

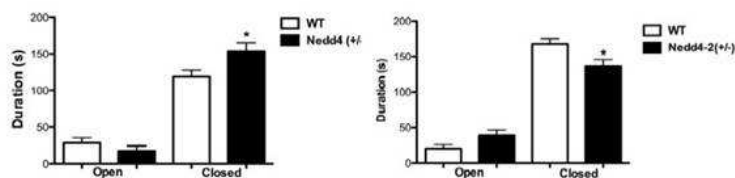
Nedd4 and Nedd4-2 heterozygosity leads to opposing anxiety behaviour in mice

D. Bongiorno,^{1,2} N. Boase,³ S. Kumar³ and P. Poronnik,^{1,2} ¹School of Medical Sciences, RMIT University, Bundoora 3083, ²Health Innovations Research Institute (HIRi), RMIT University, Bundoora 3083 and ³Centre for Cancer Biology, SA Pathology, Frome Road, Adelaide 5000.

Introduction: Nedd4 and Nedd4-2 (Neural precursor cell Expressed Developmentally Down-regulated 4 and 4-2) are two closely related ubiquitin ligases (E3) that have distinct substrates within the central nervous system (CNS) (Yang and Kumar, 2010). The absolute physiological implications of which have yet to be determined. Therefore it is not surprising that Nedd4 and Nedd4-2 knockout mice are not viable and die shortly after birth due to developmental or physiological defects. In this study we investigated whether a single copy deletion of either Nedd4 or Nedd4-2 (by using heterozygous mice) is sufficient to alter anxiety related behaviours *in vivo*. We hypothesize that although Nedd4 and Nedd4-2 are closely related E3s, they are likely to be functionally different due to unique substrate profiles, and that their loss may have differential effects on animal behaviour.

Method: Elevated plus maze (EPM) was used to assess anxiety in adult (8-12 week old) Nedd4 heterozygous (+/-, n=14), Nedd4-2 heterozygous (+/-, n=8) and wild type (WT) littermate controls (n=8 for each group). The EPM is composed of two open and two closed arms, and mice are given 5 minutes to explore the maze. The proportion of time spent in the open versus closed arm is an indicator of anxiety. For example, increase in duration spent in closed over open arm indicates increased anxiety, and conversely a decrease in time spent in open over closed arm indicates decreased anxiety.

Results:



Results from this study show that when a single copy of the gene encoding for Nedd4 or Nedd4-2 is deleted, significant alterations in anxiety is produced. Interestingly, as shown in the figure, Nedd4 heterozygous mice spend a significantly longer duration in the closed arm of the maze compared to wild-type littermate controls, suggesting increased anxiety. Conversely, Nedd4-2 heterozygous mice spend significantly less time in the closed arm, and subsequently increase the time spent exploring the open arm suggesting reduced anxiety in these mice compared to wild type littermate controls.

Conclusion: Nedd4 and Nedd4-2 may modulate neuronal function by targeted degradation of specific substrates that may contribute to the observation in anxiety changes. Further investigations are required to define precise E3 substrates that modulate anxiety behaviours in these mice.

Yang, B. and S. Kumar (2010). "Nedd4 and Nedd4-2: closely related ubiquitin-protein ligases with distinct physiological functions." *Cell Death and Differentiation* **17**(1): 9.