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TRANSGENIC LABELING OF PARVALBUMIN-EXPRESSING NEURONS WITH TDTOMATO

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Abstract—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.

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Key words: parvalbumin, interneuron, psychiatric disorders, transgenic, tdTomato, BAC.

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Abbreviations: BAC, bacterial artificial chromosome; BGHpA, bovine growth hormone polyadenylation; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PFGE, pulsed field gel electrophoresis; PVALB, parvalbumin; TRN, thalamic reticular nucleus; WPRE, woodchuck hepatitis virus posttranscriptional regulatory element.

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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 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, post-mortem 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 bacterial artificial chromosome (BAC) transgenic reporter line, where PVALB 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 the cerebellum and TRN, but not in the 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 (Fig. 1, Ting and Feng, 2013, 2014, 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 Ift27 gene-coding region from the BAC clone (Ting and Feng, 2014).

tdTomato is faithfully expressed in PVALB 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, TRN, hippocampal CA3, CA1 and dentate gyrus, and the cerebellum (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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

PVALB-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 four mice) in the somatosensory cortex and found fast-spiking suprathreshold firing patterns, sub-threshold membrane potential oscillations, and large amplitude fast after-hyperpolarizations (Fig. 3C), which are characteristic of PVALB-expressing neurons (Kawaguchi et al., 1987; Koós and Tepper, 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 (Fig. 3D, E). Similar results were obtained for neurons recorded in the CA1 region of the hippocampus and the striatum (Figs. 4D, E, 5D, E, $n = 8$ from four 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) and resting 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.