# Molecular mechanisms of drug-induced histone modifications in the medium spiny neurons of the striatum

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My thesis is about the mechanisms of histone H3 modification in medium spiny neurons (MSN) in mouse dorsal striatum and nucleus accumbens (NAc) in response to amphetamine, an antidepressant drug and dopamine transmission enhancer and haloperidol, an antipsychotic drug and dopamine D2/D3 receptor antagonist. The dysfunction of dopaminergic system is thought to underlie various pathologic conditions, including Parkinson's disease, drug abuse and many mental disorders while chromatin modifications are gene-expression-control mechanisms which can react to acute stimuli and may have accumulative consequences. Among the various site-specific modifications, we chose histone H3 serine 28 phosphrylation and H3 lysine 27 trimethylation coupled with serine 28 phosphrylation because trimethylation at H3 lysine 27 is the marker for gene silencing mediated by Polycomb group (PcG) complex which is regarded stable after development, while histone phosphorylation is generally a gene-activating mechanism. We hope phosphorylation on neighboring serine 28 may activate those genes (will be confirmed by my colleague with ChIP-seq and qPCR).

Because both in striatum and NAc there are two groups of MSNs expressing either D1 or D2 receptor which react differently to dopamine and are implicated in different neuronal circuits (less segregated in NAc), so I employed Drd1a: EGFP and Drd2: EGFP (EGFP expressed with D1 or D2 promoter) transgenic mice and by immunohistochemistry, to exploit how these two types of MSNs react to these two drugs. The result indicates, the response to amphetamine is mainly mediated by D1 neurons while the response to haloperidol is mainly mediated by D2 neurons.

And then with transgenic mice with DARRP-32 knock-out in MSNs expressing D1 receptor or MSNs expressing D2 receptor, by western blot, I observed the haloperidol-induced increase of H3S28p and H3K27me3S28p was blocked by DARRP-32 knock-out in D2 MSNs and the amphetamine-induced increase was blocked by DARRP-32 knock-out in D2 MSNs, thus I confirmed the specific localization (in D1 or D2 neuron) of these drug responses and their reliance on cAMP/DARRP-32 pathway.

Then I explored the time course of histone H3 marks induced by acute administration of drugs and found different peaking time of H3 phosphorylation and H3 trimethylation coupled with phosphorylation in both treatments.

At last, I tested the chronic effect of these two drugs, and found reduced response of H3 phosphorylation and H3 trimethylation coupled with phosphorylation in concomitance with deceased H3 trimethylation level in both chronic treatments. Intriguingly, desensitization of PKA signaling was only present in samples with chronic haloperidol. There must be different mechanisms underlying.

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