Comparison of median and ulnar nerve morphology of Danish landrace pigs and Göttingen mini pigs

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Abstract

The success of a nerve electrode is often measured by how well it selectively stimulates or records from individual fascicles within a peripheral nerve. Our objectives of the present study were to characterize the nerve morphology of landrace pigs and mini pigs and to evaluate the possible implications of the choice of animal model on testing the performance of peripheral nerve interfaces. The median and ulnar nerves were harvested form six Danish landrace pigs and eight Göttingen mini pigs. Nerve specimens were selected for analysis. The number of fascicles was counted and the fascicle diameters were measured. The average fascicle diameter was slightly greater in mini pigs compared to landrace pigs. The maximum numbers of fascicles were found just above the elbow joint for landrace pigs, and at the most proximal point for the mini pigs. The pig is an attractive animal model due to its close resemblance to humans, however, the difference in morphology between the two types of pigs implicate that the choice of animal model for testing nerve electrode performance must be carefully considered and should be taken into account when interpreting the electrode’s performance.

Keywords: landrace pig, mini pig, peripheral nerve interface, morphology, H&E

Introduction

The aim of a peripheral nerve electrode is to provide an interface between the peripheral nerve cells in the body and a neuroprosthetics device. Many neural prosthesis applications rely on the ability to interface with individual or specific populations of nerve fibers, typically motor or sensory. The success of peripheral nerve electrodes is therefore often measured in how well it selectively stimulates or records from individual fascicles within a peripheral nerve. The peripheral nerves are cable-like structures which consist of bundles of axons located within fascicles. Therefore, one important factor that influences the selectivity is the degree of invasiveness of the electrode [1]. Badia et al. recently studied the stimulation selectivity of the transverse, intrafascicular electrode (TIME) implanted in the sciatic nerve of the rat, which is composed of three major fascicles (peroneal, sural and tibial) [2]. Their results indicated that TIME could independently activate muscles that are innervated by the major fascicles. In our laboratory, we recently investigated the stimulation selectivity of the TIME implanted in the large, poly-fascicular median nerve of landrace and mini pigs [3-5]. In the large nerve model we were only able to selectively activate a small subset of muscles in the forelimb and with a higher activation current in comparison to what was used in the rats. We believe that this could partly be explained by the placement of the electrode in the nerve and the nerve morphology. The choice of animal model and knowledge of the peripheral nerve morphology are clearly important when evaluating the selectivity of peripheral nerve interfaces. Our main objective of the present study was therefore to characterize the morphology of the median and ulnar nerves in landrace pigs and mini pigs and evaluate the possible implications of the choice of animal model on testing the performance of peripheral nerve interfaces. We have previously provided preliminary information on the morphology of the landrace pigs [6].

Materials and methods

Harvesting of peripheral nerve segments

Experimental procedures were approved by the animal experiment inspectorate under Danish Ministry of Justice. Nerve specimens were harvested from both forelimbs from six Danish landrace pigs (female <50
kg) and from the right forelimbs of eight Göttingen mini pigs (female ≤ 35 kg). The animals were placed under general anesthesia and access to the nerves in the upper limb was created through the axilla. The median and the ulnar nerves were carefully exposed and freed from the surrounding tissue. The pigs were then euthanized using an overdose of sodium pentobarbital. Within few minutes after confirmed death of the animals the nerves were harvested. The median and ulnar nerves were selected for analysis since these nerves contain motor and sensory fibers innervate the hand in humans, and as such, are the potential target for implant of peripheral nerve electrodes for neural prosthetic devices.

**Data analysis**

Digital photomicrographs were first obtained of all stained specimens. Axio Vision Vs40 v. 4.6.30, Carl Zeiss Imaging solutions GmbH, Germany) was used for image analysis. It is a well-known fact that tissue samples immersed in formalin shrink \[19\]. In the present study, we found a significant difference between formalin-processed and frozen sections (t-test, \(p_{\text{median}}<0.001\) and \(p_{\text{ulnar}}<0.001\)). To allow comparison between formalin-embedded samples and frozen samples, a simple correction factor was defined as the relation between the average fascicle diameter of formalin processed and frozen sections (calculated to 1.25). After correction there was no significant difference (t-test, \(p_{\text{median}}=0.954\) and \(p_{\text{ulnar}}=0.743\)). The effect of applying the correction factor on the formalin-embedded samples is also shown in Figure 2.

![Figure 2. Comparison of the average diameter of fascicles (mean±std) in mm for the median and the ulnar. Grey bars: formalin processed nerves. Black bars: frozen sections. White bars: after applying the correction factor.](image)

To compare the number of fascicles, the fascicles were visually identified and manually counted. The visual inspection of the fascicles showed that the fascicles were oval rather than round. To estimate the fascicle diameters we therefore used an average of the short and long axis of the oval shapes. Statistical evaluation was performed in SPSS. Individual statistical analysis was performed for the median and ulnar nerve. We found no statistical difference between the nerves harvested from the left and right side of the pigs (\(p=0.957\)), and therefore the samples both sides were pooled during the further analysis. A two-way, repeated measures ANOVA was used to test whether there was any difference between the two types of pigs and the nerve morphology (Level (1, 2 or 3) was a ‘within subject’ factor, type of pig (landrace-pig or mini pig) was a ‘between subjects’ factor, number of fascicles and diameter of fascicles were the dependent variables). The whole nerve diameter was also measured, and comparison of the whole nerve
diameter between the landrace and the mini pigs for both median and ulnar nerve for all three levels was performed with t-tests.

Results

The number of fascicles and fascicle diameters are shown in Figure 3. The average number of fascicles across the three levels examined was 34 ± 5 (median) and 22 ± 5 (ulnar), for the mini-pigs, whereas for the landrace pigs the counts were 23 ± 7 (median) and 18 ± 3 (ulnar).

The ANOVA analysis revealed a significant difference between the groups of data from the two types of pigs. However, in the post-hoc analysis we found only a significant difference between the number of fascicles for the ulnar nerve among the three levels ($p = 0.016$).

To further compare the fascicle diameters, the probability distribution of the nerve diameters were plotted in Figure 4. This plot shows that the fascicle diameters for the median and ulnar nerves are similar.

Whole nerve diameter

Table 1 shows the mean diameter of ulnar and median nerves for both types of pigs on different levels. We found no significant difference between the whole nerve diameters in the landrace pig and mini pig for median ($p = 0.365$) and ulnar ($p = 0.098$) nerves.

Discussion

Methodological considerations

In both acute and chronic experiments it is not feasible to implant nerve electrodes just at the elbow joint, due to very limited space and movement of the elbow joint will likely cause mechanical stress to be translated to the nerve electrode or lead-out wires. Experiments performed by Rossini et al and Dhillon et al in humans, the intra neural electrodes were implanted just above the elbow joint at a distance of 3 to 8 cm proximal to the end of the nerve stump [7, 8] . As such no nerve segments were extracted exactly at the elbow joint in the present study.

Alan et al found that the percentage of shrinkage was up to 14.5%. They studied different correction factors previously used in the literature and found that the correction factor ranged from 1.0 to 1.5. They also stated that the maximal tissue shrinkage occurred between 12 to 14 hours after immersion in formalin [9].

In the present work a group of nerves were placed in formaldehyde (4% formalin buffer) from 7 - 10 days before further processing took place. To allow comparison between formalin embedded and frozen section, a correction factor was applied as described in the methods section.
Number of fascicles

On average landrace pig has \( n_{\text{median}} = 34\pm 5 \); \( n_{\text{ulnar}} = 22\pm 5 \) fascicles, whereas mini-pigs has \( n_{\text{median}} = 23\pm 7 \); \( n_{\text{ulnar}} = 18\pm 3 \) fascicles. In comparison, we found that the number of fascicles in median nerve of the rats \( n = 3 \) [10], cats \( n = 8 \) [11], monkeys \( n = 16\pm 5 \) [12] and human \( n = 37 \) [13]. As such, the number of fascicles in the pig nerve is comparable to the human. For landrace pigs, we found that the average number of fascicles in both median and ulnar nerves was highest just above the elbow joint (level 2). This indicates that the nerve diameter decreased from the elbow region towards the distal level as expected since; the nerve starts to branch out just after the elbow to innervate forearm and finger muscles [14]. For the mini pigs, the number of fascicles decreased gradually from the proximal to the most distal level for both nerves. The median nerve always contained a higher number of fascicles than the ulnar nerve for both type of pigs. As such, the region just above the elbow joint may be an ideal place for implantation of intrafascicular electrodes, since the chance of placing an active site close to one fascicle with a specific function is higher.

Fascicle diameter

Although, for both median and ulnar nerves the average fascicle diameter for landrace and mini pig is similar at all three levels, direct comparison with that of human fascicle diameter cannot be made due to its wide range (0.02 - 2 mm) [13].

Whole nerve diameter

Diameter of human upper peripheral nerves (median and ulnar) is approximately 3 mm [15] thus very similar to that of both landrace and mini pig (Table 1).

Choice of animal model for in vivo testing of peripheral nerve interfaces

In biomedical experiments, the pig is considered as an excellent model for bearing anatomical, physiological and neurological resemblance with human and the model is being used in several experiments [16]. Both landrace and mini pigs have similar median and ulnar nerve morphology to human. Although the landrace pig bears closer resemblance to human for these two nerves, mini-pig is also a comparable model especially during chronic neural experiments due to its smaller body weight (35-40kg).

Choice of intraneural electrodes

Improvements of recording or stimulation selectivity of current inter or intrafascicular electrodes may be possible if they are implanted in the region of the nerve where more fascicles are interfaced. Also, morphological features known from this study can help to optimize the design of intraneural electrodes by determining the pitch and number of active sites that can be placed in the electrode.

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