



HAL
open science

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

Andrew J. Murray, Jonas- Frederic Sauer, Gernot Riedel, Christina Mcclure, Laura Ansel, Lesley Cheyne, Marlene Bartos, William Wisden, Peer Wulff

► **To cite this version:**

Andrew J. Murray, Jonas- Frederic Sauer, Gernot Riedel, Christina Mcclure, Laura Ansel, et al.. Parvalbumin-positive CA1 interneurons are required for spatial working but not reference memory. Nature Neuroscience, 2011, 10.1038/nn.2751 . hal-00612704

HAL Id: hal-00612704

<https://hal.science/hal-00612704>

Submitted on 30 Jul 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Andrew J Murray^{1,2}, Jonas-Frederic Sauer^{1,3}, Gernot Riedel¹, Christina McClure¹,
Laura Ansel¹, Lesley Cheyne¹, Marlene Bartos^{1,3}, William Wisden^{1,4}, Peer Wulff¹

¹ Institute of Medical Sciences, Foresterhill, University of Aberdeen, Aberdeen, AB25
2ZD, UK

² Current address: Department of Biochemistry and Molecular Biophysics, Columbia
University, New York, NY, 10037, USA

³ Current address: Institute for Physiology I, University of Freiburg, Engesserstrasse
4, 79108 Freiburg, Germany

⁴ Current address: Division of Cell and Molecular Biology, Imperial College London,
London, SW7 2AZ, UK

Correspondence should be addressed to

P.W.: p.wulff@abdn.ac.uk

W.W.: w.wisden@imperial.ac.uk

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.

Acknowledgements

We thank M. Klugmann and T. Kuner for AAV plasmids and technical advice, E. Fuchs and H. Monyer for *PV-Cre* mice, R. Yu for *tetanus toxin light chain* cDNA, D. Kuhl for the *Arc/Arg3.1* cDNA, T. Goetz for bringing the *FLEX* system to our attention, C. Black for participation in the initial cloning of AAV vectors, P. Teismann and the microscopy core facility at the University of Aberdeen for the use of microscopy equipment, L. Strachan, A. Plano and S. Deiana for help with surgeries and behavioural testing. We were supported by the Northern Research Partnership (JFS), BBSRC grant BB/H001123/1 (PW), the Royal Society (MB & PW), the University of Aberdeen (MB, WW & PW), the J. Ernest Tait Estate (WW), the Lichtenberg Award (MB) and Medical Research Council grants G0800401 (GR) and G0601498 (WW & PW).

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)

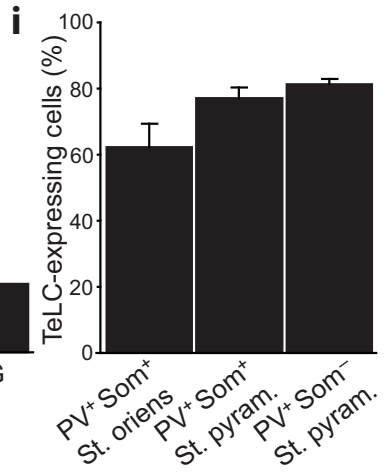
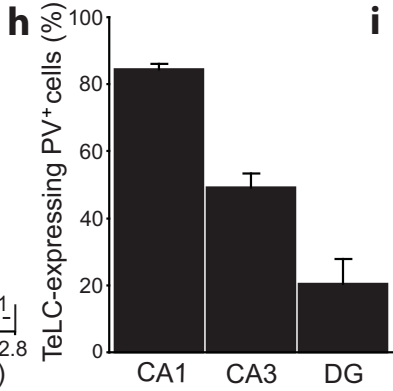
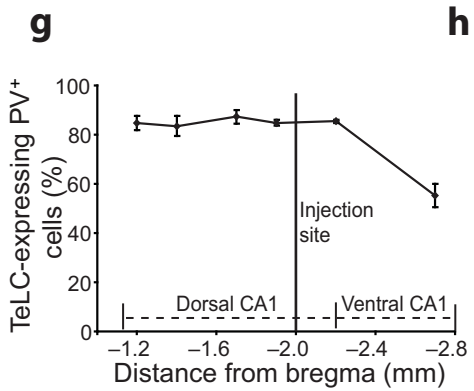
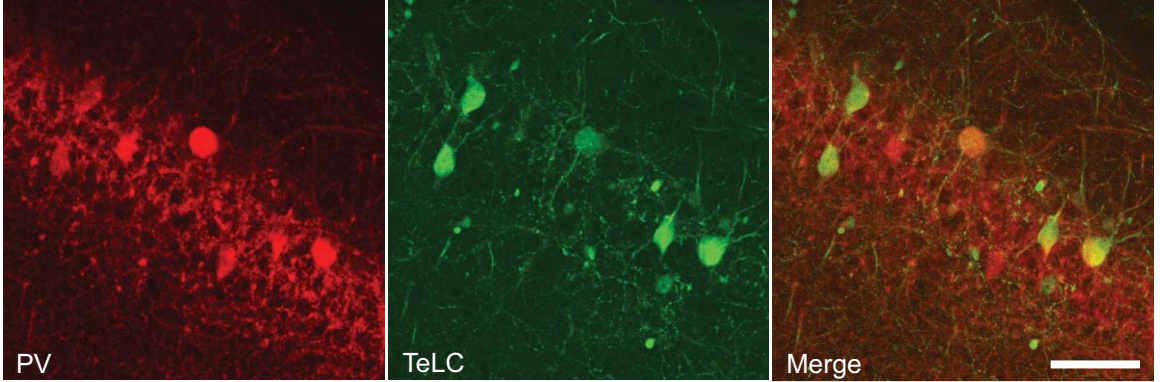
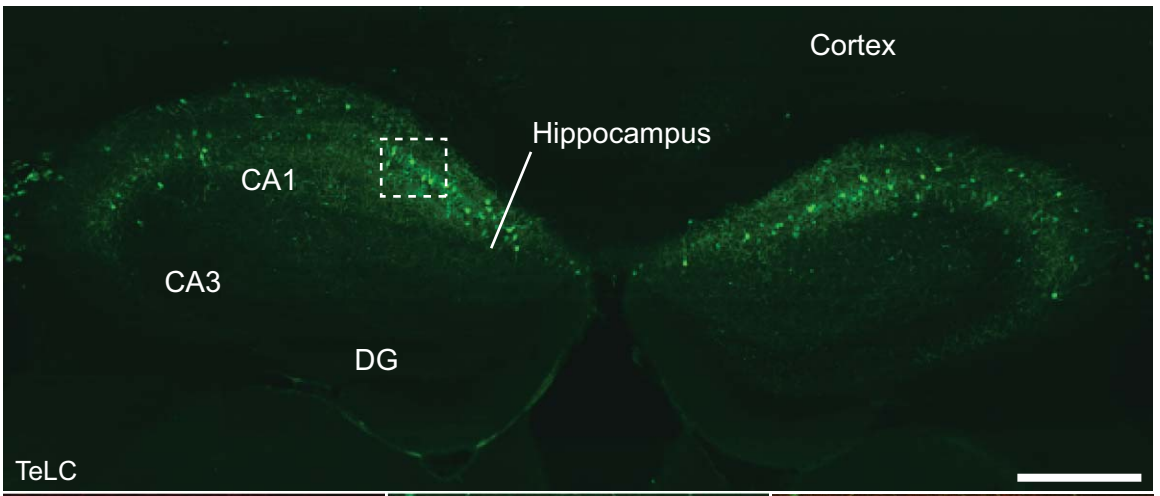
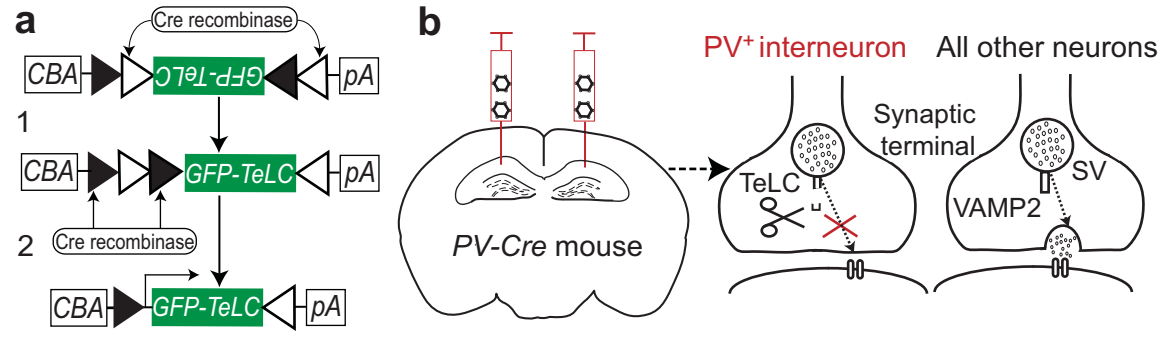


Figure 2 (Wulff)

