2011-07-12

Calcineurin is Required for TRPV1-induced LTD of CA1 Stratum Radiatum Interneurons

Tyron DeRay Jensen
Brigham Young University - Provo

Calcineurin is Required for TRPV1-induced LTD of

CA1 Stratum Radiatum Interneurons

Tyron DeRay Jensen

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

Jeffrey G. Edwards, Chair
Sterling N. Sudweeks
Scott C. Steffensen

Department of Physiology and Developmental Biology

Brigham Young University

August 2011

ABSTRACT

Calcineurin is Required for TRPV1-induced LTD of
CA1 Stratum Radiatum Interneurons

Tyron DeRay Jensen
Department of Physiology and Developmental Biology, BYU
Master of Science

Learning and memory in the brain are thought to be dependent on synaptic plasticity. In response to sensory input, synapses can be strengthened or weakened, known as long-term potentiation or long-term depression (LTD), respectively. Transient receptor potential vanilloid 1 (TRPV1) has been shown to mediate a novel form of presynaptic LTD in hippocampal interneurons. TRPV1 is currently being heavily studied in the PNS and being targeted by pharmaceuticals for its anti-nociceptive and anti-inflammatory properties. However, much less is known regarding TRPV1 function in the CNS, including the signal mechanism mediating hippocampal LTD despite its obvious importance. Here we performed whole-cell voltage clamp electrophysiology experiments from CA1 hippocampal interneurons to identify this signaling mechanism. Because calcineurin (CaN) is reported to be linked to multiple forms of synaptic plasticity, we hypothesized that TRPV1 activates presynaptic CaN, which is required for this presynaptic LTD. In order to distinguish between presynaptic and postsynaptic CaN activity we added the specific CaN inhibitors cyclosporin A (CsA) or FK-506 to the bath to block CaN activity ubiquitously in the slice, both presynaptically and postsynaptically, and to the internal solution to block CaN only in the postsynaptic neuron. CsA or FK-506 present in the internal solution, blocking only postsynaptic CaN, showed no effect on TRPV1-dependant LTD. Bath application of CsA or FK-506, inhibiting CaN in the presynaptic neuron as well, blocked LTD elicited by both a high frequency stimulation protocol ($P < 0.05$) and by direct TRPV1 activation with specific agonists resiniferotoxin and capsaicin ($P < 0.05$). This demonstrates that CsA and FK506 block both high frequency stimulation induced LTD and also TRPV1 specific depression. We are thus able to show that calcineurin is required for this form of presynaptic TRPV1 mediated LTD in the hippocampus. This finding is the first to demonstrate a TRPV1-induced signaling mechanism in CA1 hippocampus.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Declarative learning and memory in the brain are thought to be dependent on synaptic plasticity in the hippocampus. Synaptic plasticity includes the strengthening and weakening of synapses known as long term potentiation (LTP) or long term depression (LTD) respectively. Both LTP and LTD have an effect on learning and memory [1]. LTP has been studied greatly since it was discovered in the 1970's by Bliss and Lømo [2, 3]. LTD on the other hand was discovered more recently [4, 5].

LTD can be mediated either postsynaptically [6-9] or presynaptically [10-15]. Postsynaptic plasticity is mainly regulated by the number of AMPA receptors present on the postsynaptic terminal. Presynaptic plasticity on the other hand is regulated by the amount of neurotransmitter released from the presynaptic terminal.

Recently, a novel form of presynaptic LTD shown to be mediated by transient receptor potential vanilloid 1 (TRPV1) at Schaffer collateral interneuron synapses in the hippocampus [16]. It was discovered that TRPV1 is located presynaptically and activated by the retrograde messenger 12-(S)-HPETE produced in the postsynaptic cell. This depression is elicited by TRPV1 agonists or by high frequency stimulation (HFS). LTD is blocked by TRPV1 antagonists and absent entirely in TRPV1 knockout mice. This is the first time that TRPV1 has been shown to be absolutely essential for a type of plasticity.

Interestingly, TRPV1 is also a heat sensitive cation channel receptor in the PNS. It is activated by temperatures above ~43°C, decreased pH, and many different lipophilic ligands [17]. Capsaicin, the active ingredient in chili peppers is a strong, selective exogenous activator of TRPV1. TRPV1 is active in the periphery and has been said to be a molecular integrator of

1

chemical and physical noxious stimuli [18]. TRPV1 has been heavily studied for its anti-inflammatory and anti-nociceptive properties in the PNS [19-23].

In the CNS, TRPV1 was recently shown to function behaviorally. For example, TRPV1 knockout mice demonstrate reduced anxiety, fear conditioning and stress sensitization [24], showing that TRPV1 is not only important in the periphery but also plays a crucial role in the CNS. Although TRPV1 is expressed in many areas of the brain [25, 26], we are just starting to explore the physiological importance of its role in the CNS [24].

Understanding TRPV1 function in the CNS is important, especially regarding how TRPV1 modulates inhibitory hippocampal interneuron activity, because the interneurons are critical to overall hippocampal function. For example, interneurons located in stratum radiatum of the CA1 hippocampus have important regulatory functions. A single interneuron has many arbors and can innervate hundreds of pyramidal cells [27]. The wide spread inhibition from these interneurons allows synchronized firing [28, 29] and oscillatory behavior of pyramidal cells. Although interneurons are the numerical minority in the hippocampus, they regulate a number of functioning processes of pyramidal cells through their extensive and strategically wired arbors [30]. Because interneurons regulate activity of pyramidal cells, which are the output of the hippocampus, it is critical to understand how mechanisms such as synaptic plasticity alter interneuron activity, and thus affect hippocampal functions such as learning and memory.

Investigating interneuron activity and plasticity lead us to ask, how does TRPV1 induce LTD of interneurons? It is paradoxical because TRPV1 is a cation channel with high permeability for Ca^{2+}. This begs the question, how can Ca^{2+} influx through TRPV1 lead to a

decrease in glutamate release? One possible mechanism is that Ca^{2+} entry through TRPV1 activates a second messenger pathway that in turn reduces glutamate release.

An excellent candidate molecule for this second messenger pathway is calcineurin (CaN), also known as protein phosphatase 3, and formerly known as PP2B. CaN is a Ca^{2+}-dependent protein phosphatase that dephosphorylates serine and threonine residues. CaN has been connected to T cell function and is the target of immunosuppressants Cyclosporin A (CsA) and FK-506 [31]. Many studies have linked CaN activity to multiple forms of synaptic plasticity [32-37]. Interestingly, a link between TRPV1 and CaN was identified in dorsal root ganglion cells when it was shown that CaN is activated by Ca^{2+} currents through TRPV1 channels causing a reduction in neurotransmitter release [38]. Therefore CaN is an excellent candidate to examine as the second messenger required for TRPV1-induced interneuron LTD

By understanding how TRPV1 functions in the hippocampus it will give us further insight into how it may be functioning in other areas of the CNS. Also this mechanism allows for further enlightenment of TRPV1-mediated LTD, which enhances pyramidal cell LTP [39] and thus memory formation. This mechanism is also critical to understand because those who propose to manipulate TRPV1 activity for anti-inflammatory and anti-nociceptive purposes in the spinal cord should know how these changes may affect TRPV1 in the brain. Our data indicate that CaN is required for TRPV1-mediated LTD at Schaffer collateral interneuron synapses and we are the first to propose how presynaptic TRPV1 functions in CA1 hippocampus at this synapse.

METHODS

Brain Slice Preparation

Sprague-Dawley male rats (15-27 days old) were deeply anesthetized with isoflurane using a vapomatic chamber and quickly decapitated in accordance with Institutional Animal Care and Use Committee standards. The brain was rapidly removed and placed in ice-cold ringers (in mM): NaCl, 119; NaHCO$_3$, 26; KCl, 2.5; NaH$_2$PO$_4$, 1.0; CaCl$_2$, 2.5; MgSO$_4$, 0.6; glucose, 11; saturated with 95% O$_2$, 5% CO$_2$ (pH 7.4). Coronal slices (350-400 μm) were prepared and placed in a submersion chamber containing oxygenated artificial cerebrospinal fluid (ACSF) at room temperature. A surgical cut was made between the CA3 and CA1 regions to avoid bursting activity. Slices were either kept in this ACSF or transferred to oxygenated ACSF containing CaN antagonists for at least a 1 hour recovery period. Slices remained in these solutions for 1-6 hours.

Electrophysiology

Slices were then transferred to a submerged recording chamber bathed in oxygenated ACSF (28-32°C) containing elevated divalent cations to reduce epileptiform activity (4 mM CaCl$_2$ and 4 mM MgCl$_2$, replacing 2.5 mM CaCl$_2$ and 1.3 mM MgSO$_4$) as described previously. Slices were continuously perfused with ACSF at a flow rate of 2-3 ml/min. Whole-cell patch clamp recordings were made from interneurons identified visually in the CA1 stratum radiatum of the hippocampus with infrared optics, CCD camera and monitor, using an Olympus BX51WI microscope with 40x water immersion objective. A bipolar stainless steel stimulating electrode was placed in stratum radiatum, approximately 200-500 μm from the recorded cell to stimulate CA3 glutamatergic afferents of the Schaffer Collateral pathway at 0.1 Hz (stimulus intensities were typically 50-300 μA, 100 μsec). Recordings were performed in voltage clamp at -65 mV

4

using a multiclamp 700B amplifier (Molecular Devices) and EPSCs evoked by two pulses separated by 50 msec. Borosilicate glass patch pipettes (2-4 MOhms) were filled with Cs+-gluconate based internal solutions containing (in mM): NaCl, 2; $MgCl_2$, 5; HEPES, 20; ATP, 2; GTP, 0.3 and EGTA, 0.6. AMPAR-mediated currents were measured while blocking $GABA_A$ receptors with picrotoxin (100 µM). Once a stable baseline recording of AMPAR-mediated currents was obtained by stimulating through bipolar electrodes via constant current at the frequency of 0.1 Hz, either the Schaffer collateral pathway was stimulated using a conditioning HFS (2 trains of 100 Hz for 1 sec separated by 20 seconds) or TRPV1 agonists were added directly to the ACSF for 10 minutes. EPSCs were evoked and monitored for at least 15 minutes post-tetanus or post TRPV1 agonist application at 0.1 Hz.

Analysis

Data was filtered at 4 kHz and acquired with an axon 1440A digitizer (Molecular Devices) interfaced with a Dell personal computer (Optiplex 745) with pClamp10.2 clampex software (Molecular Devices). Data was analyzed using pClamp10.2 clampfit software, EPSC amplitudes were determined by measuring against a 10 msec baseline prior to evoking EPSCs. Microsoft Excel and Origin (Natwick, MA) were used to organize, average, graph and perform statistical analysis on the data, amplitude values were averaged in 1 minute intervals. To positively identify interneuron LTD, the average pre-tetanus AMPAR current from a period of 5 minutes was then compared to a 5 minute period 10-15 minutes post-tetanus or post drug application to determine statistical significance, using a t test ($p < 0.05$). To determine significance of extracellular drug affect we compared a 5 minute period 10-15 minutes post-tetanus or post drug application to the same time period of the intracellular drug application experiments. The cell input resistance was monitored throughout the experiment and if changed by more than 10%, the

5

cell was discarded. Only one experiment was performed per slice and the reported n is the number of slices. One or two slices were used per animal. Interneurons were distinguished from pyramidal cells visually, electrophysiologically (tighter cells indicated by smaller 'opening' in whole cell mode, and faster EPSCs as compared to pyramidal cells) and based on the location in the radiatum.

Materials

All salts were purchased from Sigma-Aldrich, Fisher Scientific, J T Baker or Mallinckrodt. Capsaicin, cyclosporin A, okadaic acid, picrotoxin, QX-314 bromide and resiniferatoxin were purchased from Tocris Bioscience. FK-506 was purchased from Ascent Scientific. Stock solutions of capsaicin (25 mM) and resiniferatoxin (2mM) were dissolved in DMSO and then stored at -90°C. These stock solutions were then diluted to their final concentration in ACSF.

RESULTS

CaN Is Required for HFS Induced LTD of CA1 Hippocampal Interneurons

To determine whether CaN is required for HFS-induced LTD of CA1 stratum radiatum interneurons we first used the selective CaN antagonist CsA. Bath application of CsA (40 µM) was used to block CaN activity throughout the entire slice, both in the presynaptic and postsynaptic domain. Alternatively, application of CsA in the intracellular fluid was used to only block CaN postsynaptically. Therefore, if CaN involved in LTD is present presynaptically, as we expected, only bath applied CsA should block interneuron LTD, but not when applied intracellularly. Indeed, our results demonstrated that intracellularly applied CsA had no effect on HFS-induced LTD, and showed significant depression when compared to baseline (Fig. 1A; $p < 0.05$). Bath application of CsA significantly blocked HFS-induced LTD when compared to intracellularly applied CsA (Fig. 1B; $p < 0.05$), suggesting presynaptic CaN is required for interneuron LTD. The LTD caused by HFS in the presence of intracellular CsA was not caused by a CsA-induced rundown of EPSC or poor cell health as there was no significant decrease in EPSC amplitude with CsA in the internal solution with no HFS (Fig. 1C; $p > 0.05$).

To ensure CsA was specifically targeting CaN we used an additional CaN antagonist FK-506. Intracellular application of FK-506 resulted in significant HFS-induced LTD compared to baseline (Fig. 2A; $p < 0.05$). Bath application of FK-506 significantly blocked HFS-induced LTD when compared to intracellular application of FK-506 (Fig. 2B; $p < 0.05$). There was also no significant decrease in EPSC amplitude in the presence of intracellular FK-506 and absence of HFS (Fig. 2C; $p > 0.05$).

CaN Is Required for TRPV1 Agonist Mediated Depression of CA1 Hippocampal Interneurons

Though HFS has been shown to cause LTD through TRPV1 in CA1 interneurons [16], we wanted to confirm that any action of CaN was indeed occurring through a TRPV1 receptor mediated pathway and not some other pathway that may be induced by HFS. We again used CsA (40 µM) to block CaN activity and instead of eliciting LTD with HFS we used the selective TRPV1 agonists capsaicin (1-2 µM) and resiniferatoxin (RTX; 1 µM) to induce depression. Intracellularly applied CsA had no effect on depression mediated by TRPV1 agonists at this synapse as reported previously [16], which here was significantly induced by capsaicin (Fig. 3A; $p < 0.05$) and RTX (Fig. 3C; $p < 0.05$). However, bath applied CsA blocked the depression induced by both capsaicin (Fig. 3B; $p < 0.05$) and RTX (Fig. 3D) application.

DISCUSSION

As CaN has been shown to be prominent in excitatory hippocampal neurons [40] and physiologically relevant in many forms of plasticity [32, 41, 42], it was the focus of our investigation into the mechanism of HFS-induced TRPV1-mediated LTD in CA1 interneurons. Specific CaN antagonists, CsA and FK-506, blocked HFS-induced LTD in CA1 interneurons when superfused in the slice, therefore blocking CaN in the presynaptic and postsynaptic neuron. In contrast HFS-induced LTD was elicited when these antagonists were only in the postsynaptic interneuron itself. While using two CaN antagonists confirms specificity that indeed CaN is required as part of a second messenger system required for interneuron LTD, one potential concern is that intracellular application of CsA and FK-506 does not perfuse through the interneuron to the synapse. However, this is unlikely as we have previously used intracellular blockers such as BAPTA and GDPβS and shown that LTD can be blocked with these agents, thus CsA and FK-506 should likely be able to distribute through these interneurons. Taken together, the most likely explanation of these data is that presynaptically located CaN is required for HFS-induced LTD.

Interestingly, although LTD was blocked by down-regulation of presynaptic CaN, we noticed a short-term depression in cells when we used a HFS protocol. This short-term depression mechanism is likely-separate from the CaN second messenger pathway required for LTD and may be mediated by another mechanism.

Because TRPV1 was shown to be required for LTD [16], and HFS could likely induce the activation of several signaling pathways, we needed to confirm the involvement of CaN in depression mediated by TRPV1 receptors specifically. Indeed, CaN antagonists when bath-applied, but not intracellularly-applied, blocked TRPV1 agonist-induced depression. These data

9

illustrate that CaN is specifically activated by TRPV1 receptors to induce depression, and suggest that the CaN involved is located in the presynaptic terminal. Our data are also consistent with the finding that CaN was previously shown to be involved in TRPV1 specific plasticity in the dentate gyrus [41].

Collectively, we have demonstrated that CaN antagonists block HFS-induced LTD and TRPV1-agonist mediated depression. Furthermore, the CaN required is not located postsynaptically, but presynaptically as was proposed previously [16]. Presynaptic CaN does indeed appear to be required in the signal cascade initiated by TRPV1 to induce TRPV1-mediated HFS LTD. This is the first report identifying the signaling cascade mediating TRPV1-induced interneuron LTD in CA1 hippocampus, and illustrates how TRPV1, a cation channel receptor, could counter-intuitively result in depressed neurotransmitter release.

CONCLUSION

The goal of this research is to further identify mechanisms underlying synaptic plasticity in the hippocampus. As learning and memory have been linked to it, understanding more about plasticity allows us to better understand learning and memory formation. As interneuron regulation of pyramidal cells is critical for proper pyramidal cell firing and hippocampal output, it is crucial to understand what regulates interneuron plasticity. As we come to better understand the mechanisms behind learning and memory formation we can take steps to help those with learning or memory deficits. Also, understanding the involvement of TRPV1 and its mechanisms of action in the CNS are critical to know. This is because those who study and use TRPV1 in the PNS for its anti-inflammatory and anti-nociceptive properties need to know that manipulation of TRPV1 activity may cause unwanted side effects in the CNS.

Further studies of TRPV1 mechanisms involved in plasticity are plentiful. Specifically, we could investigate the downstream consequences of CaN activation to initiate TRPV1-mediated LTD at CA1 interneurons. Identifying the target for dephosphorylation of CaN would be an interesting place to start. One possible target of dephosphorylation to look at would be voltage-gated $Ca2+$ channels. To determine if and which type of voltage gated $Ca2+$ channel is required for this LTD, we could block specific voltage gated $Ca2+$ channels looking for an effect in LTD. Also, identifying the mechanism of the short-term depression that was seen with HFS even while CaN was blocked would be interesting to study.

BIBLIOGRAPHY

1.	Martin, S.J., P.D. Grimwood, and R.G. Morris, Synaptic plasticity and memory: an evaluation of the hypothesis. Annual review of neuroscience, 2000. 23: p. 649-711.
2.	Bliss, T.V. and T. Lomo, Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol, 1973. 232(2): p. 331-56.
3.	Malenka, R.C., The long-term potential of LTP. Nature Reviews Neuroscience, 2003. 4(11): p. 923-6.
4.	Dudek, S. and M. Bear, Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proceedings of the National Academy of Sciences, 1992. 89(10): p. 4363-4367.
5.	Mulkey, R.M. and R.C. Malenka, Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron, 1992. 9(5): p. 967-975.
6.	Yang, S.N., Y.G. Tang, and R.S. Zucker, Selective induction of LTP and LTD by postsynaptic [Ca2+]i elevation. Journal of Neurophysiology, 1999. 81(2): p. 781-7.
7.	Huber, K., M. Kayser, and M. Bear, Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. Science, 2000. 288(5469): p. 1254.
8.	Snyder, E.M., et al., Internalization of ionotropic glutamate receptors in response to mGluR activation. nature neuroscience, 2001. 4(11): p. 1079-85.
9.	Xiao, M.Y., Q. Zhou, and R.A. Nicoll, Metabotropic glutamate receptor activation causes a rapid redistribution of AMPA receptors. Neuropharmacology, 2001. 41(6): p. 664-71.
10.	Clark, K.A. and G.L. Collingridge, Evidence that presynaptic changes are involved in the expression of LTP and LTD of NMDA receptor-mediated EPSCs in area CA1 of the hippocampus. J Physiol Paris, 1996. 90(5-6): p. 311.
11.	Ninan, I., et al., Early presynaptic changes during plasticity in cultured hippocampal neurons. EMBO J, 2006. 25(18): p. 4361-71.
12.	Faas, G., et al., Modulation of presynaptic calcium transients by metabotropic glutamate receptor activation: a differential role in acute depression of synaptic transmission and long-term depression. Journal of Neuroscience, 2002. 22(16): p. 6885.
13.	Watabe, A.M., H.J. Carlisle, and T.J. O'Dell, Postsynaptic induction and presynaptic expression of group 1 mGluR-dependent LTD in the hippocampal CA1 region. Journal of Neurophysiology, 2002. 87(3): p. 1395-403.
14.	Volk, L.J., C.A. Daly, and K.M. Huber, Differential roles for group 1 mGluR subtypes in induction and expression of chemically induced hippocampal long-term depression. Journal of Neurophysiology, 2006. 95(4): p. 2427-38.
15.	Rouach, N. and R.A. Nicoll, Endocannabinoids contribute to short-term but not long-term mGluR-induced depression in the hippocampus. Eur J Neurosci, 2003. 18(4): p. 1017-20.
16.	Gibson, H.E., et al., TRPV1 channels mediate long-term depression at synapses on hippocampal interneurons. Neuron, 2008. 57(5): p. 746-59.
17.	Tominaga, M. and T. Tominaga, Structure and function of TRPV1. Pflugers Arch, 2005. 451(1): p. 143-50.
18.	Cortright, D.N. and A. Szallasi, Biochemical pharmacology of the vanilloid receptor TRPV1. An update. Eur J Biochem, 2004. 271(10): p. 1814-9.

19.   Elmes, S.J.R., et al., Cannabinoid CB2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naïve rats and in rat models of inflammatory and neuropathic pain. Eur J Neurosci, 2004. 20(9): p. 2311-20.

20.   Martelli, L., et al., A potential role for the vanilloid receptor TRPV1 in the therapeutic effect of curcumin in dinitrobenzene sulphonic acid-induced colitis in mice. Neurogastroenterol Motil, 2007. 19(8): p. 668-74.

21.   Morisset, V., et al., Possible mechanisms of cannabinoid-induced antinociception in the spinal cord. European Journal of Pharmacology, 2001. 429(1-3): p. 93-100.

22.   Sinning, C., et al., Dopamides, vanillylamides, ethanolamides, and arachidonic acid amides of anti-inflammatory and analgesic drug substances as TRPV1 ligands. ChemMedChem, 2008. 3(12): p. 1956-64.

23.   Spicarova, D. and J. Palecek, The role of the TRPV1 endogenous agonist N-Oleoyldopamine in modulation of nociceptive signaling at the spinal cord level. Journal of Neurophysiology, 2009. 102(1): p. 234-43.

24.   Kauer, J.A. and H.E. Gibson, Hot flash: TRPV channels in the brain. Trends in Neurosciences, 2009. 32(4): p. 215-24.

25.   Cristino, L., et al., Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience, 2006. 139(4): p. 1405-1415.

26.   Tóth, A., et al., Expression and distribution of vanilloid receptor 1 (TRPV1) in the adult rat brain. Brain Res Mol Brain Res, 2005. 135(1-2): p. 162-8.

27.   Freund, T.F. and G. Buzsaki, Interneurons of the hippocampus. Hippocampus, 1996. 6(4): p. 347-470.

28.   Buzsáki, G., Hippocampal GABAergic interneurons: a physiological perspective. Neurochem Res, 2001. 26(8-9): p. 899-905.

29.   Cobb, S., et al., Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. 1995.

30.   Buzsáki, G., Functions for interneuronal nets in the hippocampus. Can J Physiol Pharmacol, 1997. 75(5): p. 508-15.

31.   Schreiber, S.L. and G.R. Crabtree, The mechanism of action of cyclosporin A and FK506. Immunol Today, 1992. 13(4): p. 136-42.

32.   Heifets, B.D., V. Chevaleyre, and P.E. Castillo, Interneuron activity controls endocannabinoid-mediated presynaptic plasticity through calcineurin. Proc Natl Acad Sci USA, 2008. 105(29): p. 10250-5.

33.   Mansuy, I.M., Calcineurin in memory and bidirectional plasticity. Biochemical and biophysical research communications, 2003. 311(4): p. 1195-208.

34.   Malleret, G., et al., Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell, 2001. 104(5): p. 675-86.

35.   Wang, G.-P., et al., Calcineurin contributes to spatial memory impairment induced by rapid eye movement sleep deprivation. Neuroreport, 2009. 20(13): p. 1172-6.

36.   Zeng, H., et al., Forebrain-specific calcineurin knockout selectively impairs bidirectional synaptic plasticity and working/episodic-like memory. Cell, 2001. 107(5): p. 617-29.

37.   Weitlauf, C. and D. Winder, Calcineurin, Synaptic Plasticity, and Memory. The ScientificWorld Journal, 2001. 1: p. 530-533.

38. Wu, Z.-Z., S.-R. Chen, and H.-L. Pan, Transient receptor potential vanilloid type 1 activation down-regulates voltage-gated calcium channels through calcium-dependent calcineurin in sensory neurons. J Biol Chem, 2005. 280(18): p. 18142-51.

39. Bennion, D., et al., Transient receptor potential vanilloid 1 agonists modulate hippocampal CA1 LTP via the GABAergic system. Neuropharmacology, 2011.

40. Eastwood, S.L., P.W.J. Burnet, and P.J. Harrison, Decreased hippocampal expression of the susceptibility gene PPP3CC and other calcineurin subunits in schizophrenia. Biol Psychiatry, 2005. 57(7): p. 702-10.

41. Chávez, A.E., C.Q. Chiu, and P.E. Castillo, TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. Nat Neurosci, 2010. 13(12): p. 1511-8.

42. Ghetti, A. and S.F. Heinemann, NMDA-Dependent modulation of hippocampal kainate receptors by calcineurin and Ca(2+)/calmodulin-dependent protein kinase. Journal of Neuroscience, 2000. 20(8): p. 2766-73.

FIGURE LEGENDS

Figure 1. Blocking calcineurin (CaN) with cyclosporine A (CsA) inhibits high frequency stimulus (HFS)-induced LTD. A) Intracellular application of CaN antagonist CsA (40 μM), which inhibits postsynaptic CaN, results in significant ($p < 0.05$; $n = 9$) HFS-induced LTD as compared to baseline. Alternatively, bath application of CsA (B), which also blocks presynaptic CaN, significantly ($p < 0.05$; $n = 6$) blocked HFS-induced LTD, when compared to A. C) Intracellular application of CsA (40 μM) did not significantly ($p > 0.05$; $n = 3$) decrease EPSC amplitude in the absence of HFS. These data suggest that presynaptic CaN is required for HFS-induced LTD. HFS (arrowhead; 100Hz). Error bars indicate SEM. Insets: Representative EPSCs, and series resistance (average of 15 consecutive) before (black) and after HFS (gray). Scale bar: 50 pA, 10 msec.

Figure 2. Blocking CaN with FK-506 inhibits HFS-induced LTD. A) Intracellular application of FK-506 (50 μM) to the postsynaptic cell, inhibiting postsynaptic CaN, resulted in significant ($p < 0.05$; $n = 5$) HFS-induced LTD when compared to baseline. B) Bath application of CaN antagonist FK-506 (50 μM), which inhibits presynaptic and postsynaptic CaN, significantly ($p < 0.05$; $n = 8$) blocked HFS-induced LTD as compared to A. C) Intracellular application of FK506 (50 μM) did not significantly ($p > 0.05$; $n = 4$) decrease EPSC amplitude in the absence of HFS. These data suggest that presynaptic CaN is required for HFS-induced LTD. HFS (arrowhead; 100Hz). Error bars indicate SEM.

Figure 3. Blocking CaN with CsA inhibits TRPV1 agonist-induced LTD. A) Intracellular application of CaN antagonist CsA (40 μM), blocking postsynaptic CaN, resulted in significant depression ($p < 0.05$; $n = 7$) induced by the specific TRPV1 agonist capsaicin (Cap; 1-2 μM). Capsaicin-induced depression was significantly blocked by bath application of CsA (40 μM) (B) compared to intracellularly applied CsA ($p < 0.05$; $n = 4$). C) Intracellular application of CsA (40 μM) resulted in significant ($p < 0.05$; $n = 5$) resiniferatoxin (1 μM) induced LTD compared to baseline. RTX-induced depression was blocked by bath application of CsA (40 μM) (D) compared to intracellularly applied CsA ($n = 5$). These data suggest that presynaptic CaN is required for TRPV1-induced LTD. Error bars indicate SEM.
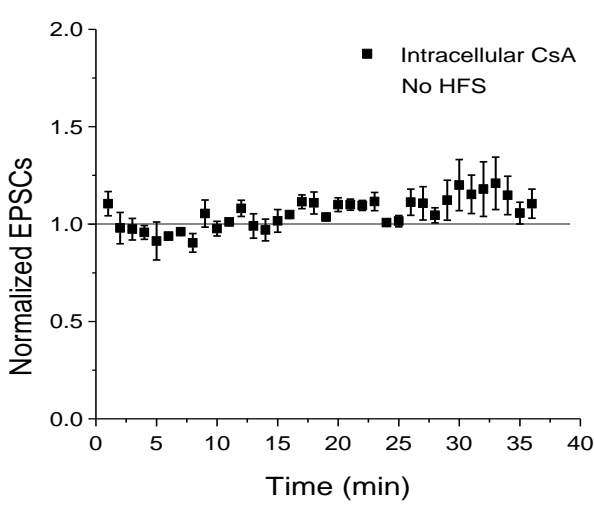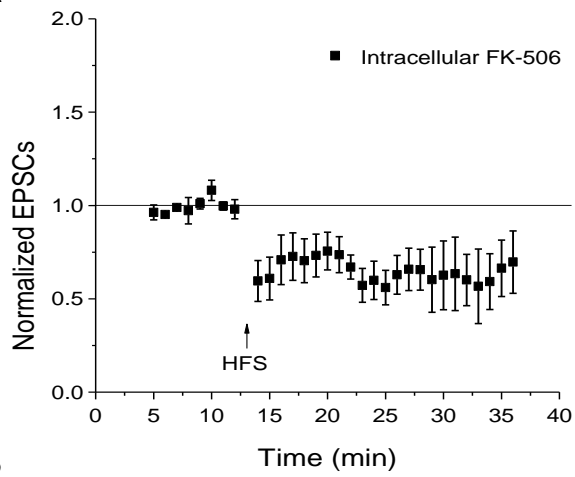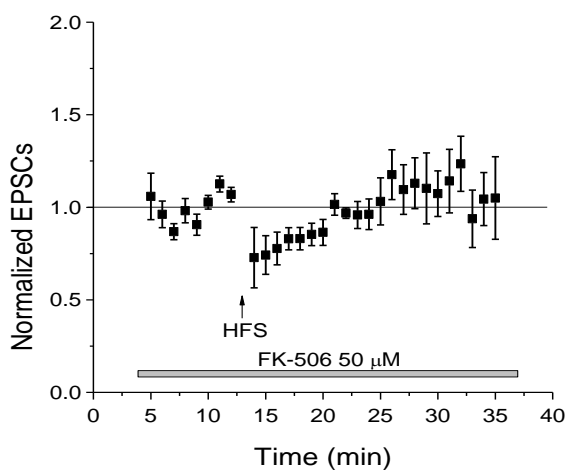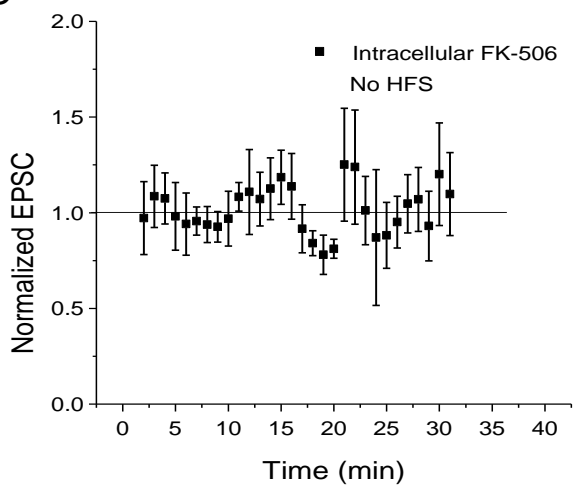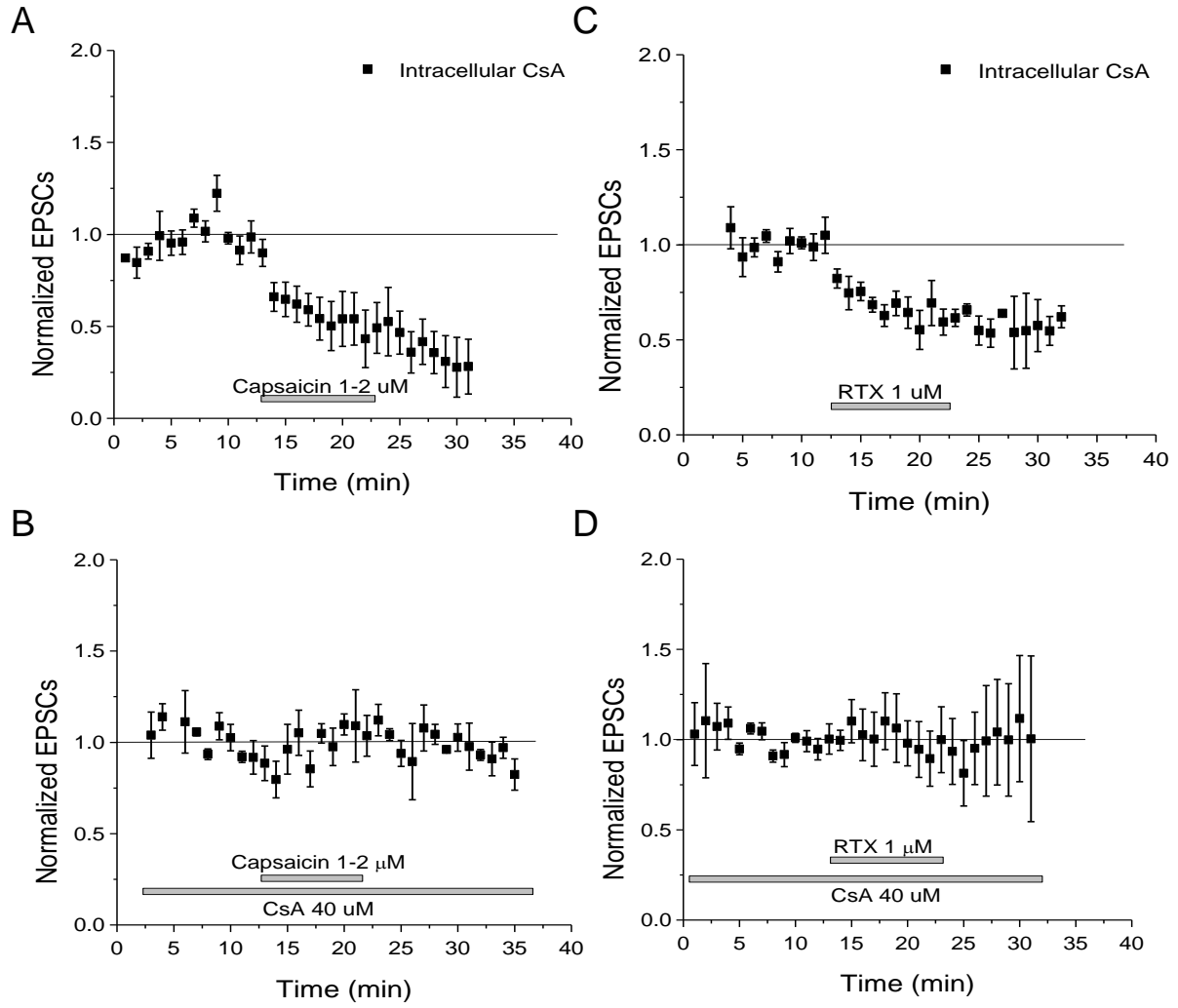
FIGURE 1

FIGURE 2

FIGURE 3

A

C

B

D
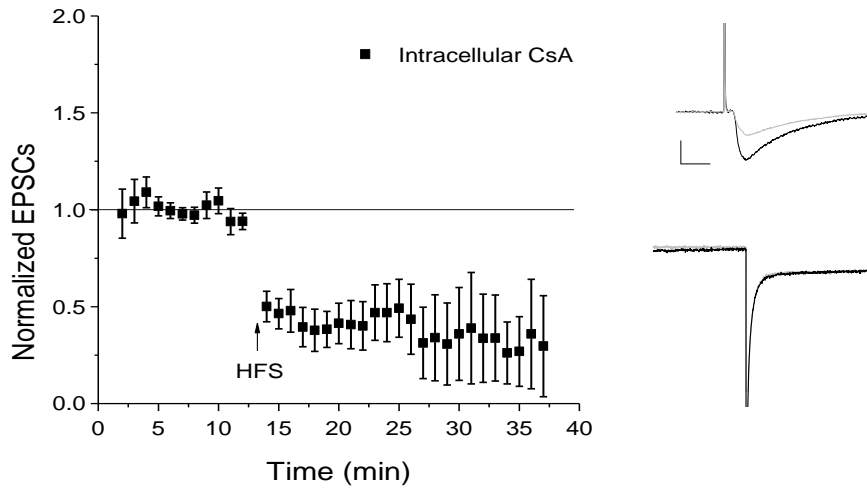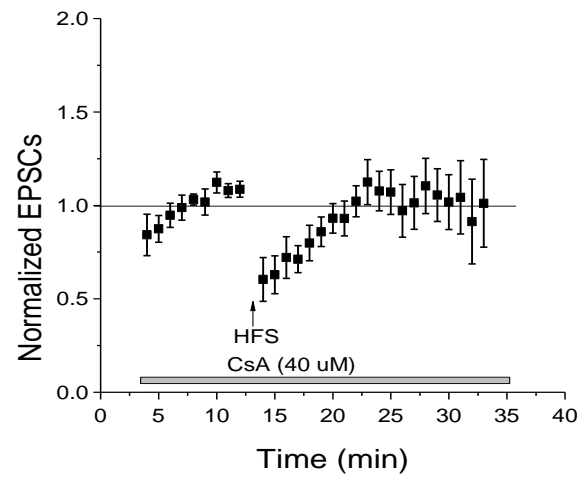
Curriculum Vitae of

Tyron D Jensen

(801)372-3516
tyronj@gmail.com

Education and Training:

B.S., *Physiology and Developmental Biology*, <u>Brigham Young University</u>   *Aug 2009*
M.S., *Physiology and Developmental Biology*, <u>Brigham Young University</u>   *Aug 2011*

Career History:

Brigham Young University   **Sept 2007 - Aug 2011**

Graduate Research Assistant   *Sept 2009 - Aug 2011*
  • Perform whole-cell patch clamp experiments

  • Analyze data

  • Mentor undergraduate research assistants

Teaching Assistant   *Sept 2009 - Aug 2011*
 Physiology and Developmental Biology 363 (Advanced Physiology Lab)
  • Teach and clarify concepts in advanced physiology

  • Grade assignments

  • Teach two or three sections a semester

Undergraduate Research Assistant   *Sept 2007 - Aug 2009*
  • Performed field electrophysiology experiments

  • Analyzed data

  • Trained new research assistants

Grants:

  • *Office of Research and Creative Activities (ORCA) Grant* - <u>Brigham Young University</u> - 2008 - "Synaptic Plasticity in Inhibitory Interneurons of the CA1 Region of the Hippocampus Measured through Miniature EPSCs" - $1500

Professional Memberships:

  • *Society for Neuroscience*   *2008 - Present*

  • *American Association for the Advancement of Science*   *2010 - Present*

Publications:

- Douglas Bennion, **Tyron Jensen**, Curtis Walther, John Hamblin, Andrew Wallmann, Jason Couch, Jacob Blickenstaff, Michael Castle, Lauren Dean, Sam Beckstead, Collin Merrill, Casey Muir, Teresa St. Pierre, Bryan Williams, Stephen Daniel and Jeffrey G. Edwards. Transient receptor potential vanilloid 1 agonists modulate hippocampal CA1 LTP via the GABAergic system. Neuropharmacology, 2011. doi:10.1016/j.physletb.2003.10.071

- Edwards, J. G., Gibson, H. E., **Jensen, T**., Nugent, F., Walther, C., Blickenstaff, J. and Kauer, J. A. (2011), A novel non-CB1/TRPV1 endocannabinoid-mediated mechanism depresses excitatory synapses on hippocampal CA1 interneurons. Hippocampus, 21: n/a. doi: 10.1002/hipo.20884

Abstracts:

- **Tyron Jensen**, Jeff Edwards. "Calcineurin is required for TRPV1-mediated LTD in the hippocampus." Intermountain Graduate Research Symposium Journal. (2011): 100.

- Tyron Jensen, Curtis Walther, Jacob Blickenstaff, Lauren Dean, John Hamblin, Jeremy Morris, Jeffrey Edwards. "Transient receptor potential vanilloid 1 modulation of hippocampal synaptic plasticity." Program No. 40.1 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online. June 2010.

- **Jensen T**, Edwards J. "Calcineurin's Role in TRPV1 Mediated LTD in the Hippocampus." Intermountain Graduate Research Symposium Journal. (2010): 138.

- Bennion D, **Jensen T**, Walther C, Mors M, Edwards J. "TRPV1 modulation of plasticity in the hippocampus." Program No. 715.7. 2009 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2009. Online. June 2009.

- Bennion D, **Jensen T**, Couch J, Castle M, Daniel S, Nelson B & Edwards J. "TRPV1 hippocampal modulation." Utah Conference on Undergraduate Research Journal. 3 (2009): 180.

- **T. JENSEN**, D. BENNION, J. COUCH, M. CASTLE, S. DANIEL, J. G. EDWARDS;. "TRPV1 modulation in the hippocampus." Program No. 737.7. 2008 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience. Online. Nov. 2008.

Presentations:

- Oral Presentation - "Calcineurin is required for TRPV1-mediated LTD in the hippocampus" - Intermountain Graduate Research Symposium 2011 - Logan, UT

- Poster Presentation - "TRPV1 mediated metaplasticity of hippocampal CA1 LTP and LTD" - Society for Neuroscience Annual Meeting 2010 - San Diego, CA

- Poster Presentation - "TRPV1 mediated metaplasticity of hippocampal CA1 LTP and LTD" - Intermountain Neuroscience Program Annual Symposium 2010 - Snowbird, UT

- Oral Presentation - "Calcineurin's Role in TRPV1 Mediated LTD in the Hippocampus" - Intermountain Graduate Research Symposium 2010 - Logan, UT

- Poster Presentation - "TRPV1 modulation of plasticity in the hippocampus" - Society for Neuroscience Annual Meeting 2009 - Chicago, IL

- Poster Presentation - "TRPV1 hippocampal modulation" - Utah Conference on Undergraduate Research 2009 - Salt Lake City, UT

- Poster Presentation - "TRPV1 modulation in the hippocampus" - Society for Neuroscience Annual Meeting 2008 - Washington, DC

- Poster Presentation - "TRPV1 modulation in the hippocampus" - SfN Faculty for Undergraduate Neuroscience Poster Competition 2008 - Washington, DC