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Transgenic labeling of parvalbumin-expressing neurons with tdTomato

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Summary

Parvalbumin (PVALB)-expressing fast-spiking interneurons subserve important roles in many brain regions by modulating circuit function and dysfunction of these neurons is strongly implicated in neuropsychiatric disorders including schizophrenia and autism. To facilitate the study of PVALB neuron function we need to be able to identify PVALB neurons in vivo. We have generated a bacterial artificial chromosome (BAC) transgenic mouse line expressing the red fluorophore tdTomato under the control of endogenous regulatory elements of the Pvalb gene locus (JAX # 027395). We show that the tdTomato transgene is faithfully expressed relative to endogenous PVALB expression throughout the brain. Furthermore, targeted patch clamp recordings confirm that the labeled populations in neocortex, striatum, and hippocampus are fast-spiking interneurons based on intrinsic properties. This new transgenic mouse line provides a useful tool to study PVALB neuron function in the normal brain as well as in mouse models of psychiatric disease.

Introduction

Parvalbumin (PVALB) is a calcium-binding protein that is expressed in about 40–50% of GABAergic interneurons (Wonders and Anderson, 2006, Xu et al., 2010, Rudy et al., 2011). Physiologically, synchronous activity of these fast-spiking interneurons generates neuronal oscillatory waves at 30–80 Hz, called gamma rhythms (Buzsáki and Draguhn, 2004, Cardin

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Author contributions
T.K., J.T.T. and G.F. conceived the study and oversaw the project. J.T.T. generated the transgenic mice. T.K. performed the characterization and T.K. and P.M. analyzed the data. T.K. and G.F. wrote the manuscript with inputs from all authors.
et al., 2009, Sohal et al., 2009), which have been hypothesized to play an important role in attention and cognition (Jensen et al., 2007). In human pathophysiology, postmortem studies with tissue from schizophrenic patients indicate GABAergic interneuron defects and a reduced number of GABAergic synapses (Woo et al., 1998), as well as reduced PVALB expression in the neocortex (Hashimoto et al., 2003). In addition, animal model studies support the idea that defects in PVALB neurons may be linked to cognitive abnormalities in neuropsychiatric diseases (Lewis et al., 2005, Belforte et al., 2010, Peñagarikano et al., 2011, Uhlhaas and Singer, 2012, del Pino et al., 2013, He et al., 2014).

Recent large-scale genetic studies implicate deleterious mutations across many genes in psychiatric disease, a substantial fraction of which are synaptic (Fromer et al., 2014, Purcell et al., 2014). In the future, many of these genes will be studied in mouse models, and elucidating PVALB neuron circuit function in these models will critically depend on means to identify PVALB-expressing neurons. However, faithful fluorescent reporter lines that would facilitate the direct identification of PVALB neurons in the brain are currently unavailable from common repositories and crossing of a Pvalb-Cre line to a Cre-dependent fluorescent reporter line is time consuming and expensive, in particular for the study of disease-associated mutations in animal models. Here, we report the generation and characterization of a BAC transgenic reporter line, where parvalbumin neurons express the red fluorophore tdTomato (JAX # 027395). We show that tdTomato is expressed throughout the brain and that the labeling is bright and confined to PVALB-expressing neurons. We further show that tdTomato-expressing neurons in the cortex, striatum and hippocampus display a fast-spiking interneuron electrophysiological signature. This Pvalb-tdTomato reporter line provides a valuable tool for the study of PVALB neuron function.

Results

Generation of the BAC-transgenic tdTomato reporter line

PVALB is expressed in various regions throughout the brain encompassing the somatosensory cortex, motor cortex, entorhinal cortex, striatum, hippocampus, thalamic reticular nucleus (TRN), globus pallidus and cerebellum (Celio, 1990). Our previously generated Pvalb-ChR2-EYFP BAC transgenic line displayed functional transgene expression in cerebellum and TRN, but not in neocortex (Zhao et al., 2011). In order to drive expression of the red fluorophore tdTomato specifically in PVALB-expressing neurons in multiple brain regions including the neocortex, we adopted an improved BAC transgenesis approach (Figure 1, (Ting and Feng, 2013, 2014, Ting and Feng, 2015). Specifically, we chose the Pvalb-spanning BAC clone RP24-306A6, which harbors more endogenous 5’ sequences and thus is more likely to contain all Pvalb regulatory elements. We employed BAC recombineering to insert a tdTomato cDNA followed by the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) and a bovine growth hormone polyadenylation signal (BGHpA). The addition of the WPRE upstream of the polyadenylation site improves mRNA stability and protein expression level (Du et al., 2010). The entire transgenic expression cassette was inserted directly downstream of the Pvalb translation initiation codon, a strategy that avoids PVALB overexpression. Furthermore, to prevent additional expression of unwanted nearby genes and potential
confounding consequences on neurophysiology and behavior (Kolisnyk et al., 2013), we used a recently described BAC trimming method to remove the unwanted Ifi27 gene-coding region from the BAC clone (Ting and Feng, 2014).

**tdTomato is faithfully expressed in parvalbumin neurons**

Two distinct Pvalb-tdTomato BAC transgenic founder lines were established following pronuclear injection of the linearized BAC DNA. It was initially determined that the transgene expression patterns of the two lines were not identical, presumably owing to random BAC DNA insertion at different genomic loci and position effect variegation (Feng et al., 2000, Yang and Gong, 2005).

In order to test the level and precision of tdTomato expression in PVALB-expressing neurons, we performed immunohistochemistry to label endogenous PVALB. One founder line (line 9) displayed relatively low expression in the neocortex (data not shown) and was not further analyzed. In the second line (line 15), the fluorophore tdTomato was highly expressed throughout various brain regions including the somatosensory cortex, motor cortex, striatum, thalamic reticular nucleus, hippocampal CA3, CA1 and dentate gyrus, and the cerebellum (Figure 2).

To examine how faithfully tdTomato expression matches the pattern of PVALB expression in the mouse brain, we quantified the percentage of tdTomato-expression neurons that are also PVALB positive by anti-PVALB antibody staining, and the percentage of PVALB-positive neurons that are labeled with tdTomato. We found that 96.8% ± 2.1% of the tdTomato expressing cells in the somatosensory cortex are PVALB-expressing neurons and 91.2% ± 2.1% of PVALB-expressing neurons in the somatosensory cortex are labeled by tdTomato (Figure 3A, B). In the hippocampus, we examined tdTomato labeling in CA3 and CA1 regions and found that 98.6% ± 2.4% of tdTomato-labeled cells are actually PVALB-expressing neurons and 93% ± 2.8% of the PVALB-expressing neurons are revealed by tdTomato expression (Figure 4A, B). Similarly, faithful labeling was achieved in the striatum with 96.9% ± 1% of tdTomato expression confined to PVALB-expressing neurons and labeling of 98.1% ± 3.2% PVALB-expressing neurons with tdTomato (Figure 5A, B). Together, these data indicate that Pvalb-tdTomato line 15 transgenic mice generated with the new trimmed BAC clone nicely recapitulate the endogenous expression patterns of PVALB, thus providing a useful tool for identifying and studying PVALB-expressing neurons in the mouse brain.

**Electrophysiological characterization of tdTomato-expressing neurons**

Parvalbumin-expressing neurons are fast-spiking neurons that display characteristic electrophysiological signature. To test whether tdTomato-expressing neurons show these characteristics, we used whole-cell patch clamp in acute brain slices to examine passive and active membrane properties of tdTomato-expressing neurons in three representative brain regions. We targeted tdTomato-positive neurons (n=8 from 4 mice) in the somatosensory cortex and found fast-spiking suprathreshold firing patterns, sub-threshold membrane potential oscillations, and large amplitude fast after-hyperpolarizations (Figure 3C), which are characteristic of PVALB-expressing neurons (Kawaguchi et al., 1987, Koós and Tepper, 1999).
1999, Tepper and Bolam, 2004). We further found a linear relationship between current injection and membrane potential (I–V) and sustained high frequency firing during 500 ms current injection steps, which are expected for PVALB-expressing neurons in this brain region (Figure 3D,E). Similar results were obtained for neurons recorded in the CA1 region of the hippocampus and the striatum (Figure 4D,E, Figure 5D,E, n=8 from 4 mice for each region).

Next, we measured the half-width of the action potential using the first action potential train at rheobase current. We detected narrow action potential half-widths in tdTomato-expressing neurons in somatosensory cortex, striatum and hippocampal CA1 neurons. Other parameters measured, such as time constant of current decay (987.63 ± 34.07 µs in cortex, 1272.50 ± 25.28 µs in striatum, and 1271.75 ± 62.06 in hippocampal CA1 neurons), membrane time constant (7.23 ± 0.15 ms in cortex, 9.53 ± 0.46 ms in striatum and 12.15 ± 0.52 ms in hippocampal CA1 neurons), membrane resistance (118.88 ± 4.08 MΩ in cortex, 112.00 ± 2.26 MΩ in striatum, 177.34 ± 9.05 MΩ in hippocampal CA1 neurons), membrane potential (−65.36 ± 0.66 mV in cortex, −77.04 ± 0.74 mV in striatum, and −61.40 ± 0.64 mV in hippocampal CA1 neurons) were in accordance with known characteristics of PVALB-expressing neurons (Table 1). Taken together, these data show that tdTomato-expression in the Pvalb-tdTomato reporter mouse line is precisely confined to PVALB-expressing fast-spiking interneurons.

Discussion

PVALB neuron dysfunction is implicated in neuropsychiatric diseases such as autism and schizophrenia (Lewis et al., 2005, Uhlhaas and Singer, 2012), but studying these neurons in animal models is significantly complicated by the lack of simple means to identify them. To date, three lines with green spectrum fluorescent protein expression driven by the Pvalb promoter have been reported (Hazama et al., 2002, Meyer et al., 2002, Zhao et al., 2011). The Pvalb-EGFP and the hIL-2R–GFP BAC transgenic lines (Hazama et al., 2002, Meyer et al., 2002) are valuable and well validated tools, but have not been made widely available and has not been deposited to any public repository. The third line is the BAC transgenic EYFP-expressing line Pvalb(H134R)-ChR2-EYFP (JAX # 012355), which can be used to identify PVALB-expressing neurons in the cerebellum and thalamic reticular nucleus, but not the striatum, cortex and hippocampus due to insufficient transgene expression in these regions (Zhao et al., 2011). Alternatively, PVALB-expressing neurons can be identified using Pvalb-Cre mice crossed to a Cre-dependent fluorescent reporter line (Kuhlman and Huang, 2008, Madsen et al., 2010, Pfeffer et al., 2013). However, in order to interrogate parvalbumin neuron function in brain circuits of homozygous knockout or mutant mice, a highly complex breeding strategy to derive triple transgenic animals would be required, which is time consuming, inefficient and costly. In this context, use of BAC transgenic mice with direct expression of tdTomato in the target neuronal population would be preferable.

We therefore sought to generate an improved BAC transgenic line expressing a red fluorescent protein in PVALB-expressing neurons and make it widely available for distribution to researchers through the Jackson Laboratories (JAX) mouse repository. In our previous efforts to develop BAC transgenic mouse lines with cell type-specific expression of
ChR2-EYFP in the nervous system, the selected Pvalb-spanning BAC clone failed to drive sufficient levels of transgene expression in the neocortex (Zhao et al., 2011), which may be related to transcription-regulatory elements inherent to the BAC construct or post-translational processes (folding, membrane-trafficking). To solve this problem, we chose a cytosolic fluorophore and selected a different Pvalb-BAC clone that contains more 5’ sequences and is thus more likely to cover all regulatory elements that are critical determinants of PVALB expression in vivo. This strategy worked very well, as we were able to generate a new BAC transgenic mouse line in which tdTomato is highly expressed in all major regions of the brain that normally express PVALB. In addition, we used a BAC-trimming approach to remove a nearby gene in the BAC clone to avoid undesirable overexpression of this gene (Ting and Feng, 2014). This is an important step that has not been carried out in previously generated BAC transgenic mice and has resulted in lines with potential confounding and unintended overexpression of unwanted genes (Kolisnyk et al., 2013, Crittenden et al., 2014).

One important aspect of the new Pvalb-tdTomato transgenic mice (line 15, JAX # 027395, http://jaxmice.jax.org/strain/027395.html) is the high level tdTomato expression in the neocortex. Cortical PVALB-expressing neurons have been implicated in several psychiatric disorders, particularly schizophrenia and autism (Hashimoto et al., 2003, Lewis et al., 2005, Peñagarikano et al., 2011). Thus, this mouse line will be a valuable tool for studying not only cortical inhibitory circuits in normal brain function, but also in animal models of schizophrenia and potentially other psychiatric disorders. The high level of tdTomato expression in this specific line may be due to the choice of alternative BAC clone, the shorter BAC clone as a result of BAC trimming, the addition of the WPRE sequence, the cytosolic fluorophore or a combination of the above factors. Additionally, the use of a red fluorophore will facilitate the implementation of other sophisticated experimental paradigms in which one seeks to probe the function of PVALB-expressing neurons in the context of intact neural circuits, such as multicolor labeling in conjunction with green fluorescent protein-based tools, optical neural control with blue light using ChR2 and related opsin variants, or GCaMP imaging.

Materials and Methods

Animal work statement

All animal-related work was performed in accordance with the guidelines from the Division of Comparative Medicine (DCM) under protocol # 0513-044-16 approved by Committee for Animal Care (CAC) of Massachusetts Institute of Technology, and in consistency with the Guide for Care and Use of Laboratory Animals, National Research Council 1996 (institutional animal welfare assurance no. A-3125-01).

Bacterial artificial chromosome transgenesis

Targeting vector construction—A pBlueScript-derived vector iTV1 was used to generate BAC targeting vectors. iTV1 contains cloning sites for A and B homology arms which flank a large multiple cloning site, bovine growth hormone polyadenylation (BGHpA) signal, and FRT flanked neomycin resistance cassette (FRT-NEO-FRT). First,
iTV1 was modified by addition of the WPRE upstream of the BGHpA to create iTV1-WPRE. This modification was chosen to improve transgene protein expression level and transgene mRNA stability. *Pvalb* specific homology arms A and B (400–600 bp each arm) were PCR amplified from *Pvalb*-spanning template BAC DNA (clone RP24-306A6) and cloned into iTV1-WPRE to make iTV1-PvalbA/B-WPRE. The tdTomato cDNA was then sub-cloned into the multiple cloning site of iTV1-PvalbA/B-WPRE to complete the BAC targeting vector iTV1-Pvalb-tdTomato-WPRE. In this final cloning step great care was taken to ensure minimal disruptions to the junction between the A Box and the start of the tdTomato sequence, except for addition of a strong Kozak consensus sequence.

**Pvalb** A homology arm:

5’-

tgatgtccaccaacaacctcctctctctggtgtgcctgtggttcttttcaggatttttcgcggcataaatttgcatccagga
cagaagcagactacagagtcttctcatcagatgctgctcccttgtacctgtgctgctgtctgtgttggtggaggtgtgtgata
ttttttttccccggcagctgtctgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgataagttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
Critical regions of all constructs were verified by DNA sequencing. The targeting vectors were linearized by restriction digestion and purified in preparation for subsequent use in BAC recombineering steps. Care was taken to ensure adequate duration of restriction digestion (or in some cases two sequential rounds of digestions were performed) such that there was no contaminating uncut plasmid carried over into the electroporation step.

Additional information including complete DNA sequences of the targeting vectors and modified BAC clones used in this study are available upon request.

**BAC recombineering**—BAC clone RP24-306A6 that spans the *Pvalb* gene plus 50Kb of 5’ and 3’ flanking regions was selected from the RP23/RP24 C57 mouse BAC library following a detailed analysis using the Esembl Genome Browser (www.Ensembl.org) and was obtained from the Children’s Hospital Oakland Research Institute (CHORI). The intact BAC DNA was isolated from the original host DH10B strain by BAC mini-prep and verified by restriction digestion and pulsed field gel electrophoresis (PFGE). Successfully verified BAC DNA was transferred into the EL250 strain in preparation for recombineering steps and maintained as a glycerol stock stored at −80°C.

The first strategic recombineering step was BAC trimming to delete the unwanted region of BAC DNA harboring the extra gene *Ift 27*. 20 mL of LB media plus chloramphenicol were inoculated with EL250 cells containing the target BAC clone and placed in a shaking incubator at 32°C for 2 hours until the cells reached an early log growth phase. The culture was induced for 15 min at 42°C in a shaking water bath to permit transient expression of genes required for homologous recombination. Cells were rapidly chilled, pelleted, and washed three times with ice-cold MilliQ water to remove salts. Approximately 10–100 ng of linearized BAC trimming vector was added to 50 µL of induced cells in a 1 mm gap cuvette and the mixture was immediately electroporated (1.75 kV, 25 µF, 200 Ohms). The cells were recovered and plated onto LB plus chloramphenicol and carbenicillin to select for successful homologous recombination events, thereby indicating insertion of the AmpR cassette in place of the targeted region. Double resistant clones were picked for further analysis to verify the deletions.

In a second round of targeting the virtually identical steps were performed to insert the tdTomato transgene expression cassette at the initiating methionine start codon of the *Pvalb* gene within the RP24-306A6 BAC clone by homologous recombination. In this case, successfully modified clones were resistant to chloramphenicol, carbenicillin, and kanamycin due to insertion of the FRT-NEO-FRT in the targeting vector. (Note: although it is easily feasible to perform the first and second targeting events in a single step due to the insertion of two different selection cassettes, it is preferable to do these steps in series in order to save a glycerol stock of the successfully trimmed BAC in EL250 cells for future reuse and re-targeting of alternative transgenes). The NEO cassette was then excised from the modified BAC by arabinose induction of flp recombinase in the EL250 cells. Modified
BAC clones were extensively screened for accuracy and the correctly targeted BAC DNA was grown in large scale and purified using the BAC100 kit (Clontech). 10–15 µg of BAC DNA was restriction digested with NotI or AscI to liberate the BAC vector from insert and the linear fragments were separated by PFGE. The intact BAC insert band was excised from the PFG, electroeluted, and spot dialyzed against fresh microinjection buffer.

**Pronuclear injection and identification of transgenic founders**—Transgenic mice (pure C57BL/6 Taconic) were generated by pronuclear injection of the highly purified intact BAC DNA into fertilized oocytes at a concentration of 0.5–2.0 ng/µL. Transgene positive founders/offspring were determined by PCR from mouse tail DNA samples using the following line-specific primers: Pvalb-genotype-F, 5’- actgcagcgctggtcatatgagc-3’; tdTomato-R, 5’- ACTCTTTGATGACCTCCTCG-3’; positive reaction yields a 130bp band. Mice that had the transgene integrated in the genome were kept as founders to establish distinct lines by mating to C57BL/6J mice. Two unique Pvalb-tdTomato BAC transgenic mouse lines (line 9 and line 15) were established and selected for further analysis. Only hemizygous mice maintained on a pure C57 background were used for experiments in this study.

**Immunofluorescence analysis**—Two-months-old mice were transcardially perfused using 4 % Paraformaldehyde (PFA). Following perfusion, the mouse was decapitated and the brain surgically dissected. Extracted brains were then subject to 12–24 h post-fixation in 4 % PFA. Next, sagittal sections were cut on a vibratome (Vibratome 1000plus sectioning system) at a thickness of 50 µm. During all of the following treatments, sections were agitation using an orbital shaker at room temperature, unless otherwise noted. Sagittal sections were then washed in PBS and treated with 1.2 % Triton-X100 (Sigma-Aldrich) for 15 min to permeabilize the tissue. After washing, sections were washed three times 5 min with PBS. Then, the tissue was blocked using 2 % BSA, 5 % normal goat serum, 0.2 % Triton-X100 in PBS for 1 h. Blocked sections were incubated for 14 h with the primary antibody for parvalbumin (PV 235, mouse, Swant, 1:5000) or RFP (600-401-379, rabbit, Rockland, 1:1000) diluted in blocking buffer. Next, sections were washed three times 10 min per wash with PBS and incubated with a secondary antibody that was raised against mouse and conjugated with Alexa488 or Alexa555 (anti-PVALB detection) or a secondary antibody that was raised against rabbit and conjugated to Alexa555 (anti-RFP detection). Following secondary antibody incubation, the sections were washed three times 10 min with PBS and mounted using a Polyvinyl-alcohol based antifading mounting medium (PVA-DABCO, Sigma-Aldrich). The sections were then imaged using a BX61WI fixed stage confocal microscope equipped with 4X, 10X, 20X and 60X oil immersion objective lenses. For the Pvalb-tdTomato co-expression analysis, the maximum projection of z-stacks of three 20X high-power fields (approximately 150 cells per region) was counted manually (considering shape and spatial extent of the fluorescence signal) from three mice using the native tdTomato fluorescence and anti-PVALB immunolabeling.

**Slice preparation and whole-cell patch clamp**—Acute slices were prepared from 1.5 to 3-month-old mice. Animals were anesthetized by averin intraperitoneal injection (tribromoethanol, 20 mg/ml, 0.5 mg/g body weight) and transcardially perfused with cutting
sucrose-based solution (mM): 194 Sucrose, 30 NaCl, 4.5 KCl, 1.20 NaH2PO4, 26 NaHCO3, 10 glucose, 0.2 CaCl2·2H2O, 8 MgSO4·7H2O (~350 mOsm, 7.2–7.4 pH). Following decapitation, brains were removed for coronal sectioning (300 µm) using a Vibratome 1000 Plus, Leica Microsystems, USA. Slices were then recovered in carbogenated regular aCSF (mM): 119 NaCl, 2.5 KCl, 1.2 NaH2PO4, 24 NaHCO3, 12.5 glucose, 2 MgSO4·7H2O, 2 CaCl2·2H2O (~300 mOsm, 7.2–7.4 pH) at 32–34°C for 10 min and transferred to room-temperature carbogenated regular aCSF. Slices were allowed to recover at least 1 h prior to all recordings. Slices were transferred into a recording chamber (RC-27L, Warner Instruments) and constantly perfused at room temperature (20–24°C) with carbogenated regular aCSF at a rate of approximately 2 ml/min. Borosilicate glass recording microelectrodes (King Precision Glass) were pulled on a P-97 horizontal puller (Sutter Instruments) and backfilled with KGlu internal recording solution (in mM: 145 K-Gluconate, 10 HEPES, 1 EGTA, 2 Mg-ATP, 0.3 Na2-GTP, and 2 MgCl2). Internal pH was adjusted to ~7.3 with KOH and osmolarity adjusted to ~300 mOsm with K2SO4. The electrode tip resistance in the bath when filled with this internal solution was 3–5 MΩm.

Cells were visually identified based on the tdTomato fluorescence signal under a BX-51WI microscope (Olympus) equipped with a mercury arc lamp. After seal rupture and initial stabilization and equilibration of the whole-cell configuration (3 min to allow dialysis of the internal recording solution), recordings were initiated and only cells with series-resistance values <20 MΩ were included in the analysis. Current clamp traces were recorded with 50 sweeps of current injections, each lasting 500 ms and starting from −150 pA with increments of 15 pA (cortex, hippocampus) or 25 pA (striatum). Signals were filtered at 10 kHz, digitized at 10 kHz and data acquired using a MultiClamp 700B amplifier and a Digidata 1440A. All analysis was performed using pCLAMP10 (Axon Instruments, Molecular Devices).

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Highlight

1. Generated a line of parvalbumin-tdTomato BAC transgenic mice.
2. The expression of tdTomato faithfully matches endogenous parvalbumin expression.
3. Functional properties of parvalbumin-tdTomato neurons are confirmed by electrophysiology.
Figure 1. Pvalb-tdTomato BAC trimming and recombineering strategy

(A) Diagram of the unmodified Pvalb-spanning BAC clone RP24-306A6. The BAC clone also spans the extra gene Ift27. The positions of two homology arms selected for BAC trimming are shown (green arrows). (B) Diagram of the BAC clone following BAC trimming, which removes the Ift27 gene. Note that BAC trimming steps eliminated the loxP site present in the BAC vector. (C) Diagram of BAC recombineering steps employed to insert the tdTomato cDNA sequence immediately downstream of the putative promoter region of the Pvalb gene. Note the addition of a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) to improve transgene expression.
Figure 2. tdTomato is expressed in various regions throughout the brain that contain PVALB-expressing neurons

“(A) tdTomato expression visualized with an antibody against RFP to enhance signals in relatively dimmer regions (GP, hippocampus, neocortex) for this low magnification montage. Note that tdTomato is expressed throughout the brain including the cortex, globus pallidus, thalamic reticular nucleus, hippocampus and cerebellum and also shows ectopic expression in ependymal cells around the fimbria. The signal appears dim in the striatum due to low number of PVALB-expressing neurons (1–2% of neurons). Scale bar 1 mm. (B) Magnified view of tdTomato expression (red) in PVALB-expressing neurons (green) in the..."
thalamic reticular nucleus. Note the high degree of co-expression (yellow). Scale bars in B–D 100 µm. (C) Magnified view of the globus pallidus. (D) Magnified view of the cerebellum.
Figure 3. Co-labeling and electrophysiology show that the Pvalb-tdTomato reporter line faithfully labels cortical PVALB-expressing fast-spiking neurons
(A) Laser scanning confocal microscopy images show the co-localization of tdTomato (red) and PVALB (green) in cortical neurons. Scale bars 50 µm. Double-labeled cells are shown in yellow. (B) Quantification of the fidelity (fraction of tdTomato- and PVALB-expressing cells of tdTomato-expressing cells) and completeness (fraction of tdTomato- and PVALB-expressing cells of PVALB-expressing cells). (C) Representative trace from a whole-cell patch clamp recording experiment. Several current injection steps are shown. A series of 50 current injection steps were applied with increments of 15 pA starting from −150 pA. Note...
the suprathreshold fast spiking rate, the subthreshold membrane potential oscillations, and
the large amplitude fast after-hyperpolarization, which are typical for PVALB-expressing
neurons. (D) linear current-voltage (I–V) plot generated from current injections as shown in
C (n=8 cells from 4 mice). The current-firing rate (I-f) plot shows the instantaneous firing
frequency upon current injections as shown in C (n=8 cells from 4 mice). Mean ± SEM.
Figure 4. The Pralb-tdTomato reporter line reliably labels hippocampal PVALB-expressing neurons

(A) Native tdTomato (red) expression and anti-PVALB immunofluorescence labeling (green) is shown. Double-labeled cells are shown in yellow. Scale bars 50 µm. (B) Quantification of the fidelity (fraction of tdTomato- and PVALB-expressing cells of tdTomato-expressing cells) and completeness (fraction of tdTomato- and PVALB-expressing cells of PVALB-expressing cells). (C) Representative trace from a whole-cell patch clamp recording experiment. Several current injection steps are shown. A series of 50 current injection steps were applied with increments of 15 pA starting from −150 pA.
the suprathreshold fast spiking rate, the subthreshold membrane potential oscillations, and the large amplitude fast after-hyperpolarization, which are typical for PVALB-expressing neurons. (D) I–V plot generated from current injections as shown in C (n=8 cells from 4 mice). I-f plot showing the instantaneous firing frequency upon current injections as shown in C (n=8 cells from 4 mice). Mean ± SEM.
Figure 5. Striatal PVALB-expressing neurons are readily and specifically labeled by the Pvalb-tdTomato reporter line

(A) Native tdTomato (red) expression and anti-PVALB immunofluorescence labeling (green) is shown. Double-labeled cells are shown in yellow. Scale bars 50 µm. (B) Quantification of the fidelity (fraction of tdTomato- and PVALB-expressing cells of tdTomato-expressing cells) and completeness (fraction of tdTomato- and PVALB-expressing cells of PVALB-expressing cells). (C) Representative trace from a whole-cell patch clamp recording experiment. Several current injection steps are shown. A series of 50 current injection steps were applied with increments of 25 pA starting from −150 pA. Note
the suprathreshold fast spiking rate, the membrane potential oscillations, and the large amplitude fast after-hyperpolarization, which are typical for PVALB-expressing neurons. (D) I–V plot generated from current injections as shown in C (n=8 cells from 4 mice). I-f plot showing the instantaneous firing frequency upon current injections as shown in C (n=8 cells from 4 mice). Mean ± SEM.
Table 1

The electrophysiological properties of tdTomato-expressing neurons are characteristic of parvalbumin-expressing fast-spiking interneurons.

<table>
<thead>
<tr>
<th>Membrane property (n=8)</th>
<th>Striatum Mean ± SEM</th>
<th>Cortex (S1) Mean ± SEM</th>
<th>Hippocampus (CA1) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>rheobase current (pA)</td>
<td>278.13 ± 8.42</td>
<td>166.88 ± 4.06</td>
<td>75.00 ± 4.37</td>
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<tr>
<td>time constant of current decay (µs)</td>
<td>1272.50 ± 25.28</td>
<td>987.63 ± 34.07</td>
<td>1271.75 ± 62.06</td>
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<tr>
<td>membrane time constant (ms)</td>
<td>9.53 ± 0.46</td>
<td>7.23 ± 0.15</td>
<td>12.15 ± 0.52</td>
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<tr>
<td>membrane capacitance (pF)</td>
<td>83.79 ± 3.39</td>
<td>64.09 ± 2.08</td>
<td>74.29 ± 3.41</td>
</tr>
<tr>
<td>membrane resistance (MΩ)</td>
<td>112.00 ± 2.26</td>
<td>118.88 ± 4.08</td>
<td>177.34 ± 9.05</td>
</tr>
<tr>
<td>resting membrane potential (mV)</td>
<td>-77.04 ± 0.74</td>
<td>-65.36 ± 0.66</td>
<td>-61.40 ± 0.64</td>
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<tr>
<td>half-width of AP (ms)</td>
<td>0.60 ± 0.01</td>
<td>0.50 ± 0.01</td>
<td>0.58 ± 0.02</td>
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<tr>
<td>AHP amplitude (mV)</td>
<td>-13.94 ± 0.24</td>
<td>-20.04 ± 0.26</td>
<td>-18.74 ± 0.38</td>
</tr>
</tbody>
</table>

The tdTomato-expressing neurons in three representative regions exhibit physiological properties that are characteristic for PVALB-expressing neurons. The values were obtained from whole-cell patch clamp experiments performed at room temperature. Data is shown in mean ± SEM. n=8 cells from 4 mice per region. Compare to (Kawaguchi et al., 1987, Plenz and Kitai, 1998, Koós and Tepper, 1999, Galarreta and Hestrin, 2002, Tepper and Bolam, 2004, González-Burgos et al., 2005, Derchansky et al., 2008, Gittis et al., 2010, Zemankovics et al., 2010, Schiff and Reyes, 2012).