Selective Loss of Huntingtin Associated Protein-1 (HAP1) in Orexin Neurons Leads to Abnormal Feeding and Locomotor Behavior

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ABSTRACT

HAP1 is a neuronal protein that associates with the Huntington disease (HD)protein huntingtin (htt). Increasing evidence suggests that both htt and HAP1 participate in microtubule-dependent intracellular trafficking. Unlike htt that is ubiquitously expressed, HAP1 is enriched in brain and expressed abundantly in the hypothalamus, a brain region that is affected in HD and plays a critical role in regulating appetite and energy homeostasis. Here, we demonstrate that mice with selective deletion of Hap1 in orexin neurons in the hypothalamus display decreased food intake and body weight. The locomotor activity of those Hap1-deficient mice was also significantly reduced. Immunostaining revealed that orexin neurons were poorly differentiated when HAP1 expression is eliminated. Subcellular fractionation studies showed that HAP1 depletion alters the distribution of dynactin p150, kinesin light chain and Htt, suggesting that HAP1-mediated trafficking is critical for the normal function of orexin neurons and hypothalamic regulation of body weight and locomotor activity.

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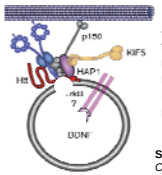
INTRODUCTION



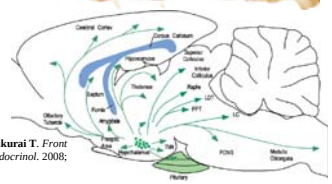
More and more evidence indicates that both HAP1 and huntingtin (Htt) are involved in intracellular trafficking. Recently many researchers have suggested a model of microtubule-dependent vesicle trafficking machinery which includes HAP1 as a component. However it is still not clear how HAP1 participates in this motor complex.

Salinas S et al, *Curr Opin Cell Biol* 2008

In mice, HAP1 is highly expressed in brain, especially in the hypothalamic region, which regulates feeding, sleeping and many other important life activities. HAP1-null mice displayed feeding disability and developmental delay. Usually they die within 3 days after birth, suggesting that HAP1 is required for postnatal survival.

It has been wondered whether reducing HAP1 expression in hypothalamic neurons in adult mice also lead to neurological phenotypes, since the whole body HAP1-KO mice die so early. Therefore we use conditional knockout mice to study.

Sheng G et al, *J Clin Invest* 2008



Ohno K, Sakurai T. *Front Neuroendocrinol*. 2008;

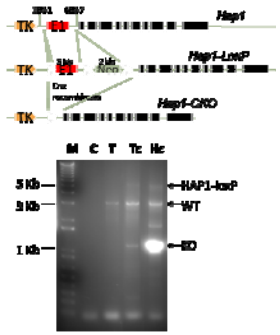
Orexin neurons are located in the lateral hypothalamic area (LHA), which is also expressing HAP1. These neurons project to almost all parts of the brain except the cerebellum. Orexins are neurotransmitters that regulate feeding, locomotor activity and sleeping. We focus on orexin neurons because they are important and orexin-cre mice are commercially available.

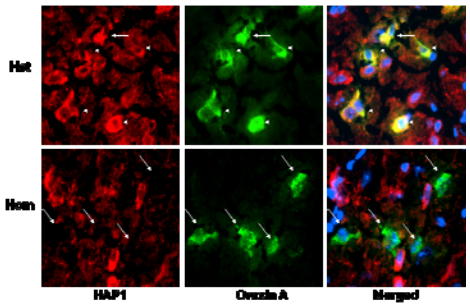
METHODS & RESULTS

Conditional knockout of *Hap1* in *Orexin*-expressing neurons

LoxP sites were introduced and flanking exon 1 of Hap1 gene. After cre recombination, the Hap1 gene expression would be silenced in orexin-expressing cells. Genotyping using samples from either the mouse tail or hypothalamus indicated high specificity of Hap1 gene deletion in hypothalamus.

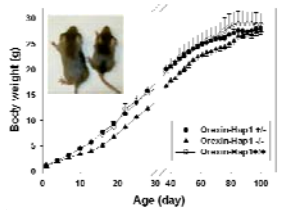
To confirm that HAP1 protein is depleted in orexin neurons, we did immunofleorescence double labeling with HAP1 and orexn A antibodies. In heterozygous mouse brain, HAP1 expressed in every orexin producing cell, suggesting a role for HAP1 in those neurons, while in homozygous orexin-Hap1 knockout mouse brain, HAP1 expression was eliminated in orexin neurons, indicating efficient Hap1 gene deletion.

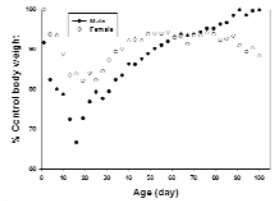




Orexin-Hap1 KO mice grow more slowly

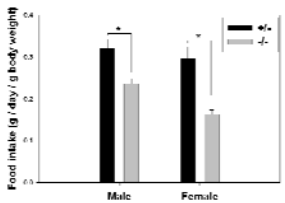
Significantly Orexin-HAP1 KO mice showed decreased body weight when compared to those controls. The greatest differences are around 2 to 4 weeks old for both males and females.

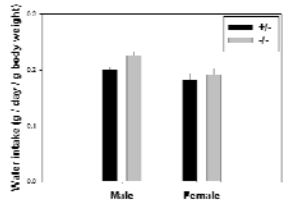




Orexin-Hap1 KO mice eat less

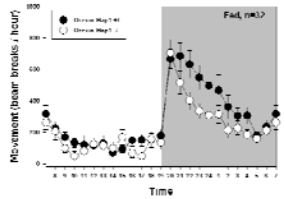
Monitoring their feeding behavior, we found that KO mice eat less. It is consistent with orexin deficiency phenotype although the water consumption was not significantly different between the two groups.

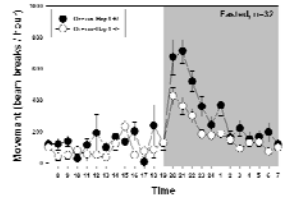




Orexin-Hap1 Ko mice perform lower locomotor acitivity

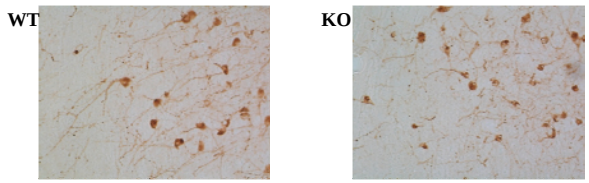
Orexin-Hap1 KO mice also had lower locomotor activities, especially during the night. Food removal made the KO mice worse, implicating a linkage between locomotor activity and feeding behavior.





HAP1 deficiency in orexin neurons impairs neurite extension

When we took out the brains and stained them with immunohistochemistry using orexin antibody, we found that HAP1 depletion reduced neurite extension of orexin neurons. It is consistent with previous observation that HAP1 is required for neurite outgrowth in a cell line. And now we are able to demonstrate it in the brain.



CONCLUSION

In summary, HAP1 deficiency causes intracellular trafficking defects, and then impairs neurite extension, which then causes dysfunction of orexin neurons and changes in hypothalamic activities.