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LETTER TO THE EDITOR

Selective Targeting of Perirhinal Cortex Projection to Hippocampal CA1 Interneurons

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Dear Editor,

The perirhinal cortex (PER) is conceptually important in the recognition memory, especially in familiarity discrimination, whereas the hippocampus is important for association and recollection [1]. This notion is known as the dual-process model of recognition memory. An alternative idea suggests that, like the hippocampus, the PER contributes to both familiarity and recollection [2], known for the unitary process model. Both models have made strong emphasis on a vital role of the PER and hippocampus in recognition memory, suggesting that they are highly interconnected. Indeed, previous animal studies using anterograde and retrograde tracers revealed direct anatomic projections from the PER to hippocampus in rodents and monkeys [3–5]. However, the latter projection is less prominent compared to that of PER to entorhinal cortex (EC) or that of EC to hippocampus. This may implicate that information flow from PER to hippocampus is primarily through an indirect path, where the EC is a major intermediator [3–6]. Consistently, the *in vivo* electrical stimulation results suggested a weak functional projection from PER directly to hippocampal dentate gyrus (DG) and CA1 areas, respectively [7, 8].

It remains unknown how the PER projections innervate different types of hippocampal CA1 neurons. To address this question, we used the channelrhodopsin-2 (ChR2) assisted circuit mapping (CRACM) method [9, 10] to map direct functional connections from the PER to dorsal hippocampal CA1 (d-HP CA1). Similar to our recent study that mapped the EC direct projection to dCA1 [10], we firstly injected the *AAV-hsynapsin-ChR2-mcherry* into the PER in the wild-type C57BL/6 mice at postnatal days 14–16, in order to achieve selective expression of light-activated optogenetic channels channelrhodopsin-2 (ChR2) in the PER neurons. After 4 weeks, ChR2(-mcherry) expression could be detected in the PER neurons and their projection axons within the DG molecular (MO) layer and the CA1 stratum lacunosum-moleculare (SLM) layer in the dorsal hippocampus (Fig. 1A), which is consistent with the previous observation [6].

In the CRACM experiment as previously described [10], activating those ChR2-expressing PER axons in the dCA1 with a 10 × 10 grid of laser beam stimuli on acute hippocampal slices allowed us to specifically map out their synaptic connections to targeted hippocampal neurons. The grid of 473-nm laser spot stimuli spaced by 40-μm intervals, covering the CA1 *SLM* and DG *MO* layers, was used to activate ChR2 channels on the PER axons (Fig. 1B). The resultant excitation by 0.5 mW–2 mW laser pulse (1-ms duration) could elicit synaptic transmission of direct PER-CA1 connections on distal apical dendrites of a dCA1 neuron so that excitatory postsynaptic currents ($EPSC_{CRACM}$) were detected by whole-cell recording on the soma. The mean latency of evoked $EPSC_{CRACM}$ was 5.84 ± 0.40 ms (n = 5), and averaged $EPSC_{CRACM}$ amplitudes of corresponding stimulation locations were

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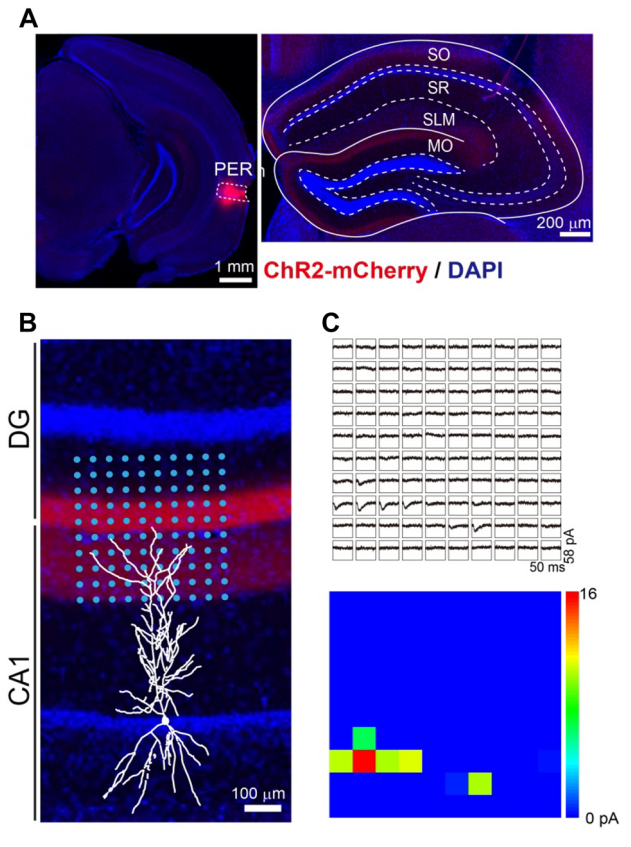

Fig. 1 Viral expression of ChR2 in the PER and CRACM of the PER-dCA1 connections. **A** Fluorescence images showing ChR2(-mcherry) expression in the PER 4 weeks after a stereotaxic injection of *AAV-hsynapsin-ChR2-mcherry* into the PER (left) and the distribution of PER axons in the dorsal hippocampus (right). **B** Diagram of CRACM experiment with a 10 × 10 grid of laser spot stimulation. **C** Averaged $EPSC_{CRACM}$ traces of 5 sweeps of laser stimulation to individual locations (upper) and the heat-map of $EPSC_{CRACM}$ amplitude (bottom) for a recorded CA1 PC.

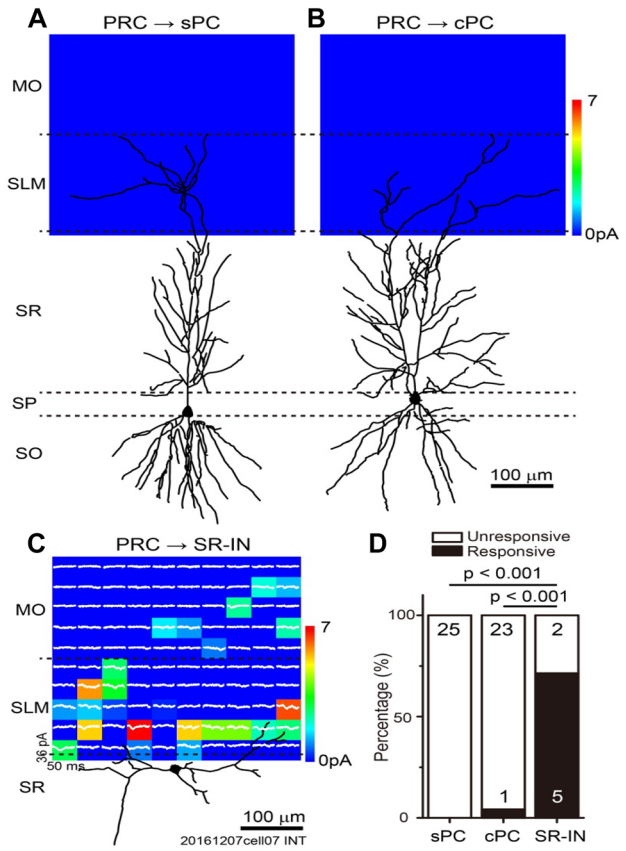

Fig. 2 PER axons selectively innervate dCA1 inhibitory interneurons. **A–C** The responsive dendritic heat-maps for the PER inputs ($EPSC_{CRACM}$ activated by laser spots at 2-mW) overlaid on the reconstructed morphologies of excitatory sPC (**A**) or cPC (**B**) within the *SP* layer or an inhibitory IN in the *SR* layer (**C**). **D** Percentages of sPCs, cPCs and *SR*-INs that were responsive (with $EPSC_{CRACM}$, black) or unresponsive (white) to laser activation of ChR2-expressing PER axons at maximum 5 mW power. The *P* value: Fisher exact test; *n*: number of examined cells.

transformed into a spatial heat-map showing dendritic field of the synapse (Fig. 1C). Cells with no apparent $EPSC_{CRACM}$ under laser stimuli at maximal 5 mW laser power were defined as the unresponsive cells, indicating no direct functional connections.

Based on post-recording construction of neuronal morphology, the recorded excitatory pyramidal cells (PCs) were located mainly within the CA1 stratum pyramidal (SP) layer and were further differentiated into two major subtypes, the simple PC (sPC) and complex PC (cPC) according to our recent study [10]. Typically, sPCs had a single apical dendritic trunk and their cell body preferentially resided in the deep SP layer, while cPCs distinctively exhibited twin apical trunks that branched out a position near the soma (Fig. 2A). Meanwhile, the recorded inhibitory interneurons (INs) were defined mainly based on reconstructed neuronal morphology and their location within the SR-layer [11]. In total, we recorded 25 sPCs, 24 cPCs and 7 inhibitory INs located within the SR and SLM layers (SR-IN) in the dCA1, respectively, in the hippocampal slices prepared from 4 mice four weeks after stereotaxic injection of *AAV-hSyn-ChR2* in the PER. We found that no $EPSC_{CRACM}$ was detected in all examined sPCs (100% non-responsive, $n = 25$) and in nearly all cPCs (95.8% non-responsive, $n = 24$) under stimulation of laser beam at maximal power (Fig. 2A, B, D). In contrast, evoked $EPSP_{CRACM}$ events could be detected in 5 out of 7 recorded inhibitory SR-INs. It was noted that the soma of responsive INs resided mainly along the border of SLM and SR layers (Fig. 2C, D; Fig. S1). These results suggest that in the dCA1, PER projection axons preferentially form their direct excitatory synapses on the inhibitory SR-INs, but innervate very few excitatory PCs. Such distinct PER-CA1 connections can result in a feed-forward inhibition, rather than excitation, to the CA1 principal PC population when the PER is activated.

This finding of selective targeting of the PER projection to hippocampal CA1 inhibitory INs can provide an circuit basis for a previous finding that electric stimulation to the PER elicited a rapid field potential sink (or excitation) in the DG MO layer only, but not in the CA1 SLM layer [7, 8]. These results together support a notion that the PER provide very few excitatory inputs to most CA1 PCs. Anatomically, PER projection axons were found to be sparely dispersed within the CA1 SLM layer and substantially less dense in other hippocampal areas compared to the EC projections [3–6].

In addition to its direct projection to hippocampal CA1 INs (Fig. 2), the PER also indirectly transfer synaptic information into the CA1 *via* the EC-hippocampus path. It is supported by the observation of dense PER projection axons in the superficial layers of both lateral and medial parts of EC [5, 6, 12, 13] . EC granule cells and PCs in the superficial 2/3 layers are known to send their excitatory projections into hippocampal DG and CA1 areas, respectively [10, 14]. Moreover, the direct PER projection could also be observed to the subiculum [8, 15], one of major downstream recipients of hippocampal CA1 outputs. Thus, it is of interest to further elucidate how the PER is interwired with other parts of hippocampal network and how these PER-associated circuits differentially contribute to hippocampus-dependent recognition memory.

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