# Review of: "Kv3.3 subunits control presynaptic action potential waveform and neurotransmitter release at a central excitatory synapse"

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The study by Amy et al. investigated the role of Kv3.3 subunits in modulating action potential and its effect on neurotransmitter release at the calyx of Held synapse. The authors found that Kv3.3 deletion showed a significant inhibitory effect at calyces, including broadening the half-width of AP, enhancing short-term depression, and impairing the ability to sustain MNTB AP firing at high-frequency stimulation. They also showed the underlying physiological implication that Kv3.3 plays a crucial role in response to sound stimulation. Overall, this is an interesting story, and I have only a few concerns listed below.

Major concerns:

1. Figure 2A shows that deletion of Kv3.1 does not decrease neurotransmitter release. However, in Figures 3E and F, the deletion of Kv3.1 increases the plateau of normalized EPSC amplitude and reduces STD. How to explain the different effects of Kv3.1 KO between two experiments?
2. In Figures 6E and 6F, the sustained depolarized plateau amplitude of Kv3.1 KO is higher than that of WT, making it easier to evoke an AP. However, Kv3.1 KO shows a decrease in the success rate of AP firing at high-frequency stimulation in Figure 6A, which seems to contradict the higher plateau amplitude. How to explain?
3. It is inferred through computational modeling that Kv3.3 KO increases the release probability and speeds a fast component of vesicle recycling in Figure 5. We suggest adding a presynaptic recording of the release probability and vesicle recovery, which may help verify the computational model.
4. The role of Kv3.1 subunit is not clarified in this article, nor is there a detailed analysis of its physiological functions. For example, Figures 2F, G, and H show EPSC traces from WT, Kv3.3 KO, and Kv3.1 KO mice. What is the purpose of setting Kv3.1 KO group, and what conclusion can be drawn from these figures?

Minor concerns:

1. Some figures can be improved. For example, the three figures in figure 1D can also be overlapped together to reflect the changes in AP shape. Additionally, it would be better to provide an inset figure enlarging the time scale (0 to 10 seconds) of Figure 4D to show the difference in fast time-constant between Kv3.3 KO, Kv3.1 KO, and WT more clearly.
2. The effects of TEA or Kv3.3 deletion are implemented on presynaptic terminals in Figure 1, but the success rate of AP is implemented on the postsynaptic MNTB neurons in Figure 6B. It would be better to

complement Figure 6B with experiments on presynaptic terminals to provide more direct evidence.