Assessing motor and gait phenotypes in Nedd4 and Nedd4-2-heterozygous mice

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Introduction: Nedd4 and Nedd4-2 (**N**eural precursor cell **E**xpressed **D**evelopmentally **D**own-regulated 4 and 4-2) are E3 ubiquitin ligases that share high degree of homology but distinct substrates within the central nervous system (CNS)(Yang & Kumar, 2010). Although the number of substrates has grown substantially, their *in vivo* roles are not well characterized. Previous studies in Nedd4 knockout embryos identified abnormalities in neuromuscular junctions and a reduction in motor neurons in the spinal cord (Liu *et al.*, 2009). In addition, the gene for a novel interacting protein for Nedd4, Brain expressed associated with Nedd4 (BEAN), is disrupted in patients with spinocerebellar ataxia type 31 (SCN31) (Sato *et al.*, 2009). These findings point to a potential role in motor function and gait. Nedd4-2 on the other hand, has been shown to regulate dopamine transporter (DAT) (Sorkina *et al.*, 2006) and therefore may also contribute to motor function. Nedd4 and Nedd4-2 knockout mice are not viable and die shortly after birth due to growth (Cao *et al.*, 2008) and lung defects (Boase *et al.*, 2011), therefore in this study we wanted to determine whether a single copy deletion of either Nedd4 or Nedd4-2 (by using heterozygous mice) was sufficient to alter motor function and gait behaviours. Expression of Nedd4 and Nedd4-2 in the brain with particular localization to regions associated with motor function and control was also investigated.

Methods: Gait and motor function were assessed in young (2 month old) and old (6 month old) Nedd4 and Nedd4-2 heterozygous mice and age matched wild-type littermates (n = 8-14). RotaRod measures the latency to fall from rotating drum over an accelerated speed for a maximum of 5 minutes. Gait analysis was examined using the DigiGait system. Mice were placed on a transparent motorized treadmill and required to take 4-6 complete strides. The analysis software was then used to calculate a number of gait parameters. Standard immunohistochemical methods were used to identify the expression of Nedd4 and Nedd4-2 in the brain.

Results: Nedd4 and Nedd4-2 levels in whole brain lysates from heterozygous animals were reduced by at least 50%. Nedd4 and Nedd4-2 were strongly expressed in Purkinje neurons of the cerebellum. Nedd4-2 however, was also found to be strongly expressed in dopaminergic neurons of the *substantia nigra*. Gait analysis in Nedd4 heterozygous mice revealed abnormalities that became more pronounced with age. At 6 months, Nedd4 heterozygous mice have significant alterations in stride, swing and brake duration with a concomitant decrease in stride length frequency. Furthermore, stride length and stride length variability were also significantly increased. Paw angle dynamics showed an open splayed phenotype compared to wild-type littermates. In contrast Nedd4-2 heterozygous mice showed no changes in gait at 2 months, and only minor differences at 6 months. No change in motor function was found in Nedd4 heterozygous mice at both time points as assessed by RotaRod. Motor function was normal in 2 month old Nedd4-2 heterozygous mice, however by 6 months there was a significant reduction in motor capacity with a reduction in latency to fall from the RotaRod.

Conclusion: Motor function is impaired in older Nedd4-2 heterozygous mice, with no change evident in the gait. This impairment may be attributed to its role in regulating dopamine re-uptake. Nedd4 heterozygous mice conversely show significant and profound changes in gait with normal motor function capacity. The expression of Nedd4 and Nedd4-2 in the cerebellum was not surprising as this region is largely responsible for motor function and control. Furthermore, identifying strong expression of Nedd4-2 in dopaminergic neurons of the *substantia nigra* suggests that these two ligases affect motor function.

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