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Parvalbumin-positive CA1 interneurons are required for spatial working but not for reference memory

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Abstract

Parvalbumin-positive GABAergic interneurons in cortical circuits are hypothesized to control cognitive function. To test this idea directly, we functionally removed parvalbumin-positive interneurons selectively from hippocampal CA1 in mice. We found that parvalbumin-positive interneurons are dispensable for spatial reference, but are essential for spatial working memory.

How the diverse types of cortical GABAergic interneurons influence behavior is unknown¹. Interest has concentrated on interneurons expressing parvalbumin, as they are selectively damaged in schizophrenia and by drugs of abuse such as ketamine and phencyclidine^{2–5}. To directly test circuit-specific functions of parvalbumin-positive interneurons in behaving mice, we specifically blocked parvalbumin-cell synaptic output in hippocampal CA1 with tetanus toxin light chain (TeLC)⁶. TeLC cleaves VAMP2 and stops vesicle fusion.

We stereotactically infused adeno-associated viruses (AAVs) containing the *GFP*-tagged *TeLC* (or *GFP* alone as control) reading frame inverted in a *flip-excision* (*FLEX*) cassette (AAV-*FLEX-TeLC* and AAV-*FLEX-GFP*) into *parvalbumin* (*Pvalb*)-*cre* transgenic mice^{7,8}. Thus, viral transgene expression can only occur in parvalbumin-expressing cells⁹ (Fig. 1a,b). We tested the method by unilateral AAV-*FLEX-TeLC* injection into the globus pallidus and reticular thalamus of *Pvalb-cre* mice. As expected, TeLC expression in parvalbumin-positive cells of these structures significantly increased ipsilateral turning compared with AAV-*FLEX-GFP*-injected controls ($P = 0.02$; Supplementary Fig. 1).

Bilateral AAV-*FLEX-TeLC* injections into dorsal CA1 (CA1-PV-TeLC mice) produced TeLC expression in approximately 84% of dorsal CA1 parvalbumin-positive neurons (Fig. 1c–g). Triple-immunolabeling for TeLC, parvalbumin and somatostatin indicated that all known subtypes of parvalbumin-positive interneurons were targeted¹⁰ (Fig. 1 and Supplementary Fig. 2). In CA3 and dentate gyrus, however, fewer parvalbumin-positive cells were affected (Fig. 1). Expression of TeLC in parvalbumin-negative cells

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AUTHOR CONTRIBUTIONS A.J.M., J.-F.S., G.R., L.A., M.B., W.W. and P.W. designed the experiments. A.J.M., J.-F.S., C.M., L.A. and L.C. performed the experiments. Anatomical data were analyzed by A.J.M., L.A. and P.W. Electrophysiological data were analyzed by J.-F.S. and M.B. Behavioral data were analyzed by A.J.M., P.W. and G.R. A.J.M., G.R., J.-F.S., M.B., W.W. and P.W. co-wrote the manuscript.

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(approximately 1% of TeLC-positive cells) or outside of the hippocampus was rare. AAV-*FLEX-GFP* injections yielded similar expression of GFP (CA1-PV-GFP) and were used as controls (Supplementary Fig. 3).

VAMP2 immunoreactivity was strongly reduced in TeLC-positive terminals ($P = 0.00005$), suggesting impaired synaptic function (Supplementary Fig. 4). To test this, we performed whole-cell recordings from CA1 pyramidal neurons in slices from ventral hippocampus during extracellular stimulation in stratum pyramidale (Fig. 2a,b). Here, evoked inhibitory postsynaptic currents (eIPSCs) originate largely from interneurons with axons in stratum pyramidale, including parvalbumin-positive basket cells, parvalbumin-positive axo-axonic cells and cholecystokinin (CCK)-containing basket cells. To isolate parvalbumin-positive cell-evoked eIPSCs, we used the cannabinoid receptor agonist WIN55,212, which selectively reduces CCK-containing basket cell-evoked IPSCs by about 70% (ref. ¹¹). Independently of presynaptic activity, CA1-PV-TeLC pyramidal neurons received substantially less cannabinoid-insensitive inhibition than control CA1-PV-GFP pyramidal neurons ($P < 0.001$; Fig. 2c,d, Supplementary Fig. 5 and Supplementary Methods). Considering the 30% CCK-containing basket cell transmission expected to remain after WIN55,212 application¹¹, this indicated a successful block of parvalbumin-positive cell-mediated inhibition in CA1-PV-TeLC mice. The block developed between 6 and 10 d post-injection (Supplementary Fig. 5). Inhibition from CCK-containing basket cells was unaltered ($P = 0.282$, Mann-Whitney U test). Immediate early gene (*Arc*, also known as *Arg3.1*, and *c-Fos*) imaging revealed no differences in hippocampal excitability between CA1-PV-TeLC and CA1-PV-GFP mice (Supplementary Fig. 3).

How does disruption of CA1 parvalbumin-positive cell-mediated inhibition affect behavior? Both CA1-PV-TeLC and CA1-PV-GFP mice had no obvious neurological deficits and performed similarly in open-field and hole-board tests (Supplementary Fig. 6), suggesting that locomotion and anxiety were unchanged. Similarly, spatial reference memory (SRM), assessed in a radial arm water maze (RAWM) task with random release sites, but fixed platform location, was unchanged. Both groups showed learning over 5 training days as indicated by the shortening in swim path length (day effect, $P = 0.008$; group effect, $P = 0.1$) and progressive reductions in reference memory errors (marginal day effect, $P = 0.06$; group effect, $P = 0.13$) (ANOVA). In addition, both groups showed a clear spatial bias for the target arm during the probe test (CA1-PV-GFP, $P = 0.026$; CA1-PV-TeLC, $P = 0.0002$ relative to chance; $t < 1$ between groups; Fig. 2e and Supplementary Fig. 6). However, CA1-PV-TeLC mice had a strong deficit in spatial working memory (SWM) (re-entries into previously visited arms; $P = 0.001$, ANOVA; Fig. 2f), suggesting impaired processing of trial-specific information¹². The number of working memory errors correlated positively with the percentage of TeLC-expressing parvalbumin-positive cells in CA1 (Pearson correlation, $P = 0.04$). The integrity of SRM in CA1-PV-TeLC mice was validated in an open-field water maze (Supplementary Fig. 6). To confirm the specific deficit in SWM, we established a delayed (60 s) matching to sample/place task using a Y-maze configuration in the water maze (Supplementary Methods). The percentage of correct responses in match trials reflects the trial-unique short-term storage of information and is hippocampus dependent^{12,13}. Again, CA1-PV-TeLC mice were significantly impaired ($P = 0.025$, ANOVA; Fig. 2g). Comparable results were obtained after more restricted parvalbumin-positive cell inactivation in CA1 (Supplementary Fig. 7), underpinning a crucial role of CA1 parvalbumin-positive cells in SWM. CA1-PV-TeLC mice showed similar deficits in working memory (RAWM, $P = 0.032$; Y-maze, $P = 0.048$), but no impairment in reference memory ($P = 0.415$) when compared with uninjected wild-type mice (ANOVA). However, CA1-PV-GFP and wild-type mice did not differ in any memory test.

To control for the possibility that inactivation of CA1 parvalbumin-positive cells caused a general failure of the CA1 circuitry rather than a selective deficit, we lesioned dorsal CA1 bilaterally with ibotenic acid and tested RAWM performance. CA1-lesioned mice had similar deficits in SWM ($P = 0.002$ compared with controls) as CA1-PV-TeLC mice and also showed significant impairment in SRM ($P = 0.006$, ANOVA; Supplementary Fig. 8). To determine whether SWM also required parvalbumin-positive cells in other hippocampal regions, we made exploratory injections of AAV-*FLEX-TeLC* into dorsal dentate gyrus coordinates of *Pvalb-cre* mice. This also generated SWM deficits ($P = 0.001$). However, although AAV-*FLEX-GFP*-injected controls showed significant reductions in path length ($P = 0.0001$) and SRM errors ($P = 0.01$), AAV-*FLEX-TeLC*-injected mice did not ($P = 0.148$ and $P = 0.4$, respectively) (ANOVA), suggesting that parvalbumin-positive cells may have additional functions in other hippocampal areas.

Hippocampal lesion and inactivation disrupt both spatial reference and working memory^{12,14,15}. We identified a population of hippocampal CA1 interneurons that selectively supports encoding of spatial working, but not incremental reference memory. Further insight will require manipulating specific parvalbumin-positive cell subtypes and other interneuron subpopulations^{1,10}. The working memory deficits found in schizophrenia are usually attributed to parvalbumin-positive cell impairment in the prefrontal cortex^{2–4}. Our findings suggest that parvalbumin-positive cell dysfunction in the hippocampus⁵ could also account, at least in part, for these deficits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Selective expression of TeLC in parvalbumin-positive interneurons of the hippocampus. **(a)** AAV construct and recombination sequence for Cre-dependent TeLC expression. The coding region is inverted between sets of heterotypic anti-parallel *loxP* sites. Cre inverts the coding region (1) and locks it in the correct orientation (2). *CBA*, *CMV enhancer/chicken β -actin* promoter. **(b)** After injection of AAV-*FLEX-TeLC* into CA1 of *Pvalb-cre* mice, TeLC selectively blocks transmitter release in parvalbumin-positive neurons. All other neurons are unaffected (PV, parvalbumin; SV, synaptic vesicle). **(c)** Immunostaining for TeLC in a coronal section of an AAV-*FLEX-TeLC*-injected *Pvalb-cre* brain. DG, dentate gyrus. Scale bar represents 500 μ m. **(d–f)** Magnification of boxed area in c showing parvalbumin expression **(d)**, TeLC expression **(e)** and their colocalization **(f)**. Scale bar represents 50 μ m. **(g,h)** Percentages of TeLC-expressing parvalbumin-positive cells along the rostro-caudal CA1 axis **(g)** and in different hippocampal subfields **(h)**. **(i)** Fraction of TeLC-positive cells in different parvalbumin-positive interneuron subpopulations. Som, somatostatin; St. oriens, stratum oriens; St. pyram, stratum pyramidale. Data are mean $\pm$ s.e.m. All procedures involving experimental mice were in accordance with the UK Animals (Scientific Procedures) Act 1986 and approved by the Ethical Review Committee of the University of Aberdeen.

Figure 2.

Blocking parvalbumin-positive cell-mediated inhibition in the hippocampus impairs spatial working but not reference memory. (a) Recording configuration. Scale bar represents 100 μm . (b) Magnification of boxed area in a. Immunostaining revealed strong TeLC expression in putative parvalbumin-positive terminals. Scale bar represents 5 μm . (c) Average IPSCs evoked during control conditions (black) and during WIN55,212 (orange). (d) Summary graph of normalized eIPSC peak amplitudes during WIN55,212. *** $P < 0.001$. (e) Both CA1-PV-TeLC and CA1-PV-GFP mice acquired the platform location in the RAWM as indicated by a reduction in path length. (f) CA1-PV-TeLC mice made significantly more working memory errors over the 5 d of training. (g) Delayed matching to sample/place task. CA1-PV-TeLC mice were not different from controls in sample trials (white bars), but did not improve in match trials (gray bars). Data are mean $\pm$ s.e.m. * $P < 0.05$.