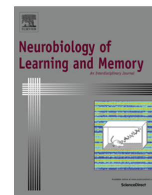
Neurobiology of Learning and Memory 142 (2017) 252–262

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme



Cerebellar learning modulates surface expression of a voltage-gated ion channel in cerebellar cortex

Jason R. Fuchs [a], Shelby W. Darlington [a], John T. Green [a], Anthony D. Morielli [b,*]

[a] Department of Psychological Science, University of Vermont, Burlington, VT 05405, United States
[b] Department of Pharmacology, University of Vermont, Burlington, VT 05405, United States

ARTICLE INFO

Article history:
Received 10 January 2017
Revised 5 May 2017
Accepted 12 May 2017
Available online 13 May 2017

Keywords:
Eyeblink conditioning
Pavlovian conditioning
Cerebellar cortex
Basket cell
Purkinje cell
Kv1.2

ABSTRACT

Numerous experiments using *ex vivo* electrophysiology suggest that mammalian learning and memory involves regulation of voltage-gated ion channels in terms of changes in function. Yet, little is known about learning-related regulation of voltage-gated ion channels in terms of changes in expression. In two experiments, we examined changes in cell surface expression of the voltage-gated potassium channel alpha-subunit Kv1.2 in a discrete region of cerebellar cortex after eyeblink conditioning (EBC), a well-studied form of cerebellar-dependent learning. Kv1.2 in cerebellar cortex is expressed almost entirely in basket cells, primarily in the axon terminal pinceaux (PCX) region, and Purkinje cells, primarily in dendrites. Cell surface expression of Kv1.2 was measured using both multiphoton microscopy, which allowed measurement confined to the PCX region, and biotinylation/western blot, which measured total cell surface expression. In the first experiment, rats underwent three sessions of EBC, explicitly unpaired stimulus exposure, or context-only exposure and the results revealed a decrease in Kv1.2 cell surface expression in the unpaired group as measured with microscopy but no change as measured with western blot. In the second experiment, the same three training groups underwent only one half of a session of training, and the results revealed an increase in Kv1.2 cell surface expression in the unpaired group as measured with western blot but no change as measured with microscopy. In addition, rats in the EBC group that did not express conditioned responses (CRs) exhibited the same increase in Kv1.2 cell surface expression as the unpaired group. The overall pattern of results suggests that cell surface expression of Kv1.2 is changed with exposure to EBC stimuli in the absence, or prior to the emergence, of CRs.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Research on learning-related changes in intrinsic excitability ("intrinsic plasticity", "non-synaptic plasticity", or "non-Hebbian plasticity") has contributed substantially to our understanding of the neurobiology of learning and memory (Daoudal & Debanne, 2003; Debanne, Daoudal, Sourdet, & Russier, 2003; Disterhoft & Oh, 2006; Frick & Johnston, 2005; Guzman-Karlsson, Meadows, Gavin, Hablitz, & Sweatt, 2014; Hansel, Linden, & D'Angelo, 2001; Mozzachiodi & Byrne, 2010; Remy, Beck, & Yaari, 2010; Sehgal, Song, Ehlers, & Moyer, 2013; Zhang & Linden, 2003). Learning-related intrinsic plasticity has been demonstrated in vertebrates, including cats, rabbits, rats, and mice and in invertebrates, including *Hermissenda* and *Aplysia*. Changes in action potential threshold, afterhyperpolarization amplitude, spike adaptation, and associated ion channel currents, such as A-type K^+ and Ca^{2+}-dependent K^+ currents, have been commonly reported after learning. These studies suggest that voltage-gated ion channels are regulated, at least transiently, during learning. The fact that regulation is typically transient (although not always, see Schreurs, Gusev, Tomsic, Alkon, & Shi, 1998) suggests that intrinsic plasticity may serve a modulatory function in memory encoding and consolidation, and in synaptic plasticity and the priming of neurons for synaptic plasticity ("metaplasticity"), rather than being part of the engram itself. On the other hand, recent research suggests that more excitable neurons can be preferentially recruited to a memory trace (Yiu et al., 2014).

Most studies demonstrating learning-related intrinsic plasticity employ electrophysiological methods, typically recording from brain slices taken from animals that have learned a task and from control animals. However, very few studies have examined the cell biological mechanisms involved in learning-related changes in voltage-gated ion channels. This is in contrast to *ligand-gated* ion channels, where measurement of cell-surface trafficking and expression have been relatively common. For example, cued fear conditioning is associated with delivery of GluA1 subunit-

* Corresponding author.
E-mail address: anthony.morielli@uvm.edu (A.D. Morielli).

http://dx.doi.org/10.1016/j.nlm.2017.05.009
1074-7427/© 2017 Elsevier Inc. All rights reserved.

containing AMPA receptors to lateral amygdala synapses (Nedelescu et al., 2010; Rumpel, LeDoux, Zador, & Malinow, 2005) while inhibitory avoidance or contextual fear conditioning involves a similar mechanism in dorsal hippocampal synapses (Matsuo, Reijmers, & Mayford, 2008; Mitsushima, Ishihara, Sano, Kessels, & Takahasi, 2011; Whitlock, Heynen, Shuler, & Bear, 2006).

Cell surface trafficking is not limited to ligand gated ion channels, and recent studies indicate that regulated trafficking is a key mechanism for modulating non-receptor ion channel function. Kv1.2 is an α-subunit within the Shaker (Kv1) family of voltage-gated potassium channels. Channels harboring Kv1.2 exist as tetramers composed entirely of Kv1.2 α-subunits, or Kv1.2 along with other Kv1 family α-subunits (Bixby et al., 1999; Chung, Shin, Kim, Lee, & Cha, 2001; Li, Jan, & Jan, 1992; Sheng, Liao, Jan, & Jan, 1993). The electrophysiological function of Kv1.2 is regulated in part by the channel's expression at the cell surface, a process governed by its endocytosis from and recycling back to the plasma membrane (Nesti, Everill, & Morielli, 2004; Stirling, Williams, & Morielli, 2009; Williams, Fuchs, Green, & Morielli, 2012). In the cerebellar cortex, Kv1.2 is primarily expressed in Purkinje cell (PC) dendrites and in basket cell (BC) axon terminals (Chung et al., 2001; Khavandgar, Walter, Sageser, & Khodakhah, 2005; McNamara, Muniz, Wilkin, & Dolly, 1993; Sheng, Tsaur, Jan, & Jan, 1992; Southan & Robertson, 1998b; Wolpaw & Lee, 1989). Expression of cerebellar Kv1.2 at the cell surface is governed by endocytosis and is modulated by the neuromodulator secretin via a mechanism involving protein kinase A (Williams et al., 2012).

The densest expression of Kv1.2 α-subunits in the cerebellum is at BC axon terminals (Chung et al., 2001; Koch et al., 1997; McNamara, Averill, Wilkin, Dolly, & Priestley, 1996; Southan & Robertson, 1998a, 1998b, 2000; Veh et al., 1995; Wang, Kunkel, Martin, Schwartzkroin, & Tempel, 1993; Wang, Kunkel, Schwartzkroin, & Tempel, 1994). Kv1.2 expression is most evident in the pinceaux (PCX) region of the BC axon terminal which lies at the base of the PC, surrounding its axon initial segment (Laube et al., 1996; McNamara et al., 1996; Wang et al., 1994). Kv1.2 is also expressed in the non-PCX region of BC axons where they descend around PC cell bodies (McNamara et al., 1996). Optogenetic activation of cerebellar molecular layer interneurons (BCs and stellate cells) inhibits PC action potentials and this inhibition consists of at least two components: an ultra-rapid (within 1 ms) inhibition of PC spiking that is Kv1-dependent and a GABA-dependent sustained inhibition that lasts 10–20 ms and is also Kv1.2 dependent (Blot & Barbour, 2014; Kole et al., 2015; Southan & Robertson, 1998a, 1998b, 2000). Kv1.2 at BC PCX affects ultra-rapid, ephaptic inhibition of PCs (Kole et al., 2015), whereas Kv1.2 in non-PCX regions of the BC axon terminal affects GABA-dependent PC inhibition (Southan & Robertson, 1998a, 1998b, 2000).

Although more difficult to detect by microscopy because of the diffuse nature of their expression, Kv1.2 in the cerebellar cortex is also expressed in PC dendrites in the molecular layer (Koch et al., 1997). Pharmacological inhibition of Kv1.2 on PC dendrites increases transient random bursts of PC spikes (Khavandgar et al., 2005), indicating that Kv1.2 is a key regulator of PC dendritic excitability. Thus, Kv1.2 influences PC function through effects on both excitatory (PC dendrite) and inhibitory (BC axon terminal) inputs.

Eyeblink conditioning (EBC) is a type of mammalian learning and memory that utilizes a discrete thalamic-brainstem-cerebellar circuit (DeZeeuw & Ten Brinke, 2015; Freeman, 2015; Thompson & Steinmetz, 2009). The basic EBC procedure involves trials in which a tone conditioned stimulus (CS) precedes (by about half a second) and co-terminates with an eye stimulation unconditioned stimulus (US). Essential sites of plasticity in the cerebellum that support expression of eye blink conditioned responses (CRs) to the tone CS include the anterior interpositus nucleus ipsilateral to the conditioned eyelid (Clark, McCormick, Lavond, & Thompson, 1984; Freeman, Halverson, & Poremba, 2005; Krupa, Thompson, & Thompson, 1993; Lavond, Hembree, & Thompson, 1985; Yeo, Hardiman, & Glickstein, 1985) and the cerebellar cortex around the base of the primary fissure ipsilateral to the conditioned eyelid (Mostofi, Holtzman, Grout, Yeo, & Edgley, 2010; Steinmetz & Freeman, 2014). We previously showed that intra-cerebellar cortical infusions of tityustoxin-Kα (TsTX), a highly selective blocker of Kv1.2 channels, facilitated EBC (Williams et al., 2012). This approach should block Kv1.2 both at BC axon terminals and PC dendrites, and thus assesses the overall effect of Kv1.2 inhibition.

Although it is clear that pharmacological inhibition of Kv1.2 affects EBC, we do not yet know if *regulation* of Kv1.2 is part of the cellular mechanism underpinning EBC. The current study addresses this question by measuring EBC-related changes in Kv1.2 surface expression around the base of the primary fissure in cerebellar cortex ipsilateral to the eye that receives the US. A great advantage of this model system is that Kv1.2 expression in cerebellar cortex is almost completely limited to BC terminals and PC dendrites and analysis can be targeted to these regions at the base of the primary fissure, a region critical for EBC (Mostofi et al., 2010; Steinmetz & Freeman, 2014). In the current experiments, cerebellar sections around the base of the primary fissure were taken from rats that had undergone EBC, explicitly unpaired stimulus presentations, or no stimulus presentations. Surface expression of Kv1.2 was analyzed by a combination of multiphoton microscopy (which allowed us to examine Kv1.2 surface expression specifically at the PCX region of the BC axon terminal) and biotinylation/western blot (which allowed us to examine Kv1.2 surface expression at BC terminals and PC dendrites combined). Our results demonstrate that EBC stimuli do induce changes in surface expression of Kv1.2 in cerebellar cortex in rats. Intriguingly, those changes are limited to animals that do not express learned eye blink responses, suggesting a role in early-phase acquisition.

2. Material and methods

2.1. Subjects

Male Wistar rats were purchased from Charles River (Quebec, Canada) and housed in pairs upon arrival with access to food and water *ad libitum*. Rats were single housed after surgery in an AAALAC-approved facility. The colony room was maintained on a 12 h light-dark cycle (lights on at 7:00 AM and off at 7:00 PM). Rats weighed 200–300 g prior to surgery. All behavioral testing took place during the light phase of the cycle and all procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont. Animal housing and experiments complied with the National Institutes of Health guide for the care and use of laboratory animals.

2.2. Surgery

Surgeries took place 4–6 days after arrival. Surgeries were performed under aseptic conditions. Rats were anesthetized with 3% isoflurane in oxygen. A midline scalp incision was made and four skull screw holes were drilled and skull screws were placed as anchors for the head stage. A bipolar stimulating electrode (Plastics One, Roanoke, VA) was positioned subdermally immediately dorsocaudal to the left eye. Two electromyogram (EMG) wires for recording activity of the orbicularis oculi muscle were each constructed of a 75-μm Teflon coated stainless steel wire soldered at one end to a gold pin fitted into a plastic threaded pedestal connector (Plastics One). The other end of each wire was passed subdermally to penetrate the skin of the upper eyelid of the left eye

Download English Version:

https://daneshyari.com/en/article/5043143

Download Persian Version:

https://daneshyari.com/article/5043143

Daneshyari.com