

D3 receptor knockout mice have altered circadian gene expression patterns in spinal sympathetic preganglionic neurons

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Circadian rhythms are important biological regulators, and we have previously shown the circadian variation of gene expression in the intermediolateral nucleus (IML) of the mouse spinal cord (Clemens et al, SFN 2005). The IML is the site of sympathetic preganglionic neurons (SPNs), and a dysfunction in descending dopaminergic projections to the IML is implicated in the circadian-related disorder Restless Legs Syndrome (RLS). We compared the circadian gene expression patterns between wild type (WT) and D3 receptor deficient mice (D3KO), using a combination of fluorescent reporters, laser-capture microdissection and DNA microarrays. Adult male mice were kept under a 12/12 day-night light regime, and spinal cords were removed in 4 hr intervals beginning 1 hr prior to daytime. Captured mRNA from the IML was amplified and hybridized with Affymetrix U74AV2 genome microarrays.

Comparing the averaged gene sets for WT at different time points, we found that the levels of gene expression changed throughout the day. Compared to the expression values at 10 pm, 45 genes expressed a > 1.5-fold increase at 10 am, whereas 93 genes expressed a > 1.5-fold decrease. Comparing WT with D3KO, we found that at 10 pm, 35 genes had a > 1.5-fold higher expression in the D3KO, while 95 genes had a > 1.5-fold reduced expression. In contrast, at 10 am, D3KO gene expression was > 1.5-fold increased for 59 genes, while additional 130 genes were reduced in their expression by > 1.5-fold.

Our data suggest that gene expression undergoes circadian changes in the IML, and that a dysfunction of the D3 receptor gene is correlated with a multitude of gene expression differences between WT and D3KO. Moreover, in the D3KO, at 10 am and 10 pm, more genes displayed a > 1.5 fold reduction in gene expression than a > 1.5-fold increase. Further studies are planned to confirm observed these findings using real-time PCR and histochemical methods.

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