

Video Article

# Exposure of the Pig CNS for Histological Analysis: A Manual for Decapitation, Skull Opening, and Brain Removal

Carsten R. Bjarkam<sup>1</sup>, Dariusz Orlowski<sup>2</sup>, Laura Tvilling<sup>2</sup>, Johannes Bech<sup>2</sup>, Andreas N. Glud<sup>2</sup>, Jens-Christian H. Sørensen<sup>2</sup>

<sup>1</sup>Department of Neurosurgery, Clinical Institute of Medicine, Aalborg University Hospital

<sup>2</sup>Center of Experimental Neuroscience (Cense), Department of Neurosurgery, Institute of Clinical Medicine, Aarhus University Hospital

Correspondence to: Carsten R. Bjarkam at [c.bjarkam@rn.dk](mailto:c.bjarkam@rn.dk)

URL: <https://www.jove.com/video/55511>

DOI: [doi:10.3791/55511](https://doi.org/10.3791/55511)

Keywords: Neuroscience, Issue 122, Anatomy, Brain surgery, Cranial nerves, Dura, minipig, Neurosurgery, Pituitary, Skull base, Sus Scrofa, Technique, Ungulate

Date Published: 4/13/2017

Citation: Bjarkam, C.R., Orlowski, D., Tvilling, L., Bech, J., Glud, A.N., Sørensen, J.C. Exposure of the Pig CNS for Histological Analysis: A Manual for Decapitation, Skull Opening, and Brain Removal. *J. Vis. Exp.* (122), e55511, doi:10.3791/55511 (2017).

## Abstract

Pigs have become increasingly popular in large-animal translational neuroscience research as an economically and ethically feasible substitute to non-human primates. The large brain size of the pig allows the use of conventional clinical brain imagers and the direct use and testing of neurosurgical procedures and equipment from the human clinic. Further macroscopic and histological analysis, however, requires postmortem exposure of the pig central nervous system (CNS) and subsequent brain removal. This is not an easy task, as the pig CNS is encapsulated by a thick, bony skull and spinal column. The goal of this paper and instructional video is to describe how to expose and remove the postmortem pig brain and the pituitary gland in an intact state, suitable for subsequent macroscopic and histological analysis.

## Video Link

The video component of this article can be found at <https://www.jove.com/video/55511/>

## Introduction

Translational neuroscience studies in pigs have become increasingly popular during the last two decades. The large size of the pig brain enables the use of conventional clinical brain imagers and the direct use and testing of neurosurgical procedures and equipment from the human clinic<sup>1,2,3,4,5,6,7,8</sup>. In the last 20 years, pigs, especially minipigs (e.g., Göttingen minipig), have been used to examine neuromodulatory treatment modalities, such as stem cell transplantation; viral vector transfection; and deep brain stimulation directed towards Parkinson disease, obesity, depression, and Alzheimer disease<sup>2,6,9,10,11,12,13,14,15,16,17</sup>. This has been followed by the development of stereotaxic and surgical approaches to manipulate the minipig CNS<sup>3,18,19,20,21</sup>. The instituted CNS changes have been evaluated in live animals using brain imaging (PET<sup>10,13,22,24</sup> and MR<sup>23</sup>), cystometry<sup>11,12,25</sup>, gait analysis<sup>17</sup>, neurological evaluation<sup>9,17</sup>, and postmortem examination based on histology and stereological analysis<sup>14,15,17,26,27,31</sup>. However, postmortem analysis requires the exposure and removal of the pig brain, which is not an easy task, as a thick, bony skull and a fibrous dural covering surround the pig brain.

The goal of this paper and instructional video is to describe how the postmortem pig brain and pituitary may be exposed and removed in an intact state in 15-20 min using non-motorized surgical tools. The instructional video and photographic illustrations show male minipigs (age: 6 months, bodyweight: 20-25 kg) used for an anatomical study on the minipig pituitary gland.

## Protocol

Animal anesthesia and euthanasia was performed in accordance with "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) and approved by the Danish Council for Animal Research Ethics.

## 1. Instruments

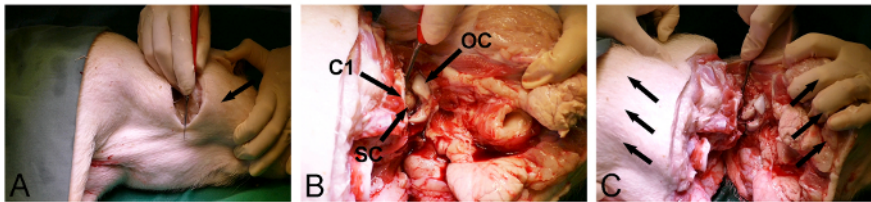
1. Collect the instruments presented in the video and listed in the **Table of Materials**.

## 2. Decapitation

NOTE: Anesthesia was induced by an intramuscular injection of 5 mL of midazolam (5 mg/mL) and 5 mL of ketamine (25 mg/mL). 5-10 min later, when the animal was deeply sedated, an ear vein was cannulated and a lethal overdose (100 mg/kg of bodyweight) of sodium pentobarbital (200 mg/mL) was given intravenously. To ensure that the animal was completely euthanized, the interdigital pain reflex was tested as shown by Ettrup *et al.* (2011)<sup>20</sup>. Complete euthanasia was ensured as described in the ethics statement above and followed by a transcardial perfusion with 5 L

of isotonic saline, as demonstrated by Ettrup *et al.* (2011)<sup>20</sup>. All the demonstrated procedures are performed postmortem, precluding the need for the welfare precautions necessary for long-term anesthesia and postprocedural survival.

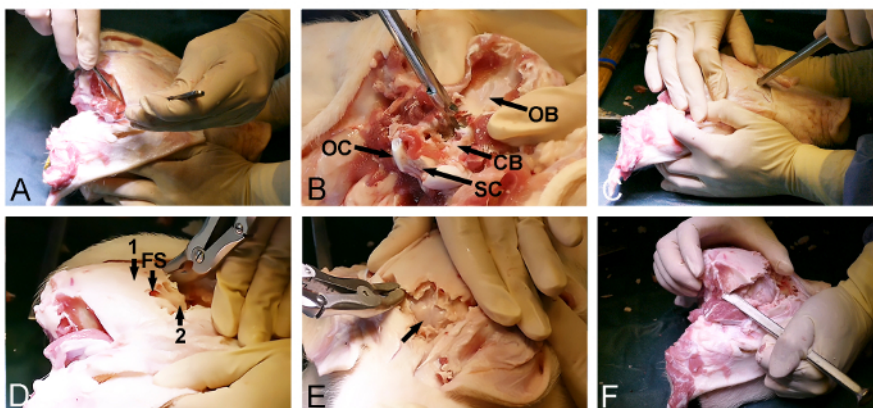
1. Decapitate the pig by a high circular cervical incision, using a surgical scalpel, just below the mandibular angle (**Figure 1A**).
2. Still with the surgical scalpel, continue the incision anteriorly through the soft tissue of the neck, including the larynx and the esophagus, until the bony spinal column is reached, approximately at the level of the craniocervical junction.
3. Advance the cut with a surgical scalpel from the anterior side of the craniocervical junction, above the anterior arc of atlas, and through the anterior atlantooccipital membrane, thereby exposing the spinal canal and the spinal cord (**Figure 1B**). Simultaneously ask an assistant to pull the pig body away from the pig head to ease the access between the skull base and the first cervical vertebra.
4. Continue the surgical incision through the dura sac and the spinal cord (**Figure 1B**). Take special care to ensure that a complete transverse section of the spinal cord is achieved.  
NOTE: Failure to perform the previous step may result in unwanted traction on the spinal cord and brain during the following steps of the decapitation process.
5. Forcefully extend the cranocervical junction at the section level (**Figure 1C**). At the same time, use the surgical scalpel to section the remaining atlantooccipital ligaments to release the articulation between the occipital condyles and the upper articulate process of atlas. Separate the pig head from the body.



**Figure 1: Minipig decapitation.** (A) Neck incision (arrow, mandibular angle). (B) Incision through the atlantooccipital ligaments and the dura-surrounded spinal cord (SC) at the craniocervical junction (C1, anterior arc of atlas; OC, occipital condyle). (C) The posterior part of the atlantooccipital articulation is released by a forceful extension (arrows) at the section level. [Please click here to view a larger version of this figure.](#)

### 3. Skull Opening

1. Position the pig head on a table.
  2. Make a dorsal longitudinal incision with a surgical scalpel through the skin and the underlying soft tissue from the back of the snout, over the vertex of the head, and down through the posterior part of the occipital region.
  3. Expose the dorsal and posterior part of the skull by removing the soft tissue located lateral to the initial incision with a surgical scalpel.
  4. Release the temporal muscle bilaterally from the skull (**Figure 2A**) with a surgical scalpel. Make sure that the posterior occipital bone is cleaned of soft tissue.
  5. Use the posterior entrance of the foramen magnum to remove the occipital bone with a Kerrison bone punch and bone rongeurs and expose the dura-covered cerebellum (**Figure 2B**).
  6. Return to the exposed anterior side of the skull and select an entry point in the frontal bone, just in front of the eyes. At this point, use a bone chisel with a hammer to penetrate the skull and enter the frontal sinus (**Figure 2C**).
  7. Use the extent of the frontal sinus to further the dorsoposterior removal of the outer skull lamina with a bone rongeur or bone punch and expose the inner, thin bony skull lamina covering the cerebrum (**Figure 2D**).
  8. Gently open the inner bony skull lamina anteriorly with a hammer and a bone chisel to expose the dura-covered cerebrum (**Figure 2E**).
  9. Continue the bone removal laterally using a bone chisel and a bone rongeur through the temporal and parietal bone in order to release the final dorsoposterior part of the skull, located between the already exposed parts of the dura-covered cerebrum and cerebellum (**Figure 2F**).
- NOTE: It is often possible, during the final step of this procedure, to use the chisel to break open the remaining posterior skull bone from one side, just like one opens a door.

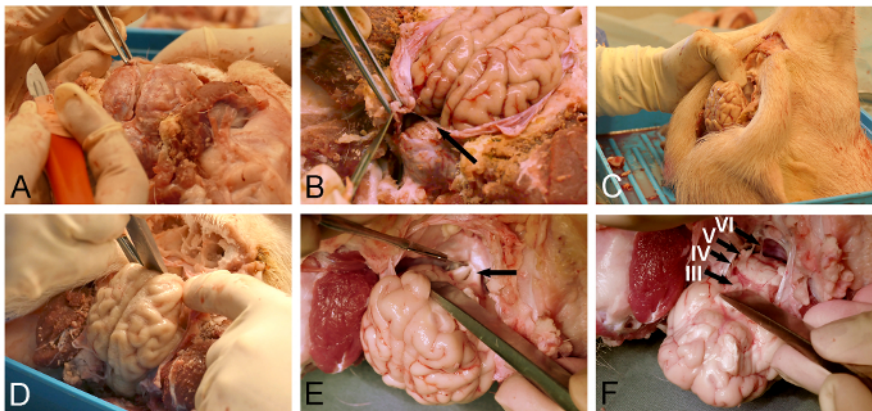


**Figure 2: Minipig skull opening.** (A) Exposure of the dorsoposterior skull surface, including the removal of the occipital and temporal muscles. (B) Removal of the occipital bone (CB, dura-covered cerebellum; OB, occipital bone; OC, occipital condyle; and SC, spinal cord). (C) A hammer and a bone chisel are used to penetrate the skull anteriorly and to enter the frontal sinus at the level of the eyes. (D) The extent of the frontal sinus (FS) is

used to remove the outer thick skull bone (1), exposing an inner thin bone lamina (2) covering the cerebrum. (E) Removal of the thin bone lamina, exposing the dura-covered cerebrum (arrow). (F) Finally, a hammer and a bone chisel are used to laterally connect the anterior and the posterior skull openings. [Please click here to view a larger version of this figure.](#)

## 4. Brain Removal

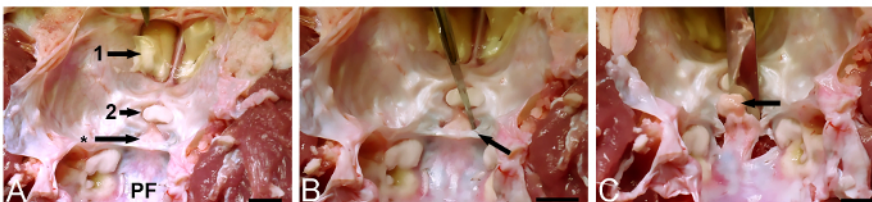
1. Use surgical forceps to lift the dura and create a gentle incision close to the venous superior sagittal sinus using a fine surgical scalpel (**Figure 3A**).
2. Use micro-scissor or a dura-knife to further open the dura covering the dorsal surface of the brain.  
NOTE: Special care must be taken when removing the dura corresponding to the cerebellar tentorium (Figure 3B), as preservation of this dural leaf will prevent subsequent brain removal.
3. Position the pig head vertically (**Figure 3C**).
4. Use the bone chisel or a dissector to release the ventroanterior cerebrum by blunt dissection of the olfactory bulb from the dura-covered floor of the cranial cavity (**Figure 3D**).
5. Use a fine surgical scalpel to section the exposed optic chiasm (**Figure 3E**). Expose and section the pituitary stalk and the oculomotor nerves.
6. Release the ventral brainstem by sectioning the lower cranial nerves (**Figure 3F**) with a fine surgical scalpel. Ensure that the dural cerebellar tentorium was completely incised (**Figure 3B**), as this dural leaf will otherwise cut through the brainstem during the release process.



**Figure 3: Minipig brain removal.** (A) Dural opening with surgical forceps and a dura knife. (B) Care must be taken to completely incise the dural leaf (arrow), located between the cerebrum and the cerebellum. (C) The pig head is positioned vertically for better visualization of skull base structures and in order for gravity to assist in the intended displacement of the brain. (D) A dissector or a bone chisel is used to relieve the olfactory bulb by blunt section from the dura-covered skull base. (E) The dissection is continued in a posterior direction along the skull base for exposure and sectioning of the optic chiasm (arrow), infundibular stalk, and oculomotor nerves. (F) The brain release is completed with the section of the lower cranial nerves as they depart from the ventral surface of the brainstem (III, oculomotor nerve; IV, trochlear nerve; V, trigeminal nerve; and VI, abducens nerve). [Please click here to view a larger version of this figure.](#)

## 5. Pituitary Removal

1. Identify the pituitary stalk and its surrounding dural leaf (the diaphragma sellae) in the skull floor (**Figure 4A**).
2. Incise the dural leaf lateral to the pituitary stalk using a fine surgical scalpel (**Figure 4B**).
3. Use a dissector to release the pituitary and lift it out of the pituitary fossa (**Figure 4C**).



**Figure 4: Minipig pituitary removal.** (A) The pituitary fossa (\*) is identified in the skull floor (1, olfactory bulb; 2, optic chiasm; and PF, posterior cranial fossa). (B) The dural covering (sellar diaphragm, arrow) is incised laterally. (C) The pituitary (arrow) is released with a dissector and lifted out of the pituitary fossa. Scale bar (A-C) = 10 mm. [Please click here to view a larger version of this figure.](#)

## Representative Results

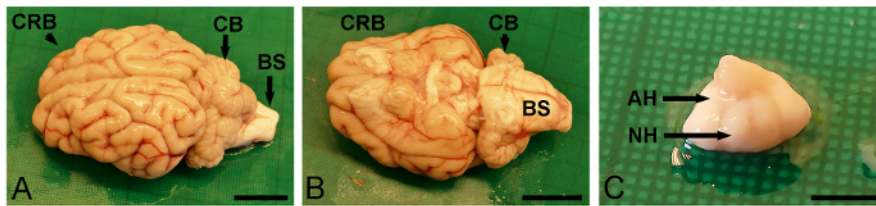
To prevent the tissue material from drying out, it is recommended to store the removed brain and pituitary in a jar filled with fixative or isotonic saline immediately after macroscopic analysis has been performed. The tissue material may be stored in the fixative for years, whereas storage in isotonic saline, even in a refrigerator, will lead to tissue decay with time.



The removed pituitary may also be directly frozen by immersion into dry ice-cooled liquid 2-methylbutane, whereas the intact pig brain is too large for direct freezing<sup>28</sup>. Instead, it is recommended to slice the pig brain, as previously demonstrated<sup>28</sup>, into 9-15 mm thick parallel coronal tissue slabs that can be frozen *in toto* and cryostat-sectioned into 40  $\mu$ m-thick sections<sup>5,18,26,28</sup>. Alternatively, specific brain areas can be free-dissected from the removed intact brain or sliced brain slab and submitted to further histological processing after vibratome sectioning<sup>30</sup>, paraffin/methacrylate embedding and microtome sectioning<sup>6,17,27</sup>, or freezing and cryostat sectioning<sup>6,14,15,25</sup>. In our setting, the pig carcasses are finally placed in specified plastic containers and stored in a dedicated cold storage room until they are collected and transported to a biological degrading facility.

With the use of non-motorized surgical tools (table of materials), the described technique (**Figures 1-3**) enables, in approximately 15-20 min, the removal of the intact pig brain (**Figure 5AB**), while the severed cranial nerves and pituitary remain connected to the skull floor (**Figure 4A**). Likewise, the pituitary may be simply removed, intact, after removing the overlying brain and releasing the dural sellar diaphragm (**Figure 4 and 5C**).

The resulting brain and/or pituitary (**Figure 5**) may subsequently be submitted to macroscopic analysis that, apart from direct visual inspection, may include size and volume measurements<sup>31</sup>. This can be followed by oriented sectioning into smaller brain slabs<sup>28,29</sup> suitable for chemical analysis and/or further histological preparation, staining, and microscopic analysis<sup>6,14,15,17,25,26,27</sup>.



**Figure 5:** The minipig brain (**A** and **B**) and pituitary (**C**). (**A**) Brain, laterodorsal view (BS, brainstem; CB, cerebellum; and CRB, cerebrum). (**B**) Brain, ventral view (BS, brainstem; CB, cerebellum; and CRB, cerebrum). (**C**) Pituitary, posterior view (AH, adenohypophysis and NH, neurohypophysis). Scale bar (A and B) = 10 mm, (C) = 5 mm. [Please click here to view a larger version of this figure.](#)

## Discussion

Most experimental neuroscience studies are performed in small animal species, such as mice and rats, where access to the CNS is facilitated by a thin skull- and dural-thickness. However, in larger experimental animals like pigs<sup>1,4,8</sup>, sheep<sup>32</sup>, and non-human primates, the considerable thickness of these structures necessitates the use of robust instruments (table of materials) and proper entry points for skull bone removal (**Figure 2**). Knowledge of restricting dural leaves (**Figures 3 and 4**) is required before the CNS can be accessed and the brain safely removed.

It is recommended to leave the dura intact during the skull bone removal, as this will protect the underlying brain from damage. Previous transcardial fixation<sup>20</sup> may likewise harden and slightly shrink the brain, allowing the bone and dural removal process to be performed with more ease and safety. A special feature of the pig skull, in contrast to the sheep and non-human primate, is the progressive expansion of the frontal sinus with age, which may be advantageous in the skull bone removal process (**Figure 2**). The presented technique may accordingly be used on all large animal species, but only in pigs, especially those older than 6 months, will the frontal sinus be developed enough to provide aid in the skull bone removal process. Finally, complete sectioning of the spinal cord during the decapitation process (**Figure 1B**) and complete sectioning of the dural cerebellar tentorium (**Figure 3B**) before the final brain release are absolutely required in order to avoid subsequent damage to the brainstem.

In some studies, it may be advantageous to have a part of the rostral cervical spinal cord attached to the brain. This can be obtained by placing the initial decapitation incision (**Figure 1A**) more caudally on the neck, allowing access to the spinal cord through an intervertebral cervical disc instead of the craniocervical junction, as demonstrated in the current video. The posterior bone removal will then have to start from the exposed caudal lamina. Apart from this, the technique will be similar, so it is important to remember that the spinal cord must be completely sectioned before the decapitation is completed with forceful extension at the section level (**Figure 1**). The current procedure is demonstrated on unfixed animals, as the actual background study necessitated HPLC analysis of the derived pituitary. Note, however, that the exact same technique is used on animals transcardially fixated with paraformaldehyde<sup>3,5,14,15,16,17,18,19,20,25,26,27,28,31</sup>, although all procedures in such a case must be performed under good ventilation provided by a fume hood<sup>20</sup>. The choice of native dissection versus *in toto*-fixation and subsequent dissection should therefore purely depend on the required post-processing procedure (e.g., conventional histology, immunohistochemistry, HPLC, and FISH)<sup>32</sup>.

As discussed in the next paragraphs, we have preferred to use non-motorized surgical tools. The skull of pigs aged above 1-2 years may, however, be so robust that skull removal with the demonstrated instruments not is possible, necessitating the use of motorized instruments, such as craniotomes, oscillating saws, and electrical drills<sup>32</sup>. In that case, it is still recommended to follow the protocol steps indicated above, thereby taking advantage of the naturally occurring skull entry points and frontal sinus development. The presented anterior access to the skull base is elegant and easy, but the described blunt release of the olfactory bulbs must be performed without direct visual guidance (**Figure 3D**), in contrast to the more posterior release of the brain (**Figure 3EF**). The ventral part of the olfactory bulbs may therefore suffer some degree of uncontrolled damage that only can be avoided, if needed, by drilling the anterior skull base out ventral to the bulbs before the release process is initiated. Note that the manipulation, especially of unfixed brain tissue during skull removal, has been indicated to result in the histological dark neuron artifact, which may lead to erroneous conclusions in neurotoxicological studies<sup>33</sup>.

The skull bone removal process can also be performed with machinery like craniotomes, oscillating saws, and electrical drills<sup>32</sup>. These may speed up the process, but they will also increase the risk of damage to the underlying neural structures. Such equipment can also be costly

and will most likely be unavailable to most laboratories. We have therefore preferred to demonstrate the current procedure using non-motorized surgical equipment (**Table of Materials**) that is easy to access and use.

The described and illustrated technique will, when properly used, enable the exposure and removal of the postmortem pig brain, pituitary, and/or cervical spinal cord (**Figure 5**), resulting in tissue pieces that are well suited for further macroscopic analysis<sup>5,6,19,26,31</sup>, sectioning into brain slabs<sup>28</sup>, and subsequent chemical analysis and/or histological processing<sup>6,14,15,16,17,25,26,27,31</sup>.

## Disclosures

The authors have nothing to disclose.

## Acknowledgements

The authors acknowledge with gratitude the skillful assistance of Mrs. Trine W. Mikkelsen, Mrs. Lise M. Fitting, and the staff at Pâskehøjgaard. The Danish Medical Research Council, the Lundbeck Foundation, and the Novo Nordisk Foundation financially supported the study.

## References

1. Lind, M.N., Moustgaard, A., Jelsing, J., Vajta, G., Cumming, P., Hansen, A.K. The use of pigs in neuroscience: Modeling brain disorders. *Neurosci Biobehav Rev.* **31**, 728-751 (2007).
2. Bjarkam, C.R., *et al.* Neuromodulation in a minipig model of Parkinson disease. *British J Neurosurg.* **22**(Suppl. 1), S9-S12 (2008).
3. Bjarkam, C.R., Cancian, G., Glud, A.N., Ettrup, K.S., Østergaard, L., Sørensen, J.C. MRI-guided stereotaxic targeting in pigs based on a stereotaxic localizer box fitted with an isocentric frame and use of SurgiPlan computer-planning software. *J Neurosci Methods.* **183**(2), 119-126 (2009).
4. Sauleau, P., Lapouble, E., Val-Laillet, D., Malbert, C.H. The pig model in brain imaging and neurosurgery. *Animal.* **3**(8), 1138-1151 (2009).
5. Bjarkam, C.R., *et al.* Safety and function of a new clinical intracerebral microinjection instrument for stem cells and therapeutics examined in the Göttingen minipig. *Stereotact Funct Neurosurg.* **88**(1), 56-63 (2010).
6. Fjord-Larsen, L., *et al.* Long-term delivery of nerve growth factor by encapsulated cell biodelivery in the minipig basal forebrain. *Mol Therapy.* **18**(12), 2164-72 (2010).
7. Sørensen, J.C., *et al.* Development of neuromodulation treatments in a large animal model - Do neurosurgeons dream of electric pigs?. *Prog Brain Res.* **194**, 97-103 (2011).
8. Dolezalova, D., *et al.* Pig models of neurodegenerative disorders: utilization in cell replacement-based preclinical safety and efficacy studies. *J Comp Neurol.* **522**(12), 2784-2801 (2014).
9. Mikkelsen, M., Møller, A., Jensen, L.H., Pedersen, A., Harajehi, J.B., Pakkenberg, H. MPTP-induced Parkinsonism in minipigs: A behavioral, biochemical, and histological study. *Neurotoxicol Teratol.* **21**, 169-175 (1999).
10. Danielsen, E.H. *et al.* The DaNEX study of embryonic mesencephalic, dopaminergic tissue grafted to a minipig model of Parkinson's disease: Preliminary findings of effect of MPTP poisoning on striatal dopaminergic markers. *Cell Transplant.* **9**(2), 247-259 (2000).
11. Dalmose, A., Bjarkam, C.R., Sørensen, J.C., Jørgensen, T.M., Djurhuus, J.C. Effects of high frequency deep brain stimulation on urine storage and voiding function in conscious minipigs. *Neurol Urodyn.* **23**(3), 265-72 (2004).
12. Dalmose, A., Bjarkam, C.R., Djurhuus, J.C. Stereotactic electrical stimulation of the pontine micturition center in the pig. *Br J Urol.* **95**, 886-889 (2005).
13. Andersen, F., Watanabe, H., Bjarkam, C.R., Danielsen, E.H., Cumming, P., The DaNeX Study Group. Pig brain stereotaxic standard space: Mapping of cerebral blood flow normative values and effect of MPTP-lesioning. *Brain Res Bull.* **66**(1), 17-29 (2005).
14. Glud, A.N., *et al.* Direct gene transfer in the minipig CNS using stereotaxic lentiviral microinjections. *Acta Neurobiol Exp.* **70**(3), 1-8, (2010).
15. Glud, A.N., *et al.* Direct MRI-guided stereotaxic viral mediated gene transfer of alpha-synuclein in the minipig CNS. *Acta Neurobiol Exp.* **71**(4), 508-18 (2011).
16. Ettrup, K.S., Sørensen, J.C., Rodell, A., Alstrup, A.K.O., Bjarkam, C.R. Hypothalamic deep brain stimulation influences autonomic and limbic circuitry involved in the regulation of aggression and cardiocerebrovascular control in the minipig. *Stereotact Funct Neurosurg.* **90**(5), 281-291 (2012).
17. Nielsen, M.S., *et al.* Continuous MPTP intoxication in the minipig results in chronic parkinsonian deficits. *Acta Neurobiol Exp.* **76**, 198-210 (2016).
18. Bjarkam, C.R., *et al.* A MRI-compatible stereotaxic localizer box enables high-precision stereotaxic procedures in pigs. *J Neurosci Methods.* **139**(2), 293-8 (2004).
19. Bjarkam, C.R., Jørgensen, R.L., Jensen, K.N., Sunde, N.Aa., Sørensen, J.C.H. Deep brain stimulation electrode anchoring using BioGlue®, a protective electrode covering, and a titanium microplate. *J Neurosci Methods.* **168**, 151-155 (2008).
20. Ettrup, K.S., *et al.* Basic Surgical Techniques in the minipig: Intubation, Transurethral Bladder Catheterization, Femoral Vessel Catheterization, and Transcardial Perfusion. *J Vis Exp.* **52**, e2652 (2011).
21. Ettrup, K.S., Tornøe, J., Sørensen, J.C., Bjarkam, C.R. A surgical device for minimally invasive implantation of experimental deep brain stimulation leads in large research animals. *J Neurosci Methods.* **200**(1), 41-46 (2011).
22. Danielsen, E.H., *et al.* Positron emission tomography of living brain in minipigs and domestic pigs. *Scand J Lab Anim Sci Suppl.* **25**(1), 127-135 (1998).
23. Røhl, L., *et al.* Time evolution of cerebral perfusion and ADC measured by MRI in a porcine stroke model. *J Magn Reson Imaging.* **15**(2), 123-129 (2002).
24. Cumming, P., Gillings, N.M., Jensen, S.B., Bjarkam, C.R., Gjedde, A. Kinetics of the uptake and distribution of the dopamine D2/3 agonist (R)-N-[1-11C]n-propylnorapomorphine in brain of healthy and MPTP-poisoned Göttingen miniature pigs. *Nucl Med Biol.* **30**(5), 547-553 (2003).

25. Jensen, K.N., Deding, D., Sørensen, J.C., Bjarkam, C.R. Long-term implantation of deep brain stimulation electrodes in the pontine micturition centre of the minipig. *Acta Neurochir.* **151**(7), 785-794 (2009).
26. Rosendal, F., *et al.* Does chronic low dose treatment with ciclosporin influence the brain? A histopathological study in pigs. *Transplantation Proc.* **37**(8), 3305-3308 (2005).
27. Nielsen, M.S., Sørensen, J.C., Bjarkam, C.R. The substantia nigra pars compacta of the minipig: An anatomical and stereological study. *Brain Struct Funct.* **213**(4-5), 481-488 (2009).
28. Sørensen, J.C., Bjarkam, C.R., Simonsen, C.Z., Danielsen, E., Geneser, F.A. Oriented sectioning of irregular tissue blocks in relation to computerized scanning modalities. Results from the domestic pig brain. *J Neurosci Methods.* **104**, 93-98 (2000).
29. Bjarkam, C.R., Pedersen, M., Sørensen, J.C. New strategies for embedding, orientation and sectioning of small brain specimens enable direct correlation to MR-images, brain atlases, or use of unbiased stereology. *J Neurosci Methods.* **108**, 153-159 (2001).
30. Bjarkam, C.R., Sørensen, J.C., Geneser, F.A. Distribution and morphology of serotonin-immunoreactive axons in the hippocampal region of the New Zealand white rabbit. I. Area dentata and hippocampus proper. *Hippocampus.* **13**(1), 21-37 (2003).
31. Bjarkam, C.R., Glud, A.N., Orlowski, D., Sørensen, J.C., Palomero-Gallagher, N. The telencephalon of the minipig, cytoarchitecture and cortical surface anatomy. *Brain Struct Funct.* e-pub ahead of print. (2016).
32. Boltze, J., Nitzsche, B., Geiger, K.D., Schoon, H-A. Histopathological investigation of different MCAO modalities and impact of autologous bone marrow mononuclear cell administration in an ovine stroke model. *Transl Stroke Res.* **2**, 279-293 (2011).
33. Jortner, B.S. The return of the dark neuron. A histological artifact complicating contemporary neurotoxicologic evaluation. *Neurotoxicology.* **27**, 628-634 (2006).