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Genetic control of neuronal activity in mice conditionally expressing *TRPV1*

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Abstract

Here we describe a knock-in mouse model for Cre-*loxP*-based conditional expression of TRPV1 in central nervous system neurons. Expression of Cre recombinase using biolistics, lentivirus or genetic intercrosses triggered heterologous expression of TRPV1 in a cell-specific manner. Application of the TRPV1 ligand capsaicin induced strong inward currents, triggered action potentials and activated stereotyped behaviors, allowing cell type-specific chemical genetic control of neuronal activity *in vitro* and *in vivo*.

An important advance in mapping functional neural circuits has been the ability to simultaneously label and electrically activate targeted neurons^{1–5}. Controlled expression of heterologous receptors has proven effective for such experimental manipulations^{1–4,6–8}. Recent use of light-gated ion channels has provided optical control of neuronal activity on millisecond time scales^{2,3,5,8,9}, but this approach requires direct optical access to brain tissue. Chemical genetic strategies for sensitizing neurons to exogenous ligands by engineered expression of heterologous receptors^{1,4} would allow an alternate approach for activation of deep brain structures, disperse neuronal populations, multiple brain regions or tissues otherwise inaccessible to light.

Here we describe a mouse model in which the rat vanilloid receptor TRPV1 (ref. 10) is conditionally expressed upon cell-specific recombination mediated by Cre recombinase. In recombined neurons, low concentrations of the TRPV1 ligand capsaicin elicited robust cation currents and action potential firing both *in vitro* and *in vivo*. This activation persisted for several seconds, could be repeatedly elicited in single neurons and was sufficient to drive behavioral responses.

To generate a conditional genetically encoded activator, we targeted the *Gt(ROSA)26Sor* (also known as *ROSA26*) locus¹¹ for the insertion by homologous recombination. We inserted a *loxP*-flanked transcriptional termination cassette upstream of the rat *Trpv1* cDNA, followed by an internal ribosomal entry site fused to enhanced cyan fluorescent protein (*IRES-ECFP*; Fig. 1a and Supplementary Fig. 1a online). We identified correctly targeted embryonic stem cell clones by Southern blot (Supplementary Fig. 1b and Supplementary Methods online) and used these clones to generate germline-targeted mice. To determine

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whether the conditional *TRPV1-IRES-CFP* was expressed upon removal of the stop cassette, we biolistically transfected plasmids encoding Cre recombinase into slices of main olfactory bulb from postnatal day 14 conditional TRPV1 mice. By 48 h, we observed overlapping expression of both TRPV1 and ECFP in neurons expressing Cre (Fig. 1b–d).

To test the function of the conditional *Trpv1* allele, we performed whole-cell patch clamp recordings from cortical neurons derived from *ROSA-stop^{flox}-TRPV1-IRES-ECFP* mice. To trigger *TRPV1-IRES-CFP* expression, we infected dissociated cortical neurons from postnatal day 1 conditional mice after 7 d *in vitro* with a lentiviral vector engineered to simultaneously express both yellow fluorescent protein (YFP) and Cre (Cre-IRES-YFP; Supplementary Methods). Positive immunoreactivity for TRPV1 in YFP-expressing cells verified Cre-mediated excision of the stop cassette ($n = 100$; Fig. 1e–g).

While blocking action potentials and all fast synaptic transmission (1 μ M tetrodotoxin, 30 μ M bicuculline, 25 μ M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 50 μ M 2R-amino-5-phosphonovaleric acid (APV)), we locally applied small volumes of 1 μ M capsaicin by a micropipette positioned near either virally infected Cre-expressing (YFP-positive) or uninfected (YFP-negative) cells, and recorded capsaicin-evoked currents. Local application of capsaicin elicited inward currents in all YFP-positive cells (mean peak current, 242 ± 13 pA; Fig. 1h). The average time to peak after local capsaicin application was 1.8 ± 0.1 s, and the time to baseline recovery was 9.4 ± 2.0 s (s.e.m.; $n = 17$; Fig. 1h). We never observed capsaicin-evoked currents in YFP-negative cells ($n = 15$; Fig. 1i,j). TRPV1-expressing neurons exhibited normal morphologies, resting membrane potentials and potassium currents to stepwise changes in membrane potential (Fig. 1e–g and Supplementary Fig. 2 online).

We next crossed *ROSA-stop^{flox}-TRPV1-IRES-ECFP* conditional mice with mice harboring a transgenic *nestin-Cre* driver, allowing pan-neuronal TRPV1 expression¹². All resulting offspring were morphologically and behaviorally indistinguishable from wild-type littermates. We identified *trans*-heterozygous mice by PCR genotyping (Supplementary Fig. 1c and data not shown), and using immunohistochemical analysis we verified Cre-mediated recombination by the widespread co-expression of TRPV1 and CFP throughout the brain (Fig. 2a–d). To test TRPV1 function, we monitored capsaicin-induced electrophysiological responses in acute neocortical slices from P21 *trans*-heterozygous mice. We performed whole-cell current clamp recordings on layer 5 cortical pyramidal neurons during bath perfusion of capsaicin (250 nM) while blocking fast synaptic transmission (10 μ M CNQX, 50 μ M APV and 10 μ M bicuculline). Application of capsaicin elicited action potentials in recombined cells (mean capsaicin-evoked firing rate above baseline, 7.9 ± 1.8 Hz, s.e.m.; $n = 40$; Fig. 2e), whereas recordings from cells in control slices derived from *ROSA-stop^{flox}-TRPV1-IRES-ECFP^{+/-}* mice not expressing Cre showed no response to capsaicin ($n = 15$; Supplementary Fig. 3a online). In response to capsaicin, neurons from *nestin-Cre* recombined *ROSA-stop^{flox}-TRPV1-IRES-ECFP^{+/-}* slices elicited dose-dependent depolarizing currents with an average half-maximal response at 350 nM (Fig. 2f). Acute application of capsaicin (1 μ M) to individual cells using a pneumatic micropipette resulted in reproducible bursts of capsaicin-evoked action potentials with similar increases in firing rate (Fig. 2g). Recordings from activated cells showed normal resting membrane potentials (-65.6 ± 0.3 mV), spike shape and evoked firing patterns (Fig. 2e,g) as previously shown for this cell type¹³. However, the frequency of action potential firing was dependent on both the duration of application and capsaicin concentration. Changing these parameters elicited a range of responses from single capsaicin-evoked spikes to high-frequency firing and channel desensitization (Supplementary Fig. 4 online). In the absence of Cre, cells from *ROSA-stop^{flox}-TRPV1-IRES-ECFP^{+/-}* mice did not respond to capsaicin (Supplementary Fig. 3a) but maintained glutamate responses (Supplementary Fig. 3b), indicating that the absence of

capsaicin activation was not due to impaired excitability. Moreover, we did not observe any sensitivity to changes in bath pH over physiologically relevant ranges, a potential concern given the proton-gating nature of TRPV1 (ref. 10). Specifically, shifting pH from 7.4 to 6.0 produced no differences in pH-evoked currents between wild-type and TRPV1 mice (Supplementary Fig. 5a online; wild-type, -75 ± 35 pA; TRPV1, -72 ± 24 pA; $P > 0.9$) and had no measurable effects on excitability (Supplementary Fig. 5b).

We next targeted cortical pyramidal neurons *in vivo* for capsaicin stimulation in anesthetized conditional *ROSA-stop^{fllox}-TRPV1-IRES-ECFP* mice harboring the *nestin-Cre* driver. We placed an extracellular recording electrode fixed to a small injection pipette containing 1 μ M capsaicin in the dorsal cortex of mice through a small craniotomy. We isolated single unit responses from individual neurons ($n = 20$, from 4 different mice) by stereotaxic targeting and monitoring of baseline neuronal activity (Supplementary Methods). In response to capsaicin application, nearly all ($n = 18$) cortical neurons fired high-frequency bursts of action potentials (Fig. 3a). Capsaicin-evoked spikes subsided after several seconds, could be repeatedly elicited with multiple capsaicin injections (Fig. 3b) and were dose-dependent (half-maximal effective concentration of 500 nM).

We next determined whether this technology could be used to activate genetically targeted populations of neurons in awake behaving mice. We unilaterally implanted cannulas into the striatum of either *nestin-Cre::ROSA-stop^{fllox}-TRPV1-IRES-ECFP* mice or control *ROSA-stop^{fllox}-TRPV1-IRES-ECFP* mice lacking Cre and delivered 0.5 μ M capsaicin by acute infusion. Exploratory and motor behavior in both *nestin-Cre::ROSA-stop^{fllox}-TRPV1-IRES-ECFP* and control mice was unaffected after recovery from surgery or infusion of control saline solution (data not shown). In contrast, stereotyped contralateral circling behavior occurred within 5 min of capsaicin infusion in mice harboring the conditionally expressed TRPV1 receptor ($n = 6$). Control *ROSA-stop^{fllox}-TRPV1-IRES-ECFP* mice lacking Cre showed no apparent capsaicin-mediated behaviors ($n = 6$; Fig. 3c,d and Supplementary Movies 1 and 2 online).

To determine whether local infusion of capsaicin into striatal tissues resulted in cellular excitotoxicity, we performed cell death assays. At capsaicin concentrations used to elicit circling (500 nM), we observed no cell death, but infusion of much higher concentrations of capsaicin (5–10 μ M) resulted in a dose-dependent excitotoxicity, likely resulting from excess calcium influx (Supplementary Fig. 6 online). Thus, in addition to ligand-induced neuronal activation, the conditional *TRPV1* mice provide a new model system for focal excitotoxicity in genetically defined neuronal populations.

Finally, to test whether similar behaviors could be elicited in mice in which TRPV1 expression was induced focally in the brain, we expressed TRPV1 in small subsets of striatal neurons by stereotactic injection of *YFP-IRES-Cre* lentivirus in *ROSA-stop^{fllox}-TRPV1-IRES-ECFP* mice (Supplementary Fig. 7 online). One week after unilateral lentiviral Cre expression in the striatum, infusion of capsaicin recapitulated the stereotyped contralateral circling behavior (Supplementary Movie 3 online). In all experiments, circling behavior subsided within 15 min after capsaicin infusion (Fig. 3c,d and Supplementary Movies 1 and 3), and infusions did not cause any observable lasting behavioral effects. In no cases did we observe ipsilateral circling after capsaicin infusion into *ROSA-stop^{fllox}-TRPV1-IRES-ECFP* mice expressing Cre in striatal neurons, indicating that circling behaviors were not due to nonspecific motor disorientation. Thus, conditional expression of TRPV1 in conjunction with infusion of modest amounts of capsaicin allows for acute, transient and genetically targeted neuronal activation, and associated behavioral responses *in vivo*.

Here we described a mouse model designed for chemical genetic manipulation of neuronal activity both *in vitro* and *in vivo*. Targeting the *ROSA26* locus for *TRPV1* knock-in afforded the unique opportunity to express a potentially activated ion channel in a controllable manner *in vivo*. Regulating the dose of capsaicin applied to TRPV1 expressing cells provided a range of control over neuronal activity from single action potentials to excitotoxic insult and neurodegeneration. Moreover, capsaicin was delivered to deep brain structures or disperse neuronal populations by standard methods of intracranial infusion.

A limitation of the system is the relatively coarse temporal resolution afforded by depolarizing neurons through TRPV1 channel activation. Unlike ChR2-mediated activation, which shows millisecond time scale activation *in vivo*^{2,3,5}, capsaicin-induced firing is on the order of seconds. Nevertheless, the broad utility of this chemical genetic conditional system for *in vivo* applications, including activation of deep brain regions and disperse neuronal populations, provide a unique capability to reliably modulate neuronal activity in a manner complementary to optical methods^{3,14} and ligand-induced inhibition⁴. Photoreleasable forms of capsaicin might be used in a unique manner, possibly allowing finer temporal control, but still require light access similar to that for ChR2. Advantages of this system that complement existing mouse models include the genetic targetability via the *Cre-loxP* system, robust neuronal activation with low-level channel expression and the ease of *in vivo* manipulations afforded through standard cannula infusion techniques. Expanding the genetic tool kit targeting neuronal subtypes for ectopic stimulation will allow investigations into mammalian brain circuitry and circuit-driven behaviors.

All experimental procedures were conducted in accordance with guidelines and standards of the Duke University Institutional Animal Care and Use Committee.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Conditional expression of TRPV1 by Cre recombinase. (a) Diagram of the conditional *stop^{fllox}-TRPV1-IRES-ECFP* allele targeted to the *ROSA26* locus. SA, splice acceptor; arrowheads, *loxP* sites. (b–d) TRPV1-IRES-ECFP reporter expression in a brain slice from a *ROSA-stop^{fllox}-TRPV1-IRES-ECFP^{+/+}* mouse after introducing a Cre-expressing plasmid via biolistics, assayed by immunohistochemical detection of the conditionally expressed rat TRPV1 (b) and the CFP reporter (c). Colocalization of TRPV1 and CFP is shown in d. Scale bar, 20 μ m. (e–g) Conditional expression of YFP (e) and TRPV1 (f) in cultured neurons from *ROSA-stop^{fllox}-TRPV1-IRES-ECFP^{+/+}* mouse after infection with a lentivirus expressing *YFP-IRES-Cre*. Colocalization of YFP and TRPV1 is shown in g. Scale bar, 10 μ m. (h,i) Capsaicin-evoked currents recorded from a lentivirus-infected (YFP⁺) neuron expressing Cre (h) and a non-infected control cell (YFP⁻; i). Deflections in the top traces indicate capsaicin injections. (j) Currents measured after local application of capsaicin to single neurons expressing TRPV1 (YFP⁺) or control cells (YFP⁻) not expressing TRPV1. Black bar, mean peak current; error bars, s.e.m. ($n = 15,17$).

Figure 2.

Conditional neuronal expression of *TRPV1-IRES-ECFP* *in vivo* by genetic intercross with mice harboring the *nestin-Cre* driver. **(a)** A Nissl-stained section of brain. **(b–d)** Immunohistochemical detection of TRPV1 **(b)** and CFP **(c)** and colocalization of the two markers **(d)** in the neocortex (region boxed in **a**) of P7 mice harboring both the *ROSA-stop^{fllox}-TRPV1-IRES-ECFP* (*ROSA-TRPV1*) and *nestin-Cre* alleles. DAPI staining indicates nuclei of both neuronal and non-neuronal cells. Insets, immunohistochemical detection of the same markers in *ROSA-stop^{fllox}-TRPV1-IRES-ECFP* mice not crossed with *nestin-Cre*. Scale bars, 50 μ m (75 μ m in inset). **(e)** Action potentials in TRPV1-expressing cortical neurons in acute brain slices from *nestin-Cre::ROSA-stop^{fllox}-TRPV1-IRES-ECFP* mice upon bath application of capsaicin (indicated by the gray bar). **(f)** Dose-dependent current response to bath applied capsaicin in TRPV1-expressing cells. **(g)** Bursts of action potentials in TRPV1-expressing cortical neurons in acute brain slices with repeated local injections of capsaicin (deflections in top trace). Inset, magnified view of a capsaicin-evoked burst of action potentials.

Figure 3.

In vivo activation of neurons that conditionally express *TRPV1*. **(a)** Electrophysiological response from a cortical cell conditionally expressing TRPV1 upon local capsaicin injection (top trace deflection). **(b)** Peristimulus time histogram of firing rate binned at 1.0-s intervals, assembled from multiple trials of capsaicin-evoked stimulation of neurons conditionally expressing TRPV1. Top, raster sweeps of spikes from individual trials. **(c)** Capsaicin-induced circling behavior in awake *nestin-Cre::ROSA-stop^{fllox}-TRPV1-IRES-ECFP* mice implanted with a ligand delivery cannula unilaterally in the dorsal left striatum. Image of recording arena (left). Tracked locomotor behavior from capsaicin-infused *ROSA-stop^{fllox}-TRPV1-IRES-ECFP* (control) mice lacking Cre expression (middle), and from capsaicin-infused *nestin-Cre::ROSA-stop^{fllox}-TRPV1-IRES-ECFP* conditional expression mice (right). Gray traces, pre-infusion movement; red traces, post-infusion movement; black dotted traces, movement after a 15-min recovery period. Arrowheads indicate the direction of movement (see Supplementary Movies 1 and 2). **(d)** Number of contralateral circling events made by either TRPV1 conditional or control mice upon infusion with capsaicin. Data represent means $\pm$ s.e.m.; $n = 6$ mice each. $**P < 0.001$; t -test.