



HAL
open science

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

Karine Badonnel, Boubet Camille, Aurélie Chaulot-Talmon, Hélène Kiefer, Christine Baly, Hélène Jammes

► To cite this version:

Karine Badonnel, Boubet Camille, Aurélie Chaulot-Talmon, Hélène Kiefer, Christine Baly, et al.. EPI-OLF project: Epigenetic programming of the olfactory bulb in relation to the maternal environment: mapping of 5-methylation and 5-hydroxymethylation. Journées d'animation EpiPhASE, Jun 2022, Rennes, France. hal-04378839

HAL Id: hal-04378839

<https://hal.inrae.fr/hal-04378839>

Submitted on 8 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

MeCP2
UMR1198

Karine Badonnel, Camille Boubet, Aurélie Chaulot-Talmon, Hélène Kiefer, Hélène Jammes, and Christine Baly

Université Paris-Saclay, UVSQ, INRAE, INRAE, BREED UMR1198, Bat. 230, Domaine de Vilvert, 78350 Jouy-en-Josas, France

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).

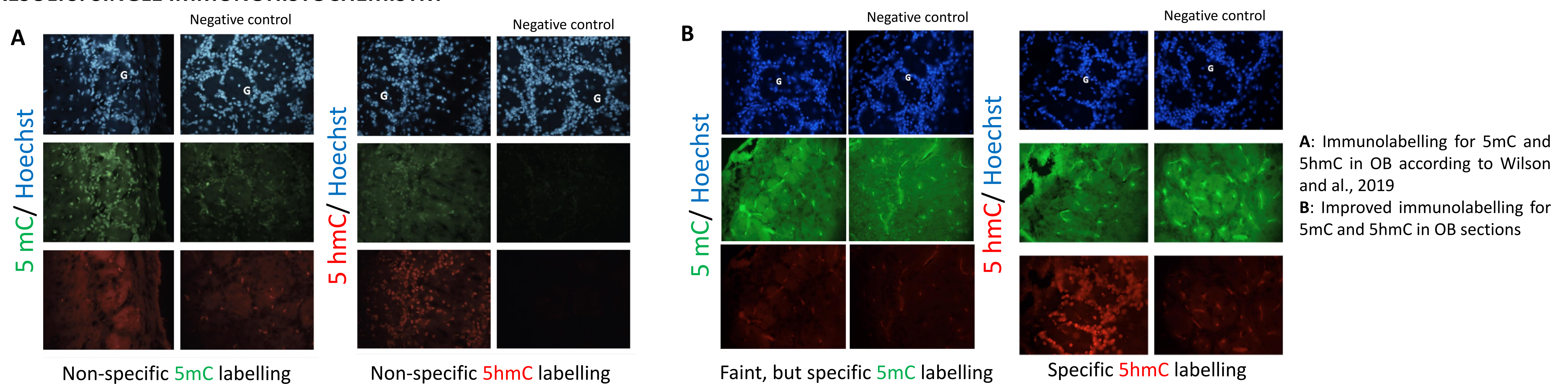
METHODS

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. Immunolabelling 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.

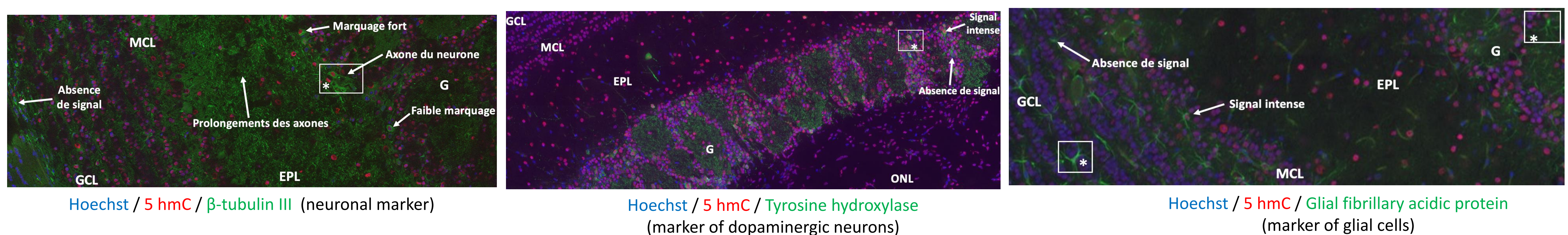
5 mC: Primary antibody: mouse anti-5mC (AbCam, 1/500- ; 1:2000)- secondary antibody: goat anti-mouse Alexa 488: 1:3000)

5 hmC: Primary antibody: rabbit anti-5hmC (Active motif; 1:2000)-secondary antibody: donkey anti-rabbit Alexa 546 : 1:3000)

RESULTS: SINGLE IMMUNOHISTOCHEMISTRY



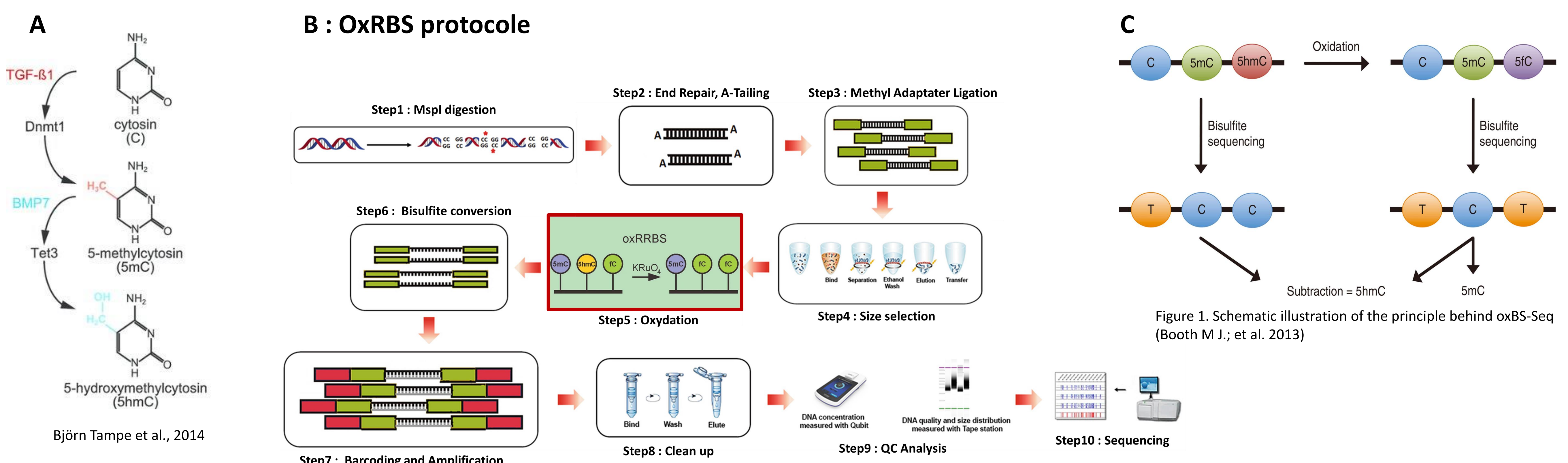
RESULTS: DOUBLE IMMUNOHISTOCHEMISTRY



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.



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 circumvented 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.