EPI-OLF project: Epigenetic programming of the olfactory bulb in relation to the maternal environment: mapping of 5-methylation and 5-hydroxymethylation

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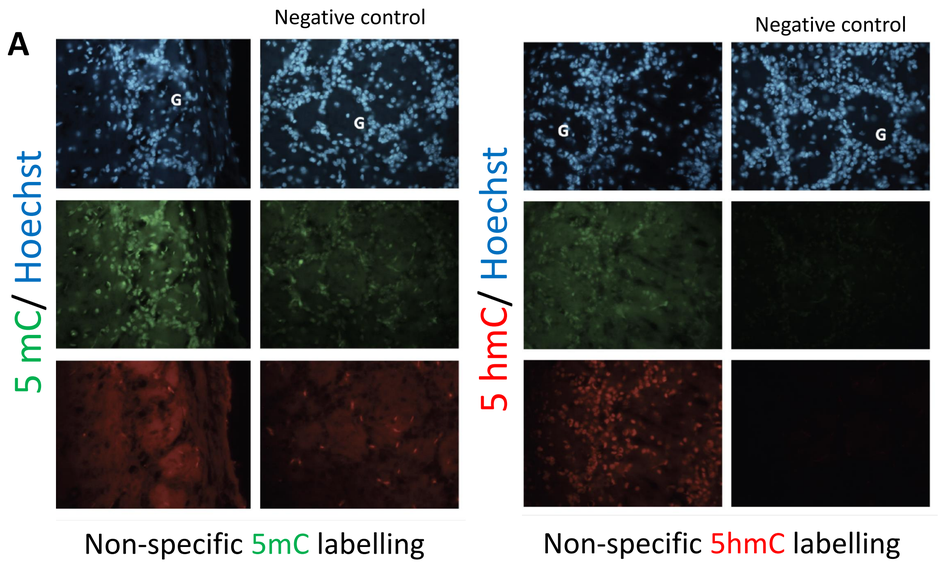
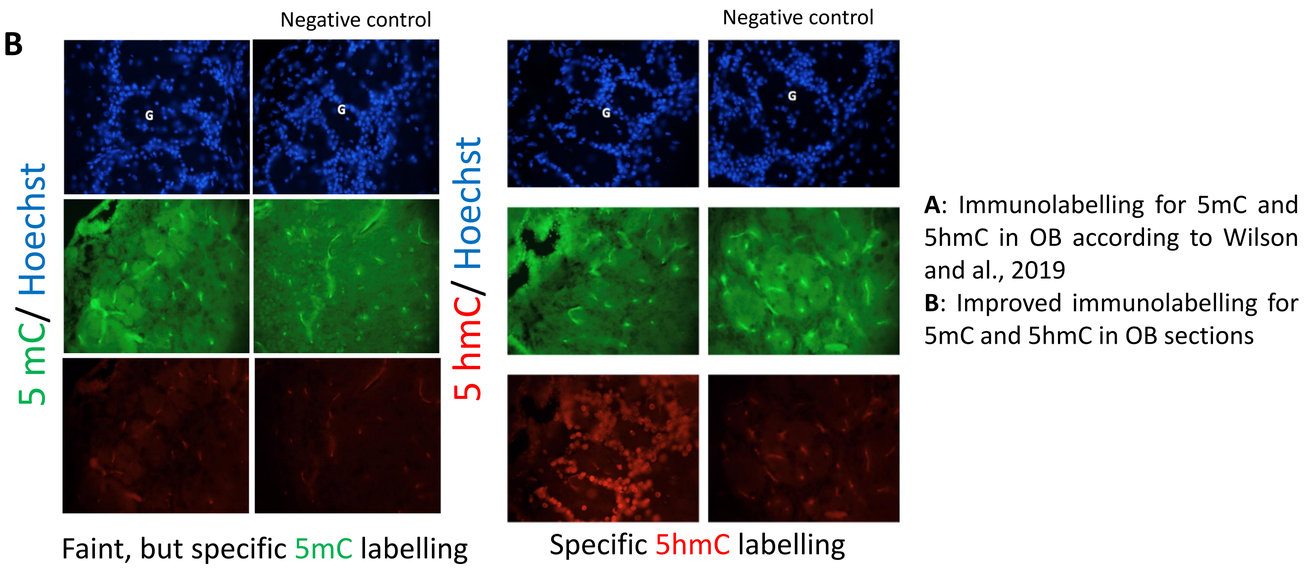
ABSTRACT
Epigenetic marks are established according to the environment and individual experiences. During brain development, they participate to the activity of neural circuits and contribute to the establishment and maintenance of several behaviours. Among them, cytosine methylation (5mC), one of the most studied modifications in the brain, is particularly sensitive to adverse environments. Recently, cytosine hydroxymethylation (5hmC), an abundant and stable mark derived from 5mC found in synaptic genes and enriched in fetal brain, has emerged as another major contributor to behavioral disturbances. These 2 important epigenetic markers constitute plausible molecular substrates in embedding the long-term effects of a maternal early experience on gene expression in brain structures linked to olfaction and odor-mediated behaviors that deserve to be studied. However, the quantification of 5hmC is still a bottleneck in our hands. This project aims to develop new tools to 1) map them by immunolabeling in olfactory tissues and 2) develop a method to quantify their variations in the olfactory bulb of offspring born a mother fed or not an obesogenic diet that display olfactory deficits (Panchenko et al., 2019).

METHODS

Immunohistochemistry: OB were dissected out from experimental samples, fixed 48h in PFA, cryoprotected in sucrose 30% for 48h, embedded in Tissue-Tek, then cut at 14 µm. Imunolabelling processing was adapted from Wilson et al., 2021, with few adjustments: slides were treated with glycine 0.1M for 30 min before demasking,, DNA was denaturated with 2N HCl, non specific sites were saturated with 2% BSA.
5 mC: Primary antibody: mouse anti-5mC (AbCam, 1/500- ; 1:2000)- secondary antibody: goat anti-mouse Alexa 488: 1:3000)
5 hmC: Primary antibody: rabbit anti-5hmC (Active motif; 1:2000)-secondary antibody: donkey anti-rabbit Alexa 546 : 1:3000)

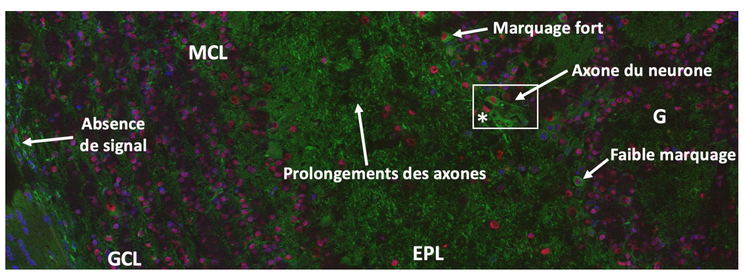
RESULTS: SINGLE IMMUNOHISTOCHEMISTRY



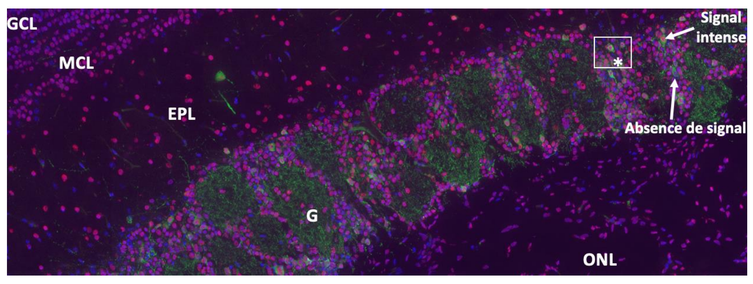


A: Immunolabelling for 5mC and 5hmC in OB according to Wilson and al., 2019
B: Improved immunolabelling for 5mC and 5hmC in OB sections

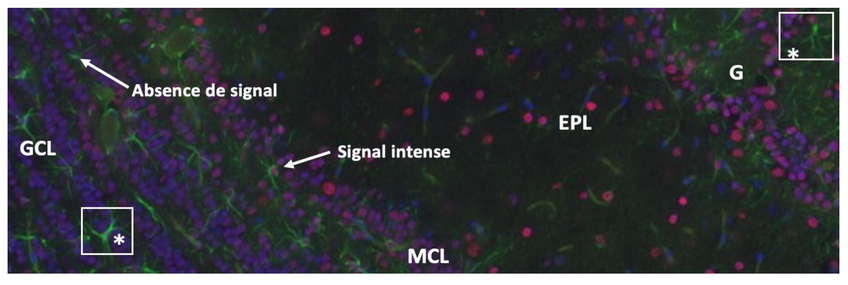
RESULTS: DOUBLE IMMUNOHISTOCHEMISTRY



Hoechst / 5 hmC / β-tubulin III (neuronal marker)



Hoechst / 5 hmC / Tyrosine hydroxylase
(marker of dopaminergic neurons)

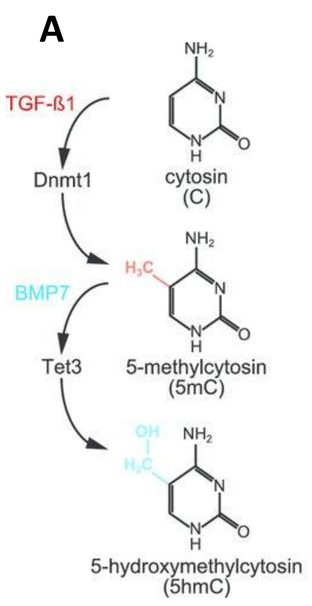


Hoechst / 5 hmC / Glial fibrillary acidic protein
(marker of glial cells)

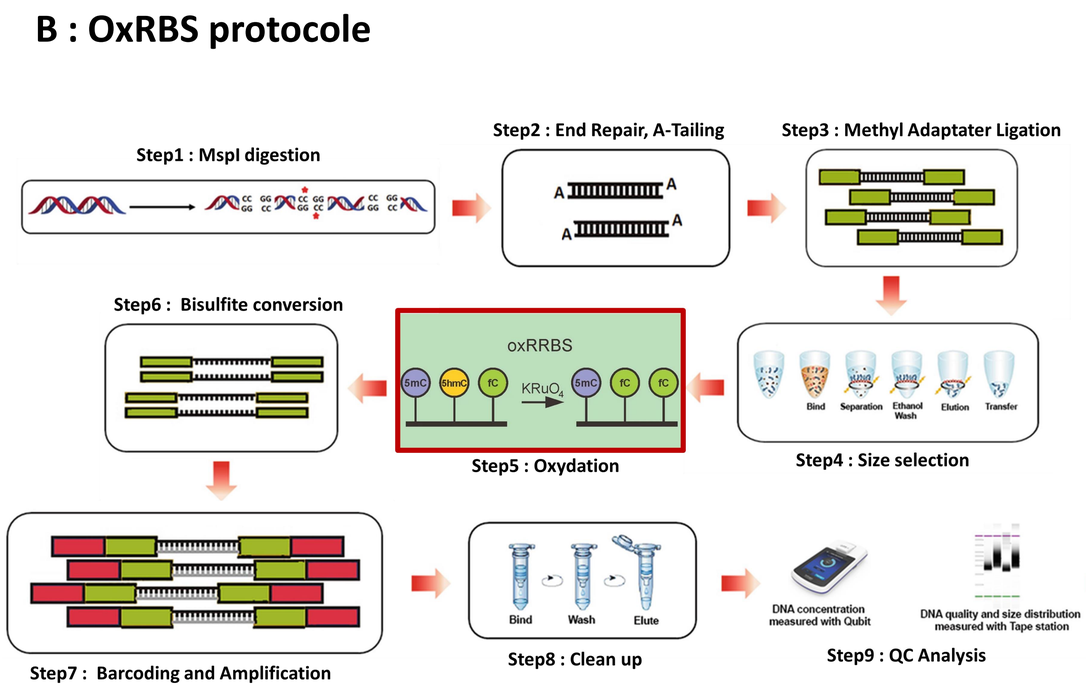
OB cells are heterogeneously labelled by 5 hmC

RESULTS: ox-RRBS

Quantification for 5 hmC: 5hmC, which is derived from 5 mC (A), is transformed in 5 formyl cytosine (5fC) upon chemical oxydation with $KRuO_4$, while 5mC is unchanged (C). Sodium bisulfite treatment of oxidized 5hmC results in its deamination to uracil which, upon sequencing, is read as a T, whereas 5mC is read as a C. Comparison of oxydated and non oxydated bisulfite-treated libraries will identify cytosines which are hydroxymethylated.



Björn Tampe et al., 2014



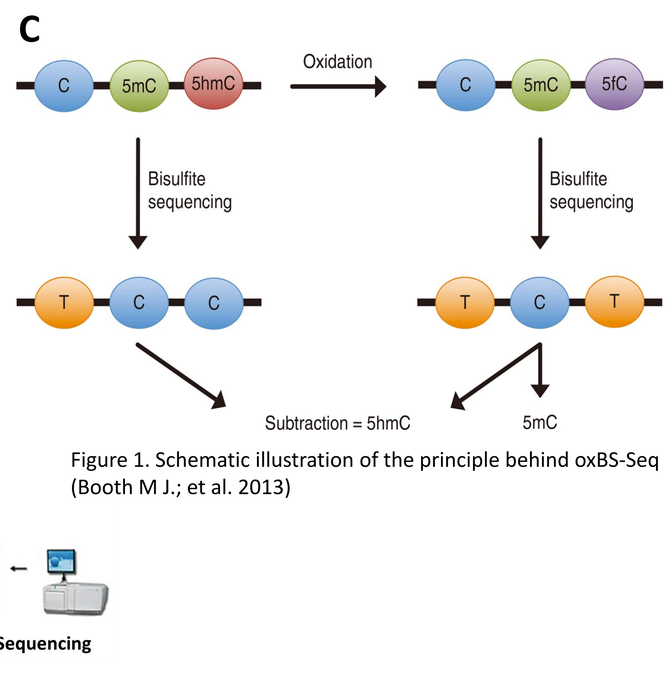


Figure 1. Schematic illustration of the principle behind oxBS-Seq (Booth M J.; et al. 2013)

DISCUSSION:

This methodological challenge has still number of uncertainties, with both technical and scientific hazards. Immunolocalization of methylated and hydroxymethylated sites in the olfactory bulb reveals heterogeneous labeling and does not suggest a selective expression of 5hmC in any particular cell type. IHC is a limitation for the field, given that epigenetic processes differ across central nervous system (CNS) cell types at the level of chromatin organization and DNA modifications. It could be partly circumwaited using enriched cell populations by flow sorting or single-cell approaches, a further step to demonstrate if 5 hmC supports epigenetic maternal programming of offspring olfactory phenotypes.
We pursue the development of the oxRRBS with new adjustments to obtain a complete and reproducible oxidation of cytosines and we develop the computer tools with the help of Luc Jouneau and Anne Aubert-Frambourg (BREED) for the processing of sequencing data, before considering the processing of the biological samples of interest. The investment to develop this method turns out to be greater than expected, especially since the literature takes little account of the associated technical hazards, leading us to develop internal standards, e.g. oxidized spikes as controls of the oxidation step.

Booth et al., 2013, Nat Protoc Oct;8(10):1841-51. doi: 10.1038/nprot.2013.115.
Panchenko et al., 2019, Nutrients Apr 26;11(5):948. doi: 10.3390/nu11050948
Tampe et al., 2014, Nephr Dialysis Transpl, Volume 29: https://doi.org/10.1093/ndt/gft025
Wilson et al., 2019: Epigenetics Jul;14(7):660-671doi: 10.1080/15592294.2019.1609866

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