Voltage-Sensitive Calcium Channels in the Brain: Relevance to Alcohol Intoxication and Withdrawal

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Contents

Abstract

Voltage-sensitive Ca^{2+} (Ca_V) channels are the primary route of depolarization-induced Ca^{2+} entry in neurons and other excitable cells, leading to an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The resulting increase in $[Ca^{2+}]_i$ activates a wide range of Ca^{2+}-dependent processes in neurons, including neurotransmitter release, gene transcription, activation of Ca^{2+}-dependent enzymes, and activation of certain K^+ channels and chloride channels. In addition to their key roles under physiological conditions, Ca_V channels are also an important target of alcohol, and alcohol-induced changes in Ca^{2+} signaling can disturb neuronal homeostasis, Ca^{2+}-mediated gene transcription, and the function of neuronal circuits, leading to various neurological and/or neuropsychiatric symptoms and disorders, including alcohol withdrawal induced–seizures and alcoholism.

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Alcohol exposure · Alcohol intoxication · Alcohol withdrawal seizures · Calcium signaling

1 Introduction

In neurons, voltage-sensitive Ca^{2+} (Ca_V) channels serve as the primary route of Ca^{2+} entry in response to membrane depolarization, driving a localized increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The driving force for Ca^{2+} entry arises from the steep electrochemical gradient maintained between extracellular and intracellular Ca^{2+} concentrations, which are typically on the order of 1 mM and 100 nM, respectively; thus Ca^{2+} entry can change membrane potential and can therefore affect neuronal excitability. In neurons, low $[Ca^{2+}]_i$ is maintained by a variety of mechanisms and processes, including Ca^{2+} efflux via a Na^+/Ca^{2+} exchange protein and a Ca^{2+}-ATPase located at the plasma membrane, as well as the sequestration of intracellular Ca^{2+} into in Ca^{2+} stores (e.g., via the sarco-endoplasmic reticular ATPase pump) or by Ca^{2+}-buffering proteins (Berridge 2012).

The Ca_V-mediated localized increase in $[Ca^{2+}]_i$ in neurons activates a variety of downstream processes, including Ca^{2+}-induced Ca^{2+} release from intracellular Ca^{2+}-gated Ca^{2+} stores, activation of Ca^{2+}-activated K^+ channels, Ca^{2+}-activated chloride channels and Ca^{2+}-dependent enzymes, and other Ca^{2+}-dependent processes such as gene transcription and neurotransmitter release. In addition, Ca^{2+} entry following relatively mild membrane depolarization (e.g., depolarization induced by activation of *N*-methyl-D-aspartate receptors) can give rise to low-threshold Ca^{2+} spikes, which can further depolarize the plasma membrane, causing voltage-gated Na^+ channels to open and initiating the repetitive firing of action potentials (Cain and Snutch 2010). Thus, Ca_V channels play a wide range of important roles under both physiological and pathophysiological conditions, including a variety of diseases associated with neuronal excitability. In the central nervous system (CNS), Ca_V channels are also an important molecular target for alcohol, and changes in neuronal Ca^{2+} signaling induced by alcohol exposure and subsequent withdrawal can lead to alcoholism and alcohol withdrawal–induced seizures, (AWSs).

2 Structure, Diversity, and Localization of Voltage-Sensitive Ca^{2+} Channels in the CNS

2.1 Structure and Diversity of Ca_V Channels

Ca_V channels are large protein complexes comprised of a pore-forming α1 subunit and up to three auxiliary β, α2/δ, and γ subunits (Simms and Zamponi 2014). In addition to providing the pore through which Ca^{2+} flows, the α1 subunit of Ca_V channels also confers the channel's electrophysiological and pharmacological

properties; in contrast, the auxiliary subunits modulate the channel's biophysical properties and regulate the channel's trafficking to the plasma membrane. In human, nine distinct genes encode the α_1 subunits (designated α_{1A} through α_{1I}), all of which are expressed in the CNS (Simms and Zamponi 2014). Based on their responsiveness to changes in membrane potential, these nine Ca_V channels are broadly classified as either low voltage–activated (LVA, comprising the Ca_V3 family) channels or high voltage–activated (HVA, which include the Ca_V1 and Ca_V2 families) channels. Activation of LVA channels and HVA channels produced transient and sustained currents, respectively.

HVA Ca_V channels have both distinct and overlapping voltage dependence and kinetics, making it difficult to differentiate HVA Ca_V currents based solely on their biophysical properties. Fortunately, however, HVA Ca_V channels have unique pharmacological profiles, which have been used to confirm the heterogeneity of the channels expressed in the CNS. Moreover, based largely on their sensitivity to various Ca_V channel blockers, HVA Ca_V channels currents have been further classified into the following five types: L-type $Ca_V1.2$ (α_{1C}), L-type $Ca_V1.3$ (α_{1D}), N-type $Ca_V2.2$ (α_{1B}), P/Q-type Cav2.1 (α_{1A}), and R-type $Ca_V2.3$ (α_{1E}) channels, encoded by the *CACNA1C*, *CANA1D*, *CANA1B*, *CANA1A*, and *CACNA1E* genes, respectively (Ertel et al. 2000; Randall and Tsien 1995). In the CNS, P/Q-type Cav2.1 channels can give rise to both P-type and Q-type currents; this distinction is likely due to a combination of factors, including the Ca_V-β subunit and/or alternative splicing of the *CACNA1A* gene that encodes the channels (Richards et al. 2007).

Molecular analyses revealed that the LVA family of Ca_V channels consists of three distinct α_1 pore-forming subunits, namely $Ca_V3.1$ (α_{1G}), $Ca_V3.2$ (α_{1H}), and $Ca_V3.3$ (α_{1I}), encoded by the *CACNA1G*, *CANA1H*, and *CACNA1I* genes, respectively (Cribbs et al. 1998; Lee et al. 1999; Perez-Reyes et al. 1998). Interestingly, unlike HVA Ca_V channels, the α1 subunit of LVA Ca_V channels does not require auxiliary subunits to form a fully functional channel, although LVA Ca_V channels can be regulated by auxiliary subunits (Klöckner et al. 1999). Finally, the three genes that encode the $Ca_V3.x$ subunits can undergo alternative splicing, giving rise to a wide diversity of functional LVA Ca_V channels (Swayne and Bourinet 2008). The Ca_V-α_1 subunit is comprised of four transmembrane domains, which are connected by cytoplasmic linkers (Simms and Zamponi 2014; Turner and Zamponi 2014). The N and C termini are located in the cytoplasmic side and they contained important sites for protein–protein interactions such as with G-protein and protein kinases (Simms and Zamponi 2014; Turner and Zamponi 2014). Interestingly, phosphorylation by PKA or PKC alters the voltage dependence and kinetics of Ca_V currents (Gray and Johnston 1987; Nagao and Adachi-Akahane 2001; Sculptoreanu et al. 1993; Stea et al. 1995).

2.2 Localization and Function HVA Ca_V1 Channels

Although L-type $Ca_V1.x$ channels are expressed widely throughout brain, each channel subtype has a unique cellular and subcellular distribution. For example, L-type $Ca_V1.3$ channels are distributed relatively evenly, whereas L-type $Ca_V1.2$ channels are localized in clusters (Hell et al. 1993; Tippens et al. 2008). Moreover, L-type $Ca_V1.2$ and $Ca_V1.3$ channels are located predominantly on the cell soma (where they regulated depolarization and Ca^{2+}-dependent pathways that control gene expression), proximal dendrites, and in some interneurons in the olfactory bulb, cerebral cortex (pyramidal neurons), hippocampus (pyramidal neurons in the CA1–CA3 areas), dentate gyrus (granule neurons), amygdala, inferior colliculus, cerebellum (granule layer, molecular layer, Purkinje cells), and spinal cord (Hell et al. 1993). Unlike L-type $Ca_V1.3$ channels, $Ca_V1.2$ channels are expressed in astrocytes in the CA3 area of the hippocampus (Tippens et al. 2008; Westenbroek et al. 1990). The distribution of $Ca_V1.2$ and $Ca_V1.3$ channels throughout the CNS has been confirmed by RT-PCR analysis, which shows that the levels of *CACNA1C* and *CACNA1D* mRNA matches the protein levels of Ca_V-α1C and Ca_V-α1D subunits, respectively (Sinnegger-Brauns et al. 2009; Schlick et al. 2010). In the striatum, *CACNA1C* and *CACNA1D* mRNA are co-expressed in medium-sized spiny neurons (Olson et al. 2005). Interestingly, L-type $Ca_V1.3a$ (but not $Ca_V1.3b$) isoform co-localizes with Shank protein and the synaptic protein PSD-95 in medium spiny neurons at excitatory synapses (Olson et al. 2005). In the CNS, approximately 80% and 20% of L-type Ca_V1 channels are $Ca_V1.2$ and $Ca_V1.3$ channels, respectively (Hell et al. 1993; Sinnegger-Brauns et al. 2009). With respect to function, evidence suggests that L-type $Ca_V1.3$ channels activate with less depolarization and inactivate more slowly than $Ca_V1.2$ channels (Koschak et al. 2001; Xu and Lipscombe 2001). Given their unique set of biophysical properties, L-type $Ca_V1.3$ channels likely play an important role in controlling Ca^{2+}-dependent firing; moreover, L-type $Ca_V1.3$ channels help sustain Ca^{2+} influx at membrane potentials at which $Ca_V1.2$ channels are closed.

$Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$ channels (i.e., P/Q-type, N-type, and R-type, respectively) are also expressed throughout the CNS. P/Q-type $Ca_V2.1$ channels are primarily concentrated in presynaptic terminals and dendritic shafts, N-type $Ca_V2.2$ are found mainly in dendrites and some cell bodies of neurons, and R-type $Ca_V2.3$ channels are found mainly in the cell soma in most sites with variable expression in dendrites (Westenbroek et al. 1992, 1995; Yokoyama et al. 1995). These Ca_V channels are found primarily in the olfactory bulb, cerebral cortex (pyramidal neurons), striatum (medium-sized spiny neurons), amygdala, hippocampus (pyramidal neurons in CA1–CA3 areas), dentate gyrus (granule neurons), thalamus, globus pallidus, hypothalamus, inferior colliculus, and cerebellum (Purkinje cells) (Hillman et al. 1991; Westenbroek et al. 1992, 1995; Volsen et al. 1995; Yokoyama et al. 1995; Day et al. 1996; Xu et al. 2010). In the cortex and hippocampus, there is barely detection of R-type $Ca_V2.3$ channels in proximal dendrites, while other structures such as olfactory bulb, amygdala, and cerebellum have intense expression of these channels in the dendrites, the

prominent sites of Ca^{2+} entry, causing transient increase in cytosolic Ca^{2+}. Molecular and biochemical analyses have confirmed that mRNA levels match the corresponding protein for $Ca_V2.1(\alpha_{1A})$, $Ca_V2.2(\alpha_{1B})$, and $Ca_V2.3$ (α_{1E}) (Mori et al. 1991; Soong et al. 1993; Day et al. 1996; Ludwig et al. 1997; Schlick et al. 2010).

At synaptic terminal, the rapid release of neurotransmitters requires tight coupling between presynaptic $Ca_V2.x$ channels to the release machinery. In addition to regulating vesicle fusion, members of the $Ca_V2.x$ channels also control neuronal excitability. For example, P/Q-type $Ca_V2.1$ and N-type $Ca_V2.2$ channels interact both physically and functionally with large-conductance, Ca^{2+}-activated K^+ channels, providing the Ca^{2+} influx needed to activate these channels (Faber and Sah 2003; Berkefeld et al. 2010); thus, P/Q-type $Ca_V2.1$ and N-type $Ca_V2.2$ channels control neuronal excitability by regulating K^+ conductances.

2.3 Localization and Function LVA Ca_V3 Channels

Like HVA Ca_V channels, LVA Ca_V3 channels are also distributed throughout the CNS; however, their expression is restricted to the cell body and dendrites of neurons primarily in the olfactory bulb (granule layer), cerebral cortex (pyramidal neurons, GABAergic interneurons), striatum, amygdala, hippocampus (CA1–CA3 pyramidal neurons), dentate gyrus (granule cells), thalamus (large neurons, GABAergic interneurons), substantia nigra, inferior colliculus, superior colliculus, inferior olive, cerebellum (granule layer, molecular layer, Purkinje cells), and spinal cord (Craig et al. 1999; Talley et al. 1999; Yunker et al. 2003; McKay et al. 2006; Kovács et al. 2010; Liu et al. 2011; Kanyshkova et al. 2014).

As discussed above, LVA Ca_V3 channels are activated upon weak depolarization and carry depolarizing currents; therefore, similar to L-type $Ca_V1.3$ channels, LVA $Ca_V3.x$ channels also play an important role in controlling neuronal excitability. LVA $Ca_V3.x$ channels also inactivate at a fast rate. Thus, a combination of low threshold of activation with fast inactivation kinetics results in transient Ca^{2+} influx, giving rise to the so-called "low-threshold Ca^{2+} potentials," which initiate the burst-firing process (Cain and Snutch 2010; Contreras 2006; Jahnsen and Llinas 1984; Lee et al. 2003; Yazdi et al. 2007; Xu and Clancy 2008). The burst-firing mode in the CNS contributes to the generation of physiological events such as sleep spindles, and pathological conditions such as epileptic seizures (Cain and Snutch 2010, 2012). In addition, LVA $Ca_V3.x$ channels generate a so-called "window current" near the neuron's resting membrane potential, thereby regulating Ca^{2+} homeostasis (Dreyfus et al. 2010). In the CNS, LVA $Ca_V3.x$ channels are also associated both with voltage-gated K^+ channels and with Ca^{2+}-activated K^+ channels (Anderson et al. 2010; Rehak et al. 2013), giving LVA $Ca_V3.x$ channels the ability to activated K^+ channels and regulate neuronal firing.

3 Effects of Acute Alcohol Exposure on the Expression and Function of Ca_V Channels

Oakes and Pozos (1982a, b) reported that alcohol exposure decreased Ca_V currents (and voltage-gated K^+ currents but not voltage-gated Na^+ currents) in dorsal root ganglia neurons. This effect was not associated with change in the resting membrane potential and spike amplitude. However, the duration of the action potential (AP) was decreased, and AP threshold was increased (Oakes and Pozos 1982a, b). A large body of experimental evidence indicates that acute alcohol exposure suppresses K^+ depolarization–induced and AP–evoked Ca^{2+} transients in several CNS neurons including inferior colliculus, cerebellar, and hippocampal neurons (Gruol et al. 1997; Mah et al. 2011; Morton and Valenzuela 2016; our unpublished data). Consistent with these findings, we found that acute alcohol exposure inhibits the current carried by HVA Ca_V channels in inferior colliculus neurons (our unpublished data). Furthermore, acute alcohol exposure suppresses currents through L-type $Ca_V1.x$ channels at neurohypophysial terminals, in supraoptic neurons, and hippocampal neurons (Wang et al. 1991, 1994; Widmer et al. 1998; Zucca and Valenzuela 2010). On the other hand, P-type $Ca_V2.1$ channels in Purkinje cells are unaffected by acute alcohol exposure (Hall et al. 1994). Thus, in the CNS, L-type $Ca_V1.x$ channels appear to be particularly sensitive to the acute effects of alcohol exposure.

Interestingly, LVA $Ca_V3.x$ channels are also an important target for alcohol. For example, acutely exposing rodent thalamic neurons to a low or high alcohol concentration increases or decreases, respectively, LVA $Ca_V3.x$ currents (Mu et al. 2003; Joksovic et al. 2005). Furthermore, the inhibitory effect of alcohol on LVA $Ca_V3.x$ currents appears to be mediated by protein kinase C (Shan et al. 2013). In contrast, acute exposure to either low or high alcohol concentration inhibits LVA $Ca_V3.x$ currents in the inferior olive in primates (Welsh et al. 2011). Thus, the increase in LVA $Ca_V3.x$ currents in response to low alcohol concentration in rodents – but not in primates – suggests species-specific differences in the underlying mechanisms.

The inhibition of HVA Ca_V channels and LVA Ca_V channels (Fig. 1), and downstream Ca^{2+}-related signaling following acute alcohol exposure suggests that this mechanism may induce a compensatory upregulation of HVA Ca_V channels and LVA Ca_V channels during chronic alcohol intoxication; this upregulation would be masked by the inhibitory effect alcohol, but would then be revealed during alcohol withdrawal.

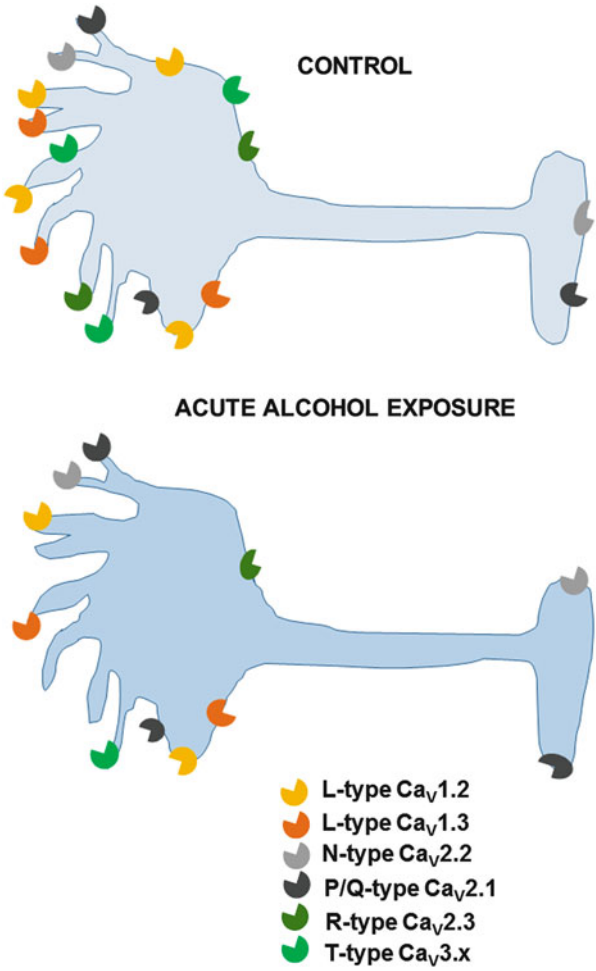

Fig. 1 Acute alcohol intoxication downregulated Ca_V channels in the brain. In normal conditions, the expression of LVA and HVA Ca_V channels is tightly regulated whereas following acute alcohol intoxication, a dysregulation occurs leading to a downregulation of LVA and HVA Ca_V channels (L-type $Ca_V1.x$)

4 Effects of Chronic Alcohol Exposure on the Expression and Function of Ca_V Channels

Several lines of evidence indicate that chronic alcohol exposure alters Ca^{2+} signaling in the CNS. For example, chronic alcohol exposure increases AP–evoked Ca^{2+} transients in hippocampal neurons (Mulholland et al. 2015), possibly by upregulating of Ca_V channels. Consistent with this notion, P-type $Ca_V2.1$ current is increased in the cerebellum during chronic alcohol exposure (Gruol and Parsons 1994). On the other hand, chronic alcohol intoxication by inhalation did not alter the protein levels of P/Q-type $Ca_V2.1$ (α_{1A}) protein levels in cortical neurons (Katsura et al. 2005). Similarly, the protein levels of the P/Q-type α_{1A} subunit were

unchanged in the central nucleus of the amygdala following chronic intermittent alcohol exposure (Varodayan et al. 2017a). Increased protein levels of L-type $Ca_V1.3$ (α_{1D}) channels were measured in cortical neurons in mice following chronic alcohol exposure by inhalation (Katsura et al. 2005). However, in the model of chronic intermittent alcohol exposure, the protein levels of the L-type $Ca_V1.2$ (α_{1C}) subunit were decreased in the central nucleus of the amygdala (Varodayan et al. 2017b). The dihydropyridine binding sites, which represent L-type $Ca_V1.x$ channels, were increased in ethanol-dependent brains (Dolin et al. 1987). Accordingly, chronic alcohol exposure increased total Ca_V currents including L-type $Ca_V1.x$ in hippocampal neurons in ethanol-tolerant long-sleep mice compared to short-sleep mice; this effect was not associated with changes in the biophysical properties of the channels, suggesting an increase in the number of functional L-type $Ca_V1.x$ channels (Huang and McArdle 1993). L-type $Ca_V1.x$ channels are also implicated in alcohol-mediated neurodegeneration, as inhibition of these channels attenuated cytotoxicity related to chronic alcohol exposure of neocortical cell cultures (Ruhe and Littleton 1994).

Finally, the protein levels of N-type $Ca_V2.2$ (α_{1B}) channels were unchanged in cortical neurons following chronic alcohol administration (Katsura et al. 2005), whereas McMahon et al. (2000) reported an increase in the number of N-type $Ca_V2.2$ channels in the frontal cortex and hippocampus in AWS-prone mice following chronic alcohol administration. Thus, the increase in N-type $Ca_V2.2$ channel expression may be specific to certain brain structures, and this increase may be related to the genetic predisposition of AWS-prone mice to these seizures. Importantly, mice that lack functional N-type $Ca_V2.2$ channels have reduced alcohol consumption (Newton et al. 2004). Similarly, mice treated with blockers and/or agonists of L-type $Ca_V1.x$ channels have reduced alcohol consumption (Rezvani and Janowsky 1990; Rezvani et al. 1991; De Beun et al. 1996a, b). These findings suggest that the anti-alcohol effect may not be related to antagonistic activity at L-type $Ca_V1.x$ channels; alternatively, the anti-alcohol effect may be restricted to specific brain sites. The amygdala appears to be one of the brain sites underlying this behavioral effect, as blocking of L-type $Ca_V1.x$ channels in the central nucleus of the amygdala reduces alcohol intake in rodents (Varodayan et al. 2017b). Taken together, these findings suggest that both L-type $Ca_V1.x$ channels and N-type $Ca_V2.2$ channels might serve as viable therapeutic targets for treating of alcoholism. The mechanisms underlying changes in L-type $Ca_V1.x$ channels and N-type $Ca_V2.2$ channels are not fully understood (Fig. 2). Nevertheless, chronic alcohol exposure increases the expression of protein kinase C (PKC) isoforms, including PKC delta (PKCδ) and PKC epsilon (PKCε); moreover, chronic alcohol exposure upregulated L-type $Ca_V1.x$ channels and N-type $Ca_V2.2$ channels via PKCδ- and PKCε-dependent mechanism, respectively (Gerstin et al. 1998; McMahon et al. 2000).

Interestingly, in primates, chronic alcohol exposure decreases and increases LVA $Ca_V3.x$ in the thalamus and inferior olive, respectively (Carden et al. 2006; Welsh et al. 2011). In contrast, no changes in the mRNA levels or current density of LVA $Ca_V3.x$ channels were seen in thalamic neurons in a mouse model of chronic alcohol exposure (Graef et al. 2011); however, the steady-state inactivation of LVA $Ca_V3.x$ channels was altered in these neurons during alcohol intoxication suggesting a change in Ca^{2+} currents carried by these channels (Graef et al. 2011).

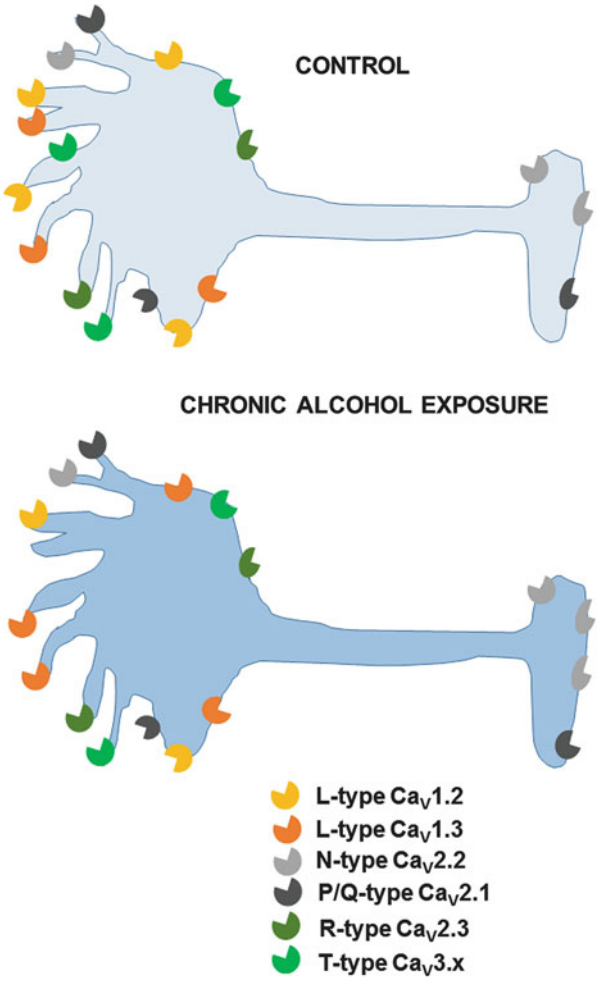

Fig. 2 Chronic alcohol intoxication upregulates Ca_V channels in the brain. During chronic alcohol exposure, a compensatory mechanism to the inhibitory effect of alcohol exposue occurs leading to an upregulation of Ca_V channels including L-type $Ca_V1.3$ (but downregulation of $Ca_V1.2$); moreover, the function and/or expression of these channels is masked by the inhibitory effect of alcohol. LVA Ca_V channels are either upreglated or downregulated in various brain sites

5 Effects of Alcohol Withdrawal on the Expression and Function Ca_V Channels

Alcohol withdrawal triggers increase in the expression of early gene c-fos throughout the CNS at the time at which the seizure susceptibility peaked (Bouchenafa and Littleton 1998). The increased expression of c-fos was prevented by inhibition of L-type $Ca_V1.x$ channels, suggesting an important role of Ca^{2+} influx in the mechanisms underlying AWS susceptibility (Bouchenafa and Littleton 1998). In addition, withdrawal from chronic alcohol exposure induced neuronal hyperexcitability in the hippocampus; this epileptiform activity was mediated, in part, by L-type $Ca_V1.x$ channels (Riplet et al. 1996; Whittington and Little 1991, 1993; Whittington et al. 1992, 1995). Seizures are usually the most severe symptoms

associated with alcohol withdrawal syndrome. Typically, these AWSs are generalized tonic-clonic seizures, which are initiated in the brainstem. In our model of acoustically evoked AWSs, neurons in the IC play a critical role in initiating AWSs, whereas the cortex, hippocampus, and amygdala play a role in propagating these seizures (Faingold et al. 1998; Takao et al. 2006; Faingold 2008; Newton and N'Gouemo 2017). In this model, K^+ depolarization–induced Ca^{2+} transients were increased in inferior colliculus neurons when the susceptibility to AWS peaks (our unpublished data). The influx of Ca^{2+} into neurons plays an important role in the neuronal hyperexcitability that underlies seizures, as $[Ca^{2+}]_i$ rises – and extracellular $[Ca^{2+}]$ decreases – during epileptiform activity (Heinemann et al. 1977; Albowitz et al. 1997; Delorenzo et al. 2005). Thus, inhibition of Ca^{2+} influx into neurons is a promising therapeutic approach for various types of seizures, including AWSs. Interestingly, pharmacologically blocking L-type $Ca_V1.x$ channels suppressed acoustically evoked AWSs (Little et al. 1986). These findings suggest that altered L-type $Ca_V1.x$ channels – at least in the IC – play a key role in initiating these seizures. Consistent with this notion, currents through HVA Ca_V channels are increased before the onset of AWS susceptibility and when the prevalence of AWSs peaks, but they returned to control levels after AWS susceptibility has returned to baseline (N'Gouemo 2015; N'Gouemo and Morad 2003). Thus, the increase in HVA Ca_V currents measured in IC neurons *prior* to the onset of AWS susceptibility cannot be a consequence of seizure activity. Interestingly, alcohol withdrawal increased HVA Ca_V currents in dentate granule neurons in AWS-prone mice but not in AWS-resistant mice (Perez-Velazquez et al. 1994), suggesting that genetic differences in the genes encoding HVA Ca_V channels may contribute to differences in AWS susceptibility and the expression of HVA Ca_V channels.

Alcohol withdrawal-induced upregulation of L-type $Ca_V1.x$ channels in the brain was also reported in a mouse model (Brennan et al. 1990; Guppy et al. 1995; Watson and Little 1999). In our rat model of acoustically evoked AWSs, the increased Ca^{2+} current density in IC neurons mediated by L-type $Ca_V1.x$ channels and P-type $Ca_V2.1$ channels occurs during peak AWS susceptibility (N'Gouemo 2015; N'Gouemo and Morad 2003). These findings suggest a possible causal relationship between the upregulation of L-type $Ca_V1.x$ channels and P-type $Ca_V2.1$ channels in IC neurons and the occurrence of AWSs. L-type $Ca_V1.x$ channels and P-type $Ca_V2.1$ channels play important roles in synaptic plasticity and glutamate release, respectively (Thiagarajan et al. 2005; Ermolyuk et al. 2013). Thus, an increase in currents through L-type $Ca_V1.x$ channels and/or P-type $Ca_V2.1$ channels in IC neurons is likely to increase both firing and transmitter release, leading to increased AWS susceptibility. Consistent with this notion, blocking L-type $Ca_V1.x$ channels in the IC suppressed AWS susceptibility, whereas inhibiting P-type $Ca_V2.1$ channels only reduced AWS severity (N'Gouemo 2015). Moreover, the protein levels of L-type $Ca_V1.3$ (α_{1D}) channels – but not L-type $Ca_V1.2$ (α_{1C}) channels or P/Q-type $Ca_V2.1$ (α_{1A}) channels – are upregulated in IC neurons when AWS susceptibility peaks (Fig. 3), but not *prior* to the onset of AWS susceptibility (N'Gouemo et al. 2015; Newton et al. 2018). However, it is important to note that the lack of change in protein levels of P/Q-type $Ca_V2.1$ (α_{1A}) channels reflects all P/Q-type channel phenotypes and may therefore masks any increase in the selective expression of P-type $Ca_V2.1$ channels occurring in some selective neuronal subtypes.

Interestingly, although mRNA expression of *CACNA1D* and *CACNA1A* (which encode the L-type α_{1D} and P/Q-type α_{1D} subunits, respectively) is increased in IC neurons prior to the onset of AWS susceptibility, their corresponding total protein levels are unchanged in these neurons (N'Gouemo et al. 2015; Newton et al. 2018). Thus, changes in cell surface expression and/or phosphorylation of these HVA Ca_V channels may account for the increased current density in IC neurons *prior* to the onset of AWS susceptibility. In support of this notion, the activity and expression of protein kinase A are increased in IC neurons *prior* to the onset of AWS susceptibility (Akinfiresoye et al. 2016). Under normal conditions, phosphorylation by protein kinase A enhances L-type $Ca_V1.x$ and P-type $Ca_V2.1$ currents (Fournier et al. 1993; Mogul et al. 1993; Davare and Hell 2003), while activation of PKC inhibits the activity of N-type $Ca_V2.2$ channels, but increases other types of Ca_V currents (Diversé-Pierluissi and Dunlap 1993; Rane and Dunlap 1986; Rane et al. 1989). Interestingly, alcohol acts on L-type $Ca_V1.x$ channels by inhibiting calmodulin-dependent activity of the channel (Canda et al. 1995). Thus, increase in L-type $Ca_V1.x$ currents *prior* to the onset of AWS susceptibility may be due to phosphorylation of the channels. Similarly, downregulation of N-type $Ca_V2.2$ channels seen in IC neurons at the time at which AWS susceptibility peaks may be due to enhanced PKC activity.

On the other hand, the protein levels of N-type $Ca_V2.2$ (α1B) subunit are decreased in IC neurons when AWS susceptibility peaks (N'Gouemo et al. 2006) (Fig. 3). Interestingly, activation of PKC inhibits the activity of N-type $Ca_V2.2$ channels, but increases other types of Ca_V currents (Diversé-Pierluissi and Dunlap 1993; Rane and Dunlap 1986; Rane et al. 1989), suggesting increased PKC activity in the IC following alcohol withdrawal at the time at which the susceptibility to AWS peaked. The downregulation of N-type $Ca_V2.2$ channels may contribute to AWS susceptibility by reducing Ca^{2+}-dependent inhibitory mechanisms, as Ca^{2+} influx contributes to the activation of Ca^{2+}-activated K^+ current, which initiates repolarization and underlies the afterhyperpolarization, an intrinsic neuronal inhibitory mechanism (Faber and Sah 2003; Loane et al. 2007; Berkefeld et al. 2010; N'Gouemo and Morad 2014). Interestingly, some Ca^{2+} channel types have been shown to provide the necessary Ca^{2+} influx required to activate small-conductance, and/or large-conductance, Ca^{2+}-activated K^+ channels in the brain (Faber and Sah 2003; Berkefeld et al. 2010). Thus, there appear to be significant differences in coupling between Ca^{2+} channels and Ca^{2+}-activated K^+ channels, suggesting a functional role for the Ca^{2+} channels in driving the activity of Ca^{2+} microdomains.

In primates, alcohol withdrawal decreases LVA $Ca_V3.x$ currents in inferior olive neurons (Welsh et al. 2011). In a mouse model of alcohol withdrawal, thalamic neurons have increased mRNA levels of the genes encoding the LVA $Ca_V3.2$ and $Ca_V3.3$ channel subtypes, but not $Ca_V3.1$ channel subtype (Graef et al. 2011). Despite these changes in mRNA levels and in the steady-state inactivation of LVA $Ca_V3.1x$ channels, alcohol withdrawal does not cause a change in LVA $Ca_V3.1x$ currents in thalamic neurons (Graef et al. 2011). However, ethosuximide, a potent blocker of LVA $Ca_V3.x$ channels commonly used to treat absence seizures, suppresses susceptibility to AWSs in a mouse model (Riegle et al. 2015), suggesting these channels may have therapeutic applications beyond the treatment of absence seizures.

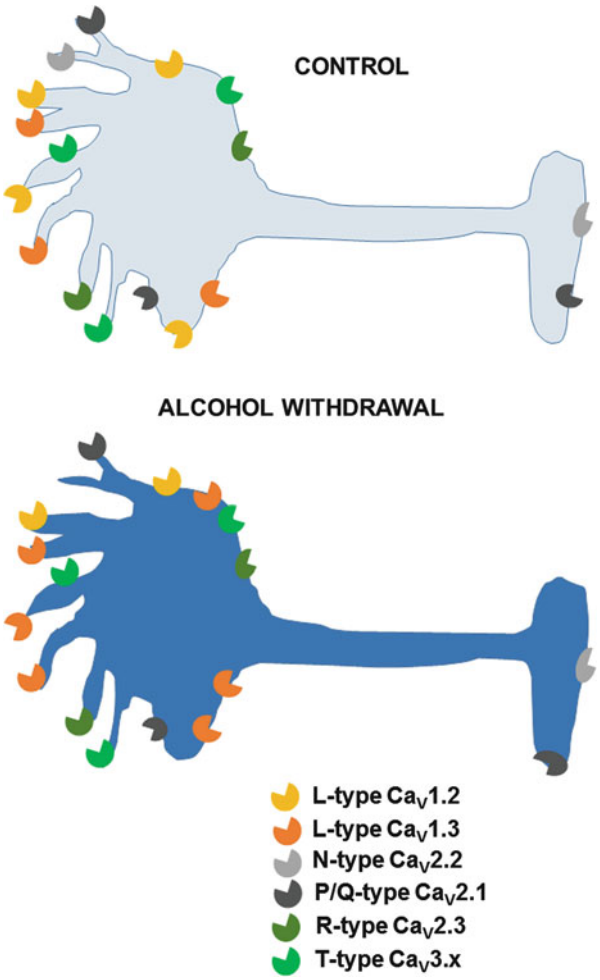

Fig. 3 Alchol withdrawal upregulates Ca_V channels. Alcohol withdrawal following chronic alcohol exposure unmasks the upregulation of Ca_V channels mainly L-type CaV1.3 channels and downregulation of L-type CaV1.2 and N-type $Ca_V2.2$ channels. LVA Ca_V channels are either upreglated or downregulated in various brain sites

6 Conclusion

In the CNS, Ca_V channels play an important role in regulating neuronal excitability, and changes in their activity and/or expression contribute to a wide variety of pathological conditions, including seizures. In keeping with their central role in CNS excitability, Ca_V channels are also an important target for alcohol, and both acute and chronic alcohol exposure, as well as alcohol withdrawal, can alter the function of Ca_V channels, giving rise to an array of symptoms and disorders, including alcohol abuse, alcoholism, and AWSs. Paradoxically, there is a positive relationship between increased Ca_V channel function/expression and increased susceptibility to AWSs, yet downregulating Ca_V channels can also cause seizures, as

some Ca_V channels are functionally coupled to K^+ channels and/or chloride channels. From this review, it becomes clear that HVA $Ca_V1.x$ (i.e., L-type) channels and HVA $Ca_V2.2$ (i.e., N-type) channels are promising targets for treating alcohol abuse and alcoholism; in contrast, L-type $Ca_V1.3$ – and to some extent LVA $Ca_V3.x$ (i.e., T-type) – channels are promising targets for treating AWSs. Moreover, the alcohol-related changes in the function and/or expression of various Ca_V channels vary among brain structures, suggesting the need for targeted therapeutic approaches, reflecting the notion that localized changes in specific Ca_V channels induce distinct sets of symptoms associated with alcoholism and the alcohol withdrawal syndrome.

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