The Göttingen minipig in translational neuroscience

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Introduction

Animal models are essential in the development of novel treatment paradigms for most diseases, and for decades rodent models of various diseases have been imperative. The translation of results and treatment options from these small animals to humans often requires the use of a large animal as an intermediate step. Non-human primates have been used for this purpose, as this species shares many similarities with human anatomy and physiology. The use of non-human primates may present both ethical and economical obstacles, which is why alternatives to this large animal model species are necessary.

For the past fourteen years, the CENSE-group has worked to establish the Göttingen minipig as a research animal for neurological and neurosurgical disorders. The aim of our translational research is to examine mechanisms of action and develop new treatment paradigms of neuromodulation in a large, non-primate animal model. The Göttingen minipig has a large gyrencephalic brain (approx. 4x5x6 cm) enabling the use of conventional imaging modalities as well as operating techniques and neuromodulatory devices intended for human use.[1-12] At our centre, we have the option of using CT, MRI and PET imaging in our studies, and scanning modality depends on the hypothesis and the experimental paradigm.[1;5;6;8;12-16]

Neurosurgery in the Göttingen minipig

To facilitate surgery and stereotaxic procedures, the head of the minipig is placed in a head holder that also functions as an MRI-compatible localiser box. The head is fixated in the holder by means of titanium screws in the os zygomaticum. Access to the minipig brain is achieved using standard neurosurgical techniques and instruments, and for high-precision stereotaxic placement of deep brain stimulation (DBS) electrodes and intracerebral microinjections of tracer, toxins or stem cells, a stereotaxic MRI is performed prior to surgery. Stereotaxic coordinates for the target site can be calculated by means of an implanted fiduciary marker or by external fiducials in the side plates of the head holder, the latter allowing import of the MRI into standard neuro-navigation systems to achieve a level of precision in the stereotaxic targeting equal to that in routine clinical use.[2;3;17;18]

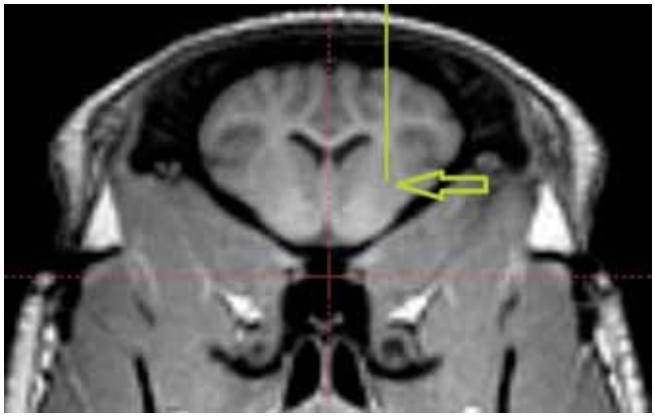

Figure 1 shows the stereotaxic MRI loaded into the coordinate calculating system. The arrow points toward the target, which in this case is the right putamen. The retrograde tracer FluroGold was injected to visualise the afferent connections of this striatal structure. The vertical green line indicates the injection tract.

Anatomical studies in the Göttingen minipig

Figures 1 and 2 show the principle of the stereotaxic systems used by our group in the minipig. The precision of the stereotaxic procedure allowed us to inject tracers into small brain areas to visualise the connections of the minipig brain structures, such as the putamen, the prefrontal cortex and the nucleus accumbens. Anatomical studies are, furthermore, made post mortem by immunohistological analysis, and we have inter alia characterised the hypothalamus, the pars compacta of the substantia nigra and the subthalamic nucleus in the Göttingen minipig.[13;19;20]

Parkinson's disease in the Göttingen minipig

A Parkinson's disease (PD) model has been established in the minipig using the toxin MPTP.[22] We have continued to work with this model and showed a progressive nature of disease symptoms by means of a pump inserted on the back of the pig which continuously injects the toxin. The animals showed symptoms of progressively reduced motor performance and activity, and post-mortem examination showed a reduced density of dopaminergic neurons on the pars compacta of the substantia nigra.

Currently, we are working to establish the rotating 6-hydroxydopamine (6-OHDA) rotating model of PD in the minipig. This model, where the selectively monoaminergic toxin 6-OHDA is injected into the nigrostriatal pathway, has been well established in rodents and primates for several decades. A unilateral lesion is made, causing asymmetry in the dopamine system, which causes the animal to rotate when stimulated with amphetamine and apomorphine, respectively. The unlesioned side of the brain serves as a normal internal control and the animals' rotational behaviour can be used to measure the treatment effect in future studies.[23-25]

Neural stem cell transplantation in the Göttingen minipig

We have tested the feasibility of neural stem cell transplantation in an MPTP model of Parkinson's disease, grafting neural tissue from the ventral mesencephalon of 28-week old pig-embryos and placing them in the dopamine depleted striatum of adult minipigs with PD. PET imaging revealed increased fluorodopa uptake in the transplantation sites and post-mortem examination revealed grafts surviving after 7 months.[5;8]

Subsequent studies have included implantation of stem cells using a newly developed intracerebral microinjection device, and

Figure 2 shows a 6-month-old minipig fixated in the MRI-compatible head holder. Stereotactic coordinates for the injection of a tracer into the putamen have been calculated, the burr hole has been made and the stereotactic arch system allows for high-precision injection.

stereotaxic implantation of lentiviruses carrying an alpha-synuclein construct into the substantia nigra to overexpress alpha-synuclein locally in transfected nigral neurons.[4;21]

Deep brain stimulation in the Göttingen minipig

In order to examine the mechanisms of action of DBS for PD, we implanted a DBS system unilaterally in the chronic MPTP model of the disease. This resulted in an improvement of motor performance on the side contralateral to the stimulation, leading to rotational behaviour.[18] PET studies of the animals revealed increased blood flow around the stimulation site in the subthalamic nucleus and increased oxygen uptake in the motor cortex, leading us to the hypothesis that DBS for PD results in a normalisation of the neural signalling in the basal ganglia system.[11]

Likewise, we have targeted the ventral hypothalamus with DBS to induce the satiety as a potential treatment of obesity, as well as the subgenual area as a potential DBS target for treatment of depression. Finally we have targeted the pontine micturition centre and achieved central control of voiding in the minipig.[7;10;26] Future studies will involve DBS targeting of the limbic structure of the nucleus accumbens in an attempt to establish a model of depression.

Spinal cord stimulation in the Göttingen minipig

Spinal cord stimulation is an established clinical treatment for severe chronic pain syndromes. Its mechanisms of action, however, have yet to be clarified. The treatment involves placing an electrode in the epidural space over spinal cord segments to innervate the dermatome inflicted by chronic pain. Accordingly, we have set out to map the dermatomes of the minipig and intend to use this anatomical knowledge in addressing the mechanisms of action of the treatment by functional imaging modalities.[11]

Conclusion

The Göttingen minipig provides a unique translational platform on which to develop and test innovative therapeutic approaches, both pharmacological and surgical, and the minipig is increasingly recognised as a useful animal model for translational neuroscience.

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