UPMC
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DOCTORAT DE L'UNIVERSITE PIERRE ET MARIE CURIE

Spécialité : Neuroscience

Ecole doctorale de rattachement ED3C

RAPPORT

De M. : Jan JAKUBÍK

Qualité : Researcher Academy of Science

Lieu d'exercice : Prague

Ce rapport devra être transmis

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Et copie à

Mme.

Président(e) de la commission des thèses de

Adresse :

Sur la thèse présentée par

M. : FARAR

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

Rapport :

Thesis "Adaptation of the central nervous system to the absence of acetylcholinesterase" by Vladimír Farár has 198 standard A4 pages (including 36 Figures, 16 Tables, **astonishing** 24 pages of references and 38 pages of supplements – copies of **3 published original articles**). Thesis explores the effects of knock out of proline rich membrane anchor (PRiMA) that leads to absence of principal form of acetylcholinesterase (AChE) in the central nervous system (CNS) of mice. Volume of the data substantially surpasses what is common for PhD thesis. Introduction is extensive and clearly and understandably overviews in the detail cholinergic transmission and genetic models targeting cholinesterases and summarizes current knowledge of the role of anchoring proteins of cholinesterases. Methodologically, thesis asses broad range of biochemical, pharmacological, physiological and behavioral changes in experimental animals *in vivo*, *ex vivo* as well as *in vitro*. Methods are well described in sufficient detail. The fundamental part of the thesis was published in 3 original articles in esteemed journals including the top in its field Journal of Neurochemistry proving quality of the results. Conclusions drawn are well substantiated by results. I have only several minor comments (see below) that do not in any sense undermine quality of the work and may well remain for discussion during the thesis defense. Overall the presented thesis fulfills and in many aspects vastly surpasses requirements for PhD thesis therefore **I do encourage it for defense**.

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Minor comments:

- On Pg. 26 author states that "*There are only sparse data concerning cognitive function of M4 KO mice, ...*". However it could be noted that (although mainly pharmacological) there is accumulating evidence for M_4 receptors playing a crucial role in cognitive processes and in pathology of schizophrenia.
- The statement on Pg. 29 "*It is suggested that BChE serve as ... a scavenger of ... AChE inhibitors.*" is unfortunate because it suggests that BChE has an ability to inactivate, decompose or otherwise rid off AChE inhibitors.
- The statement on Pg. 30 "*... questioning the physiologically relevant contribution of BChE to ACh breakdown (Mohr et al., 2013)*" in my opinion may be little bit misleading as one of conclusions of the cited paper that states: "*These findings suggest that treatment of AD related cognitive impairment relies, indeed, on AChE inhibition in early to moderate stages, while specific BuChE inhibition may*

only be useful in stages where AChE is reduced by more than 50% which, however, does not exclude the validity of mixed enzyme inhibition."

- Regarding Methods, namely radiography of muscarinic receptors (Pg. 56): How concentrations of [^{3}H]-pirenzepine and [^{3}H]-AFDX384 were chosen? Was the cross-reactivity of 5 nM [^{3}H]-pirenzepine with non-M_1 receptors and of 2 nM [^{3}H]-AFDX384 with non-M_2 receptors determined / estimated?

- Regarding behavioral tests (Pg. 71), taking into account that muscarinic receptors are involved in cognitive processes where they play mainly modulatory role and muscarinic agonists have beneficial while antagonists deteriorating effects, what was expected effect of PRiMA KO in behavioral especially learning tests?

- On Pg. 78 author states that ambient concentration of ACh in striatum reaches 3 µM and then speculates: "*These high extracellular ACh concentrations exceed the EC50 of MR ... but not that of most NRs. ... It is therefore not surprising that the main adaptation of ACh receptors concerns MR.*" However situation is actually opposite: Acetylcholine EC_{50} at NRs is nanomolar (e.g. Moroni et al., 2006) and at MRs is in micromolar range (e.g. Machová et al., 2008).

- Unfortunately thesis is full of formatting and grammatical errors and typos spoiling aesthetic impression of otherwise nice piece of work: Namely, according to the Guide to Receptors and Channels by the Nomenclature Committee of IUPHAR receptor subtypes should be indicated in subscript (subscripts e.g. M_1, β_2, D_2, etc. are missing throughout the thesis). Number of atoms in molecule formula should be indicated in subscripts (subscripts are missing in Methods). In some instances tritium (^{3}H) misses superscript. From grammatical errors lack of subject-verb agreement and missing article are the most occurring ones.