Hypothalamic cell models for an integrative and holistic assessment of endocrine-related disruptions for human health and environment

Context

The endocrine system, which accounts hormones conserved across vertebrates, plays key roles in physiological processes from early development to late life stages. During the last decades, increasing concerns raised about the **fertility decline** and the disruption of other **endocrine-related processes**, such as metabolism, thyroid function, circadian rhythm, behavior etc, **in** both **human and wildlife**. Given the diversity of these endocrine systems and endpoints, as well as environmental challenges to consider, huge efforts from scientists and regulators are still needed for a better protection of human and environmental health.

This project aims to target these challenges by proposing a relevant in vitro hypothalamic cell exposure model that tackles the complexity of endocrine systems, endpoints and mixtures of endocrine disrupting chemicals (EDCs) to which humans and wildlife are exposed to. First, the hypothalamus is a central node, regulating a wide range of endocrine systems (steroids, thyroid hormones, glucocorticoids, metabolic hormones…), which in turn exert feedbacks to the hypothalamus. Additionally, the hypothalamus governs numerous functions important for individual and species survival like reproduction, metabolism, sleep, circadian rhythm, and regulates a wide range of behaviours (cognition, anxiety, and motor activity, etc). Deficiency of the hypothalamus can simultaneously impair all these endocrine activities and related endpoints. Currently, regulatory agencies carry out chemical risk assessment on a substance-by-substance and endpoint by endpoint basis. However, scientific evidence clearly shows that human and animal populations are exposed to mixtures of these substances that can target multiple endocrine systems and endpoints. In this context, the co-supervisory teams (Sorbonne Université-SU, MNHN) have accumulated during the last years a great number of data on *in-vivo* exposure models (mice, amphibians) to single and mixtures of EDCs. The research has investigated the adverse effects on reproduction, metabolism, cognitive and anxiety-related behaviors, motor activity and the underlying endocrine mode(s) of action involving the thyroid and sex steroid systems (Adam et al., 2021; Ahmadpour et al., 2021; Ducroq et al., 2023a, 2023b; Fini et al 2017; Caporale et al., 2022; Leemans et al 2023).

Scientific objectives. This project aims:

- To use an in vitro human model of hypothalamic neurons derived from human induced pluripotent stem (hiPS) cells set up in the laboratory of SU co-supervisor and the Xenopus model used by the MNHN co-supervisor (Naulé et al., 2023; Leemans et al 2023) to **identify relevant gene targets** for the exposure to two mixtures representative of Human exposure.

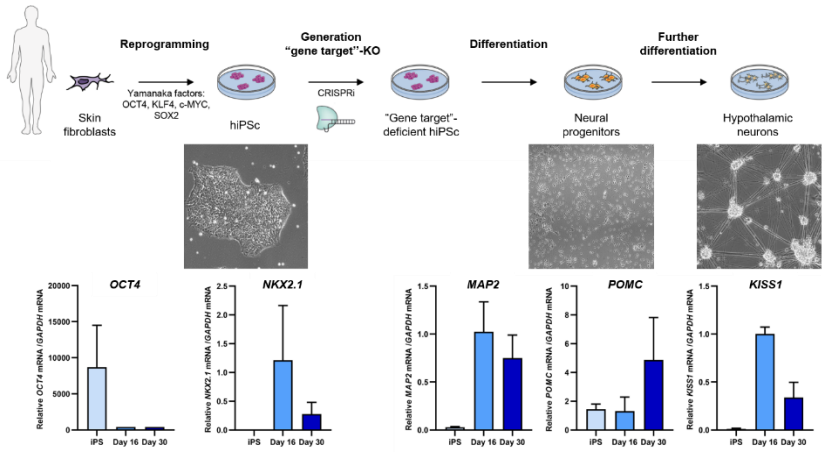

Figure 1 shows the steps of generation of mature hypothalamic neurons, which express key neuropeptides such as Kisspeptin (reproduction) and POMC (metabolism).

- To compare the **gene networks identified in the two models exposed to the same mixtures**, as well as from mice as currently developed by the co-supervisors, to characterize relevant networks or targets for the exposure.
- To use the obtained data for standard operating procedures (**SOPs)** and adverse outcome pathways (**AOPs**). These SOPs and AOPs will inform on risk assessment for human and environmental health, encompassing various species. This approach aligns with the One-Health concept.

Justification of the methodology approaches

WP1- (Year 1, Sorbonne Université lab). The postdoc will expose the neural progenitors generated from male hiPSCs (Figure 1) to vehicle alone (group#1) agonists and antagonists of major hormonal axis (Estrogen, Androgens, Thyroid hormones, and Glucocortoids) at known basal levels. Then, cells will be exposed to complex mixtures reflecting our exposome with the mix found in human breast milk (PCB153, DDE, 2,3,7,8-TCDD, Diledrin, Arsenic V, Hexachlorohexan, Heptachlor, PFOS; group#2) or in amniotic fluid (DEHP, DBP, PCB153, 4-4'DDE, Perchlorate, Triclosan, BPA, Hexachlorobenzen, BDE209, Benzophenon3, PFOS, PFOA, Naphtol, Lead and Mercury; group#3) for a repeated dose treatment of 14 days for long-term effects during the key phases of differentiation into mature neurons. Cells will then be used for neurotoxicity test battery to measure key neurodevelopmental processes such as proliferation, differentiation, dendritic arborization, neuroplasticity as shown on cortical cells (Fritsche et al. 2018a; Davidsen et al. 2021). Samples will be collected from the three treatment groups for transcriptomic analyses.

WP2- (Year 2, MNHN lab). The postdoctorant will analyze the samples collected from hypothalamic neurons using a non-biased approach of RNAseq thanks to the expertise in this field of MNHN lab. In parallel, he or she will expose the new ELEA (Early exposure Late Effect Assay) developed by the MNHN supervisor *on Xenopus* in the EU ATHENA, EnDpoiNTs and PARC projects where the two human EDC mixtures (groups#2, 3) will be used during development. The candidate will assess hypothalamic gene expression by RNAseq.
The obtained data from the human *in-vitro* and xenopus models as well as from mice will be compared to identify relevant biomarkers that could apply whatever the vertebrate species considered for EDC mixtures.

WP3. The data generated from this project will be used to construct the SOP for the inclusion of human hypothalamic in vitro model and be submitted to the PEPPER platform to accelerate its use and validation. The aim is to integrate the new hypothalamic assay into the DNT test batteries but also in battery of assays (including fish, amphibians or mice) to decipher modes of actions for a better consideration of the One-Health concept. The candidate will also build the AOP that could help for risk assessment. In addition, during these two years, the postdoc will be actively involved in the valorization, publication and the dissemination of data related to the project to i) both the scientific community through oral communications and posters in national and international meetings (Gordon Conference on Endocrine disruptors, Society of Endocrinology, Society of Neuroendocrinology, ARET…), ii) towards regulators and stakeholders, and iii) to large audience through articles in national languages and participation to local and national initiatives (Semaine du Cerveau, Fête de la Science…). The internationally recognized expertise of co-supervisor's expertise in i) the fields of neuroendocrinology and endocrine disruption, ii) risk assessment and interaction with regulators (both are experts at the ANSES and EFSA and are members of European consortia), and iii) dissemination towards the large public will be valuable for the recruited postdoctorant.

Relevance to the themes indicated in the call

The interdisciplinary project combines methodologies including the recently developed hiPS cell technology amphibian larvae study, cellular and High throughput molecular approaches, and the SOP and AOP development. This project will allow to capture complex integrations of the exposome at central level and to determine the molecular endocrine disruption. This will bring together disciplines related to neuroendocrinology, developmental neurotoxicity, endocrine disruption and risk assessment for human health and the environment. This diverse and interdisciplinary expertise makes this project among the state-of -the art projects currently developed in the field of risk assessment of EDC mixtures, and in compliance with the 3R rules of animal experimentation. Understanding whether and how the exposome and lifestyle or access to natural resources can lead to these changes at the individual and populational levels is a huge challenge for scientists and regulatory authorities. This project paves the way for subsequent studies analyzing for example markers related to other environmental factors that impact the hypothalamus such as stress, Western life diet alone or in combination with chemicals.