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Circadian variation in tyrosine-hydroxylase levels in sympathetic intermediolateral nucleus of spinal cord & greatly reduced staining in D3 receptor knockout mice: An animal model of RLS?

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Restless legs syndrome (RLS) is a CNS disorder with abnormal sensations that are reduced during motor activity, worsen at rest, & have a marked circadian pattern. Treatments increase CNS dopaminergic (DA) activity. The hypothalamic A11 region is the sole source of spinal DA & is implicated in RLS since hypothalamus modulates autonomic function & exhibits circadian rhythmicity, features of the RLS phenotype. While DA in brain has circadian rhythmicity, no such study has been performed in the spinal cord. Here, we used tyrosine-hydroxylase (TH) content as a marker of descending catecholaminergic activity to examine circadian differences in expression in spinal sympathetic preganglionic nuclei (SPN) in wildtype (WT) & D3 receptor knockout mice (D3KO).

Adult male mice were isolated 1 week prior to the experiment, under a 12/12 day-night light regime & i.p. injected with Fluorogold to retrogradely label SPNs. Mice were sacrificed in 4-hr intervals, starting one 1hr before subjective daylight. Spinal cords were removed; frozen & thoracic cord was cryostat-sectioned then immersed in Lana's fix. We processed for TH immunohistochemistry, followed by a DAB reaction to quantify TH expression levels.

In both WT & D3KO mice, we found that TH immunolabeling was most intense in the SPN. The SPN in WT mice express circadian variations in TH levels with peaks at 10:00 & 14:00 hours (inactive period). In contrast, TH staining is significantly reduced in D3KO mice (to 34% of WT) with a blunted circadian profile. We conclude that: (i) TH levels are regulated in a circadian manner in the spinal cord and suggest that sympathetic preganglionic neurons are modulated accordingly by descending catecholaminergic system(s). (ii) D3KO mice have greatly reduced labeling - Since DA actions on the SPN are largely inhibitory, reduced spinal DA actions would increase sympathetic arousal consistent with the behavioral phenotype in these animals (increased activity, hypertension) and consistent with RLS.