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Research report

The porcine corticospinal decussation: A combined neuronal tracing and tractography study

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ABSTRACT

Background: Pigs and minipigs are increasingly used as non-primate large animal models for preclinical research on nervous system disorders resulting in motor dysfunction. Knowledge of the minipig pyramidal tract is therefore essential to support such models.

Aim and methods: This study used 5 female Göttingen minipigs aging 11–15 months. The Göttingen minipig corticospinal tract was investigated, in the same animals, with in vivo neuronal tracing and with postmortem diffusion weighted MRI tractography to provide a thorough insight in the encephalic distribution of this primary motor pathway and its decussation at the craniocervical junction.

Results: The two methods similarly outlined the course of the pyramidal tract from its origin in the motor cortex down through the internal capsule to the craniocervical junction, where both methods displayed an axonal crossover at the pyramid decussation. The degree of crossover was quantified with unbiased stereology, where 81–93% of the traced corticospinal fibers crossed to the contralateral spinal cord. Accordingly, in the upper cervical spinal cord the corticospinal tract is primarily distributed in the contralateral lateral funiculus and in close relation to the gray matter, wherein some direct terminations on large ventral column gray matter neurons could be identified.

Discussion: The combination of neuronal tracing and tractography exploited the strengths of the respective methods to gain a better understanding of the encephalic distribution and craniocervical decussation of the Göttingen minipig corticospinal tract. Moreover, a quantification of the crossing fibers was obtained from the tracing data, which was not possible with tractography. Our data indicate that the porcine corticospinal system is quite lateralized down to the investigated upper cervical levels. However, further elucidation of this point will require a full examination of the corticospinal tracing pattern into the caudal spinal cord combined with an analysis of the direct versus indirect termination pattern on the lower motor neurons.

1. Introduction

Pigs and minipigs (sus scrofa) have been increasingly used as non-primate large research animals for preclinical research on nervous system disorders resulting in motor dysfunction (Bjarkam et al., 2008; Christensen et al., 2018; Cumming et al., 2001; Dolezalova et al., 2014; Glud et al., 2011; Nielsen et al., 2016; Schubert et al., 2016; Tanaka et al., 2008), as they have a large gyrencephalic brain relative to their body size permitting the use of conventional neurosurgical equipment and clinical scanners (Bjarkam et al., 2008; Glud et al., 2010; Lind et al., 2007). Moreover, pigs are easy to handle, and their anatomy and physiology closely resembles that of humans (Fang et al., 2012). Also, they have both economical and ethical advantages compared to the use of non-human primates (Bjarkam et al., 2009; Glud et al., 2010).

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However, the neuroanatomical knowledge of the porcine corticospinal tract (CST) is sparse, as it generally has been expected that this fiber system in hoofed animals (ungulates) was less developed reflecting the lacking ability for skilled precision movements of the distal extremities (Lassek, 1942). This belief was supported by an anatomical study of the landrace pig pyramidal tract indicating that corticospinal fibers were not seen caudal to the first cervical metamere (Palmieri et al., 1986). More recent studies using fMRI (Fang et al., 2005), muscle motor evoked potentials (Tanaka et al., 2008), and neuronal tracing (Leonard et al., 2017) have, however, questioned the classical view on the porcine corticospinal system. Thus, passive movement of the pig hind limb will result in activation of pig cortical motor areas (Fang et al., 2005), just as lesion of the internal capsule will abolish limb muscle motor evoked potentials contralateral to the lesion (Tanaka et al., 2008). Neuronal tracing from the pig motor cortex (MC) have likewise demonstrated that the pig corticospinal tract can be seen in the cervical spinal cord, where it is located in the lateral fiber column similar to humans, but in contrast to the position of the rodent corticospinal tract in the dorsal column (Leonard et al., 2017). It is, however, noteworthy that the CST is not demonstrated directly in the former two studies (Fang et al., 2005; Tanaka et al., 2008), while the study by Leonard et al. (Leonard et al., 2017) do not mention the degree of pyramidal decussation and neither indicate whether their demonstrated unilateral cervical CST is located ipsilateral or contralateral to the tracer injection in the motor cortex.

In order to understand white matter tracts, neuronal tracing has been used for years to investigate neuronal connections and is currently the gold standard (Dyrby et al., 2007; Vercelli et al., 2000). Biotinylated dextran amine (BDA) has been successfully used for anterograde neuronal tracing across animal species (Brandt and Apkarian, 1992; Reiner et al., 2000; Veenman et al., 1992), e.g. for tract tracing of the canine CST (Han et al., 2012). The invasive nature of this technique, however, limits its applicability. On the other hand, tractography derived from diffusion weighted MRI is a non-invasive method that can be performed both in and *ex vivo* to study neuronal connections (Anaya Garcia et al., 2015; Dyrby et al., 2011) in animals as well as in humans. Contrary to neuronal tracing, tractography is not a labelling of individual axons, but the imaging technique is based on the anisotropic water diffusion inside and between the axons. There have been obstacles due to factors as low spatial resolution, low signal-to-noise ratio and complex white matter regions with multiple crossing fibers (Calabrese et al., 2015; Tournier et al., 2004), where the latter has been described to cause problems with both false positives and false negatives (Tournier et al., 2012). To handle such complex local microanatomy, local crossing fiber models can be used (Behrens et al., 2007).

This study aims, accordingly, to describe the Göttingen minipig corticospinal decussation combining, in the same animal, neuronal tracing and tractography. Additionally, the laterality of the CST will be quantitatively assessed by the use of unbiased stereology (West, 2012a).

2. Materials and methods

2.1. Pilot study

The neuronal tracing procedure, techniques and survival time was determined based on a pilot study in rats and existing literature (Lazarov, 2013; Reiner et al., 2000).

2.2. Main study

2.2.1. Animals

5 female Göttingen minipigs (*Ellegaard Göttingen Minipigs, Dalmose, DK),* JBG-1 to JBG-5, aging 11–15 months and weighing 22.6–28.0 kg were used in this study as approved by the Danish National Council of Animal Research Ethics (protocol number 2015-15-0201-00965).

2.2.2. Materials

BDA tracing solution was freshly made prior to each injection using lyophilized BDA powder (NeuroTrace™ BDA-10.000 Neuronal Tracer Kit, ThermoFischer, Waltham, MA, USA) dissolved in 0.01 M phosphate buffered saline (PBS) with pH 7.4 yielding a 10% BDA solution.

2.2.3. Surgical preparation and neuronal tracer injection

Animals were sedated with 10 ml sedative mixture (6 ml midazolam 5 mg/mL, 4 ml ketamine 25 mg/mL, i.m.). Intravascular access was obtained through cannulation of an ear vein and animals were further sedated before being intubated as previously described (Ettrup et al., 2011) permitting isoflurane anaesthesia and respirator ventilation. Animals received buprenorphin (*Temgesic®*) analgesic and antibiotic prophylaxis bolus (1.5 g *Cefuroxim "Fresenius Kabi"*) and were placed in a MRI-compatible headframe providing stability and attachment of stereotaxic equipment (Bjarkam et al., 2004). A midline incision was made subsequent to a subcutaneous local anaesthetic injection and a fiducial marker was inserted in a skull burr hole. Anatomical scans for fiducial marker visualization and stereotaxic planning were then acquired on a 3 T Siemens Magnetom Trio MR scanner (Tim Trio) using a Turbo Flash 3D T1-weighted sequence with slice thickness 1 mm, voxel size $1 \times 1 \times 1\ mm^3$, 176 slices, FOV = 256, TR = 2420 ms, TE = 3.7 ms, 2 averages, TI = 960 ms, and flip angle = 9°. During the scan animals were placed in a prone position on a 24 element spine matrix coil with a 6 element body matrix coil on top of the stereotaxic frame.

The position of the Göttingen minipig motor cortex known from a previous study (Bjarkam et al., 2017a) could now be estimated in relation to the MRI-visualized skull fiducial marker (Glud et al., 2017) and a burr hole craniotomy was then placed over the estimated position of the right motor cortex. The dura was opened with a dura knife revealing the dorsal surface anatomy of the right hemisphere. It was now possible, as indicated on Fig. 1, to identify the longitudinal cerebral fissure, and the ansate, the cruciate and the coronal sulci, which delineate the motor cortex (Bjarkam et al., 2017a). The BDA solution was slowly pressure injected (0.1 µL/min) using a 10 µL Hamilton microsyringe placed in a manual micromanipulator system. To ensure a sufficient amount of traced neurons from the exposed brain surface, the right motor cortex was injected at three separate sites separated by 1 mm in the anterior-posterior direction at three depths (surface −3 mm, surface −2 mm, and surface −1 mm) yielding the same total dose of 4.5 µL tracer in all animals and covering an injection area for each animal as indicated on Fig. 1. After each injection, the syringe was left for 5 min to prevent reflux of the tracer along the needle track (Nance and Burns, 1990). The dura was replaced and covered with BioGlue® (*CryoLife, Kennesaw, Georgia, US*) and the scalp re-sutured.

2.2.4. Tissue fixation

After 26–31 days, animals were euthanized by an intra-cardial pentobarbital overdose after sedation. The brain was trans-cardially perfusion fixated with 5 l phosphate buffered paraformaldehyde (PFA) in a 4% solution (pH 7.4) using a pressurized container with a catheter tip placed in the ascending aorta through the left ventricle (Ettrup et al., 2011). The fixated brain and the upper spinal cord were removed subsequent to surgical skull removal and laminectomy (Bjarkam et al., 2017b). The cortical injection area was labeled with red tissue staining (India Ink®) through the surgical dura opening. The removed brains were initially placed in 4% PFA solution until at least a week prior to ex vivo DWI scanning where they were transferred to a neutral 0.01 M PBS solution.

2.2.5. Diffusion weighted magnetic resonance imaging

Ex vivo DWI was acquired on a 4.7T Agilent MR scanner at Danish Research Centre for Magnetic Resonance (DRCMR). The preparation of the brains for ex vivo imaging used the procedure previously described by Dyrby et al. (Dyrby et al., 2011) allowing a long-time imaging protocol without artefacts from respiration movements and influence of