Effects of treatment with Doxycycline on Behavior, Neuroinflammation and Brain Pathology in rTg4510 mouse model

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INTRODUCTION

rTG 4510 mice express h4R0N P301L Tau downstream of a tetracycline-operon-responder construct under control of Ca2+-calmodulin kinase II promoter, that allows control of gene expression by feeding of doxycycline (tet-off; Ramsded et al, 2005). The mice express transgenic tau mainly in forebrain structures. The pathology forms very early, showing pre-tangles consisting of hyper-phosphorylated tau already at 2.5m and agyrophilic NFTs at 4m in cortex and at 5.5m in hippocampus (Spires et al, 2006). NFTs are furthermore congophilic and Thioflavin S positive. These mice also display significant degeneration of CA1 hippocampal neurons at 5.5m and gross atrophy of the forebrain and hippocampus being present at 4mo. This is also the age to develop significant behavioral deficits (learning/memory). It is important to note that transgene suppression by doxycycline stops neuronal death, decreases levels of tau and p-tau, and reverses the cognitive disturbance (Santacruz et al., 2005), which provides a robust control for any treatment study. Only little is known to the effect of Dox treatment on total Tau levels and related gliosis from histological quantifications, the here presented data shall fill this gap.

METHODS

Animals:

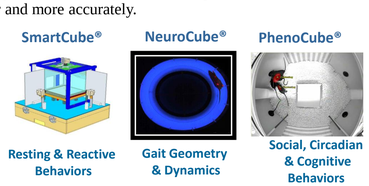
Mice were generated crossing a transcriptional operon-Tau mouse (FVBn background) with a CamKII-transcriptional activator (tTA in a C57B6/J background) mouse. Three genotypes were tested: Tau-negative/CamK-negative (double negative; WT), Tau-negative/CamK-positive (tTA) and Tau-positive/ CamK-positive (Tg4510). Upon receipt, animals were group for the duration of the study. Animals were maintained on a 12 hr /12 hr light/dark cycle with the light on at 7:00 a.m. EST. Room temperature was maintained at 20-23°C with relative humidity maintained at 30-70%. Prior to testing, all animals were examined on a regular basis, handled, and weighed to assure adequate health and suitability. In each test, animals were randomly assigned across testing and treatment groups.

Behavioral Assessments:

PhenoCube: Extensively customized Intellicage boxes (New Behavior AG) fitted with proprietary video analysis equipment. Animals were evaluated in 48 h test sessions, being placed in the PhenoCube environment after a 16 h water deprivation period in the home cage. The cages were maintained on a 12:12 light/dark cycle, with white light during the day and red light during the night, maintaining a low subjective light level for the subjects during the night period. While inside the cage, water was only available from within the PhenoCube corners, while food was freely available on the cage floor at all times. Where possible, mice were left undisturbed during the course of experimental sessions. In both test sessions, the test animals initially received magazine training through a simple 'Habituation' protocol, allowing them to freely retrieve water from the PhenoCube corners. Prior to lights-out on day 1, after 6 h in the cage, the protocol was switched to a training protocol described as 'Alternation', requiring the animals to visit specific locations to retrieve water and to alternate between potentially reinforced locations.

SmartCube: Mice were placed in a custom built apparatus where multiple challenges were presented over the course of each test session. Digital videos of the subjects were processed through computer segmentation algorithms to fit geometrical models to each mouse frame image. The resulting fitted parameters were then analyzed using behavioral classifier algorithms to extract behavioral states such as rearing, locomotion and immobility. The data obtained in this way were used to define a phenotypic signature.

NeuroCube: The NeuroCube® system is a platform that employs computer vision to detect changes in gait geometry and gait dynamics in rodent models of neurological disorders, pain & neuropathies. This platform is unique for gait testing as is completely automated and thus removes any bias or subjectivity. The sensitivity of the computer vision and bioinformatics allow PsychoGenics to capture symptoms of the disease model earlier and more accurately.



Data Analysis and bioinformatics

For SmartCube and NeuroCube, the most dominant of the features collected that define the phenotype (symptom descriptors) are identified and ranked using complex proprietary bioinformatics algorithms and an overall discrimination index is calculated for all features. Graphical representations of the datasets corresponding to the groups compared are derived and a p-value is calculated to assess the statistical significance of the discrimination ratios obtained. PhenoCube data are presented as mean SEM. ANOVA or t-test were used for analysis

BEHAVIOR

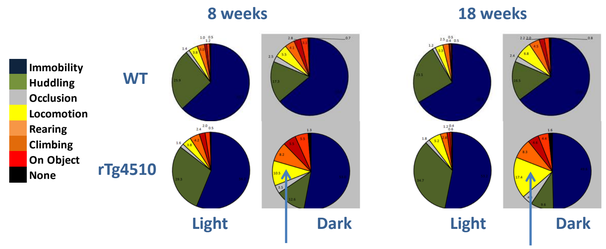


Figure 1: PhenoCube testing showed increased activity of the rTG4510 mice during dark cycle. In addition locomotion was increased compared to WT mice at both ages

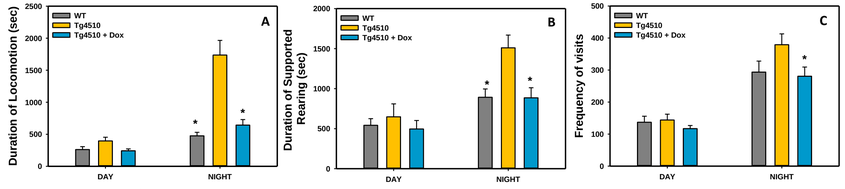


Figure 2: rTg 4510 female mice (6 months) showed increased locomotor and rearing behaviors during their dark cycle. Treatment with doxycycline (200ppm) in chow for 2 months attenuated these behaviors. P<0.05 compared to Tg4510

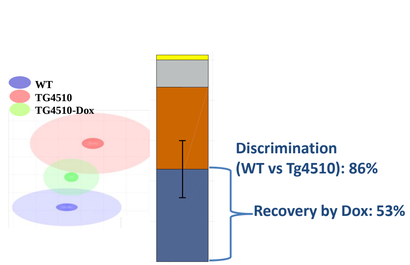


Figure 3: Cloud Graphical Representation showing differences between 6 month old female WT, Tg4510 and Tg4510 + Dox groups in SmartCube. Tg4510 (no Dox) mice showed a were more elongated body, increased mobility, decreased immobility, scanning, sniffing and rearing behaviors. The discrimination in between WT and Tg4510 was 86%. Treatment with doxycycline partially recovered (53%) some of these features. (n=12-14).

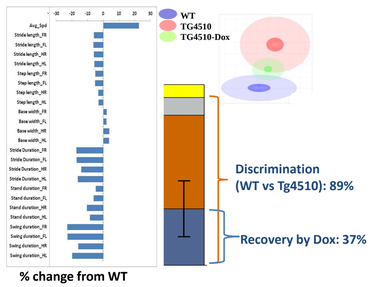


Figure 4: rTg4510 female mice (6 months) showed significant changes in gait as compared to WT mice. They showed shorter stride and step length and shorter swing and stride durations compared to WT mice. The discrimination in gait features between WT and Tg4510 was 89%. Treatment with doxycycline recovered (37%) some of these features. (n=12-14)

SUMMARY

Both SmartCube and PhenoCube showed that rTg4510 mice exhibit increased activity, decreased immobility that were attenuated with Dox treatment. Gait differences were also seen between WT and Tg4510 that were partially reversed with Dox. Histological assessments showed severe astrogliosis paired with microglial activation in rTg4510 mice. Treatment with Dox reversed pathological Tau species as found in NFTs and dystrophic neurites. Human Tau is still produced in the presence or absence of Dox. Howeber the pathological consequences, NFT load, neurite dystrophy and gliosis, are reversed to the levels seen in WT mice

HISTOLOGY

METHODS

In short mice were flushed with 0.9% saline to remove RBCs, then brains were harvested, divided at midline and the left hemisphere was post fixed for 48 hours in 4% PFA. Thereafter they were cryo-protected in sucrose until sunk and frozen in tissue freezing medium within cryo-molds in dry-ice cooled liquid Isopentane. A uniform systematic random set of seven sections, ten micron thick and reaching through the whole cerebral cortex and hippocampus was immunohistochemically labeled with Tau13 + GFAP + Iba1, counterstained with DAPI. Whole sections were imaged on an Axio.Scan Z1 slide scanner at 10x magnification. Quantifications using Image Pro Premier v9.1 were automated and rater independent using constant evaluation parameters for each marker. IR was generally measured above an intensity based threshold and using size restrictions.

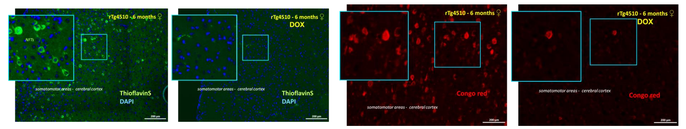


Figure 5: rTg4510 female mice (6 months) show beta-sheet structured ThioflavinS positive NFTs (left panel). They are also congophilic (right panel). Both are strongly reversed by Dox treatment.

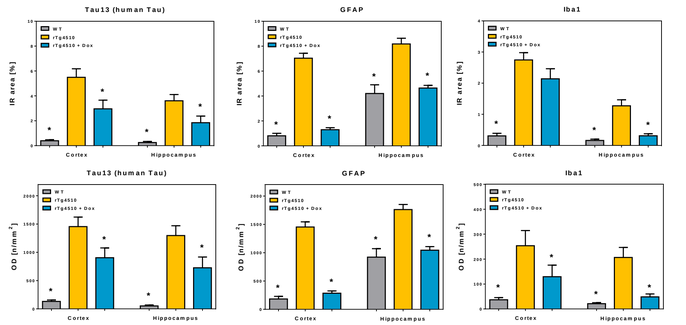


Figure 6: Human Tau13 IR Tau (left), GFAP IR astrogliosis .(middle) and Iba1 IR microgliosis (right) in the cerebral cortex and hippocampus of female WT, rTg4510 and rTg4510 + Dox groups. Tg4510 (no Dox) display strong overproduction of human mutated Tau leading to an enormous astrogliosis and microgliosis in both investigated brain regions. Dox treatment reduces total human Tau load by approximately 40-50%, which is sufficient to totally halt pathological astrogliosis. Also hippocampal microglia is reversed to levels of WT controls, however, cortical microglial activation remains active. Data are shown for IR area percentage (upper lane) and object density (lower lane; n=4-5/ group).

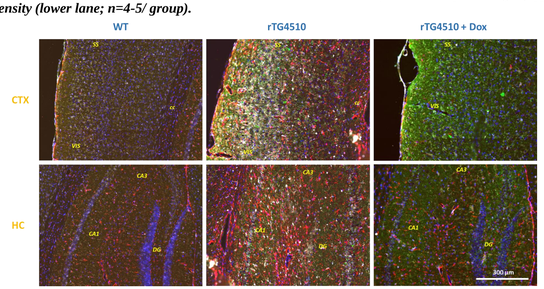


Figure 7: Representative images of human Tau13 IR Tau (green), GFAP IR astrogliosis (red) and Iba1 IR microgliosis (white) in the cerebral cortex and hippocampus of female WT, rTg4510 and rTg4510 + Dox groups. hTau is absent in WT controls. rTG4510 display immense astro- and microgliosis, which is reverted by Dox treatment. Note that human Tau is still overexpressed also in Dox groups, however, the gliosis is widely normalized.