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DOCTORAT DE L'UNIVERSITE PIERRE ET MARIE CURIE

Spécialité : Cerveau, cognition, comportement

Ecole doctorale de rattachement ED3C

RAPPORT

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Sur la thèse présentée par M. FARAR

Ayant pour sujet : Adaptation du système nerveux central à l'absence d'acétylcholinestérase

The PhD work of Mr. Vladimir Farar concerns the adaptation of the brain to mutations (PRiMA KO and AChE del5+6 mice) that induce the elimination of membrane-bound acetylcholinesterase (AChE). AChE is a key player of cholinergic transmission as it hydrolyses acetylcholine (ACh) thus participating to termination of cholinergic signalling. The membrane-bound, multimeric form of AChE is the main variant of AChE in the central nerve system (and in muscle). Brain ACh is involved in several behaviours, including locomotor activity, spatial reference or motor skill learning.
Mr. Farar thus analysed these behaviors in mutant mice, to detect whether absence of PRiMA-anchored AChE had an impact on them. He also assessed adaptation of the brain by analysing cholinergic markers as well as markers of other neurotransmitter systems that are known to interact with cholinergic neurons. Altogether Mr. Farar's thesis points surprisingly little deficits and adaptations of PRiMA KOs and ACHE del5+6 mutant mice, and discusses this observation.

M. Farar has used a diversity of techniques: behavioural tests, biochemical analysis, and a variety of radioligand binding. His thesis also presents and discusses results from complementary approaches performed by colleagues.

The *Introduction* of the manuscript is relatively concise (33 pages). It first describes the central cholinergic system and the different forms of cholinesterases. It then presents the different genetic models that are available to study the implication of cholinesterases. In this regard, it could have been interesting to also describe AChE Tg mice that, although they are not used in the experiments, are extensively referred to in the discussion. Besides that, the *Introduction* is overall clearly written and gives a good overview of Mr. Farar's research topic.

The *Methods* section (10 pages) describes the experimental procedures used. It is overall easy to read.

The *Results* section (10 pages) is organized in small paragraphs recalling the objective of each experiment and describing its results. The data are well illustrated and quantified in tables.

The behavioural phenotype of PRiMA KO mice is first described. Mr. Farar demonstrates that PRiMA KO have similar locomotor activity, and almost unperturbed static and dynamic gait as compared as wild-type (WT) animals. These mice also have intact motor skill and spatial learning.

The absence of strong behavioural phenotype led to the questioning of how cholinergic levels are actually modified in these mice. Mr. Farar performed microdialysis in vivo in the striatum and demonstrated that ACh level are indeed 200-300 times increased in comparison to WT mice.

A third set of experiments was performed to compare the expression of cholinergic markers to assess the adaptation of the brain to high levels of ACh. These experiments involve analysis of muscarinic binding sites in membrane preparations from various brain areas, as well as autoradiography of muscarinic, nicotinic receptors and of the vesicular transporter for ACh (VAChT). The density of muscarinic receptors appeared reduced, the one of heteromeric nicotinic receptors slightly reduced too but the density of VAChT was unchanged.

Alterations in muscarinic signalling were further analysed by behavioural experiments. Muscarinic agonists or antagonists are known to affect locomotor activity, body temperature control, heart rate, and to induce epileptic seizures. All of these effects were altered in PRiMA KO mice.

Neurochemical experiments were performed to test the high-affinity choline-uptake (HACU) capacity of synaptosomes from diverse brain areas, or the activity of ACh synthesizing enzyme ChAT in homogenates from the same regions. HACU and ChAT activity were unchanged in PRiMA KO mice.

Mr. Farar then determined the relative abundance of glutamatergic (AMPA, kainate and NMDA), GABA-A, and dopaminergic (D1 and D2) receptors by autoradiography, and demonstrated that none of them was affected in the mutants.

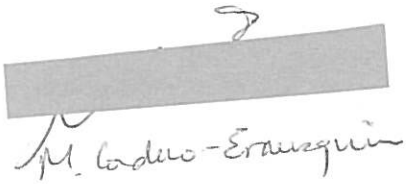
Finally, he performed an analysis of the developmental regulation of muscarinic binding sites (on plasma membranes from whole brain) and of AChE and butyrylcholinesterase activity (on whole brain homogenates). He demonstrates alterations in the ontogeny of these two parameters until P30.

In the *Discussion* section (14 pages), Mr Farar discusses his results in the context of the international literature in the field and also includes relevant unpublished data. The section discussing the lack of changes in preterminal cholinergic markers is slightly more difficult to read, in part due to the lack of proper description of AChE Tg mice as mentioned earlier. This complex section could have benefited from an illustration (figure or table). The rest of the *Discussion* is well written and relatively easy to follow.

This PhD work has directly led to two articles that Mr. Farar has signed as first author; in addition, M. Farar has contributed to three additional articles in relation with his PhD work.

In conclusion, Mr. Farar performed a high quality research work using a variety of experimental techniques. The results obtained by Mr. Farar are interesting and contribute to a better understanding of the role of acetylcholinesterases in the brain. This work also opens important research avenues on the role of peripheral AChE.

I think that this work is worth being defended in front of the thesis committee.



Ce rapport devra être transmis

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