



Mechanical nociceptive thresholds of dorsal laminae in horses after local anaesthesia of the palmar digital nerves or dorsal branches of the digital nerve

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ABSTRACT

We examined the hypothesis that the palmar digital nerves (PDNs), but not the dorsal branches (DBs) of the digital nerves, innervate the sensitive dorsal laminae of the equine foot by evaluating the effects of perineural anaesthesia of the PDNs and DBs separately on pain sensation evoked via mechanical stimulation of the dorsal laminae and other regions of the equine foot. Six clinically normal mares were used in a crossover design. A portable dynamometer was used to evaluate mechanical nociceptive thresholds at different points on the dorsal laminae, bulbs of the heel, coronary band and sole before and after the horses underwent perineural injection of PDNs or DBs with a local anaesthetic solution (treated group) or an isotonic saline solution (control group). Cornified tissue was removed from the sole and the dorsal aspect of the hoof wall before evaluations of mechanical nociceptive thresholds.

Anaesthetising PDNs distal to the DBs increased mechanical nociceptive thresholds compared to baseline values ($P < 0.001$) at sites assessed in the dorsal laminae, sole, and the bulbs of the heels. Anaesthetising DBs increased mechanical nociceptive thresholds compared to baseline values ($P < 0.01$) only at sites assessed at the most proximal aspect of the foot (i.e., coronary band sites). In conclusion, PDNs, not DBs, are primarily responsible for pain signal transmission evoked by pressure in the dorsal laminae of the foot of clinically normal horses.

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Introduction

Anaesthesia of the palmar digital nerves (PDNs) at the level of the ungular cartilages has been reported to desensitise only the palmar/plantar third or half of the foot (Adams, 1974; Stashak, 1987). However, more recent studies demonstrated that anaesthesia of the PDNs also desensitised the entire sole (Schumacher et al., 2000) and distal interphalangeal joint (Easter et al., 2000).

The contribution of the PDNs to nociception in the dorsal hoof wall has not been documented despite progress in establishing the structures of the equine foot that are desensitised by PDN blocks. Numerous authors have stated that anaesthesia of the palmar/plantar digital nerves, at the level of or distal to the proximal border of the ungular cartilages (i.e., distal to the dorsal branches of the palmar digital nerves, DBs), does not desensitise the dorsal laminae

of the foot (Stashak, 2002; Kaneps, 2004; Bassage and Ross, 2010; Moyer et al., 2011; Schumacher et al., 2013), and the dorsal laminae are only desensitised by anaesthetising the DBs (Schumacher et al., 2007; Bassage and Ross, 2010; Moyer et al., 2011). However, this theory has not been tested experimentally.

The current study examined the hypothesis that the PDNs, but not the DBs, innervate the sensitive dorsal laminae of the equine foot, by evaluating the effects of perineural anaesthesia of the PDNs and DBs separately on pain sensation elicited via mechanical stimulation of the dorsal hoof wall and other regions of the equine foot.

Materials and methods

Horses and experimental design

The Universidade Federal de Minas Gerais Ethics Committee in Animal Experimentation approved this study (Approval No. 56, 9 April 2014). Eight crossbred mares with mean \pm standard deviation (range) age of 12.8 ± 4.4 (5–16) years, mean body condition score (Henneke et al., 1983) of 6.2 ± 0.8 (5–7) and mean bodyweight of 360 ± 17.7 (340–380) kg were enrolled. All horses were healthy based on physical examination and haematological analyses. None of the horses was observed to be

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lame when examined while walking and trotting on a hard surface in a straight line and circled in both directions. All of the horses had a normal response to hoof testers.

The effects of anaesthetising the PDNs (distal to the branching of the DBs) were compared to the effects of anaesthetising the DBs in the same horse using a cross-over design. Only the forelimbs were used, with one limb being randomly chosen as the treated limb by the flip of a coin. Local anaesthesia in the treated limb was performed as described below using a perineural injection of a 2% lidocaine hydrochloride solution (Xylestesin, Cristalia) adjacent to the medial and lateral PDNs and the medial and lateral DBs. PDNs were anaesthetised first in four of the horses, and the DBs were anaesthetised first in the remaining horses to mitigate the potential effect of treatment order.

An isotonic saline solution (NaCl 0.9%) was injected in the control limbs in an identical manner as the treated limb, adjacent to the PDNs or DBs. The same re-

searcher (C.P.) performed all perineural injections and was masked to the type of solution injected.

Perineural infiltration of the PDNs and the DBs was performed on the same day, but the second nerve injection was performed only when the painful sensation of the foot had completely returned to the baseline values. A minimal interval of 4 h was established between perineural injections of PDNs and DBs. Lidocaine without epinephrine was used to limit the duration of anaesthesia.

Perineural blocks

Perineural injections of PDNs were performed as described by Moyer et al. (2011), with the distal portion of the forelimb held off the ground. A 25-gauge, 1.6-cm needle was directed distally through the skin approximately 1 cm proximal to the proximal border of the ungular cartilage of the distal phalanx on the palmar aspect of the neurovascular bundle.

Perineural injections of the medial and lateral DBs were performed with the distal portion of the limb held off the ground, using an approach similar to the one previously described for semi-ring block (Moyer et al., 2011), in which a 23-gauge 2.0-cm needle was inserted at the level of the middle third of the proximal phalanx in a dorsal direction (perpendicular to the long axis of the pastern) into the subcutaneous tissue dorsal to the neurovascular bundle.

A 1 mL volume of lidocaine or saline solution was used for each PDN injection, and 2 mL was used for the DB injections due to the less predictable anatomical location of these nerves. The perineural injection of PDNs with a local anaesthetic solution was considered accurate when the sensitivity to focal pressure at the bulbs of the heel and the solar corium (Schumacher et al., 2000) was lost, but sensitivity remained at the dorsal aspect of the coronary band. Perineural injections of DBs of the medial and lateral digital nerves with local anaesthetic solution were considered accurate when the sensitivity to focal pressure at the dorsal aspect of the coronary band was lost, but the sensitivity to focal pressure applied to the bulbs of the heel remained. Sensitivity was considered present when a withdrawal response to pressure applied using the tips of closed Kelly haemostatic forceps on the assessed site was observed. The same researcher performed all of the tests in a standard manner. Horses were excluded from the experiment when anaesthetic accuracy was not achieved using the pre-established lidocaine volumes.

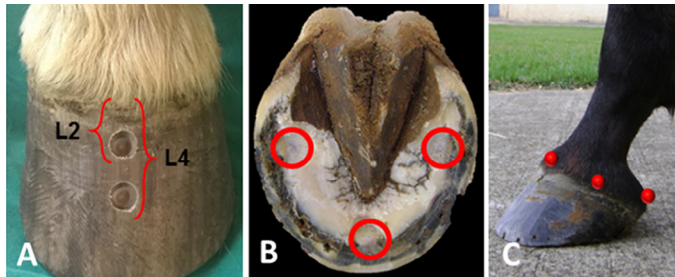


Fig. 1. Sites assessed for mechanical nociceptive thresholds in horses subjected to perineural injection with a local anaesthetic solution (treatment group) or isotonic saline solution (control group) around the palmar digital nerves or the dorsal branches of the digital nerves. (A) Proximal (L2) and distal (L4) dorsal laminae. (B) Dorsal, palmarolateral and palmaromedial aspects of the sole. (C) Heel bulb and lateral, medial and dorsal aspects of the coronary band.

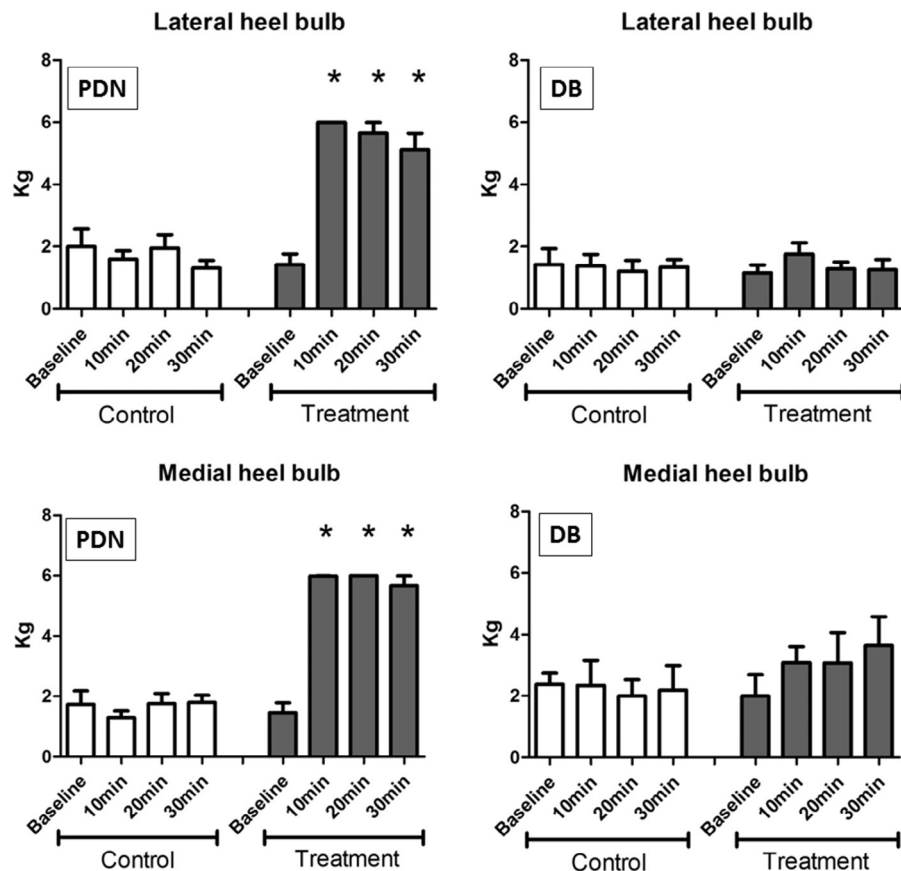


Fig. 2. Mean (\pm standard error of the mean) mechanical nociceptive threshold (MNT) expressed as kg of force (Kg, Y axis) at the lateral and medial bulbs of the heel of horses before (baseline) and 10, 20, and 30 min after (X axis) perineural injections of a local anaesthetic solution (treatment group) or isotonic saline solution (control group) of the palmar digital nerves (PDN) or the dorsal branches of the digital nerves (DB). The asterisk indicates a significant difference compared to baseline values in the same group or the control group value at the same time point ($P < 0.001$).

Algometry

Effects of the perineural deposition of the local anaesthetic or isotonic saline solution on sensitivity within various regions of the foot were evaluated using a portable dynamometer with a 12-cm-long stem (ITFG-5020, Instrutemp), as described in previous studies in horses (Haussler et al., 2008; Zarucco et al., 2010; Jordana et al., 2014) and dogs (Harris et al., 2015). The stem was applied at a 90° angle to various regions of the foot and coronary band with continuously increasing force. A 7 mm diameter flat tip was fixed to the stem to test the sensation at the coronary band, and a conical-shaped tip (base diameter, 7 mm) was fixed to the stem to test sensations in the keratinised areas of the hoof wall and sole.

Baseline mechanical nociceptive thresholds (MNTs) for all assessment sites were established on both feet prior to perineural injections with anaesthetic or saline solutions. The MNT was defined as the minimal force required to stimulate the horse to move the limb. MNTs were measured using a dynamometer 10, 20, and 30 min after the perineural injection of the local anaesthetic or isotonic saline solution to

assess the effect of treatment. The test was discontinued if the force reached 6 kg to avoid tissue injury from the dynamometer (Zarucco et al., 2010).

One researcher (C.P.) performed all MNT assessments. Two experienced researchers (J.M. and H.M.) recorded the dynamometer force at the exact moment the horse moved. We obtained two recordings of force by consensus of two assessors for each area examined. The mean of these two values was considered the result. All of the researchers who assessed the response to injections were masked to limb treatment.

Assessment sites

A 10 mm diameter circular section of keratinised tissue (horn) was removed from the dorsal aspect of the hoof, 2 cm (site L2) and 4 cm (site L4) distal to the coronary band, to assess the pressure sensitivity in dorsal laminae before and after perineural injections (Fig. 1). Horses were restrained in stocks without sedation to remove the circular section of horn. The dorsal area of the hoof was scrubbed and

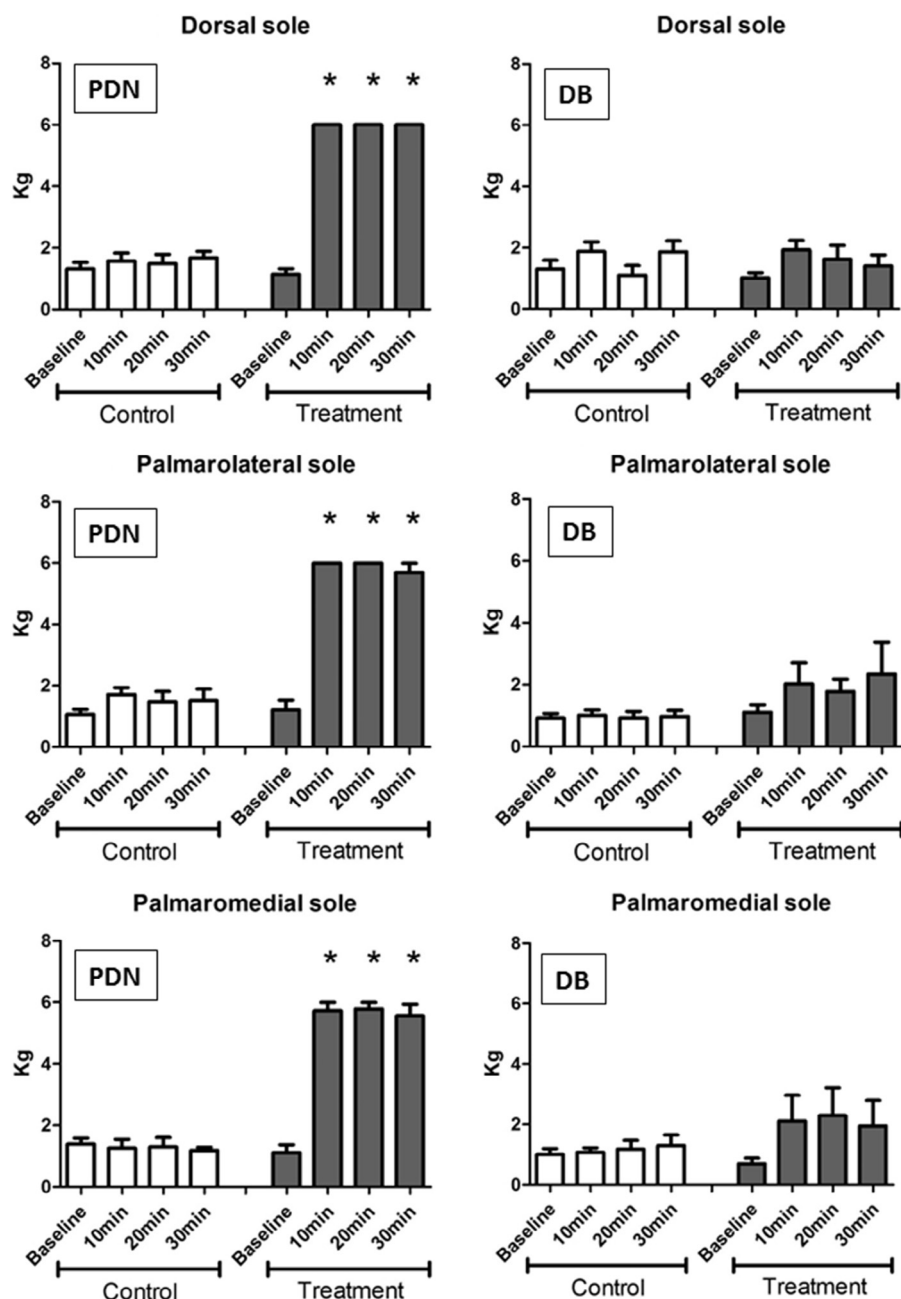


Fig. 3. Mean (\pm standard error of the mean) mechanical nociceptive threshold (MNT) expressed as kg of force (Kg, Y axis) at the palmarolateral, dorsal, and palmaromedial aspects of the sole of horses before (baseline) and 10, 20, and 30 min after (X axis) perineural injections of a local anaesthetic solution (treatment group) or isotonic saline solution (control group) of the palmar digital nerves (PDN) or the digital dorsal branches of digital nerves (DB). The asterisk indicates a significant difference compared to the baseline value in the same group or the control group value at the same time point ($P < 0.001$).

a sterile, 10 mm diameter stainless steel drill bit mounted on an electric screwdriver was positioned at a 90° angle to the surface of the hoof wall and advanced at low speed until resistance to drilling suddenly decreased and the remaining keratinised tissue could be depressed using a haemostat. There was no bleeding or damage to the sensitive laminae, but a foot withdrawal response could be obtained by the application of focal pressure to the hoof defect.

A 1 cm² area of keratinised tissue was removed at the dorsal, palmarolateral and palmaromedial aspects of the sole to assess the solar sensitivity to pressure before and after perineural injections (Fig. 1). Keratinised tissue was removed until the soft horn was exposed. There was no bleeding of the solar corium, but a limb withdrawal response could be obtained by the application of focal pressure to the sole defect.

The sensitivity of the skin to the pressure was assessed in the lateral and medial bulbs of the heel and three regions of the coronary band: lateral, dorsal, and medial aspects (Fig. 1).

Statistical methods

Repeated measures ANOVA was used to compare the values within each group over time, and the mean MNTs were compared using the Newman–Keuls test. Student's *t* test was used to compare group values within each time point. Log transformation was used when the data were not normally distributed. The level of statistical significance used for all tests was $P < 0.05$.

Results

Two of the eight horses selected for this experiment were not included in the analyses because anaesthesia of the PDNs failed to diminish nociception at any of the three sites tested for sensitivity on the sole despite producing insensitivity in the bulbs of the heel.

The mean baseline values (\pm standard deviation; baseline prior to any injection) of MNT of right and left front feet in six horses were 1.53 ± 0.81 , 1.50 ± 0.78 , and 1.71 ± 0.98 kg for the lateral, dorsal,

and medial aspects of the coronary band, respectively. The baseline MNTs at the bulbs of the heel were 1.71 ± 1.1 kg (lateral bulb) and 1.60 ± 0.92 kg (medial bulb). Baseline MNTs at the sole were 1.13 ± 0.46 kg (palmarolateral), 1.23 ± 0.46 kg (dorsal), and 1.24 ± 0.56 kg (palmaromedial). Baseline MNTs at the dorsal laminae were 1.48 ± 1.42 kg for the L2 site and 0.90 ± 0.49 kg for the L4 site.

Anaesthesia of the PDNs with lidocaine increased the MNTs over baseline values at all sites in the bulbs of the heel, sole and dorsal laminae ($P < 0.001$; Figs. 2, 3 and 4). MNTs did not increase over baseline values at any sites at the coronary band (Fig. 5). No withdrawal responses were observed at the L4 site of the dorsal laminae in the six horses, and no withdrawal responses were observed at the L2 site in five/six horses at the maximum force (6 kg) after PDN anaesthesia. An L2 withdrawal response was reached at a force of 3.63 kg in one horse following PDN anaesthesia.

DB anaesthesia did not produce a significant increase in MNT values at either site in the dorsal laminae or any site in the sole ($P > 0.05$). Increased MNT values over baseline were only present at the coronary band ($P < 0.01$; Fig. 5).

Discussion

The model used in this study is, to our knowledge, the only model developed to test nociception of the dorsal laminae of the equine foot. We developed this model to assess the effect of anaesthesia of two digital nerves, which are commonly blocked in lameness assessments of the hoof. We demonstrated that local anaesthetic block of the DBs did not completely desensitise the laminar region of the

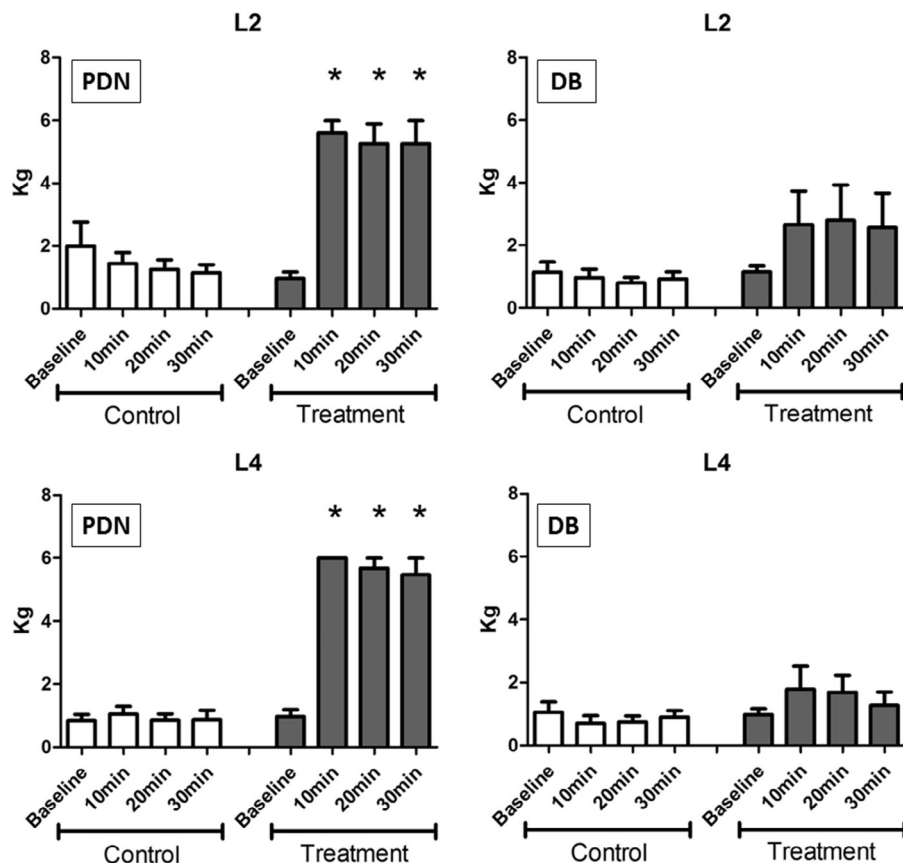


Fig. 4. Mean (\pm standard error of the mean) mechanical nociceptive threshold (MNT) expressed as kg of force (Kg, Y axis) at the proximal (L2) and distal (L4) sites of penetration to expose the dorsal laminae of the hoof of horses before (baseline) and 10, 20, and 30 min after (X axis) perineural injections of a local anaesthetic solution (treatment group) or isotonic saline solution (control group) of the palmar digital nerves (PDN) or the dorsal branches of the digital nerves (DB). The asterisk indicates a significant difference compared to the baseline value in the same group or the control group value at the same time point ($P < 0.001$).

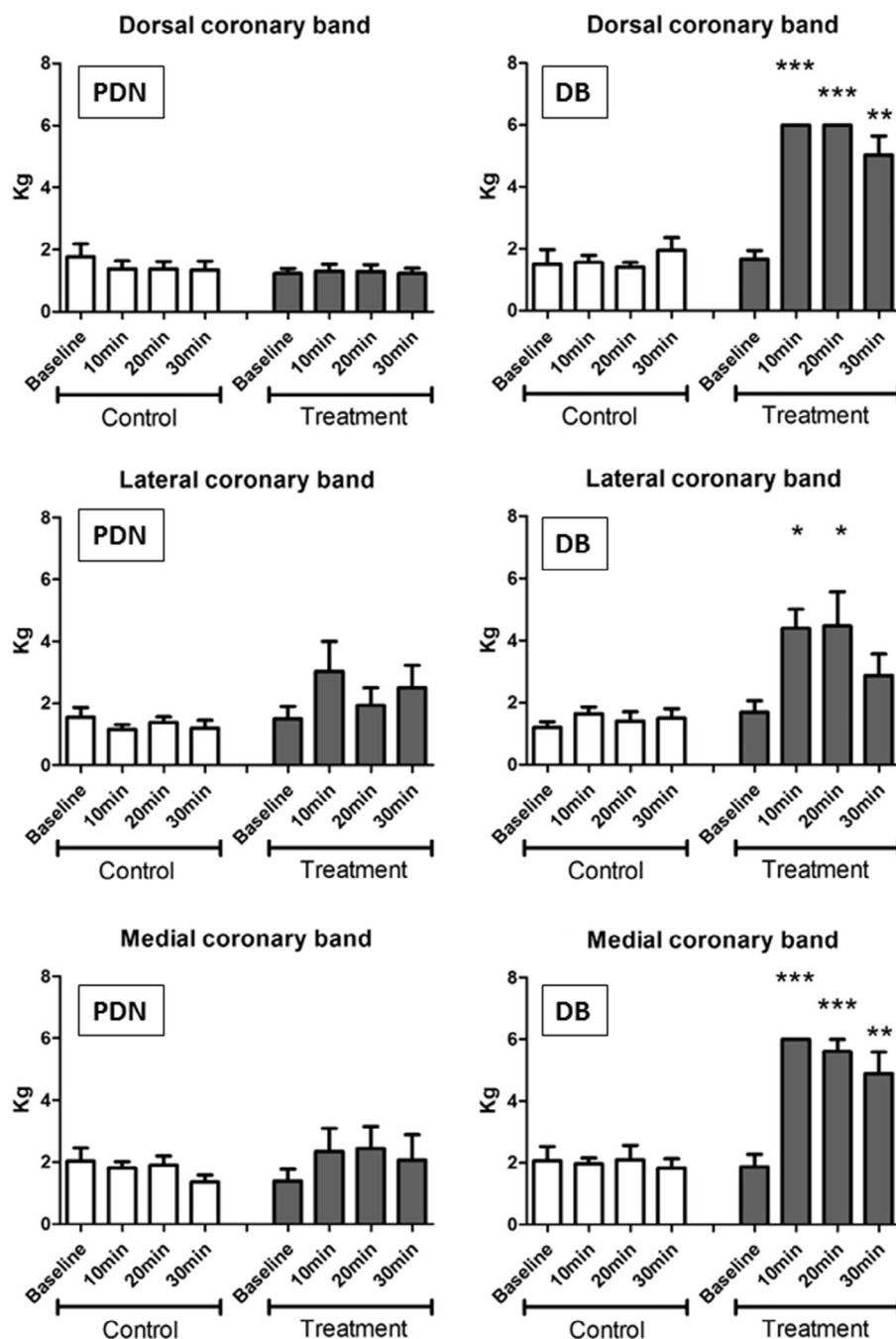


Fig. 5. Mean (\pm standard error of the mean) mechanical nociceptive threshold (MNT) expressed as kg of force (Kg, Y axis) at the lateral, dorsal, and medial aspects of the coronary band of horses before (baseline) and 10, 20, and 30 min after (X axis) perineural injections of a local anaesthetic solution (treatment group) or isotonic saline solution (control group) of the palmar digital nerves (PDN) or the dorsal branches of the digital nerves (DB). Asterisks indicate a significant difference compared to the baseline value in the same group or the control group value at the same time point (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

dorsal aspect of the hoof wall, despite desensitising the skin of the dorsal aspect of the coronary band. In contrast, anaesthetising the PDNs at the level of the ungular cartilage significantly increased the MNTs of the laminae of the dorsal aspect of the hoof wall. These findings contrast with other descriptions of the sensory innervation of the foot (Stashak, 2002; Kaneps, 2004; Bassage and Ross, 2010; Moyer et al., 2011; Schumacher et al., 2013), but they are consistent with the reported findings of an anatomical study that demonstrated that the PDNs innervate the entire laminar corium (Sack, 1975).

The increased MNTs in the solar corium in the current study confirmed the PDN innervation, which is consistent with previous studies that found that perineural anaesthesia of the PDNs ameliorated lameness caused by pain in the toe and heel regions of the sole (Schumacher et al., 2000). Other authors used solar pressure produced by hoof testers to document that the PDNs were effectively anaesthetised, because of concern that painful sensations of skin of the bulbs may be eliminated without complete anaesthesia of the deeper structures of the hoof (Dyson and Marks, 2003). Our study used a dynamometer instead of a hoof tester to apply

pressure to the solar corium, to quantify the effective elimination of solar nociception following PDN anaesthesia.

More local anaesthetic solution is commonly infiltrated adjacent to the PDN nerves to obtain adequate PDN anaesthesia in clinical situations when the horse remains sensitive to hoof testers after PDN perineural anaesthesia. However, a small amount of lidocaine (1 mL) was chosen in our experiment to minimise the possibility of proximal diffusion of the local anaesthetic solution (Nagy et al., 2009), which may desensitise structures other than those innervated by the PDNs. The use of such a small volume most likely resulted in the failure to increase MNT values in the soles of two horses in this study. A recent study reported that loss of local anaesthetic solution due to diffusion into lymphatic vessels could potentially occur when performing distal PDNs blocks (Nagy and Malton, 2015). Taken together, these findings suggest that a local anaesthetic volume of 1 mL per nerve may not completely block nociceptive conduction of the PDNs in all horses, and this volume cannot be recommended for PDN blocks in clinical situations.

The observations that: (1) perineural anaesthesia of the DBs increased MNTs at all points tested on the coronary band (Fig. 5), and (2) perineural anaesthesia of the PDNs did not increase the MNTs at any site assessed on the coronary band, indicate that the DBs primarily innervate the dorsal, dorsolateral, and dorsomedial aspects of the coronary band. We conclude that the DBs sparsely innervate the proximal region of the dorsal laminae, because anaesthesia of the DBs produced a small increase in the MNTs at the L2 site, although these were not statistically significant. Additionally, the MNT at the L2 site of one horse subjected to anaesthesia of the PDNs did not reach the maximum value (i.e., 6 kg).

Notably, the group size of six horses in this study was sufficient to fully support the statistically significant differences observed in the dorsal laminae in the PDN anaesthetic blocks, given that the power of the test was 1.00. However, the power of the tests was lower in the analysis of the dorsal branch anaesthetic blocks at the sites L2 and L4. Therefore, it is possible that differences may have been detected with a larger number of horses in the study.

The results of the current study indicate that the PDNs are the major nerves responsible for the innervation of the dorsal laminae in horses. This observation corroborates observations of previous reports that demonstrated that anaesthesia of the PDNs occasionally resolved the lameness exhibited by horses with laminitis (Ross, 1998; Barr, 2010). However, in the authors' clinical experience, the lameness exhibited by many severe laminitis cases cannot be eliminated with only a PDN nerve block. The most likely reason for this difference is that neuropathic pain, such as allodynia (i.e., pain due to a stimulus that does not normally provoke pain) and hyperalgesia (i.e., increased sensitivity to pain), which are commonly observed in humans suffering from severe, chronic pain, are likely to occur in laminitic horses, and these conditions may affect the response to perineural anaesthesia (Jensen and Finnerup, 2014). Abnormal morphology of nerves within the foot, which demonstrate changes associated with peripheral nerve injury and neuropathic pain, were described in horses with chronic laminitis (Jones et al., 2007) and supports the presence of neuropathic pain in many laminitic horses. Although perineural anaesthesia of the palmar digital nerves using a small volume of lidocaine successfully blocked experimentally produced pain in the dorsal laminae of otherwise healthy horses, this result does not necessarily apply to horses with neuropathic pain. Clinicians must keep this phenomenon in mind when performing perineural anaesthesia of the distal limb in laminitis cases.

The baseline MNT values obtained in the hoof's coronary band in this study were very similar to previous values in the same region in horses (Zarucco et al., 2010), which suggests that this methodology provides repeatable results. Removal of the outer keratinised dorsal hoof wall appeared to provide a safe means of exposing the

sensitive laminar tissue to the effects of pressure algometry, while not causing any long-term detrimental effects. The mean baseline values of the MNTs of the laminae were very similar to the values obtained in the sole and were approximately 33% lower than the values obtained at the coronary band and the bulbs of the heel. The higher sensitivity of the laminae and the sole may have occurred because of the exposure of deeper tissues (as a result of removal of keratinised hoof/sole) and the conical tip used. The fact that no horse displayed signs of painful sensitivity, bleeding or infection during or after the study supports the use of pressure algometry and MNTs as a viable experimental technique to investigate dorsal laminar pain in the equine digit.

Conclusions

Palmar digital nerves, but not the dorsal branches of the digital nerve, are primarily responsible for pain signal transmission evoked by pressure in the dorsal laminae of the foot of clinically normal horses.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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