

Peer Review

Review of: "Cholinergic Signaling Differentially Regulates Song Premotor Circuits to Stabilise Songs in Songbirds"

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The manuscript entitled “Cholinergic Signaling Differentially Regulates Song Premotor Circuits to Stabilise Songs in Songbirds” by Xu et al. describes the modulatory cholinergic system that controls song premotor neuronal circuits. They have analyzed the nicotinic and muscarinic actions on different neuronal populations that may control motor skill learning. Using different *in vitro* and *in vivo* electrophysiological techniques, they have established the mechanisms of cholinergic modulation of songbirds’ song behavior. I have only a few comments to clarify the interpretation of the results.

Page 4. First paragraph, Results. mEPSCs; although it is defined in the methods, it has to be defined the first time it is used.

Page 15. Second paragraph. Although some authors have shown a hyperpolarization effect of ACh on the cortex, other authors have shown that it depolarizes, for example, Núñez et al. Cholinergic-mediated enhancement in barrel cortex layer V pyramidal neurons. *Journal Neurophysiology* 108: 1656–1668. doi: 10.1152/jn.00156.2012. These works should be cited.

In the same paragraph, ACh also has an effect on layer 5 pyramidal neurons.

Page 23, Methods, Animals. “were older than 120 days”; please indicate a range of time.

Methods, Slice Preparation. “Following deep anesthesia, the Brain..” What was the anesthesia?

Page 24, Methods, Drugs. Define CAR as a muscarinic cholinergic agonist.

Declarations

Potential competing interests: No potential competing interests to declare.