Deepening the regulation of PDYN gene, as a function of alcohol consumption

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For several years an important role in alcoholism research has been attributed to the endogenous opioid system [Koob et al., 1998]. Different groups analyzed genes system polymorphisms and transcriptional in alcoholics and in mammals exposed to alcohol. In particular, our group observed the epigenetic regulation of prodynorphin (*PDYN*) gene via gene promoter DNA methylation (D'Addario et al., 2017; Wille-Bille et al., 2018). Pattern-based prediction of transcriptional factors (TFs) binding to *PDYN* sequence analyzed show that the CpG site resulted to be differentially modulated by alcohol both in humans and in rats, is recognized by c-Jun, a TF that in combination with c-Fos forms the AP-1 (Activator Protein 1) complex. Moreover, *PDYN* rat sequence under study is recognized also by cAMP response element-binding protein-1 (CREB1).

We here developed an in vitro assay to monitor the binding affinity of both c-Jun and CREB1 at *PDYN* gene promoter region, analyzing how DNA methylation can influence this binding usAlphaScreen® assay. In parallel, we quantitatively measured it in the Ventral Tegmental Area (VTA) of rats prenatally exposed to ethanol or vehicle, using Chromatin ImmunoPrecipitation (ChIP).

The AlphaScreen® assay show a different binding affinity of c-Jun and CREB1 with *PDYN* sequence. Moreover, CREB1 binding results significantly affected by differential DNA methylation at the CpG site contained in its recognition motif. ChIP assay confirmed the differential CREB1 binding in VTA of rats, observing an increased expression of *PDYN* in animals prenatally exposed to alcohol compared to those not exposed.

We used a new analytical method to quantitative monitor the effect of CpG methylation on the interaction of *PDYN* gene with TF in combination with an already well-characterized ChIP method to further describe the mechanisms behind *PDYN* gene regulation by alcohol. This approach would be of help for the design of new drugs targeting specific DNA sequences.