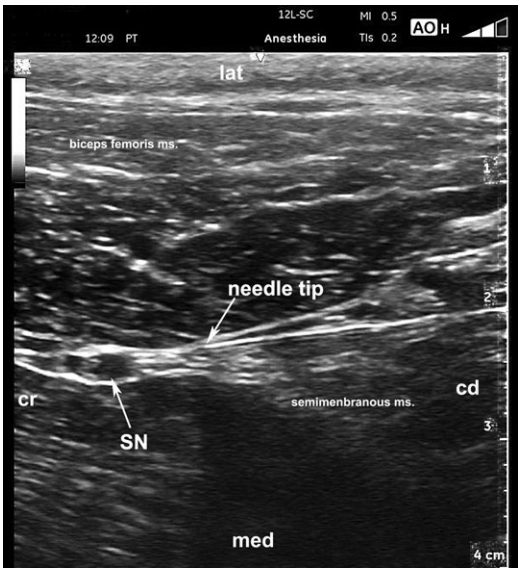

Figure 2 Lateral ultrasound view of the pelvic limb in a dog. The sciatic nerve (SN) is observed in a short axis view, while the stimulating needle is in plane with the ultrasound beam. Cr: cranial; Cd: caudal, med: medial, lat: lateral.

the MR with 2 mA the stimulating current was reduced to 1 mA and the needle further advanced until the same MR was obtained. The distance between the needle tip and the sciatic nerve was measured again by US as described above. Subsequently, the needle-to-nerve distance was evaluated when the MR was obtained with a stimulating current of 0.5 mA. Thereafter the nerve stimulator was set to 0 mA, and employing a direct ultrasound view and conserving the needle's trajectory, the tip was advanced towards the sciatic nerve until it was in contact with the epineurium. Needle-to-nerve contact was defined as a slight indentation and/or displacement of the nerve. When the needle tip had contacted the nerve, the current was gradually increased at 0.1 mA increments until a MR of the effector muscles was elicited; thus the MET using a pulse duration of 0.1 ms was registered.

In the phase-$NS_{(0.3\ ms)}$, the needle-to-nerve distances and MET were evaluated using the same methodology described in the phase-$NS_{(0.1\ ms)}$ but with a pulse duration of 0.3 ms.

Finally, in the phase-$US_{(0.1\ ms)}$, the needle was completely removed and reintroduced by a second researcher who was not aware of the point where the needle had previously contacted the nerve. In this phase, the stimulating needle, with the nerve stimulator set to 0 mA, was directed toward the caudal aspect of the sciatic nerve, only guided by direct ultrasound view. Once the needle contacted the sciatic nerve the intensity of current was gradually increased until obtaining the MET necessary to evoke the MR as described above. At least 90 seconds passed between two consecutive phases.

Both researchers performing the nerve stimulation and ultrasound measurements were skilled with regional nerve blocks under NS and US guidance.

When all measurements were recorded, sciatic and femoral nerves were blocked with 0.1 mL kg^{-1} $nerve^{-1}$ of bupivacaine 0.5% as described by Campoy et al. (2010) and Echeverry et al. (2012) respectively. Data concerning the quality of the sensory and motor blockade, the intra-operative and post-operative outcome were not included in the present study.

After intervention, a 15 days follow-up period was performed in order to detect any complication such as proprioceptive deficits or pain on puncture site.

Statistical analysis

Data were evaluated for normal distribution (Shapiro–Wilk normality test). Measured distances obtained after nerve stimulations with the different intensities of current and those obtained between the different pulse durations were analysed using a Kruskal–Wallis test and a Dunn's *post hoc* test. Comparisons between the different METs were evaluated using a Mann–Whitney test. Differences were considered significant when $p < 0.01$. Data normally distributed are presented as mean ± SD, and non-parametric data as median (range).

Results

Phase $NS_{(0.1\ ms)}$, $NS_{(0.3\ ms)}$ and $US_{(0.1\ ms)}$ were performed in 30, 15 and 21 out of 30 cases respectively. Dogs weighed 30.2 ± 4.2 kg. None of the enrolled subjects showed signs of nociception during the procedure and no complication associated to nerve stimulation or nerve blocks was observed. Recovery and follow-up period were uneventful.

The registered needle-to-nerve distances after sciatic nerve location are shown in Table 1. In phase-$NS_{(0.1\ ms)}$ the distance between the needle tip and the nerve was significantly less when the MR was obtained with 0.5 mA than with 1 and 2 mA ($p < 0.001$). In phase-$NS_{(0.3\ ms)}$, the distance between the tip of the needle and the sciatic nerve

Table 1 Median (range) of needle-to-nerve distances measured by ultrasound after peripheral nerve stimulation of the sciatic nerve with 2, 1 and 0.5 mA with a pulse duration of 0.1 and 0.3 ms in dogs

	Needle-to-nerve distance (mm)	
Intensity of current (mA)	**0.1 ms**	**0.3 ms**
2	6.0 (2.3–23.3)	7.5 (4.5–19.0)
1	4.6 (1.5–14.6)	3.9 (2.3–12.2)
0.5	2.3 (0–8.6)	2.2 (1.0–7.0)

was significantly greater when the MR was obtained with 2 mA than with 1 and 0.5 mA ($p < 0.01$). There were no significant differences between phase-$NS_{(0.1\ ms)}$ and phase-$NS_{(0.3\ ms)}$ regarding the needle-to-nerve distance at any stimulating current (i.e. 2 mA: $p = 0.7$; 1 mA: $p = 0.98$; 0.5 mA: $p = 0.85$).

Muscular response could not be obtained with 0.5 mA in two cases, one in $NS_{(0.1\ ms)}$ and one in $NS_{(0.3\ ms)}$ phase. Direct undesired needle-to-nerve contact during the electrical nerve location was observed in one case in phase-$NS_{(0.1\ ms)}$ with 0.5 mA (3.3%). Thus in 96.6% of the nerve location the MR was obtained before the needle tip contacted the nerve. Undesired needle-to-nerve contact using a pulse duration of 0.3 ms did not occur.

The minimal electrical threshold (MET) necessary to produce the MR after obtaining needle-to-nerve contact was 0.4 (0.18–1.3) mA in phase-$NS_{(0.1\ ms)}$, 0.32 (0.12–0.8) mA in phase-$NS_{(0.3\ ms)}$ and 0.7 (0.32–1.5) mA in phase-$US_{(0.1\ ms)}$ (Fig. 3). The obtained MET was significantly higher during phase-$US_{(0.1\ ms)}$ compared to phases $NS_{(0.1\ ms)}$ and $NS_{(0.3\ ms)}$; moreover MET in phase-$NS_{(0.3\ ms)}$ was significantly higher than MET in phase-$NS_{(0.1\ ms)}$ (Fig. 3). The cumulative rates of evoked MR to increased electrical currents after obtaining needle-to-nerve contact in the different phases are shown in Fig. 4.

Discussion

The use of electrical nerve location was introduced in veterinary medicine in order to reduce the subjectivity of blind blocks and to increase the accuracy and success rate of regional nerve blocks (Wenger 2004; Campoy 2006; Campoy et al. 2008; Portela et al. 2013). With the introduction of ultrasound in the field of regional anaesthesia, it is now possible to observe in real time the relationship between the needle tip and the nerve, as well as the surrounding anatomical structures and local anaesthetic distribution (Campoy et al. 2010; Echeverry et al. 2010, 2012; Shilo et al. 2010).

To the author's knowledge this is the first study in veterinary regional anaesthesia conducted to establish the relationship between electrical and ultrasound nerve location. The study evaluates how the needle tip is related to a target nerve in dogs when

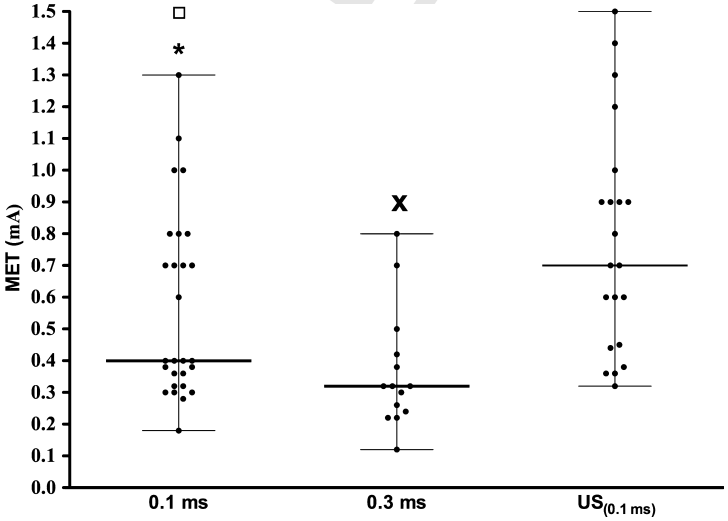

Figure 3 Minimal electrical thresholds (MET) necessary to produce the motor response after obtaining needle-to-nerve contact assisted by nerve stimulator-ultrasound (pulse duration 0.1 and 0.3 ms) or ultrasound alone (US) to guide the tip of the needle towards the sciatic nerve in dogs. □: significant differences with 0.3 ms ($p = 0.021$); * significant differences with US (0.1 ms) ($p = 0.029$); ×: significant differences with US (0.1 ms) ($p = 0.0002$).

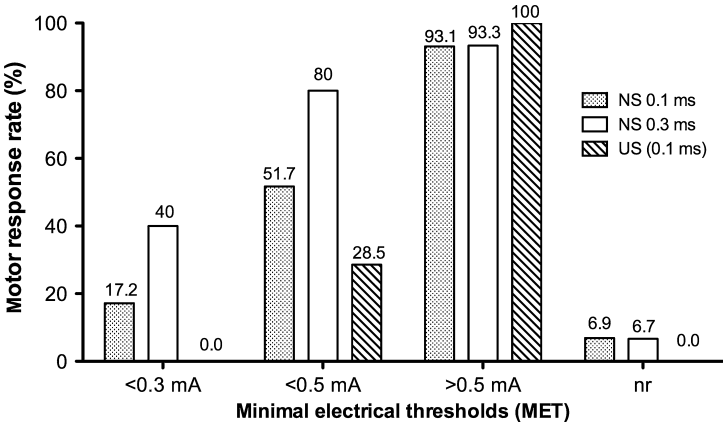

Figure 4 Cumulative rates of evoked MR (%) to increased electrical currents (<0.3 mA, <0.5 mA, >0.5 mA or without motor response [nr]) after obtaining needle-to-nerve contact assisted by nerve stimulator-ultrasound (pulse duration 0.1 and 0.3 ms) or ultrasound alone (US) to guide the tip of the needle towards the sciatic nerve in dogs.

customary sciatic nerve electrolocation is employed in a clinical veterinary setting. It was confirmed that the intensity of current necessary to obtain a motor response of the effector muscle decreases as the needle tip moves towards the nerve, according with the Coulomb's law. However, when the needle tip contacted the outermost part of the nerve (epineurium), the intensity of current necessary to obtain the motor response was 0.4 (0.18–1.3) and 0.32 (0.12–0.8) mA at 0.1 and 0.3 ms, respectively, or 0.7 (0.32–1.5) mA when the nerve was approached only by US. Therefore our hypothesis that a motor response could be elicited with a low intensity of current (i.e. <.3 mA) when the tip of the needle contacts the target nerve could not be confirmed.

Tsai et al. (2008) reported that in pigs the MR could only be obtained when the tip of the needle was located at least 1 mm away from the nerve, but when the tip was positioned at a distance between 5 and 20 mm from the nerve, the MR could not be evoked even with high stimulating currents (i.e. 2 mA, 0.1 ms). These results are in contrast with the observations in our study (Table 1). However, in the cited study, the nerve stimulation was performed in an open sciatic nerve model, in which the desiccation of the exposed tissues may have altered their impedance, changing the conductive properties. Other reports conducted in cats and rabbits show how the stimulating current necessary to evoke a motor response decreases as the tip of the needle gets closer to the target nerve, which is in agreement with the findings in our study (Ford et al. 1984; Sung 2004).

The recommended endpoint for a successful nerve block is the achievement of a specific motor response with a stimulating current of 0.5 mA (Magora et al. 1969; De Andrés & Sala-Blanch 2001; Urmey 2006), but in order to avoid an intraneural injection, it has been suggested that there should be lack of motor response with 0.2 mA (Voelckel et al. 2005). Despite these suggestions, several reports show how dissociation between needle-to-nerve contact and motor response can occur (Choyce et al. 2001; Moon 2002; Urmey & Stanton 2002; Bollini et al. 2003; Hogan 2003; Perlas et al. 2006). In 2003, Bollini et al. conducted a study in people showing that after obtaining a correct MR with a customary approach at the interscalene level, and advancing the needle until elicitation of paraesthesia, a motor response could only be obtained in 61.9% of the patients with an electrical current ≤0.5 mA, while in the remaining 38.1% the MR only occurred after slight withdrawal of the needle. In another study, using ultrasound direct view, Perlas et al. (2006) put the tip of the insulated needle in direct contact with nerves of the brachial plexus and observed that the sensitivity of nerve stimulation to detect needle-to-nerve contact with low stimulating currents (≤0.5 mA and pulse duration of 0.1 ms) resulted in 75%, therefore a 25% of 'false-negative' cases occurred in which no muscle response could be elicited. Furthermore, studies in laboratory animals showed that even with an intraneural needle placement under direct visualization, the MR might be absent (Chan et al. 2007; Tsai et al. 2008). Likewise, the study reported here

showed that after the needle tip contacts the nerve wall, the MR with a stimulating current below 0.3 mA could only be obtained in 17.2 and 40% of the cases with pulse duration of 0.1 and 0.3 ms respectively (Table 1; Fig 4). Moreover, after obtaining needle-to-nerve contact, the MET was higher (Fig 3) than the minimal electrical threshold of 0.2 mA recommended to avoid intraneural injections (Voelckel et al. 2005). It is interesting to observe that the MET differs on the basis of the method employed for nerve location (Fig 3, Fig 4). The different METs observed between phase-$NS_{(0.1\ ms)}$ [0.4 (0.1 –1.3) mA] and phase-$NS_{(0.3\ ms)}$ [0.32 (0.8–1.2) mA] could be explained by the density of electrical charge in the needle tip, in which higher pulse duration can evoke a MR with a lower intensity of current (Hadzic et al. 2004a; Sauter et al. 2009).

As shown in these results, after the needle tip contacted the nerve wall, only in few cases the MR could be elicited with a low current (Fig 4). Possible explanations have been formulated for this occurrence (Tsai et al. 2008; Sauter et al. 2009; Li et al. 2011). One ascribes this phenomenon to the different tissue impedances that the needle tip crosses through its path toward the nerve. The electrical current follows the 'way' of minimal electrical resistance (low impedance), therefore if the perineural tissue has low impedance, it could deviate the electrical flow away from the nerve. Sauter et al. (2007, 2009) demonstrated in people that nerves surrounded by muscles (low impedance) show higher MET compared with nerves surrounded by connective or fat tissues (high impedance). Therefore the connective tissue that normally covers a peripheral nerve could modify the response to the nerve stimulation and it could also explain the large variance in needle-to-nerve distances registered at any current intensities (Table 1). Nerve stimulators that measure the tissue impedance could be helpful to better understand and clinically apply the electrical nerve location. Another possible cause regarding the lack of MR after direct needle-to-nerve contact could be attributed to the nerve hyperpolarization consequent to repeated electrical stimulations, known as conduction block phenomenon (Li et al. 2011). Therefore lack of motor response with low intensity of current during electrical nerve location cannot exclude that the needle tip is already contacting the nerve wall. However, Bigeleisen et al. (2009) reported that when the needle tip is positioned intraneurally the MET was lower (0.30 ± 0.19 mA) than the MET observed when the needle tip is contacting the nerve wall outside the epineurum (0.60 ± 0.37 mA).

The aforementioned studies on needle-to-nerve relationship did not exploit the main advantage of electric nerve stimulation, which is the possibility to gradually approach the nerve, correlating the motor response to the intensity of current. In the present study it emerged that although MR could be absent when the tip of the needle contacts the epineurium, while the needle was moving towards the nerve, 0.5 mA elicited the MR in 96.6% (0.1 ms) and 100% (0.3 ms) of cases before the tip of the needle contacted the target nerve.

An MR with 0.2 mA and the resistance to injection have been proposed as indicators of possible intraneural injections (Hadzic et al. 2004b; Voelckel et al. 2005). However these concepts are still unclear. As noticed in the present and other studies, there is an electrical 'dark' zone surrounding the nerve, in which the electrical stimulus could be unable to depolarize the nerve. Thus the lack of MR with low intensity of current is not a reliable indicator of needle-to-nerve contact (Johnson et al. 2007; Sauter et al. 2007; Tsai et al. 2008; Robards et al. 2009).

In a clinical veterinary setting, after positioning the insulated needle, the proper MR can sometimes be difficult to obtain, even when the needle is correctly located. This happens most frequently with relatively superficial nerves, for example when the sciatic nerve is approached between the greater trochanter of the femur and the ischiatic tuberosity (Campoy 2006). In such cases, when the needle pierces the skin and passes the thin muscular plane, its tip could already be located in the electrical 'dark' zone or actually in direct contact with the nerve wall, hence the muscular response could be absent. This issue should be considered, especially in patients with poor muscular masses in which the peripheral nerves could be relatively shallow, in order to avoid unnecessary attempts to elicit the proper muscular response with potential nerve trauma. Further studies are necessary to better characterize this occurrence in dogs.

Interestingly in our study, when the MET was determined only using the US guide [Phase-$US_{(0.1\ ms)}$], the minimal electrical threshold to obtain the MR was much higher [0.7 (0.32–1.5) mA] than that obtained when the needle was directed towards the nerve using the nerve stimulation (Fig 3) and MR could only be obtained in 28.5% of cases with a

stimulating current below 0.5 mA (Fig 4). Although two different researchers performed these determinations, the differences could better be explained by the different methods used to approach the nerve. Using the PNS, the advancement of the needle tip is guided by the MR feedback, therefore it will be directed essentially toward the motor component of the mixed nerve. When the needle is guided only by US, its tip is directed to an arbitrary point of the epineurium, which is not necessarily related to the motor components of the nerve (Hogan 2003; Bollini & Cacheiro 2007). The internal structure of the nerves may not be homogeneous, as described for rat sciatic nerve, in which only 6% of the axons in the mid-thigh are myelinated motor axons (Schmalbruch 1986). Therefore, the heterogenic distribution of the motor components of the nerve could have influenced the different METs obtained when the needle tip contacted the sciatic nerve wall.

The effectiveness of the nerve block was not evaluated in the present study, but a report in people shows that ultrasound-guided interscalene needle placement produces successful anaesthesia regardless of elicitation of the effector motor response above or below 0.5 mA (Sinha et al. 2007).

Our study had several limitations: first ultrasound is an operator-dependent technique with an important subjective component, especially regarding the measurement of needle-to-nerve distances and determination of contact between needle tip and nerve wall. Although the measurements were performed by experienced clinicians, some individual and subjective interpretation cannot be ruled out. Moreover, even if the elapsed time between two consecutives nerve stimulations was about 90 seconds, multiple attempts can theoretically result in hyperpolarization of the nerve and influence subsequent stimulations (Bhadra & Kilgore 2004; Sauter et al. 2007; Li et al. 2011). Another possible limitation was that the needle was introduced three times toward the nerve. The minimum trauma that the needle produces during its passage through the tissues can create oedema or haemorrhage, that act as conductive solutions, increasing the tip's conductive area and perhaps modifying the response to nerve stimulation. Nevertheless, in a clinical setting several attempts before finding the correct needle position might be needed, especially at the beginning of the learning curve for nerve blocks.

In conclusion when the sciatic nerve is approached at the level of the proximal thigh in dogs using peripheral nerve stimulation, the electrical current necessary to obtain the effector muscular response decreases as the needle moves toward the nerve. When the needle tip is in contact with the epineurium, a motor response may not be elicited at low current intensities (0.3 mA), and the clinician should be aware of this factor. However, when the needle is advanced towards the nerve, the effector muscle response can be evoked before the needle tip contacts the target nerve in almost all cases. Therefore, during the combined ultrasound-electrical stimulation nerve blocks, lack of muscular response must be interpreted carefully and assessing the correct needle tip position is more important than making unnecessary needle movements in order to search a muscular response with currents below 0.5 mA.

Further studies are required in order to better understand the relationship between the electrical nerve location and the muscular response of specific nerves in dogs and the clinical implications of the electrical 'dark zone' of the nerves during electrolocation.

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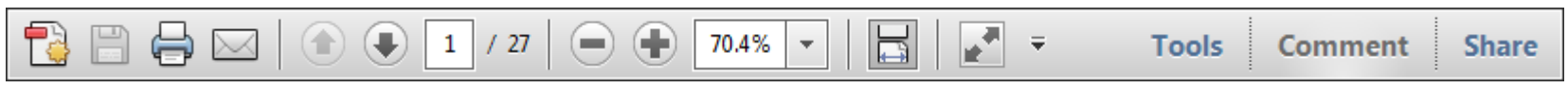

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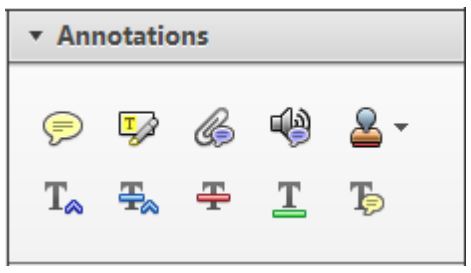

1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
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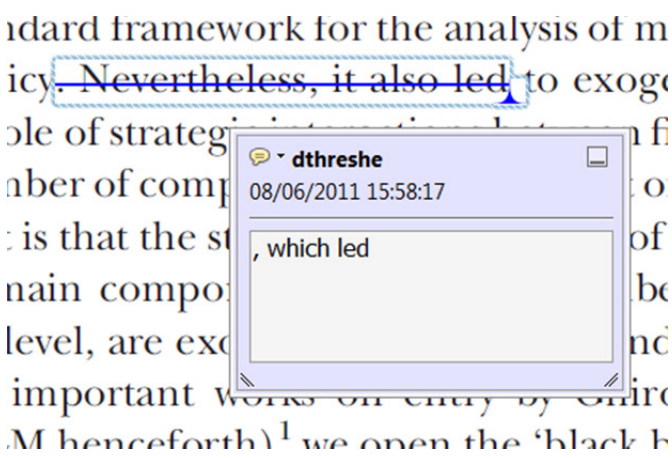
- Type the replacement text into the blue box that appears.

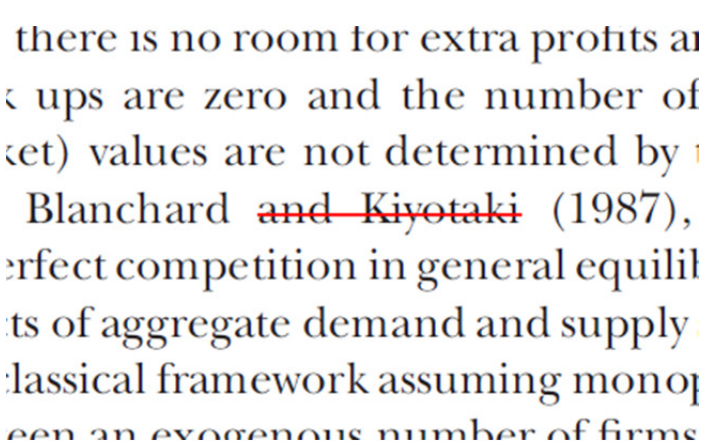


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.



3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
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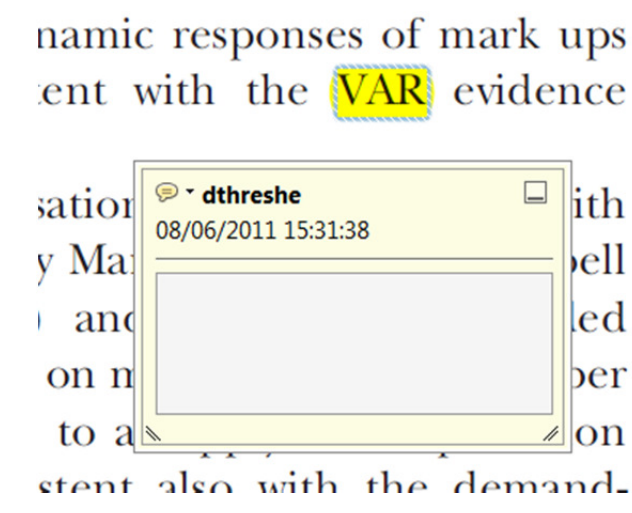

4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
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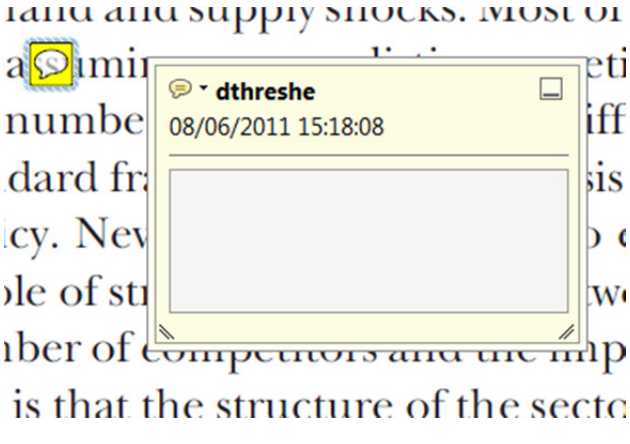

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5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
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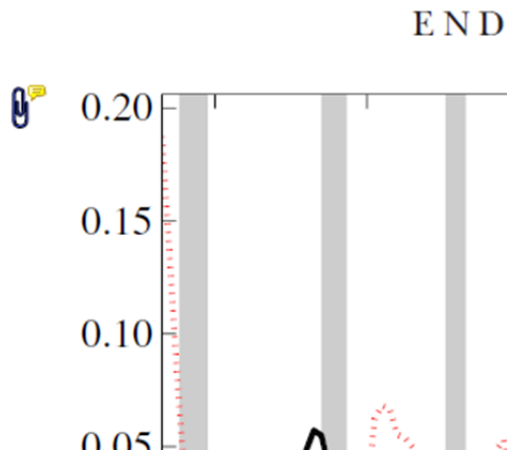

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Inserts a selected stamp onto an appropriate place in the proof.

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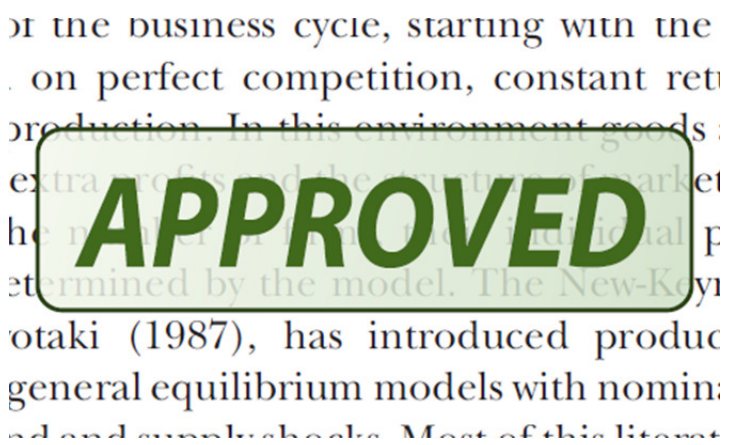

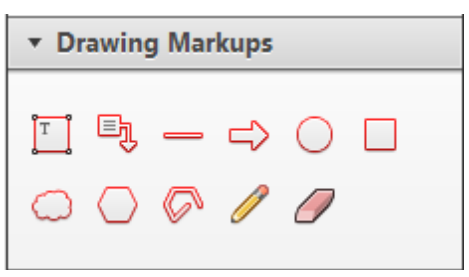

7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
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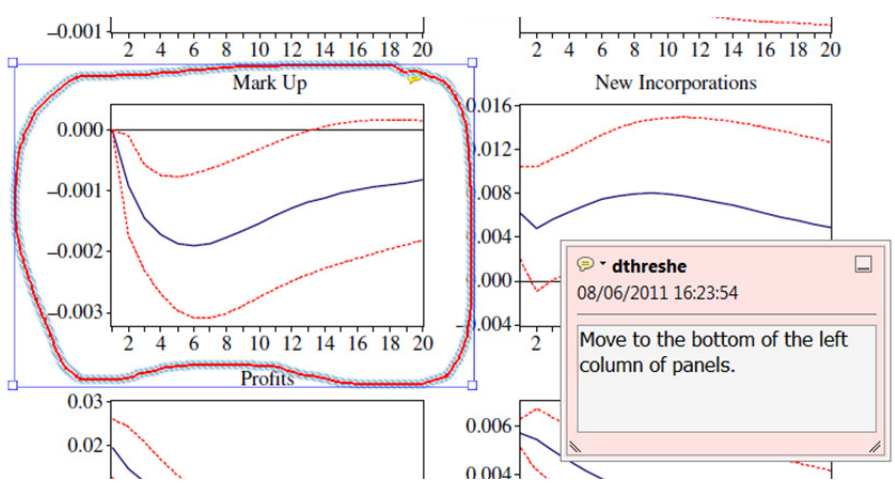
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



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