Addictive vs Pro-Cognitive Psychostimulants: Analysis of Methamphetamine and Modafinil Differential Epigenetic Targets in the Prefrontal Cortex

Betina González, PhD

Instituto de Investigaciones Farmacológicas (ININFA-UBA-CONICET)
Junín 956, 5th floor (1113), Buenos Aires City, Argentina.
Telephone: (+54) 11-4961-6784/5949
Email: betina.gonz@gmail.com

Research Report

Brief project description and aims

There is limited information on the mechanisms by which methamphetamine (METH) can induce cognitive deficits while modafinil can improve cognition. Accumulating evidence indicates that cognitive functions that relay in the prefrontal cortex (PFC) play a critical role in the neurobiology of drug abuse [1]. Although both METH and modafinil are psychostimulants that increase dopamine signaling, the have opposed profiles: METH is highly addictive, detrimental to PFC function and cognition and toxic in dopaminergic areas of the CNS [2,3,4,5], as opposed to modafinil which has very low abuse liability, improves PFC activation and cognition and has a neuroprotective profile [6,4]. In the last years, many epigenetic mechanisms have been found as mediators of the enduring drug-induced changes in gene expression. Upon cellular stimuli, histones 3 and 4 of the nucleosome can be modified to either loosen chromatin and allow for transcriptional initiation or condense chromatin and deny promoter access. We hypothesized that METH and modafinil would induce changes in long lasting epigenetic marks that alter the transcriptional capacity of PFC circuits and cellular components, such as histone 3 and 4 acetylation (H3ac and H4ac), indicative of transcriptional activation.

In recent years several powerful techniques have been developed to evaluate epigenetic status in both physiologic and pathologic conditions. Chromatin immunoprecipitation (ChIP), that involves an antibody to a specific histone modification or other DNA-binding protein of interest is used, lies at the heart of these technologies. ChIP assays can be evaluated by standard methods like quantitative real time PCR, or by next generation sequencing (ChIP-seq), allowing the identification of genome-wide DNA binding sites for transcription factors and other proteins bounded to DNA such as acetylated histones.

This study is aimed to investigate the effect of METH and modafinil on the global targets of histone acetylation in medial PFC, by performing ChIPseq assays using antibodies specific for pan-acetylated H3ac and H4ac.

Outcome

Experiments were set as follow: male C57 mice received 7-day-repeated METH (1 mg/kg), modafinil (90 mg/kg) or vehicle treatment, and were sacrificed at day 4 after the last injection. We have previously shown in mice that this METH treatment induces PFC hypoactivation [2] and visual memory impairments associated with blunted prefrontal ERK signalling that can be rescued by modafinil treatment [3]. Two medial PFC (mPFC) per sample were processed for ChIP experiments (15 min fixation in paraformaldehyde 1%). Before tissue sonication, IgG-coupled magnetic beads (Dynabeads® Protein G 10004D Invitrogen) were incubated with anti-H3ac (5 μg, 06-599 Millipore), anti-H4ac (2,5 μg, 06-866 Millipore) or normal rabbit IgG (negative control, 2.5 or 5 μg, 12-370 Millipore) antibodies overnight at 4 °C under constant rotation in blocking solution. Chromatin shearing was carried out in a sonicator (Bioruptor Pico, Diagenode) to obtain average DNA fragment size in the range 200–1,000 bp. After sonication, 25-30 μg of chromatin were transferred to the antibody–bead conjugates and incubated over night at 4 °C and rotation. Immunoprecipitated DNA was washed and eluted from the beads, extracted with phenol-cloroform, quantified and aliquots stored at -70 °C. With

these DNA samples we performed qPCR using primers designed to analyse promoters of genes of interest, and also we sent aliquots to Macrogen (USA, Rockville, MD) to check DNA quality for up-coming massive sequencing.

Quantitative PCR analysis showed very interesting and novel results that are central to a manuscript that we are preparing for submission. We found that repeated METH distinctively decreased H3ac enrichment at NMDA glutamate receptor subunit Grin1, DA receptor Drd2, orexin receptors Hcrtr1 and Hcrtr2, and Hdac1 and Hdac2 promoters (Figure 1A). Repeated METH also caused increased H4ac enrichment at Grin1 and Drd1a promoters (Figure 1B), which correlated with increased mRNA expression (Figure 1C). Interestingly, we found increased H4ac in Hcrtr1 after repeated modafinil, showing that orexinergic system plays a distinctive role on modafinil mechanism of action in the mPFC. We found that repeated METH decreased histone acetylation at HDACs promoters, where Hdac1 showed decreased H3ac (Figure 3A) and Hdac2 both decreased H3ac and H4ac (Figure 1A and 1B). At the mRNA level we found no change in Hdac1 among groups, and decreased Hdac2 after METH compared to modafinil (Figure 1C). We also found increased AMPA glutamate receptor subunit Gria1 mRNA after repeated METH only (Figure 1C), but found no changes on H3ac and H4ac enrichment at this promoter (Figure 1A and 1B).

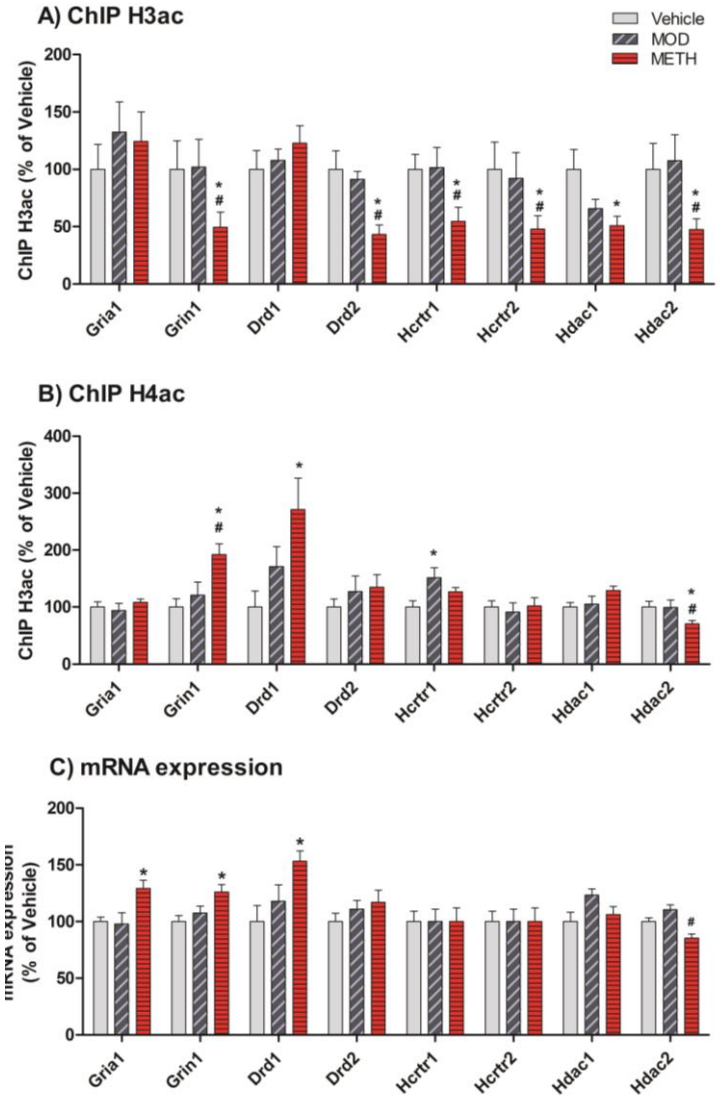

Figure 1: *Effect of repeated METH and modafinil treatment on acetylated histones enrichment at promoters and their mRNA expression*.
A) Acetylated histone 3 (H3ac) enrichment, ANOVA-Bonferroni (N=8-10). B) Acetylated histone 4 (H4ac) enrichment, ANOVA-Bonferroni (N=8-10). C) mRNA expression by RT-PCR. ANOVA-Bonferroni (N=5-6). * Different from Vehicle, # different from MOD, $p<0.05$.

These results identified for the first time epigenetic targets of METH and modafinil that we can sum-up briefly as follow: i) METH elicited specific effects decreasing H3ac enrichment

at several promoters that were not observed with modafinil, in fact we could not detect any effect of modafinil on H3ac status in the genes analyzed; ii) METH showed a specific effect increasing H4ac enrichment at NMDA subunit Grin1 and dopamine receptor Drd1, and decreasing enrichment at Hdac2, which were correlated with their respective mRNA expression; iii) Modafinil shared none of the effects we found in METH for H3ac and H4ac, and showed only one specific effect increasing H4ac enrichment at orexin receptor Hcrtr1.

To date there is no published information on epigenetic targets of modafinil in the CNS. For METH, it was described epigenetic effects only in the striatum [7] but there is no information on PFC effects. These preliminary results identified H3 acetylation as one specific epigenetic target of METH that could be linked to the detrimental effects elicited by this abused drug on cognition and PFC malfunction. Also, we were able to pin-point orexin receptor Hcrtr1 as one specific target of modafinil action in the mPFC, indicating that orexin neurotransmission could be mediating specific effects of this drug. Importantly, we have generated the DNA samples to perform the genome–wide analysis of H3ac and H4ac targets of METH and modafinil in the mPFC, and we are at the beginning of the analysis process with Macrogen ChIP-sequencing services. The ChIP-seq data will allow identifying global changes occurring after METH and modafinil administration protocols in the PFC, which could open new pathways to potential therapeutic targets to psychostimulant addiction.

We believe that the outcome of the project after one year is very satisfactory. We were able to set up the ChIP technique in our lab, and generated very novel ChIP results that are part of a manuscript in preparation, where the ISN will be properly acknowledged.

Financial Report

The funds (4118 USD) were destined to buy the following reagents:

Anti-acetyl-histone 3 antibody (06-599 Millipore) x2	954.64 USD
Anti-acetyl-histone 4 antibody (06-866 Millipore) x2	954.64 USD
Normal Rabbit IgG (12-370 Millipore) x2	280.52 USD
Dynabeads® Protein G (10004D Invitrogen)	2775.64 USD
TOTAL	**4965.44 USD**

Acknowledgments

I am deeply grateful with the Committee for Aid and Education in Neurochemistry (CAEN) chair Dr. Caroline Rae and members for financing this project. I would also like to acknowledge that CAEN financial support has also been instrumental in the development of this line of research with a CAEN1A granted in 2013, thanks to which I was able to receive training at Dr. Jean Lud Cadet's lab at the National Institute on Drug Abuse (NIDA) in ChIP techniques. These financial supports have been crucial in my first steps from post-doc toward an independent neuroscientist, and helped set a new technique in my Institution that will open new avenues of genomic research beyond the neurobiology of stimulants field.

References

1. Goldstein RZ, Volkow ND (2011) Nat Rev Neurosci. 12(11):652-69.
2. González B, Rivero-Echeto C, Muñiz JA et al. (2015) Addict Biol. doi: 10.1111/adb.12249.
3. González B, Raineri M, Cadet JL, et al. (2014) Neuropharmacology 87:188-97.
4. Raineri M, González B, Goitia B et al. (2012) PLoS One 7(10):e46599.
5. Cadet JL, Bisagno V, Milroy CM (2014) Acta Neuropathol 127(1):91-107.
6. Gozzi A, Colavito V, Seke Etet PF et al. (2012) Neuropsychopharmacology 37(3):822-837.
7. Jayanthi S, McCoy MT, Chen B et al. (2014) Biol Psychiatry 76(1):47-56.