Reviewer Comments:
Reviewer #1:
Suitable Quality?:Yes
Sufficient General Interest?:Yes
Conclusions Justified?:Yes
Clearly Written?:Yes
Procedures Described?:No
Supplemental Material Warranted?:Yes

Comments:

Overall, we are satisfied with the response of the authors to the comments from the previous round of reviews. This is a very important study and a technically excellent paper. We agree with the authors' response "that the demonstration of the links between cholinergic activity, SPW-Rs and their blockade, and memory within the same experiment presents progress and addresses unanswered questions." It is also very interesting that the data shows that cholinergic activity dips precisely at the peaks of SPW-Rs. The authors have addressed the comments about working memory. While the reviewers do not agree with their definition of working memory being involved when "the delay between encoding and using that information is long," the authors cite existing literature to support their definition that "working memory is not defined by its duration but by its single time utility." This indicates that the debate about the definition of working memory exists throughout the field. The specific comments below address a few details of the presentation. This is an important and novel paper that is highly deserving of publication.

Specific comments:

Methods and supplemental: There should be some additional description of the details of the ACh3.0 fluorescent signal fiber photometry in the Methods section (beyond what is presented in the Methods which is essentially the same in the supplemental section) because this is one of the first publications using the ACh3.0 sensor. What LED power was used for the measurements? How many mice were implanted with the 200 µm fiber, how many with the 400 µm fiber and how did the signals differ? What kind of fiber patch cords were used? Further, what kind of optical filters were used? How was the LED triggered? What kind of detector was used? Please list all details of the fiber photometry rig that might be critical for others to replicate those findings.

We thank the Reviewer for these suggestions. We have added the necessary details to the Methods section of the revised manuscript as: “The virus AAV-hSyn-ACh3.0 (Vigene Biosciences Inc) was injected into the dorsal hippocampus and a 200 µm (n = 5 mice) or 400 µm (n = 4 mice) diameter optic fiber was implanted 200-300 μm above injection site to collect the emission fluorescent signal from that area. During recording, a 400 Hz square wave train, driven LED (470nm) by a signal generator (Rigol DG4062 Arbitrary Waveform Generator) was delivered to excite ACh3.0 sensor. The LED driver (LEDD1B) and fiber coupled LED (M470F3) were obtained from Thorlabs. The applied power, measured from mono fiberoptic patchcord (FC-MF1.25) tip, was 30~60 μW, and from the optical fiber in the brain (FP400URT or FP200URT) was 80-95% of the input power (measured by PM100D from Thorlabs, in air). The emission light of ACh3.0 signal in dorsal hippocampus traveled back through the same optical fiber, bandpass filtered (500-550 nm, Minicube, FMC3-e(460-490)\_F(500-550)\_S, Doric), passed through a lowpass filter (Model 440 instrumentation Amplifier) at 20 Hz, detected by a Femtowatt Silicon Photoreceiver (Newport, 2151) and recorded using a real-time processor (CED 1401).”

Regarding the ACh 3.0 emission light difference between using 200 and 400 μm optical fibers, we did not implant both fibers into the same animal. Because the virus expression varied from mouse to mouse, we are not able to compare the difference accurately. In principle, the 400 μm optical fiber can collect twice as many emission photons from the brain area as 200 μm optical fiber. On the other hand, 200 µm fibers produce less neuronal damage. Because of the latter reason and because 200 µm fibers provided sufficient efficacy, in later experiment we used the thinner version.

In addition, we have performed a new analysis by calculating the correlation between the ACh 3.0 signal and the power of gamma oscillation. We found a robust positive correlation, in contrast to the equally strong negative correlation between the ACh 3.0 signal and ripple power. These analyses are shown in Fig. 3.

Abstract - "not only but theta mechanisms" - the word "but" seems out of place here

Corrected

Page 3 - "active cholinergic system-dependent" - sentence is still not finished though this was mentioned in previous review and the response states that "mechanism" would be added.

We apologize for this omission. It has been corrected in revised manuscript.

Page 4 - The effect of atropine on the fluorescent signal is somewhat unclear. I see from the Yulong Li paper that muscarinic antagonists block the fluorescence ACh3.0 sensor, so this is apparently a test of the sensor, but could be interpreted as an effect of atropine on ACh release mechanisms. They should indicate that they are using it to block the sensor.

We now included a clarifying sentence: ‘atropine, which is an acetylcholine muscarinic receptor antagonist’ in revised version.

Page 6 - "of cholinergic activity of during memory delay" - the word "of" should be removed before during

Corrected.

Page 7 - "recoding silicon probes" = recording silicon probes (missing "r")

Corrected.

Page 7 - "while the mouse run" - run=ran

Corrected.

Page 7 - "higher that" - higher than

Corrected.

Page 8 - "of spontaneous a spontaneous" - remove repeat

Corrected.

Page 11 - "principle component" - appears twice - should be "principal"

We have corrected the text.

**Supplemental materials**

Page 2- "connectorized" - This is not a standard English word. Does it refer to the addition of connections?

The non-word ‘connectorized’ has been replaced with ‘connection’

Page 4, Fig. 1 legend - "independent with" - usually this would be stated as "independent of"

Corrected.