Histone deacetylation during brain development is essential for permanent masculinization of sexual behaviour.


Summary:
The background of this study comes from the idea that genes involved in sex differentiation of the brain may be expressed in a sex-specific manner due to differential histone acetylation levels of their promoters established in the perinatal period. This paper focuses on histone deacetylation in early brain development and its functional implications in adulthood. In particular, the area under consideration is the MPOA – an area known to be highly sexually dimorphic and is responsible for certain male sexual behaviour. The goal is to study differences in histone deacetylation H3 and H4 in promoters of ERα and Arom (responsible for estradiol levels) in the MPOA in males and females.

Experiments were carried out in rats. The entire study can be conceptualized in two stages. The preliminary goal of the study was to isolate histone acetylation levels of ERα and Arom promoter regions in the MPOA on ED21 and PD3 in males and females. A ChIP assay was performed and results showed that the gene promoters involved in sex differentiation of the brain is differentially acetylated in males and females and that these differences between ED21 to PD3 is involved in masculinization of the brain. Now that these differences are established, the next goal was to study the effect of inhibition of HDAC on male sexual behaviour in adulthood. Therefore, pups were injected with TSA (HDAC inhibitor) on PD0 and PD1 and were tested for latency for first mount, intromission and ejaculation- once every week from 10 to 12 weeks. Results showed a significant decrease in male sexual behaviour (without an effect on other brain functions) in TSA treated rats. The goal was to isolate the subtype of HDAC involved in masculinization. In particular HDAC-2 and -4 – expressed in the developing brain, are responsible for steroid hormone signalling in other tissues. Antisense ODN against mRNA for these proteins were infused at PD0 and PD1 and the down regulation of these proteins in the POA (PD2) were confirmed using western blot technique. Antisense treated adult rats exhibited similar reduction in male sexual behaviour as in TSA treated rats. Further, quantitative real time PCR was done on PD1 rats and no difference between sexes were found in the expression of HDAC2 and -4 in the MPOA. However, a ChIP assay analysis using antibodies against HDAC2 and -4 revealed that HDAC2 binding to ERα1b promoter was seven times higher in males than in females and HDAC2, -4 binding to Arom II was also significantly higher in males. Thus the histone deacetylation (and hence suppression of gene expression) of the gene promoters ERα and Arom (responsible for estradiol levels) plays a role in sexual differentiation of male brain.

Critique:
This is an excellent paper with a strong cause-effect hypothesis. The evidence (from previous studies) reported in the introduction is well selected, providing a firm background on the specific aim of this study. The methods and techniques used are very specific towards the goals set. The present study definitely shows the significance of histone deacetylation on sexual differentiation during early postnatal period and in enduring adult behaviour. However, the effect of TSA injection and Antisense ODN treatments on HDAC levels at adulthood is unclear. In other words, does a one time antisense treatment down regulate these proteins strong enough that there is no compensatory mechanism that can over rule by the time pups become adults? Also are the HDAC levels in adulthood (at the time when male sexual behaviour is experimentally tested) the same as tested on PD2? If same, it means HDAC inhibition is irreversible in terms of its effects on masculinization. Studying the mechanism behind will also help in understanding if this is a unitary mechanism or involves other compensatory mechanism. Nevertheless the reported results open up the scope in studying different possible interactions between epigenetic modifications.