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Comments on Cui Q-Q *et al*: "Hippocampal CD 39/ENTPD 1 promotes mouse depression-like behavior . . ."

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Comment on: **Q-Q Cui *et al*** (April 2020)
See reply: **Z-L Hu *et al***

The paper of Cui *et al* (2020) published in EMBO *reports* requires further comment. First a short note on correct nomenclature: Protein and gene names are confused. CD39 (ectonucleoside triphosphate diphosphohydrolase 1, NTPDase-1) is the protein name, whereas *ENTPD1* is the name for the corresponding (human) gene. In case of the mouse gene, this would be *Entpd1* (e.g., https://www.uniprot.org/uniprot/P49961 and https://www.genenames.org/about/guidelines/). The same applies to *ENPP1-3* and *ENPP8*.

A central finding for the further development of the study of Cui *et al* concerns the increase in mRNA expression of *Entpd1* in hippocampal extracts of mice susceptible to chronic social defeat stress. In addition, the authors provide qPCR data to document gene expression of NTPDase-2 and NTPDase-3, two paralogs of NTPDase-1, which—like NTPDase-1—hydrolyze ATP to AMP. They observe a decrease in *Entpd2* mRNA, which is not further investigated but which is of relevance at least for the results concerning alterations in neurogenesis.

It is a major concern that the authors do not refer to relevant previous evidence indicating that the issue might be more complicated and might allow for alternative interpretations of their results. Cui *et al* conclude that CD39 affects hippocampal neurogenesis. Yet, they solely provide evidence for DCX-positive neuroblast but not for mature neuron formation. They refer to the paper of Lin *et al* (2007), which shows high but non-specified ectonucleotidase activity in the dentate gyrus. They do not mention previous papers which show that in the dentate gyrus, the ectonucleotidase NTPDase-2 is specifically associated with hippocampal stem and progenitor cells (Shukla *et al*, 2005) and that deletion of NTPDase-2 results in increased hippocampal progenitor cell and neuroblast proliferation (Gampe *et al*, 2015). Cui *et al* conclude that "Ectonucleotidase expressing in the SGZ of the hippocampus may serve as a brake on the proliferation of NSCs." They do not mention an earlier paper on hippocampal SGZ (and subventricular zone) neurogenesis using mice globally null for *Entpd2* (Gampe *et al*, 2015), which concludes "This suggests that NTPDase2 functions as a brake on nucleotide-mediated cell proliferation in either neurogenic niche."

Cui *et al* introduce ARL67156 as "nonspecific inhibitor" of CD39. They do not mention that ARL67156 also inhibits NTPDase2 and NTPDase3 (Lévesque *et al*, 2007), particularly when the inhibitor is applied at high excess (injection of a 100 μM solution) over the endogenous extracellular ATP substrate. It should also be noted that injected apyrase not only mimics NTPDase-1 but also NTPDase-2 and NTPDase-3.

Previous literature not mentioned in the paper of Cui *et al* could have helped to identify some of the hippocampal cellular elements involved. It has long been known that CD39/NTPDase-1 is expressed by microglia and also by the vasculature of the brain (e.g., Braun *et al*, 2000; Färber *et al*, 2008). In fact, single-cell RNA sequencing data (Zhang *et al*, 2014 and https://www.brainrnaseq.org/) show that NTPDase-1 is expressed in microglia and endothelial cells but not in neurons or astrocytes. This contradicts Appendix Fig S1A–C of Cui *et al*, which implies that NTPDase-1 immunoreactivity is expressed by essentially all hippocampal neurons. No microglia are discernible. A complete NTPDase1 Western blot lane revealing the specificity of the antibody would have been essential.

The picture of ectonucleotidase functions in chronic social defeat stress-induced depression-like behavior and hippocampal neurogenesis may therefore be more complex than inferred by Cui *et al* Microglial NTPDase-1 may be mainly involved in the NTPDase-1 effects observed. Regarding progenitor cell proliferation, progenitor cell-associated NTPDase-2 rather than NTPDase-1 is likely to contribute. Whether ATP or the final hydrolysis product adenosine is more relevant remains to be investigated.

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References

Braun N, Sévigny J, Robson SC, Enjyoji K, Guckelberger O, Hammer K, Di Virgilio F, Zimmermann H (2000). *Eur J Neurosci* 12: 4357 – 4366

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Cui Q-Q, Hu Z-I, Hu Y-L, Chen X, Wang J, Mao L, Lu X-J, Ni M, Chen J-G, Wang F (2020). *EMBO Rep* 21: e47857

Färber K, Markworth S, Pannasch U, Nolte C, Prinz V, Kronenberg G, Gertz K, Endres M, Bechmann I, Enjyoji K *et al* (2008). *Glia* 56: 331 – 341

Gampe K, Stefani J, Hammer K, Brendel P, Pötzsch A, Enikolopov G, Enjyoji K, Acker-Palmer A, Robson SC, Zimmermann H (2015). *Stem Cells* 33: 253 – 264

Lévesque SA, Lavoie EG, Lecka J, Bigonnesse F, Sévigny J (2007). *Br J Pharmacol* 152: 141 – 150

Lin JH, Takano T, Arcuino G, Wang X, Hu F, Darzynkiewicz Z, Nunes M, Goldman SA, Nedergaard M (2007). *Dev Biol* 302: 356 – 366

Shukla V, Zimmermann H, Wang L, Kettenmann H, Raab S, Hammer K, Sévigny J, Robson SC, Braun N (2005). *J Neurosci Res* 80: 600 – 610

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N *et al* (2014). *J Neurosci* 34: 11929 – 11947