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Morphology and morphometry of the ulnar nerve in the forelimb of pigs

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Abstract

The knowledge of the morphology and morphometry of peripheral nerves is essential for developing neural interfaces and understanding nerve regeneration in basic and applied research. Currently, the most adopted animal model is the rat, even though recent studies have suggested that the neuroanatomy of large animal models is more comparable to humans. The present knowledge of the morphological structure of large animal models is limited; therefore, the present study aims to describe the morphological characteristics of the Ulnar Nerve (UN) in pigs. UN cross-sections were taken from seven Danish landrace pigs at three distinct locations: distal UN, proximal UN and at the dorsal cutaneous branch of the UN (DCBUN). The nerve diameter, fascicle diameter and number, number of fibres and fibre size were quantified. The UN diameter was larger in the proximal section compared to the distal segment and the DCBUN. The proximal branch also had a more significant number of fascicles (median: 15) than the distal (median: 10) and the DCBUN (median: 11) segments. Additionally, the mean fascicle diameter was smaller at the DCBUN (mean: 165 μm) than at the distal (mean: 197 μm) and proximal (mean: 199 μm) segments of the UN. Detailed knowledge of the microscopical structure of the UN in pigs is critical for further studies investigating neural interface designs and computational models of the peripheral nervous system.

KEYWORDS
nerve morphology, peripheral nerves, peripheral nervous system, pig, ulnar nerve

1 | INTRODUCTION

Data collected from 1993 through 2006 in the United States showed injuries to the UN to be the most frequent upper extremity peripheral nerve injuries, with an average hospital charge, in 2006, of $25,311 and 40.4% of the cases requiring acute repair (Lad et al., 2010). They can result both in sensory and motor deficits in the hand (Woo et al., 2015) and, consequently, reduced quality of life and high impact on society (e.g., loss of production due to a lengthy sick leave of on average 160 days) in a relatively young population (average of 39.9 years old) (Bergmeister et al., 2020). The UN is, therefore, a key target for developing nerve repair techniques (Brown & Mackinnon, 2008; Galtrey & Fawcett, 2007). In addition to nerve repair, the UN is commonly used to develop peripheral nerve interfaces to restore sensorimotor functions after limb amputation (Micera et al., 2011; Raspopovic et al., 2014) and probe the neural circuitry (Janjua et al., 2021). Thus, in preclinical phases, investigating the UN in animal models is essential to understand and appreciate the mechanisms that lead to nerve repair and neural prosthetics to improve patient outcomes. Nevertheless, the most widely adopted animal to study peripheral nerve regeneration and peripheral nerve interfaces today is the rat (Larson & Meng, 2020; Vela et al., 2020), even though recent studies have shown that porcine models may be a more appropriate model because of their comparable size, fibre number and fascicular pattern (Stakenborg et al., 2020; Zilic et al., 2015). The advantages of a better translational animal model have direct implications for facilitating the transition from research to the clinic.

In humans, the Ulnar Nerve (UN) contains both motor and sensory axons that originate from the ventral rami of the C8 and T1 nerve roots, passing from the axilla into the medial aspect of the anterior compartment of the upper arm, passing behind the medial epicondyle at the elbow, descending to the forearm (Polatsch et al., 2007). In the forearm, the UN supplies motor branches to the flexor carpi ulnaris muscle and the flexor digitorum profundus muscle (Tubbs et al., 2006). At approximately the distal third of the forearm, the UN gives off the Dorsal Cutaneous Branch of the Ulnar Nerve (DCBUN) from the main trunk (Goto et al., 2010). The DCBUN innervates the dorsal skin of the fifth and the medial half of the fourth digits and the ulnar side of the carpus and hand; the main trunk continues to enter the hand, providing innervation to most of the hand intrinsic muscles, including the thumb adductor muscles and the palmaris brevis muscle (Woo et al., 2015).

The interest in swine as translational animal models for biomedical research has significantly increased in recent years (Swindle et al., 2012) in several fields such as nutrition (Roura et al., 2016), pain (Meijs et al., 2021), neurodegenerative diseases (Hoffe & Holahan, 2019) and brain imaging (Sauleau et al., 2009). The reason is that swine's comparative anatomy and physiology is more similar to humans than rodents (Douglas, 1972). Studies have shown that the anatomical, biochemical and cellular composition of the peripheral nerves in swine is more similar in structure to humans than in rats (Pelot et al., 2020; Zilic et al., 2015), and a recent study by Hanna et al. (2021) showed how the nerves in the brachial plexus of the Wisconsin miniature swine have a similar structure and size to humans (Hanna et al., 2021). Studies comparing the cervical and abdominal vagus nerve composition between humans, mice and pigs have also demonstrated a higher degree of similarity between the pigs and humans in terms of diameter, connective tissue, fibre number and fascicle diameter (Pelot et al., 2020; Stakenborg et al., 2020). The characteristics of peripheral nerves in swine have also been investigated regarding surgical approach, accessibility and histological characteristics. In particular, it has been shown that the UN at the forearm level may be a desirable target for regeneration studies due to the superficiality of the nerve, that does not require the dissection of deeper structures (Scholz et al., 2010).

The morphology and morphometry of the ulnar nerve have been extensively described in humans (Brill & Tyler, 2017; Oliveira et al., 2011; Schenck et al., 2015) and rodents (Barton et al., 2016; Bertelli et al., 1995; Santos et al., 2007). Nevertheless, there is a lack of knowledge of large animal models such as pigs. The internal morphology of nerves is crucial for optimizing the design of neural interfaces since the morphological structure of the nerve (e.g., fibre diameter, fascicle diameter and spatial arrangement of fascicles) significantly influence electrical activation thresholds (Grinberg et al., 2008). Similarly, information on nerve morphology directly affects the approach required for nerve repair (e.g., fascicular repair may be more appropriate for mono- or oligofascicular patterns, whereas group fascicular or epineural repair are more suitable for polyfascicular nerves) (Matsuyama et al., 2000). The morphology of the ulnar nerve in pigs has been described by Kundu et al. (2012), but only for proximal regions of the forelimb (Kundu et al., 2012). Consequently, the present study aimed to investigate the morphology and morphometry of the ulnar nerve in the distal forelimb of pigs (i.e., the DCBUN and the main trunk of the ulnar nerve at two distal segments (before and after branching)).

2 | METHODS

2.1 | Nerve dissection

All animal procedures were performed in accordance with the Danish Veterinary and Food Administration under the Ministry of Food, Agriculture and Fisheries of Denmark (protocol number 2017-15-0201-01317). Seven female Danish Landrace pigs, with a mean weight of 34.1 kg (range: 29.0–39.0 kg), were used for this study. The animals were anesthetised using sevoflurane (1.5 to 2.5% minimum alveolar concentration), propofol (2 mg/h/kg) and fentanyl (10/μg/h/kg) and then euthanised with an overdose of pentobarbital.

The animals were placed in a supine position, an incision of approximately 20 cm was made on the posterior right forelimb, and the ulnar nerve was carefully exposed and freed from the surrounding tissue. Three nerve segments of 2 mm each were selected for the analysis of the morphology and morphometry: (1) At the main trunk of the ulnar nerve, approximately 4 cm above the branching point, (2) at the main trunk, approximately 3 cm after the branching of the main trunk giving off the DCBUN, and (3) at the DCBUN at 2 cm after the branching point. Figure 1 illustrates the location of the nerve segments used for the analysis.

2.2 | Histological procedures and analysis

The nerve specimens were cut and fixed by immersion in a 4% formaldehyde solution for at least 24 h. After fixation, the specimens were embedded in paraffin and stained with haematoxylin and eosin (H&E). Histologic slices of 2.5 μm were digitized using a NanoZoomer

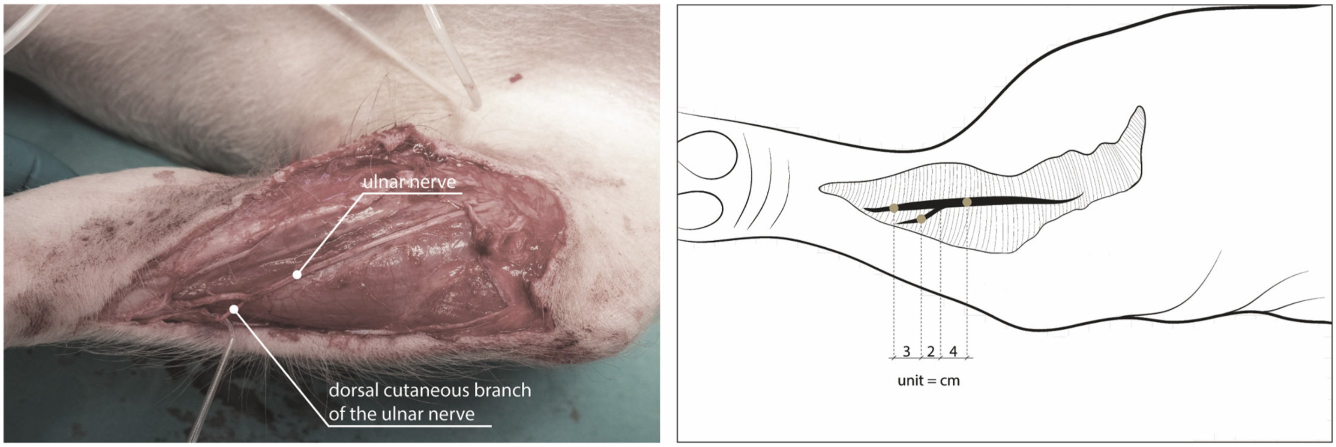

FIGURE 1 Photograph and illustration of the ulnar nerve and the dorsal cutaneous branch of the ulnar nerve (DCBUN) in the forelimb of a pig. On the left image, it is shown the proximal UN nerve and the split between the UN and the DCBUN. On the right, the orange dots highlight the location of the nerve segments used for the morphometrical analysis and the distance between the segments extracted for following analysis.

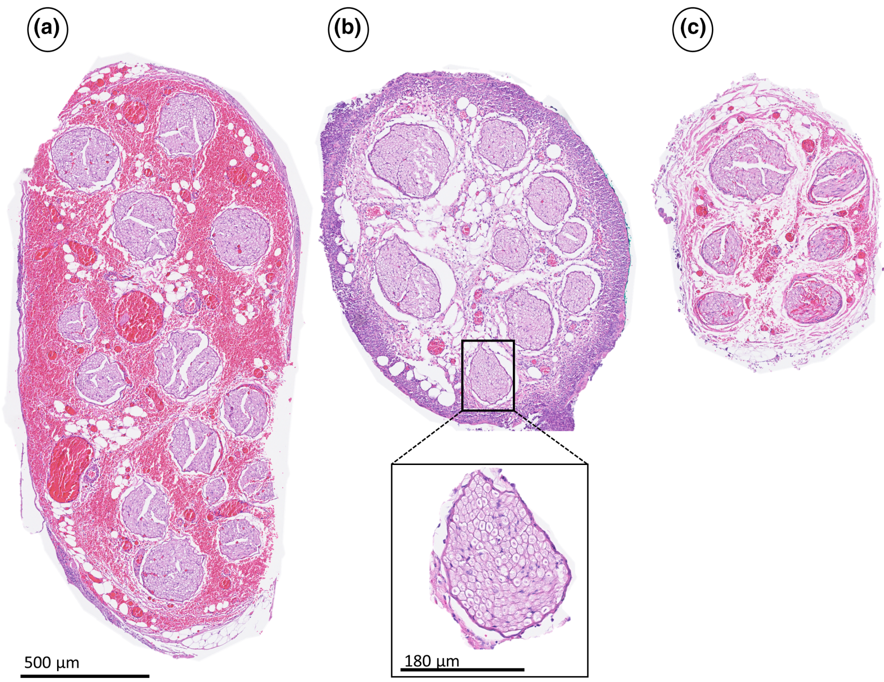

FIGURE 2 (a) Representative image of the proximal branch of the ulnar nerve with 14 fascicles, (b) image of the distal branch of the ulnar nerve with nine fascicles and (c) the DCBUN with six fascicles. Haematoxylin–eosin (H&E) stained. Scale bar = 500 μm. The insert shows one fascicle in higher magnification, where it is possible to identify the perineurium and the myelinated fibres in the endoneurium (the white rings being the ghost image of the myelin sheath). Scale bar = 180 μm.

S360 digital slide scanner (Hamamatsu Photonics) under 40× magnification.

First, the fascicles were visually identified and counted. To obtain the area and diameter values, the external boundary of the epineurium was used to measure the sectional area of the entire nerve, and the perineurium was used as a landmark to obtain the area of the fascicles. Then, the cross-sectional areas were converted into effective diameters assuming a circular cross-section. For comparison with previous reports, when the area was provided, it also converted it to effective diameter. These features were measured with the freehand selection tool from FIJI software (Schindelin et al., 2012). Additionally, the fascicles were visually identified and counted. As tissue samples undergo shrinkage when fixed in formalin, a correction factor was applied to the nerve area and fascicle diameters. The correction factor of 1.25 was selected since it has been shown to adjust for shrinkage by comparing frozen and formalin-embedded samples (Kundu et al., 2012).

Fibre count and diameter were determined according to the method described by Engelmann et al. (Engelmann et al., 2020), a semi-automatic axon quantification software carried out using the FIJI platform (Schindelin et al., 2012) with a sensitivity of 94% and a specificity of 87%. Briefly, the images were pre-processed by first increasing the contrast, and subsequently converted into binary images using a uniform threshold adjustment. At this point, axons were enclosed in white ellipses with black edges. Then, axons were automatically counted using the analyse particle tool with circularity = 0.10–1.00. A low cut-off value for the inclusion of axons was 4.0 μm, since we observed that this parameter was optimal to minimize false positives detection. A representative image of the swine's UN is illustrated in Figure 2.

2.3 | Statistical analysis

A linear mixed model was used to compare the measurements between the branches using the branches as a within-subject factor (Location: DCBUN, proximal main trunk, distal main trunk), while the animal was treated as a random effect. The normality assumption was verified through residual analysis (histograms and Q-Q plots). If not stated otherwise, results are shown as mean ± standard deviation. The adopted significance level was 0.05, and statistical analysis was performed in R software package (R Core Team, 2020).

3 | RESULTS

In seven Danish landrace pigs, the morphology of the ulnar nerve at the proximal and distal segments in the forelimb and the DCBUN in the forearm were quantified. The DCBUN from animal three was excluded from the analysis because the sample was not collected. The results of the nerve diameter, fascicle number and diameter for the three ulnar nerve segments (DCBUN, distal UN and proximal UN) are provided in Figure 3 and Table 2.

3.1 | Comparison of nerve diameter

The nerve branches' diameter was significantly different between the UN segments ($p < 0.05$); the diameter of the main trunk before giving off the DCBUN branch (1713 ± 153 μm) was significantly larger than the main trunk after the branching (1392 ± 207 μm, $p < 0.05$) and the DCBUN (1074 ± 66 μm, $p < 0.05$); there was also a difference between the diameter of the main trunk after the branching and the DCBUN ($p < 0.05$) (Figure 3a). Interestingly, Figure 3a also illustrates a consistent pattern for all animals and nerve segments: the diameter of the nerve in the proximal UN was always larger compared to the distal UN. Likewise, despite the lack of statistical significance, the diameter of the distal UN was always larger than the DCBUN.

3.2 | Comparison of fascicle number and fascicle diameter

The three ulnar nerve segments (DCBUN, Distal UN and Proximal UN) also had a significantly different number of fascicles ($p < 0.05$) (Figure 3b). The proximal main trunk contained a median value of 15 fascicles (range: 14–16), that was significantly higher than the DCBUN (median: 11, range: 6–13, $p < 0.05$) and the distal main trunk (median: 10, range: 8–13, $p < 0.05$). However, there was no difference in the fascicle number for the distal UN and the DCBUN ($p = 0.97$). Lastly, the fascicle diameters differed across the branches ($p < 0.05$). The mean fascicle diameter of the proximal main branch (199 ± 57 μm) was significantly larger than the mean fascicle diameter of the DCBUN (165 ± 67 μm, $p < 0.05$). The mean fascicle diameter of the distal branch (197 ± 57 μm) was also larger than the DCBUN ($p < 0.05$). Conversely, there was no difference in the fascicle diameters between distal and proximal main trunks ($p = 0.96$) (Figure 3c).

3.3 | Comparison of fibre count

There was a significant difference in fibre number across the three segments of the ulnar nerve ($p < 0.05$). The proximal branch of the UN contained, on average, 4040 ± 572 fibres, considerably more than the distal UN (1876 ± 384, $p < 0.05$) and the DCBUN (1174 ± 253, $p < 0.05$). The DCBUN and the distal UN also showed a different number of myelinated fibres ($p < 0.05$). Table 1 provides a summary of the parameters extracted from each animal.

4 | DISCUSSION

The morphological characteristics of porcine nerves have been most thoroughly investigated for the vagus nerve. The results have been shown to approximate the human models in terms of nerve diameter, fascicular structure and connective tissue (Pelot et al., 2020; Stakenborg et al., 2020). Recently, a study also reported a similar size and origin for the brachial plexus of swine compared to humans (Hanna et al., 2021). Nevertheless, there is still a gap in the literature regarding morphological and morphometrical data for distal segments of peripheral nerves in large animal models, even though forelimb and hindlimb nerves are the most studied for direct peripheral nerve repair (Vela et al., 2020) being of great importance for developing neural prostheses (Raspopovic et al., 2021). Anatomically, peripheral nerves, particularly the UN in swine, have shown to be a suitable model for nerve regeneration because of their easy accessibility that does not require the dissection of deeper structures (Scholz et al., 2010). The superficiality of the UN is also a desirable characteristic for testing the long-term safety and stability of peripheral nerve interfaces as it reduces surgical complexity and the likelihood of tissue damage. Therefore, we provide quantitative information on the morphometry of the UN in the forelimb of swine.

Table 2 shows data from experimental studies investigating the UN at the forearm level in humans, rats, dogs and swine. Throughout the discussion, these findings will be compared to and discussed with those obtained by the studies in Table 2 to understand how the parameters obtained in pigs relate to other species.

4.1 | Nerve diameter

Measurements from this study showed a larger diameter in the main branch of the ulnar nerve before branching into the DCBUN. Brill et al.

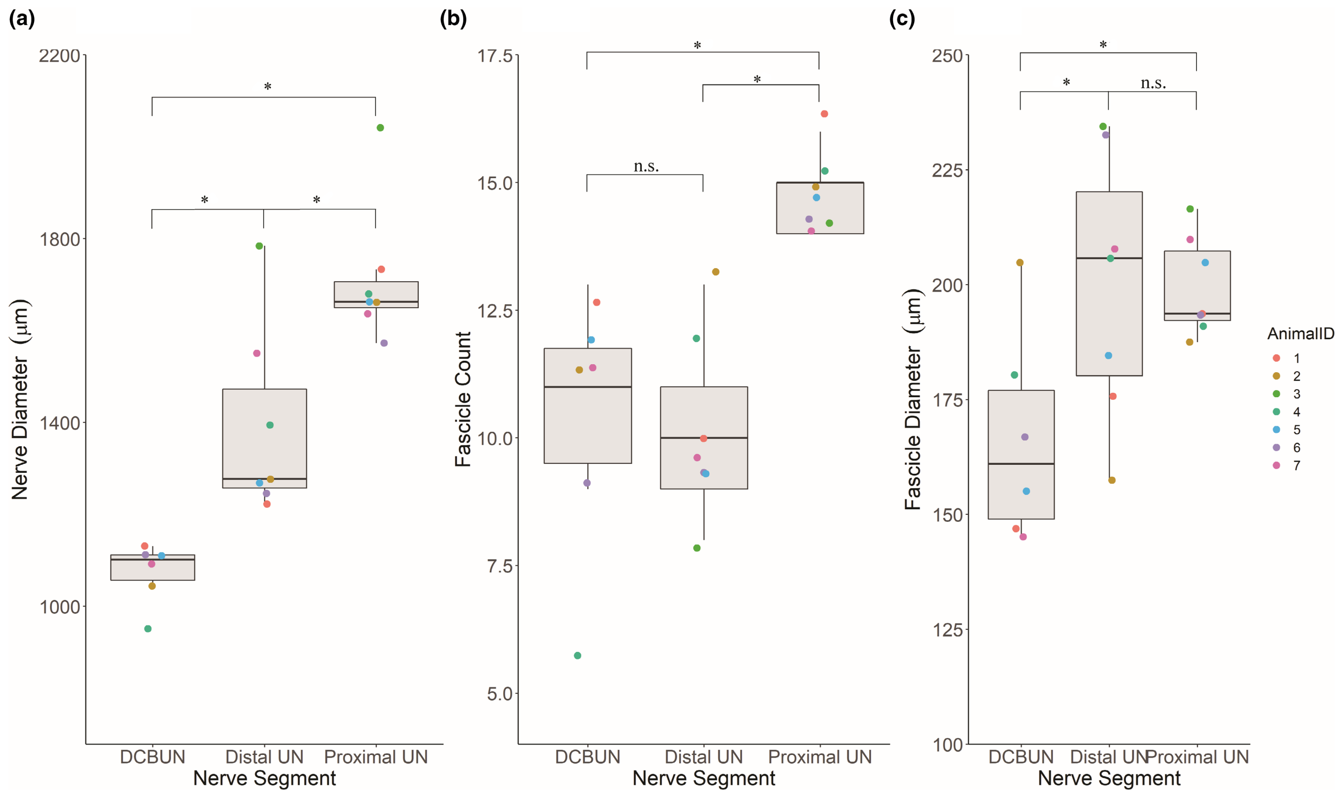

FIGURE 3 Comparison of the morphometric data of the ulnar nerve segments. (a) The nerve diameter was significantly larger in the proximal segment of the UN compared to the distal segment of the UN ($p < 0.05$) and the dorsal cutaneous branch of the UN (DCBUN) ($p < 0.05$). (b) The proximal segment of the UN presented a significantly larger number of fascicles compared to the DCBUN ($p < 0.05$) and the distal segment of the UN ($p < 0.05$). (c) The mean fascicle diameter of the DCBUN was significantly smaller compared to the distal UN ($p < 0.05$) and the proximal UN ($p < 0.05$). In all boxplots, centre lines represent the median value, and the box limits illustrate the lower and upper quartiles (25th and 75th percentiles). The upper and lower whiskers extend to ±1.5× the interquartile range and data beyond the end of the whiskers are outliers. The individual observations are also displayed, coloured by Animal ID. One asterisk indicates $p < 0.05$ while n.s. indicate non-significant differences at a significance level of 0.05. The DCBUN from animal three is not included in the analysis because the sample was not collected.

quantified, in humans, the ulnar nerve from the axilla to the wrist; the authors have also found a reduced nerve area from the elbow to more distal locations (Brill & Tyler, 2017). The same pattern was observed in rats from the arm to the wrist (Barton et al., 2016). We found the proximal main trunk to have a mean diameter of 1.71 mm, substantially larger than the 0.60 mm found in the rat forelimb (Bertelli et al., 1995) and more comparable to the 2.60 mm found in humans (Brill & Tyler, 2017). Closer to the wrist, the ulnar nerve diameter was found to be 2.82 mm in humans (Brill & Tyler, 2017), considerably larger than the 0.28 mm observed in rats (Barton et al., 2016). In pigs, we showed that the diameter of the distal UN is 1.30 mm, demonstrating, again, how the diameter of the UN in pigs is more comparable to humans than rats. For the DCBUN, human studies have found diameter sizes of 1.60 and 2.40 mm (Botte et al., 1990; Cavusoglu et al., 2011). The results from this study show that the diameter of the DCBUN in pigs is on average 1.07 mm. Comparable nerve diameters may allow for better translational success rates by designing neural interfaces that closely resemble human dimensions; the electrode-fibre distance is an essential parameter for recording neural activity since it is inversely proportional to the amplitude of a single-unit action potential (Struijk, 1997). Therefore, as the diameter of the pig UN best approximates the human UN, it may be a promising alternative for testing electrodes and stimulation parameters.

4.2 | Number of fascicles

The number of fascicles significantly decreased between the most proximal branch (i.e., the main trunk) and the more distal branches (i.e., DCBUN and main trunk after branching); a similar pattern has been observed in the ulnar nerve of pigs from 5 to 7 cm proximal to the elbow joint until 1 to 2 cm below the elbow joint (Kundu et al., 2012). The opposite pattern was observed in Wistar rats, with a single fascicle at the axilla level and a range of 1–4 fascicles at a distal level (forearm, distal 1/3) (Santos et al., 2007). On the contrary, humans have a similar number of fascicles from past the elbow to the wrist (Brill & Tyler, 2017). The main branch of the ulnar nerve had a median value of 15 fascicles, fewer than what has been found in humans at the forearm level (mean of 20.7 fascicles (Brill & Tyler, 2017)) but in a larger number than what has been observed in rats (range: 1–4, (Santos et al., 2007)). For the

TABLE 1 Animals, nerve diameters, fascicular number and diameter, and the number of fibres.

Segment	Animal ID	Nerve (μm)	Fascicle number	Fascicle diameter (μm)	Number of myelinated fibres
DCBUN	1	1131	13	176 ± 35.2	1266
	2	1045	11	205 ± 14	1238
	4	952	6	180 ± 51.1	852
	5	1111	12	169 ± 12.0	1573
	6	1113	9	167 ± 56.7	1156
	7	1093	11	145 ± 29.8	962
Distal UN	1	1223	10	226 ± 74.7	2125
	2	1277	13	168 ± 30.7	2201
	3	1785	8	269 ± 32.9	1627
	4	1395	12	206 ± 55.8	1559
	5	1269	9	207 ± 5104	1826
	6	1246	9	237 ± 25.8	1454
	7	1551	10	233 ± 49.7	2341
Proximal UN	1	1773	16	236 ± 47.2	3319
	2	1662	15	237 ± 55.8	4156
	3	2042	14	252 ± 56.3	4476
	4	1680	15	223 ± 55.3	3719
	5	1663	15	230 ± 54.1	3989
	6	1573	14	216 ± 34.7	3677
	7	1637	14	238 ± 55.7	5033

DCBUN branch, we observed a median of 11 fascicles, whereas human studies have shown a mean of 5 (Luo et al., 2018; Oliveira et al., 2011). A study using dogs has also reported a smaller number of fascicles (range: 1–4) for the cutaneous branch (Illanes et al., 1990). Interestingly, a study comparing the morphology of the vagus nerve of humans, pigs and rats has found an increased number of fascicles in pigs, around 10 times more fascicles than in humans (Pelot et al., 2020); likewise, compared to humans, a higher number of fascicles have been observed in the Wisconsin miniature swine for the median nerve (Hanna et al., 2021). The fascicular number and structure should be considered when designing neural interfaces as gradients in the extracellular field potential are dependent not only on a target fascicle but on its neighbouring fascicles (Grinberg et al., 2008); hence, using animal models with a monofascicular structure may provide inaccurate activation thresholds when results are translated to nerves with a polyfascicular structure. Furthermore, having an animal model with a comparable number of fascicles is essential for the investigation of fascicular selectivity in neuroprosthetic devices, where, ideally, the control of a particular muscle is achieved without the concomitant activation of different muscles innervated by the same nerve (Badia et al., 2011).

4.3 | Fascicle diameter

The average fascicle diameter of the DCBUN was smaller than the average fascicle diameter of the main trunk of the ulnar nerve before and after the bifurcation point. However, at the main trunk of the UN, there was no difference between the fascicle diameters proximally and distally. A study investigating the fascicle diameter of the ulnar nerve in pigs at three levels: (1) 5 to 7 cm proximal to the elbow joint, (2) 1 cm proximal to the elbow joint and (3) 1 to 2 cm distally to the elbow joint also found a similar fascicle diameter across the three levels, with an average fascicle diameter of 0.26 mm (Kundu et al., 2012). In the main trunk, we found a mean fascicle diameter of 0.20 mm and 0.19 mm for the proximal and distal segments, respectively. This result may indicate a trend that the fascicular diameter is larger in the upper limb, a result which has also been observed in a study exploring nine regions (from the axilla to the wrist) of the ulnar nerve in humans, where fascicular diameters were larger in the upper arm than they were in the forearm (Brill & Tyler, 2017). In that study, the authors reported a mean fascicular diameter of 0.38 mm in the forearm. Notably, the same effect of decreasing fascicular area for the distal segments has been observed in rats for the ulnar, median and radial nerves (Santos et al., 2007). It has been shown that fascicular diameter considerably affects electrical stimulation thresholds. Specifically, small fascicles have lower activation thresholds than large fascicles (Koole et al., 1997). These results can, therefore, be used to optimize computational tools and neural interface designs.

4.4 | Fibre number

It is well known that the number of fibres in a peripheral nerve is constant during adulthood (Jeronimo et al., 2005; Schellens

TABLE 2 Summary of studies that have provided quantitative data at the forearm level of the ulnar nerve.

Animal model	Segment location	Nerve diameter	Fascicle number	Fascicle diameter	Number of myelinated Fibres	Ref
Human—DCBUN	Above the tendon of the extensor digiti minimi	–	5 ± 2	0.74 ± 0.39 mm	2104 ± 907	Oliveira et al. (2011)
Human—DCBUN	At the level just proximal to its branching from the UN	1.65 ± 0.03 mm	–	–	–	Cavusoglu et al. (2011)
Human—DCBUN	Nerve origin	2.4 (range, 1.6 to 3.5) mm	–	–	–	Botte et al. (1990)
Human—Proximal UN	At the level just before the branching point	3.25 ± 0.04 mm	–	–	–	Cavusoglu et al. (2011)
Human—Distal UN[a]	Within Guyon's canal	1.6 ± 0.4 mm	8.6 ± 5.2	1.0 ± 0.7 mm	2900 ± 1050	Schenck et al. (2015)
Human—UN	Approximately 3 cm distal to the bifurcation from the median nerve	2.90 ± 0.40 mm	18.3 ± 2.6	0.47 ± 0.17 mm	24,449 ± 284	Hanna et al. (2021)
Human—UN[b]	Distal forelimb	2.60 ± 1.49 mm	20.7 ± 1.4	0.38 ± 0.18 mm	–	Brill and Tyler (2017)
Wisconsin Miniature Swine—UN	Approximately 3 cm distal to the bifurcation from the median nerve	3.03 ± 0.37 mm	22.3 ± 1.2	0.34 ± 0.08 mm	16,725 ± 521	Hanna et al. (2021)
Swine—UN	1 to 2 cm distally to the elbow joint	1.8 ± 0.5 mm	17 ± 7	0.25 ± 0.09 mm	–	Kundu et al. (2012)
Rat—UN	Distal (wrist)	0.28 ± 0.03 mm	–	–	869 ± 18	(Barton et al., 2016)
Rat—UN	Forearm	0.6 mm	–	–		Bertelli et al. (1995)
Rat—UN	Distal	170.8 ± 45.0 µm	Range: 1–4	–	421 ± 142	Santos et al. (2007)
Dog—DCBUN	1 cm ventral to its division in the middle of the antebrachium	–	Range: 1–3	0.62 ± 0.29 mm	1967 ± 349	Illanes et al. (1990)

Note: If not stated otherwise, values indicate mean ± standard deviation.

[a]For this study, the authors reported the total fascicle area in mm^2.

[b]The fascicle diameter value represents the average of the forearm, whereas the other measurements from this study were taken from region 7, which is midway between the elbow and the wrist.

et al., 1993; Thomas et al., 1980). Thus, the number of fibres can be one of the parameters that correlate with nerve function. The ulnar nerve and its branches were dissected at the same levels in all animals studied, and the fibre number was higher for the proximal branch, followed by the distal branch and the smallest number for the DCBUN. Information on the fibre number of the ulnar nerve in pigs is rare in the literature. Nevertheless, these results are similar to those obtained in humans (Oliveira et al., 2011) and much larger than those obtained in rats (Santos et al., 2007). Once again, indicating that the porcine model is suitable and can be more comparable to humans concerning neural interface design, data interpretation and translational studies.

4.5 | Methodological considerations

Counting small myelinated fibres is considered difficult because they are generally harder to stain and also because using manual morphometry techniques or sampling schemes, the observers might underestimate them (da Silva et al., 2007; Ellis et al., 1980; Mezin et al., 1994). There is a possibility that in the present study, the total number of fibres may have been underestimated. Three main factors should be taken into consideration for this possible underestimation. First, we used paraffin-embedded samples, which causes a disruption of the myelin sheath during the xylol baths before paraffin baths and embedding. This may cause difficulty in visualizing and identifying small, myelinated fibres. Second, the semi-automated axon quantification method used in this study (Engelmann et al., 2020) was validated in samples stained with paraphenylenediamine, which primarily stains the myelin sheath of peripheral nerves. Third, we adopted a cut-off value of 4 μm diameter for the axon count, so smaller myelinated fibres are underrepresented. Further studies involving nerve specimens epoxy resin embedding, semi-thin sectioning and computed morphometry will be performed to better investigate the number and size of the myelinated fibre in this model. Fourth, the animals investigated in this study were relatively young, and significant alterations are observed in the nerve morphometry of developing animals, such as increase in fascicular area, myelinated fibre and myelin sheath area (Jeronimo et al., 2005). Therefore, a direct comparison must consider the effect of ageing in the nerve morphometry.

The present study extracted the nerve segments with reference to the DCBUN split, as it allows to account for between-animal variations and has been used as a reference point by previous studies (Tereshenko et al., 2023). Therefore, care must be taken comparing the results to different studies that have used distinct reference points.

5 | CONCLUSION

This study develops on the knowledge of the swine ulnar nerve morphology and morphometry by quantifying key features of the ulnar nerve in the pig's forelimb at two levels of the main branch and the dorsal cutaneous branch of the ulnar nerve. The measures include nerve diameter, number of fascicles, fascicle size and myelinated fibre number, which can provide reference values for developing electrode designs, computational models and clinical procedures for nerve repair in the future.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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