Gait analysis in the Göttingen minipig model of Parkinson disease based on viral gene transfer mediated alpha synuclein overexpression in the substantia nigra

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Introduction

Parkinson disease (PD) is a serious neurological disorder resulting from an excessive loss of dopaminergic neurons in the substantia nigra (SN). Animal models have been very helpful in gaining a better understanding of the disease mechanisms and in the developing of new treatments for the disease. However, most animal models are based on acute neuronal loss and thus do not mimic the progressive neuron loss seen in patients. This may be an important part of the reason that preclinical studies of neuroprotection so far have not been predictive of results in patients. In addition, high costs, social and ethical concerns are making it increasable difficult to perform experiments on primates. In this study we have used large animals. We used Göttingen minipigs because their genome is close to the human genome, they are cheap and they are easy to train. Furthermore the large gyrated pig brain enables identification of cortical and subcortical structures by conventional MRI and permits conventional neurosurgery, including stereotaxic surgery.

WewantedtoestablishafunctionalmodelofParkinsondiseaseintheGöttingenminipigbasedonMRI-guideddirectviralmediatedgene transfer in the substantia nigra by use of lentiviral vectors encoding alpha synuclein.

Methods

Six female Göttingen minipigs were stereotaxically injected unilaterally into the substantia nigra with 6 x 2.5 µl lentivirus capable of transducing cells and mediating recombinant expression of alpha synuclein. The animals were kept 4-5 months and the behavior was observed. All animals underwent pre- and post-operative digital gait analysis, using an infrared 3-D computerized Vicon system with 6 cameras to measure the temporospatiale parameters of gait.

After sacrifice we examined the SN, striatum and other parts of the brain for the alpha-synuclein gene using PCR. We also examined the nigro-striatal expression of alpha-synuclein, to confirm targeting and spread of viral expression using immunohistochemistry and histology. The animals were transcardially perfused with 4% PFA, the brain removed, paraffin embedded, cut into 30 µm thick slices and examined histopathologically and immunohistochemically.

Results

The animals tolerated the surgical procedure well with no signs of adverse effects.

There were no observed behavioural changes postoperatively. All animals corporated well to the gait analysis.

After alpha synuclein treatment the pigs changed their gait pattern. In spite of normal gait velocity, they took significantly longer steps and raised their legs higher from the floor. Furthermore they mowed more, and there was a significantly decrease in the double limb stance phase of the hind limbs.

After sacrifice we fund a decreased neuronal TH-positive staining in the SN.

On the control side the immunohistochemically EGFP-positive neurons targeted the striatum. PCR confirmed the location of the EGFP-gene in the SN only, thus confirming that axonal transport of the gene product had occurred.

Figure 1:
The digital gait analysis. Six infrared Vicon cameras were placed around a fenced area. The pigs were trained to walk back and forth, being offered chocolate treats. With this procedure, we obtained objective measurements of gait velocity.

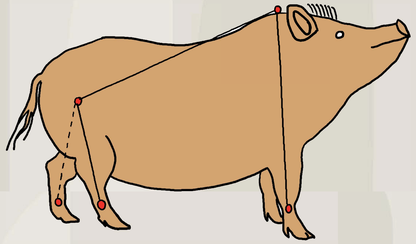

Figure 2:
Drawings of the pig with the 5 reflecting markers, as there are seen in the computer.

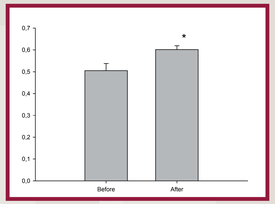

Figure 3
The figure shows the significantly increase in step length after injection of lentiviral vectors encoding alpha synuclein into the substantia nigra.

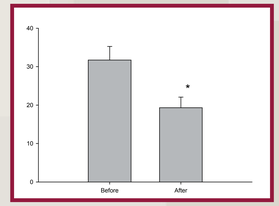

Figure 4
The figure shows the significantly decrease in double support phase of the hind limbs after injection of lentiviral vectors encoding alpha synuclein into the substantia nigra.

Figure 5:
Trajectories and injection sites were clearly visible and were seen targeting the SN in all examined animals. No signs of infection or haemorrhage were seen using GFAP, NISSL and HE staining.
The targeting of SN was confirmed using tyrosine hydroxylase (TH) immunohistochemistry.

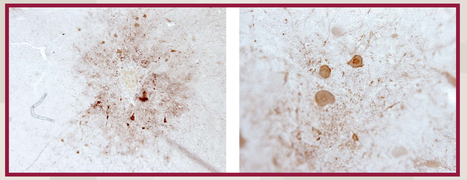

Figure 6:
The pictures shows that α-syn neurons were clearly visible around the injection site and in the medial forebrain bundle. In the striatum no positive neurons were seen, only scattered signs ofa-syn

Conclusion

Direct viral mediated gene transfer by MRI-guided stereotaxic injection of lentiviral vectors in the minipig brain, enabling alpha synuclein expression, results in distinct changes in gait pattern. We did not expect this because there was observed no behavioural changes at all. The gait velocity was also normal. However, the animals move with longer step and more raised step. They mowed with a shorter double support phase of the hind limb. In control pigs we have observed a close relation between velocity and these parameters. When the gait velocity is increased, the step length and raise of limb is increased too. Furthermore the double supports phase decreases when velocity increases. We also know from our animal MPTP studies that parkinsonian pigs walked with decrease velocity and increased raise of limb, but with normal step length. This could indicate that the pigs in this study had developed a "parkinsonian gait pattern", but the gait looked like they were in some way forced to walk faster than they would prefer. They walked with a gait pattern that we would had expected to see if the pigs move faster than they did.

The changes are small, but our results indicate that it is possible to make a large animal model of parkinsonian gait in the Göttingen minipig by injection of lentiviral vectors in the brain.

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