IMPAIRED LEARNING ABILITY IN GSK3β-OVEREXPRESSING TRANSGENIC MICE

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INTRODUCTION

Alzheimer's disease (AD), is characterized by progressive memory loss and impairments in language and behaviour. The cognitive decline is accompanied by neuronal atrophy and loss, mainly in the cortex, hyppocampus and amygdala. Neuropathological hallmarks in AD are extracellular senile plaques, deposits of amyloid fibrils, and intraneuronally aggregates of paired helicoidal filaments (PHFs), composed of hyperphosphorylated forms of the microtubule-associated protein tau. GSK3β is a ubiquitously expressed proline-directed serine/threonine kinase that is particularly abundant in the CNS. GSK3β is known to participate in multiple signalling pathways, and has been postulated in AD pathogenesis through its involvement in tau hyperphosphorylation, β-amyloid-induced neurotoxicity and mutated PS1 pathogenic effects. In view of the postulated lethality of embryonic GSK3β overexpression as well as the known role of GSK3β in development, transgenic mice were generated by using the conditional tetracycline-regulated system. In these mice, transgene expression is under the control of the CaM kinase II α-promoter to achieve substantial overexpression of wild-type GSK3β in forebrain neurons and, therefore, in more relevant regions for AD. These Tet/GSK3β mice overexpress GSK3β in hippocampal and cortical neurons, are fully viable and show many of the biochemical and cellular aspects of AD neuropathology, including tau hyperphosphorylation and somatodendritic localization, reactive gliosis and neuronal death, although they do not show tau filament formation.

Tet/GSK3β MICE OVEREXPRESS GSK3β IN THE CORTEX AND HIPPOCAMPUS

In Tet/GSK3β mice, transgene expression is driven to the forebrain by the CamK-IIα promoter and it is conditional in a tetracycline-regulated manner. To achieve the conditional transgene expression, a double transgenic approach was used in which Tet/GSK3β mice occur as a result of breeding tTA mice (CamK-IIα promoter driving expression of the tetracycline sensitive transcription factor tTA, also termed Tet-Off) with BiTetO mice (inducible bidirectional TetO promoter driving expression of myc-tagged GSK3β and β-gal cDNA genes)

Immunohistochemistry in brain sections of adult Tet/GSK3β mice shows β-galactosidase expression in the cortex, the different fields of the hippocampus and in the striatum. No expression is detected in other brain regions such as globus pallidus, thalamus, brainstem and cerebellum. Brain sagittal sections (50 µM) were stained with the following primary antibodies: anti-myc (1/1000), anti-β-galactosidase (1/5000), anti-GFAP (1/1000) and AD2 (1/1000).

NOVEL OBJECT RECOGNITION BEHAVIOURAL TEST

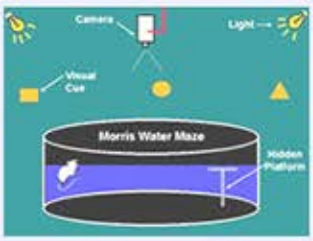
The novel object recognition test (OR) assesses the ability to recognize a novel object in a well known environment. Mice were submitted to an acquisition trial in the presence of two similar objects. A second trial was performed with a novel object together with the familiar one. The Memory Index (MI) defined the ratio of the time spent exploring the novel object over the time spent exploring both objects. Animals were considered to show recognition activity when the head of the animal was less than 2 cm close to the object.

MORRIS WATER MAZE BEHAVIOURAL TEST

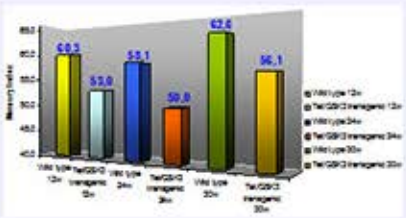
The Morris water maze (MWM) task involves placing the rodent in a pool of water where it must use visual cues to remember the location of a hidden platform just below the water's surface.

The MWM test measures spatial learning and memory. This is one of the most popular tasks in behavioural neuroscience and is sensitive to both the amnestic and memory-enhancing effects of drugs, as well as gene manipulation dependent on intact hippocampal function.

The parameters measured were: time to reach the platform (latency); distance swam; speed (distance/latency); time spent in each quadrant, and number of times crossing the virtual platform.

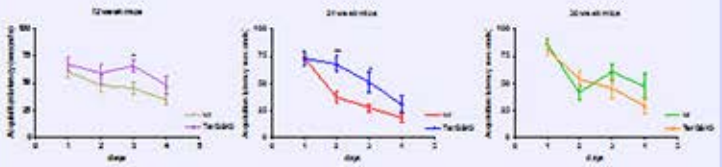


AGE AND LEARNING CAPACITY IN THE OBJECT RECOGNITION TEST



The animals were subjected to OR trial. Eight 12 weeks old mice and twelve 24 and 30 weeks old transgenic and wild type mice were tested. A memory index ($MI=t_C/(t_A+t_C)$) where t_A represents the time exploring the old object (A) and t_C the time exploring the new object (C) was calculated for each mouse. In all cases wild type mice showed higher value of MI.

AGE AND LEARNING CAPACITY IN THE MORRIS WATER MAZE



The animals were subjected to Morris water maze test. Eight 12 weeks old mice per group and twelve 24 and 30 weeks old transgenic and wild type mice were used. Animals had access to dry food *ad libitum* during the whole duration of the study. Here we represent latency (time to reach the platform). There is a statistically significant difference in terms of learning abilities between wild type and Tet/GSK3β mice aged 12 and 24 weeks in MWM, and there aren't differences between wild type and Tet/GSK3β transgenic mice aged 30 . *p<0.05 **p<0.01

CONCLUSION

Conditional Tet/GSK3β double transgenic mice show GSK3β overexpression in the brain, leading to increased tau phosphorylation levels and reactive gliosis. We have determined their performance at different ages in two standard learning and memory paradigm such as the Morris water maze and the Novel object recognition test when compared to the wild type. GSK3β overexpression in the brain of the transgenic mice leads to learning and memory deficits in both experimental paradigms. Tet/GSK3β transgenic mice might prove a unique animal model for AD and an invaluable tool for testing the therapeutic potential of selective GSK3 inhibitors.