# Review of: "Orexin receptors 1 and 2 in serotonergic neurons differentially regulate peripheral glucose metabolism in obesity"

Marcela Morales-Mulia[1]

1 Instituto Nacional de Psiquiatría

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**Review of: “Orexin receptors 1 and 2 in serotonergic neurons differentially regulate peripheral glucose metabolism in obesity”**

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**Authors**

Xing Xiao, Gagik Yeghiazaryan, Simon Hess, Paul Klemm, Anna Sieben, André Kleinridders, Donald A. Morgan, F. Thomas Wunderlich, Kamal Rahmouni, Dong Kong, Thomas E. Scammell, Bradford B. Lowell, Peter Kloppenburg, Jens C. Brüning & A. Christine Hausen.

I read your article with great interest, for several reasons:

a. my field of research is also on the orexin system but in the mesolimbic pathway.

b. I was interested in their animal models, inactivation of orexin receptors, and optogenetic stimulation.

c. I am interested in the involvement of orexins in metabolism.

d. it is a very well conducted study.

This study reinforces the important contribution of orexin signaling in metabolic diseases such as obesity-induced by consumption of high-fat food and its interaction with the serotonergic system in the raphe nucleus (RN). Furthermore, it provides us with a general description of the differential modulation exerted by orexin receptors in the control of glucose metabolic homeostasis, such differentiation has been observed and discussed in other orexin-mediated functions.

In my opinion, the authors conducted various sophisticated assays to identify the localization and expression of the two orexin receptors in neurons expressing the serotonin transporter in subregions of the RN. Although their assays at the molecular level were in very few serotonergic neurons, they compared their results of images of RNAscope in situ hybridization in the dorsal (DRD), ventral (DRV), and raphe pallidus (RPa) with published scRNA-Seq data of serotonergic neurons in the dorsal raphe nucleus, which strengthens their findings. In summary, the comparison between their results and the database agrees that there is more Ox1R in DRD while Ox2R is more expressed in DRV.

To identify the contribution of each orexin receptor on peripheral glucose metabolism and in response to the feeding high-fat diet (HFD), the authors did the following:

1. The authors generated mice with specific deletion of Ox1R or Ox2R in serotonergic neurons, and later they verified the deletions via RNAscope fluorescent in situ hybridization.
2. They characterized the differential activation of serotonergic neurons in response to orexin-A and -B with electrophysiological recordings.
3. Finally, they verified that the deletion of the receptors affected the functionality of each one of them, measuring the increase in $Ca^{2+}$ induced by the orexins.

As I mentioned earlier, although these experiments were carried out very carefully, there are important variations in relation to the percentage of positive neurons and fluorescence intensity, between mice with or without deletion of Ox1R or Ox2R in the RN subregions (Fig. 1c-f compared to Fig. 2e- h), which may help to understand the variations observed in $Ca^{2+}$ measurements in these animals, in addition to the difference in the affinity of both orexins for their receptors. However, this is neither mentioned nor disputed by the authors.

Something that strikes me is the lack of body weight gain in both controls and Ox1R or Ox2R inactivated mice in response to the high-fat diet over the 10-week period. Since these types of high-fat diets in most cases significantly increase body weight from the first weeks because of the accumulation of fat and decreased lipolysis. In addition, several pieces of evidence suggest that orexins act as a regulator of obesity by increasing both caloric intake and energy expenditure, resulting in late weight gain, between 3-5 weeks of consumption of high-fat food. The authors suggest that the observed changes in brown adipose tissue (BAT) may have a late-onset and that the exposure time to the high-fat diet was not long enough to translate these effects into significant changes in body weight. Even though animals with deletion of orexin receptors present alterations in insulin sensitivity, glucose tolerance, glucose uptake, and genes and proteins expression levels that indicate damage in mitochondrial function, oxidative phosphorylation, and thermogenesis in the liver, and BAT.

Furthermore, the authors propose that orexin signaling in serotonergic neurons could be more relevant under conditions of nutritional restriction, which is why it would have been interesting to evaluate the activation of orexinergic neurons in the lateral hypothalamus at 16 hours of fasting to relate this effect with the improvement in glucose tolerance observed in mice with deletion of Ox2R, as was done in Fig. 4a, b, when fasting was 6 hours and a decrease in glucose tolerance was observed, in mice with Ox1R inactivation.

As the authors well mentioned, there is still much to know about the cellular mechanisms by which orexin receptors present in serotonergic neurons contribute to peripheral glucose metabolism. Undoubtedly, this study provides great knowledge about the distribution of Ox1R and Ox2R receptors in the RN.