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 crude neural lesions and that do not mimic the progressive nature of the disease that has been observed in patients. This may be an important part of the reason that translational 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.

We wanted to establish a functional model of PD in minipigs. To achieve this we injected an adenovirus expressing alpha-synuclein into the substantia nigra by use of stereotaxic techniques. We wanted to test if the adenovirus reached the substantia nigra and if any behavioral changes were observable.

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 for 4-6 months and the behavior was observed. All animals underwent pre- and post-operative gait analysis, using an infrared 3-D computerized vision 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, paraffined, dehydrated and cut into 20 μm thick sections and examined histopathologically and immunohistochemically.

Results
The animals tolerated the surgical procedure well with no signs of adverse effects. There were no observed behavioral changes postoperatively. All animals performed well in the gait analysis. After alpha synuclein treatment the pigs changed their gait pattern. In control pigs we have observed a close relation between velocity and these parameters. When the gait velocity is increased, the step length and rise of limbs is increased too. Furthermore the double support phase decreases when velocity increases. We noticed that in our animal MEP studies that parkinsonian pigs walked with decreased velocity and increased raise of limb, but with normal step length. This could indicate that the pigs in the 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.

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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 behavioral changes at all. The gait velocity was not altered. However, the animals moved with longer step and more raised step. They moved 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 rise of limbs is increased too. Furthermore the double support phase decreases when velocity increases. We noticed that in our animal MEP studies that parkinsonian pigs walked with decreased velocity and increased raise of limb, but with normal step length. This could indicate that the pigs in the 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.