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**Characterization of the Dat$^{cre}$.iDTR mouse model for Parkinson's Disease**

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Idiopathic Parkinson's disease (PD) is the second most frequent neurodegenerative disease. Up-to-date medical treatments are only able to provide partial symptomatic relief of the major motor symptoms. In order to assess the effectiveness of new PD therapies, it is necessary to generate animal models that develop those motor impairments due to the apoptosis of Substantia nigra (SN) neurons. The lack of a mouse model that captures the essential clinical and major histopathological features in a stable and reliable way poses major obstacles for scientists to develop new therapies that could possibly cure the disease.
Around 90% of all PD cases are idiopathic. Only a minority of PD patients carry a familial mutation or have been exposed to toxic agents that are known to cause the disease. However common murine PD models based on these known rare causes for PD fail to fully recapitulate the key features of the disease and did not prove to be useful for testing new therapeutic approaches.

In order to obtain a stable PD-like motor phenotype in mice that is based on apoptosis of neurons in the SN, we crossed mice carrying the simian Cre-inducible diphtheria toxin receptor (iDTR) with mice carrying the Cre-recombinase under the control of the dopamine transporter (Dat). Administration of diphtheria toxin (DT) to mice carrying both transgenes (Dat$^{cre}$.iDTR mice) induces a dose-dependent dopaminergic neuron ablation and Parkinsonian motor phenotype that is reversible upon L-Dopa treatment.

Three doses of DT were tested: 3x15ng, 4x8ng and 3x8ng. After 16 days, a depletion of over 85% of TH-positive neurons was assessed in 3x15ng and 4x8ng DT-treated Dat$^{cre}$.iDTR mice. In 3x8ng DT-treated Dat$^{cre}$.iDTR mice, the depletion was less severe with around 60% in male and 30% in female Dat$^{cre}$.iDTR mice. Interestingly, several days after the DT injections, Dat$^{cre}$.iDTR mice developed an akinetic phenotype. In common rodent PD motor tests (Open Field Test, Pole Test, Accelerating Rotarod) we measured a dose-dependent severity of the deficits. As in human patients, the symptoms were less pronounced in female Dat$^{cre}$.iDTR mice. Importantly the administration of L-Dopa to DT-treated Dat$^{cre}$.iDTR mice was able to rescue all the DT-evoked deficits, demonstrating that the akinetic phenotype was specifically caused by the lack of dopamine in DT-treated Dat$^{cre}$.iDTR mice. Surprisingly, motor symptoms of DT-treated Dat$^{cre}$.iDTR mice did not only persist, but deteriorate chronically after the acute DT administration as shown for 3x8ng DT-treated Dat$^{cre}$.iDTR male mice. Moreover our data indicates that the degenerative process in the SN continues after the acute depletion of dopaminergic neurons by DT. Moreover the loss of tyrosine hydroxylase-positive cells was accompanied by the formation of α-synuclein aggregates in elder DT-treated Dat$^{cre}$.iDTR mice. Yet, we did not observe Lewy body formation.

Altogether, these findings indicate that factors independent of the DT might lead to further degeneration of the SN of Dat$^{cre}$.iDTR mice. This way the model might be a helpful tool to test new PD therapies that focus on chronic degeneration of SN neurons after an unspecific initiation point. Moreover – as compared to other preclinical PD-models – the Dat$^{cre}$.iDTR model unifies major pathological and phenotypical criteria for a PD model. In contrast to other acute toxin models, there is no recovery of motor symptoms and neurons after cessation of the toxin administration. Furthermore the Dat$^{cre}$.iDTR model offers a practical advantage as compared to common toxin-based rodent PD models: the so far used toxins do cause PD in humans, thus special security arrangements are requested for the experimenter. This is not the case with the Dat$^{cre}$.iDTR model.

The CD95 system is an attractive target for neuroregenerative therapies. Experiments that were performed in this study, indicate that CD95 and CD95L expression is upregulated in Dat$^{cre}$.iDTR mice after DT administration, analogous to what was described by Corsini et al in a model of global ischemia. There, it was furthermore shown that CD95 promotes NSC migration and integration in the injured brain. Yet, in this study such an effect could not be shown with the Dat$^{cre}$.iDTR PD model. No difference in motor performances was detected between the NSC-treated and non-treated groups. Furthermore the transplanted NSCs could not be detected in the Substantia nigra of the mice at 100 days after the transplantation. In order to obtain clear results, following experiments should imply a sham-injured group, a higher dose of DT per mouse and more mice per group.

Further studies with the Dat$^{cre}$.iDTR model might be helpful to clarify if the CD95 system is a potential target for neuroregenerative therapies, particularly for Parkinson's disease.