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

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Competing Interests Statement

The authors declare they have no competing financial interests.

Author contributions

A.J.M., J.-F.S., G.R., L.A., M.B., W.W. and P.W. designed experiments. A.J.M., J.-
F.S., C.M., L.A. and L.C. performed 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.; Behavioural 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.

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

How the diverse types of cortical GABAergic interneurons influence behaviour is unknown¹. Interest has concentrated on interneurons expressing PV, as they are selectively damaged in schizophrenia and by drugs of abuse such as ketamine and phencyclidine²⁻⁵. To directly test circuit-specific functions of PV⁺ interneurons in behaving animals we specifically blocked PV-cell synaptic output in hippocampal CA1 with tetanus toxin light chain (TeLC)⁶. TeLC cleaves VAMP2, and stops vesicle fusion.

We stereotaxically 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 *PV-Cre* transgenic mice^{7,8}. Thus viral transgene expression can only occur in PV-expressing cells⁹ (**Fig. 1a,b**). We tested the method by unilateral *AAV-FLEX-TeLC* injection into the globus pallidus and reticular thalamus of *PV-Cre* mice. As expected, TeLC expression in PV⁺ 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 PV-neurons (**Fig. 1c-g**). Triple-immunolabelling for TeLC, PV and somatostatin (SOM) indicated that all known sub-types of PV⁺ interneurons were targeted¹⁰ (**Fig. 1i** and **Supplementary Fig. 2**). In CA3 and dentate gyrus (DG), however, fewer PV⁺ cells were affected (**Fig. 1h**). Expression of TeLC in PV-negative cells (approximately 1% of TeLC-positive cells) or outside the hippocampus was rare. *AAV-FLEX-GFP* injections gave 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 (PNs) 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 PV⁺ basket cells (BCs), PV⁺ axo-axonic cells and cholecystokinin(CCK)-containing BCs. To isolate PV-cell-evoked eIPSCs we used the cannabinoid receptor agonist WIN55,212, which selectively reduces CCK-BC-evoked IPSCs by about 70%¹¹. Independent of presynaptic activity CA1-PV-TeLC PNs received substantially less cannabinoid-insensitive inhibition than control CA1-PV-GFP PNs ($p<0.001$; **Fig. 2c,d**; **Supplementary Fig. 5** and **Supplementary Methods** online). Considering the 30% CCK-BC transmission expected to remain after WIN55,212 application¹¹, this indicated a successful block of PV-cell-mediated inhibition in CA1-PV-TeLC mice. The block developed between 6 and 10 days post-injection (**Supplementary Fig. 5**). Inhibition from CCK-BCs was unaltered ($p=0.282$, Mann-Whitney U-test). Immediate early gene (*Arc/Arg3.1* and *c-fos*) imaging showed no differences in hippocampal excitability between CA1-PV-TeLC and CA1-PV-GFP mice (**Supplementary Fig. 3**).

How does disruption of CA1 PV-cell-mediated inhibition affect behaviour? 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 unchanged locomotion and anxiety. 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). Additionally, 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 PV-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 (60s) matching to sample/place task using a Y-maze configuration in the water maze (**Supplementary Methods** online). The percentage of correct responses in match trials reflects 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 PV-cell inactivation in CA1 (**Supplementary Fig. 7**), underpinning a crucial role of CA1 PV-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 un-injected wild-type mice (ANOVA). However, CA1-PV-GFP and wild-type mice did not significantly differ in any memory test.

To control for the possibility that inactivation of CA1 PV-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, but

in addition showed significant impairment in SRM ($p=0.006$, ANOVA; **Supplementary Fig. 8**). To see if SWM also required PV-cells in other hippocampal regions, we made injections of AAV-*FLEX-TeLC* into dorsal dentate gyrus (DG) coordinates of *PV-Cre* mice. This also generated SWM deficits ($p=0.001$). However, whereas AAV-*FLEX-GFP*-injected controls showed significant reduction 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 PV-cells may have additional functions in other hippocampal areas.

Hippocampal lesion/inactivation disrupts both spatial reference and working memory^{12,14,15}. Here, 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 PV-cell sub-types and other interneuron sub-populations^{1,10}. The working memory deficits found in schizophrenia are usually attributed to PV-cell impairment in the prefrontal cortex²⁻⁴. Our findings suggest that PV-cell dysfunction in the hippocampus⁵ could also account, at least in part, for these deficits.

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References

1. Klausberger, T. & Somogyi, P. *Science* **321**, 53-57 (2008).
2. Behrens, M.M. *et al.* *Science* **318**, 1645-1647 (2007).
3. Morris, B.J., Cochran, S.M. & Pratt, J.A. *Curr. Opin. Pharmacol.* **5**, 101-106 (2005).
4. Lewis, D.A., Hashimoto, T. & Volk, D.W. *Nat. Rev. Neurosci.* **6**, 312-324 (2005).
5. Zhang, Z.J. & Reynolds, G.P. *Schizophr. Res.* **55**, 1-10 (2002).
6. Yamamoto, M. *et al.* *J. Neurosci.* **23**, 6759-6767 (2003).
7. Fuchs, E.C. *et al.* *Neuron* **53**, 591-604 (2007).
8. Wulff, P. *et al.* *Proc. Natl. Acad. Sci. USA* **106**, 3561-3566 (2009).
9. Atasoy, D., Aponte, Y., Su, H.H. & Sternson, S.M. *J. Neurosci.* **28**, 7025-7030 (2008).
10. Baude, A., Bleasdale, C., Dalezios, Y., Somogyi, P. & Klausberger, T. *Cereb. Cortex* **17**, 2094-2107 (2007).
11. Glickfeld, L.L., Atallah, B.V. & Scanziani, M. *J. Neurosci.* **28**, 1824-1832 (2008).
12. Olton, D.S., Becker, J.T., & Handelmann, G.E. *Behavioral and Brain Science* **2**, 313-365 (1979).
13. Hampson, R.E., Jarrard, L.E. & Deadwyler, S.A. *J. Neurosci.* **19**, 1492-1507 (1999).
14. Reisel, D. *et al.* *Nat. Neurosci.* **5**, 868-873 (2002).
15. Riedel, G. *et al.* *Nat. Neurosci.* **2**, 898-905 (1999).

Figure Legends

Figure 1 Selective expression of TeLC in PV⁺ 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 *PV-Cre* mice, TeLC selectively blocks transmitter release in PV⁺ neurons. All other neurons are unaffected (SV, synaptic vesicle). **(c)** Immunostaining for TeLC in a coronal section of an AAV-*FLEX-TeLC*-injected *PV-Cre* brain. **(d–f)** Magnification of boxed area in (c) showing **(d)** PV expression, **(e)** TeLC expression and **(f)** their colocalization. **(g,h)** Percentages of TeLC-expressing PV-cells **(g)** along the rostro-caudal CA1 axis and **(h)** in different hippocampal subfields. **(i)** Fraction of TeLC-positive cells in different PV⁺ interneuron sub-populations. Data are mean \pm s.e.m. Scale bars: c, 500 μ m; d–f, 50 μ m. All procedures involving experimental mice were in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and approved by the Ethical Review Committee of the University of Aberdeen.

Figure 2 Blocking PV-cell-mediated inhibition in the hippocampus impairs spatial working but not reference memory. **(a)** Recording configuration. **(b)** Magnification of boxed area in (a). Immunostaining revealed strong TeLC expression in putative PV⁺ terminals. Scale bar: a, 100 μ m; b, 5 μ m. **(c)** Average IPSCs evoked during control conditions (black) and during WIN55,212 (orange). **(d)** Summary graph of normalized eIPSC peak amplitudes during WIN55,212. **(e)** Both CA1-PV-TeLC and CA1-PV-GFP mice acquired the platform location in the radial arm water maze as indicated by

a reduction in path length. **(f)** CA1-PV-TeLC mice make significantly more working memory errors over the 5 days 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 (grey bars). Data are mean \pm s.e.m. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

SI Guide

Supplementary Item & Number	Title or Caption
Supplementary Figure 1	Injection of AAV-FLEX-TeLC into the globus pallidus (GP) and reticular thalamus (RT) of PV-Cre mice causes motor deficits
Supplementary Figure 2	Classification of TeLC-expressing PV+ neurons in CA1 according to somatostatin (SOM) immunoreactivity
Supplementary Figure 3	CA1-PV-GFP control mice show similar viral transgene and immediate early gene expression as CA1-PV-TeLC animals
Supplementary Figure 4	VAMP2 immunoreactivity is strongly reduced in presynaptic terminals of TeLC-expressing neurons
Supplementary Figure 5	Loss of PV-cell-mediated inhibition develops between days 6 and 10 after virus injection and persists during high frequency action potential firing
Supplementary Figure 6	CA1-PV-TeLC mice show no significant changes in locomotion, exploratory behaviour, anxiety or spatial reference memory
Supplementary Figure 7	CA1-restricted inactivation of PV-cells causes spatial working memory deficits in a delayed matching to sample/place task
Supplementary Figure 8	Fibre-sparing lesions of dorsal CA1 with ibotenic acid disrupt spatial working and spatial reference memory in the radial arm water maze
Supplementary Methods	Supplementary Methods

Figure 1 (Wulff)

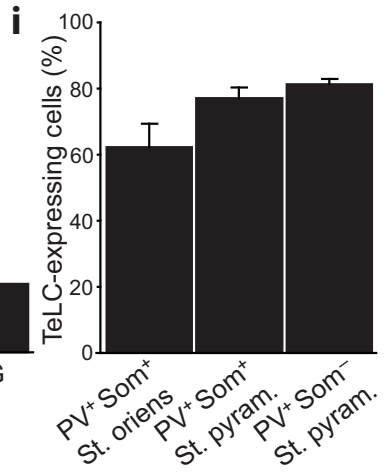
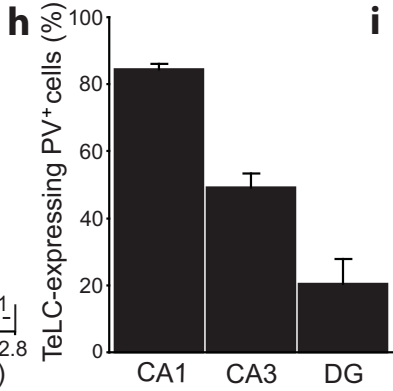
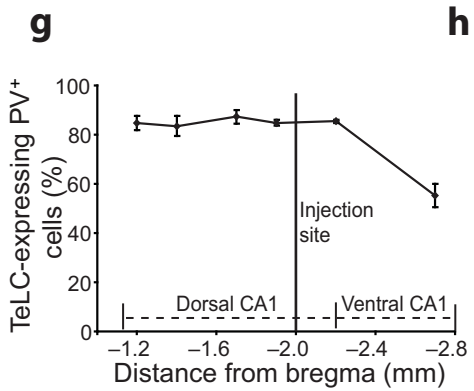
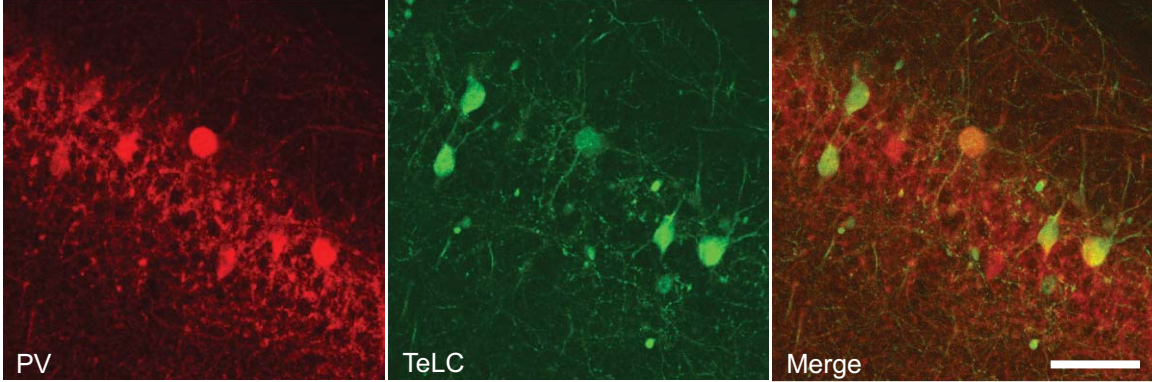
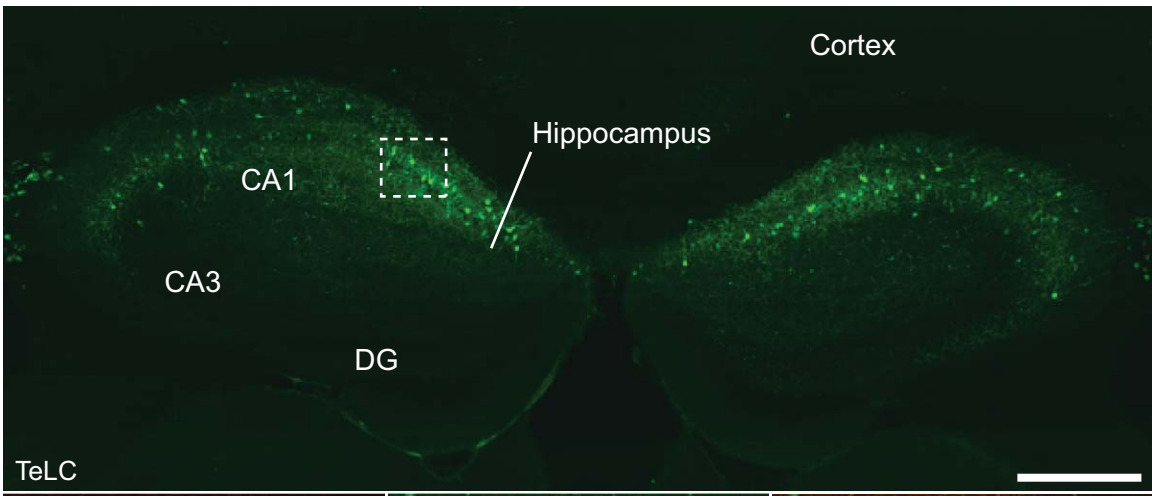
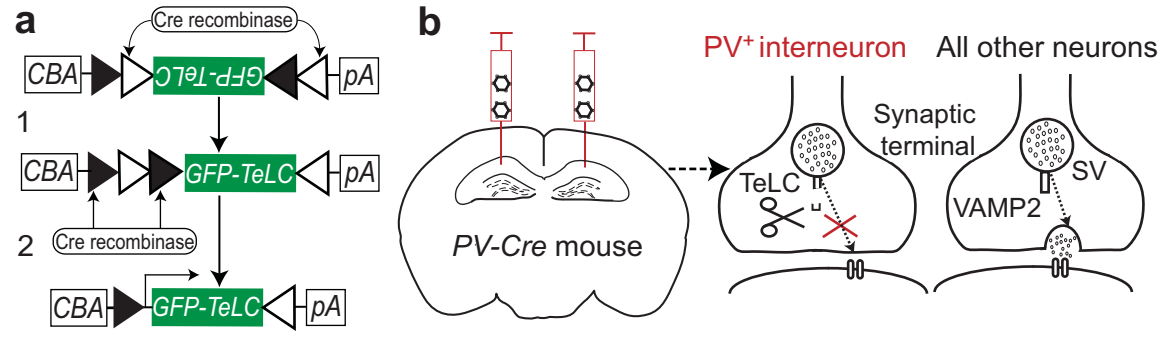


Figure 2 (Wulff)

