Neurosci. Bull. December, 2019, 35(6):1106–1109
https://doi.org/10.1007/s12264-019-00391-8

www.neurosci.cn
www.springer.com/12264

INSIGHT

# Functional Self-Excitatory Autapses (Auto-synapses) on Neocortical Pyramidal Cells

**Wei Ke[1] · Quansheng He[1] · Yousheng Shu[1]**

Received: 27 February 2019 / Accepted: 9 March 2019 / Published online: 16 May 2019


Synapses are essential structures in the nervous system and normally form between two cells. However, some occur in a single neuron between the axon and its own dendrites and soma. These special synapses are known as autapses (or auto-synapses or self-synapses) [1]. Previous studies have shown that autapses were much more abundant in cultured neurons [2–4] than in the intact brain, so there was suspicion that autapses *in vivo* may be wiring errors or redundant structures [5]. However, accumulating evidence indicates that autapses are actually abundant in certain types of neurons [6]. They selectively form on fast-spiking GABAergic interneurons but not on low-threshold spiking interneurons [6, 7]. Recently, we showed that autapses selectively occur on subcortically-projecting layer V pyramidal cells (PCs); they produced giant AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-only postsynaptic responses and promoted burst firing and coincidence detection [8]. Therefore, PC autapses are important circuit elements in the intact brain, and their role in regulating cortical information-processing needs to be considered seriously.

## Neocortical PCs Form Autapses: Physiological Evidence

Electrophysiological recordings in GABAergic neurons have revealed the occurrence of autapse-mediated inhibitory postsynaptic currents (IPSCs) immediately after action potentials (APs) [7, 9–12]. The temporal separation of APs and autaptic responses is due to the narrow AP waveforms in these neurons. However, because PCs generate much broader APs than GABAergic cells, autaptic responses might be masked by the AP waveforms [13]. Our recent study tackled this waveform-overlapping problem and provided the first piece of physiological evidence showing that neocortical PCs form functional autapses [8].

Just like paired recordings from pre- and postsynaptic cells to examine unitary synaptic transmission, we performed simultaneous recordings from the soma (postsynaptic) and the axon (presynaptic) of a single layer V PC in prefrontal cortical (PFC) slices [14] and isolated the two compartments by 2-photon laser axotomy. Therefore, the evoked axonal action potentials (APs) would not reach the soma by back-propagation and mask autaptic responses. The short onset latency of these responses (< 2 ms) agrees well with monosynaptic transmission. Since they were completely blocked by the AMPA/kainic acid receptor antagonist CNQX, they were actually autaptic excitatory postsynaptic currents (aEPSCs) mediated by ionotropic glutamate receptors. Furthermore, our experiments using $Sr^{2+}$-containing bath solution also revealed the occurrence of autapses in PCs. Since $Sr^{2+}$ delays and prolongs neurotransmitter release, aEPSCs that normally overlap with APs were unmasked and readily detectable. Indeed, we recorded desynchronized autaptic events, which only occurred in the stimulated PC, and not in unstimulated neighboring PC. These results suggest that the desynchronized events are due to monosynaptic release at autapses, not through polysynaptic transmission. If transmission was polysynaptic, the neighboring PCs in some of the paired recordings would also receive delayed synaptic inputs. Further experiments in a $Sr^{2+}$ bath containing tetrodotoxin (TTX) that blocks AP generation and thus abolishing

✉ Yousheng Shu
yousheng@bnu.edu.cn

[1] State Key Laboratory of Cognitive Neuroscience and Learning, IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing 100875, China

polysynaptic transmission showed desynchronized autaptic events, confirming monosynaptic transmission. In these experiments, $Cs^+$-containing pipette solution blocks $K^+$ channels and the recorded cell is electrically more compact, so somatic depolarization would reach the autapses and trigger neurotransmitter release.

## Selective Formation of Autapses in A Subpopulation of Neocortical PCs

Using Golgi preparations of the rabbit occipital cortex, Van der Loos and Glasers found self-innervating synapses in half of the PCs examined [1]. Later anatomical studies in electrophysiologically-recorded PCs reported inconsistent results. Most of the recorded layer V PCs (80%) in somatosensory cortical slices of juvenile rats possess autaptic contacts [13], whereas only 10% of the PCs in the superficial layers (layers II–IV) in adult cat visual cortex have autapses [6]. Typical synaptic structure of autapses was revealed by electron microscopy in both studies. Since GABAergic autapses only occur in fast-spiking interneurons but not low-threshold spiking interneurons [6, 7], it is of interest to know whether the abundance of autaptic PCs is cell-type-specific.

In our experiments, we used two approaches to investigate the abundance of PC autapses in cortical slices. In the paired soma-axon recording experiments together with 2-photon axotomy, we found that $\sim$ 55% of the layer V PCs showed autaptic responses. Adding $Sr^{2+}$ to the bath provided an efficient way to assess the abundance of autapses on PCs. In the presence of $Sr^{2+}$, the percentage of layer V PCs with autapses was similar to that in the axotomy experiments. In sharp contrast, considerably fewer layer II/III PCs ($\sim$ 12%) exhibited autaptic responses. Among the layer V PCs, subcortically-projecting cells tended to have autapses (up to 85%), whereas those projecting to the contralateral PFC tended not to form autapses (only 3%). These results indicate a selective formation of autapses in layer V PCs projecting to subcortical regions, such as the pontine nuclei and the habenula. The inconsistent anatomical observations noted above might be attributable to differences among the cortical layers. However, whether the abundance of autaptic PCs depends on species, brain region, and age needs to be further examined.

## PC Autapses Produce Giant AMPA-Only Autaptic Responses

Compared with the EPSCs between two layer V PCs, the aEPSCs were much larger, $\sim$ 5-fold greater in amplitude than PC-PC EPSCs. The paired-pulse ratio of aEPSCs ($< 1$) was lower than that of EPSCs ($> 1$), indicating a higher release probability at PC autapses. Close examination of the reconstructed PCs revealed more putative autaptic contacts than PC-PC synaptic contacts. Thus, the higher release probability and more autaptic contacts may contribute to the generation of giant aEPSCs. Anatomical observation also revealed a preference for autapse formation at the basal dendrites, relatively close to the soma ($< 100$ μm from the soma).

Previous studies have demonstrated that recurrent PC-PC synapses contain both N-methyl-D-aspartate (NMDA) and non-NMDA receptors. To determine whether autapses express NMDA receptors, we performed experiments with a $Cs^+$-based pipette solution and a modified bath solution: replacing $Ca^{2+}$ with $Sr^{2+}$ to desynchronize glutamate release; omitting $Mg^{2+}$ but adding glycine or D-serine to enhance NMDA receptor activity at negative membrane potentials; adding picrotoxin to block fast inhibitory postsynaptic potentials; and adding TTX to block APs and epileptiform activity. The $Cs^+$ pipette solution made the cell more electrically compact. Voltage pulses at the soma caused long-lasting desynchronized aEPSC events. These events were completely blocked by NBQX, a selective AMPA/kainic acid receptor antagonist, indicating the absence of NMDA components. Consistently, the NMDA receptor antagonist APV had no effect on the decay time constant of mini-aEPSCs. However, it reduced the decay time constant of mini-EPSCs (presumably from other PCs). Therefore, autapses tend not to express NMDA receptors.

Apparently, the physiological properties of autapses differ from those of PC-PC synapses. Future electron microscopic studies are required for the examination of autaptic contacts and the expression of NMDA receptors in different synapses. It remains unknown whether individual autaptic contacts have larger numbers of release sites.

## Physiological Function of PC Autapses

Autapses in GABAergic interneurons produce IPSCs immediately after individual APs [7, 9–12]. This self-inhibition suppresses AP generation [7] and increases spiking precision in neocortical fast-spiking interneurons [9]. In addition, neurotransmission at GABAergic autapses can also be asynchronous under physiological conditions [10, 11] (*i.e.* vesicle release is not tightly coupled to individual APs, similar to the $Sr^{2+}$-induced desynchronized release); delayed and prolonged asynchronous GABA release at autapses induced by high-frequency firing reduces the reliability of subsequent APs in fast-spiking interneurons [11]. These studies reveal an important role of GABAergic autapses in regulating neuronal signaling. For

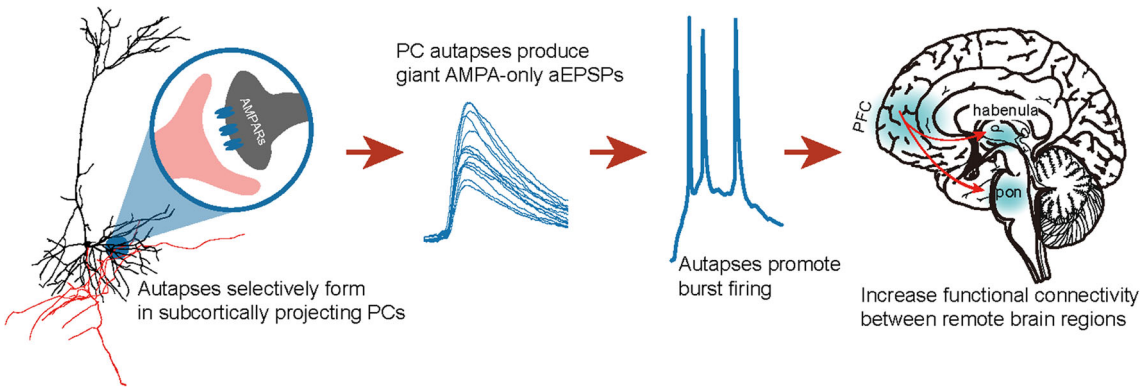


**Fig. 1** Glutamatergic autapses selectively form in layer V PCs projecting to subcortical regions. PC autapses produce giant AMPA-only autaptic postsynaptic responses, which promote burst firing and the coincidence-detection of self-activity and other inputs. PC bursts reliably cause postsynaptic responses and spiking activity (due to supralinear summation) in the target regions, thus enhancing functional connectivity between remote brain regions. Note that the axon of the reconstructed PC is shown in red

excitatory autapses, studies have shown that cholinergic self-excitation autapses produce long-lasting depolarization and persistent activity in *Aplysia* motor neurons [15]. Glutamatergic autapses have been shown to mediate interictal epileptiform activity and the paroxysmal depolarizing shift in solitary excitatory neuron microculture from rat hippocampus [4].

In layer V PCs in acute cortical slices, enhancing autaptic responses using cyclothiazide, a blocker of AMPA receptor desensitization, lowers the current threshold for the generation of a second AP. Conversely, blocking autaptic responses with kynurenic acid, an antagonist of ionotropic glutamate receptors, increases the current threshold. These results indicate that PC autapses increase neuronal responsiveness to an immediate input. Injection of artificial autaptic conductances with dynamic clamping immediately after AP generation shortens the inter-spike interval (*i.e.* increases the instantaneous firing frequency) and promotes burst firing. Loading the recorded PC with BAPTA, which inhibits autaptic responses, progressively reduces the intra-burst frequency. Together, these findings suggest an important role of autapses in promoting AP bursts in layer V PCs (Fig. 1), adding to the well-known ion channel mechanism for burst firing: an interaction between dendritic voltage-gated $Na^+$ and $Ca^{2+}$ channels and back-propagating APs [16], as well as the activation of a persistent $Na^+$ current at the first node of Ranvier [17]. Given that a single AP-induced synaptic transmission is unreliable due to synaptic failure, a burst of several APs could faithfully transmit a signal to the target cells. Thus, AP bursts may cause supralinear summation of postsynaptic potentials, increasing functional connectivity and thus synchronization between different brain regions. Considering that autapses selectively form in subcortically-projecting PCs in layer V, we speculate that PC autapses might promote the synchronization of activity between the cortex and subcortical regions (Fig. 1). In contrast, autapses may not be involved in synchronization between cortical regions in the two hemispheres because they do not form in PCs projecting to the contralateral cortex. If autapses only occur in the PFC, one would speculate that they could play critical roles in regulating PFC functions such as working memory, attention, and sociability. Compared with ion channels, autapses provide more targets for neuromodulation by transmitters including dopamine and acetylcholine.

It is surprising that PC autapses do not express NMDA receptors. Although pre- and postsynaptic APs at autapses are tightly coupled, the absence of NMDA receptors may prevent spike timing-dependent plasticity. Dynamic clamp experiments have demonstrated that AMPA-only autapses ensure strict coincidence-detection. If autapses expressed both AMPA and NMDA receptors, the NMDA component would prolong autaptic depolarization and widen the time window for a successful spiking response to inputs that arrived within this window. However, AMPA-only autapses provide a very narrow time window for subsequent synaptic inputs (*i.e.* these inputs have to arrive immediately after a particular AP), thus facilitating the coincidence-detection of self-activity and tightly-coupled inputs. Excitatory neurons would not need autaptic NMDA receptors to achieve slow excitatory feedback because polysynaptic connections can do the work. To achieve fast and immediate feedback after AP generation, they use the ideal hardware: AMPA-only autapses.

## Conclusions

Recent findings provide strong physiological evidence showing the existence of functional autapses in neocortical PCs, particularly those in layer V projecting to subcortical

brain regions [8]. PC autapses produce giant AMPA-only postsynaptic responses, which facilitate burst firing and allow strict coincidence-detection (Fig. 1). Together, these findings indicate that autapses are not wiring errors or redundant structures in the intact brain; they actually serve as an important element for cortical processing. Autapses, including those of GABAergic neurons, could be important substrates for cognitive brain functions, and should be considered in future computational models trying to reconstruct realistic neural networks. Furthermore, a list of questions should be addressed in future studies to better understand the structure, properties, and functions of autapses. For example, how autapses develop at early developmental stages, whether they express distinct synaptic molecules [18], why their postsynaptic responses are so large, whether they are subject to change in physiological [19] and disease states (*e.g.* autism [20] and epilepsy [10]), why there are no NMDA receptors, what their role is in the generation of network activity, and how brain function changes when they are eliminated or enhanced.

**Acknowledgements** This insight was supported by the National Natural Science Foundation of China (31430038, 31630029, and 31661143037).

**Conflict of interest** The authors declare that there is no conflict of interest.

## References

1. van der Loos H, Glaser EM. Autapses in neocortex cerebri: synapses between a pyramidal cell's axon and its own dendrites. Brain Res 1972, 48: 355–360.
2. Bekkers JM, Stevens CF. Excitatory and inhibitory autaptic currents in isolated hippocampal neurons maintained in cell culture. Proc Natl Acad Sci USA 1991, 88: 7834–7838.
3. Segal MM. Epileptiform activity in microcultures containing one excitatory hippocampal neuron. J Neurophysiol 1991, 65: 761–770.
4. Segal MM. Endogenous bursts underlie seizurelike activity in solitary excitatory hippocampal neurons in microcultures. J Neurophysiol 1994, 72: 1874–1884.
5. Bekkers JM. Synaptic transmission: functional autapses in the cortex. Curr Biol 2003, 13: R433–R435.
6. Tamas G, Buhl EH, Somogyi P. Massive autaptic self-innervation of GABAergic neurons in cat visual cortex. J Neurosci 1997, 17: 6352–6364.
7. Bacci A, Huguenard JR, Prince DA. Functional autaptic neurotransmission in fast-spiking interneurons: a novel form of feedback inhibition in the neocortex. J Neurosci 2003, 23: 859–866.
8. Yin L, Zheng R, Ke W, He Q, Zhang Y, Li J, *et al.* Autapses enhance bursting and coincidence detection in neocortical pyramidal cells. Nat Commun 2018, 9: 4890.
9. Bacci A, Huguenard JR. Enhancement of spike-timing precision by autaptic transmission in neocortical inhibitory interneurons. Neuron 2006, 49: 119–130.
10. Jiang M, Zhu J, Liu Y, Yang M, Tian C, Jiang S, *et al.* Enhancement of asynchronous release from fast-spiking interneuron in human and rat epileptic neocortex. PLoS Biol 2012, 10: e1001324.
11. Manseau F, Marinelli S, Mendez P, Schwaller B, Prince DA, Huguenard JR, *et al.* Desynchronization of neocortical networks by asynchronous release of GABA at autaptic and synaptic contacts from fast-spiking interneurons. PLoS Biol 2010, 8: e1000492.
12. Pouzat C, Marty A. Autaptic inhibitory currents recorded from interneurones in rat cerebellar slices. J Physiol 1998, 509: 777–783.
13. Lübke J, Markram H, Frotscher M, Sakmann B. Frequency and dendritic distribution of autapses established by layer 5 pyramidal neurons in the developing rat neocortex: comparison with synaptic innervation of adjacent neurons of the same class. J Neurosci 1996, 16: 3209–3218.
14. Shu Y, Hasenstaub A, Duque A, Yu Y, McCormick DA. Modulation of intracortical synaptic potentials by presynaptic somatic membrane potential. Nature 2006, 441: 761–765.
15. Saada R, Miller N, Hurwitz I, Susswein AJ. Autaptic excitation elicits persistent activity and a plateau potential in a neuron of known behavioral function. Curr Biol 2009, 19: 479–484.
16. Williams SR, Stuart GJ. Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. J Physiol 1999, 521: 467–482.
17. Kole MH. First node of Ranvier facilitates high-frequency burst encoding. Neuron 2011, 71: 671–682.
18. Mirza FJ, Zahid S. The role of synapsins in neurological disorders. Neurosci Bull 2018, 34: 349–358.
19. He C, Hu Z. Homeostasis of synapses: expansion during wakefulness, contraction during sleep. Neurosci Bull 2017, 33: 359–360.
20. Wang M, Li H, Takumi T, Qiu Z, Xu X, Yu X, *et al.* Distinct defects in spine formation or pruning in two gene duplication mouse models of autism. Neurosci Bull 2017, 33: 143–152.