

Targeting voltage-gated calcium channels in neurological and psychiatric diseases

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Abstract | Voltage-gated calcium channels are important regulators of brain, heart and muscle functions, and their dysfunction can give rise to pathophysiological conditions ranging from cardiovascular disorders to neurological and psychiatric conditions such as epilepsy, pain and autism. In the nervous system, calcium channel blockers have been used successfully to treat absence seizures, and are emerging as potential therapeutic avenues for pathologies such as pain, Parkinson disease, addiction and anxiety. This Review provides an overview of calcium channels as drug targets for nervous system disorders, and discusses potential challenges and opportunities for the development of new clinically effective calcium channel inhibitors.

Calcium channels

A group of membrane proteins that allow entry of calcium into cells.

Alternative splicing

A process by which one gene can create different variants of one protein.

Parkinson disease

A neurological disorder caused by a loss of dopaminergic neurons.

Epilepsy

A neurological disorder in which patients present with seizures.

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Calcium ions are critical mediators of cell signalling by virtue of their electrogenic functions (that is, calcium-induced changes in membrane potential)¹ and through their roles as intracellular messengers (that is, through activation of calcium-dependent enzymes)². Increases in intracellular calcium that are mediated either by their release from internal calcium stores, or via entry of calcium ions across the plasma membrane, have been linked to a wide spectrum of physiological processes, including neurotransmitter/hormone release³, activation of gene transcription^{4,5}, and muscle contraction⁶. Consequently, processes that result in compromised calcium signalling can lead to a range of pathophysiological conditions, such as hypertension, cardiac hypertrophy and a vast array of neurological problems⁷. One of the key mediators of calcium entry from the extracellular space are voltage-gated calcium channels — a family of membrane proteins that open in response to membrane depolarization to permit the influx of calcium along its electrochemical gradient. The mammalian nervous system expresses nine different types of calcium channels that have specific functions and subcellular distributions⁸, with additional functional diversity arising from alternative splicing events⁹ and specific association with different types of ancillary subunits and regulatory proteins¹⁰. Calcium channel dysregulation has been linked to a number of disorders, including pain, Parkinson disease and epilepsy⁷. Furthermore, mutations in calcium channel genes in human patients have been associated with pathologies such as seizure disorders, migraine and ataxia^{11,12}. Consequently, calcium channels are considered important targets for therapeutic intervention.

A number of drugs that block voltage-gated calcium channels are in clinical use (TABLE 1), with many other calcium channel blockers being in preclinical or early clinical development. This Review article highlights the role of these channels in neuronal function and assesses their growing potential as drug targets for nervous system disorders.

Subtypes and structure of calcium channels

Before the advent of molecular cloning techniques, calcium channels in the heart and brain were classified based on their voltage-dependent activation into either high voltage-activated (HVA) or low voltage-activated (LVA; also known as T-type) channels¹³, with the latter requiring smaller membrane depolarizations to open. HVA channels are subdivided further based on their pharmacological and biophysical characteristics into L-, P-, Q- and R-types¹⁴. We now know that these different subtypes of HVA and LVA channels correspond to a total of ten different Cava1 subunits that are encoded in the mammalian genome. Three of these Cava1 subunits (Cav3.1, Cav3.2 and Cav3.3) comprise the family of T-type calcium channels, and their expression in the plasma membrane is sufficient to form functional channel proteins¹⁵. There are two major families of HVA Cava1 subunits: the Cav1 family encodes four different types of L-type channels (Cav1.1 to Cav1.4), whereas the Cav2 family encompasses P/Q-type (Cav2.1), N-type (Cav2.2) and R-type (Cav2.3)⁷ channels, with P- and Q-type channels being distinguished by alternative splicing and channel subunit composition^{16,17}. Each of these $\alpha 1$ subunits is a large (~200 kDa) protein composed of four homologous transmembrane

Table 1 | Selected calcium channel blockers for the treatment of neurological or psychiatric conditions

Compound (company)*	Targets	Main indications	Possible indications	Status [‡]	Refs
Isradipine (Dynacirc; Reliant)	L-type channels	Hypertension	Parkinson disease and dependency	Approved, Phase III trial for Parkinson disease	170,207
Nimodipine (Nimotop; Bayer)	L-type channels and T-type channels	Hypertension	Febrile seizures	Approved	154
Cilnidipine (Atelec/Cilacar; Fuji/Ajinomoto)	L-type channels and N-type channels	Hypertension	Pain and tremor	Approved	70,143
Gabapentin (Neurontin; Pfizer)	Cav α 2 δ subunits	Pain and epilepsy	Anxiety	Approved	148
Pregabalin (Lyrica; Pfizer)	Cav α 2 δ subunits	Pain and epilepsy	Anxiety	Approved	82,148,221
Lamotrigine (Lamictal; GlaxoSmithKline)	R-type channels (NS)	Epilepsy and bipolar disorder	Pain	Approved	149,150
Topiramate (Topamax; Mylan)	R-type channels (NS)	Epilepsy	Weight loss, addiction and PTSD	Approved	152,153
Zonisamide (Zonegran; Eisai)	T-type channels (NS)	Epilepsy	Pain and Parkinson disease	Approved	145
Ethosuximide (Zarontin; Pfizer)	T-type channels	Epilepsy	Pain	Approved	101,140
Ziconotide (Prialt; Elan)	N-type channels	Pain	NA	Approved	86–91
Valproate (Depakene/Convulex; Abbott)	T-type channels (NS)	Epilepsy and bipolar mania	Parkinson disease	Approved	143,145
Z944 (Epirus)	T-type channels	Pain	Epilepsy	Phase II trial for pain	112,147
CNV2197944 (Convergence)	N-type channels	Pain	Anxiety and dependency	Phase II trial for pain	See the Convergence Pharmaceuticals press release
Z160 (Epirus)	N-type channels	Pain	Anxiety and dependency	Failed Phase II trial for pain	93

NA, not applicable; NS, not specified; PTSD, post-traumatic stress disorder. *The names of distributing pharmaceutical companies are shown in parentheses, although several of the compounds are now available as generic drugs. [‡]Note that Z944 and CV2197944 are in clinical trials and not yet approved.

domains, each containing six transmembrane helices plus a pore loop (p-loop) motif (FIG. 1) that forms the permeation pathway of the channel and ensures selectivity for calcium ions over monovalent ions⁷. Each of the membrane domains also contains a voltage sensor motif that allows the channel to open in response to membrane depolarization¹⁸. The amino- and carboxy-terminal regions and the linkers between the four membrane domains face the cytoplasm and serve as important targets for second messenger regulation of channel function⁷. The majority of known drug interaction sites with voltage-gated calcium channels lie either within the permeation pathway, or in channel regions that closely surround the pore of the channel, such as the fifth and sixth transmembrane helices^{19–21} (FIG. 1). All of the HVA Cav α 1 isoforms require co-assembly with ancillary calcium channel subunits to obtain properly functioning channels²².

Cav β subunits are cytoplasmic proteins that associate with the domain I–II linker region of the Cav α 1 subunit²³. The Cav α 2 δ subunits undergo proteolytic cleavage into Cav α 2 and Cav δ fragments that are then re-linked via disulphide bonds, with the Cav α 2 portion

being entirely exposed to the extracellular milieu and the Cav δ portion acting as a membrane anchor²⁴. Cav β and Cav α 2 δ subunits are each encoded by four different genes⁷. Their main functions are to regulate the biophysical characteristics of the channels and, more importantly, the export of the channel complex from the endoplasmic reticulum and the stability of the channel in the plasma membrane^{25,26}. Some HVA calcium channels also contain a Cav γ subunit (most notably the skeletal muscle L-type channels), a transmembrane protein encoded by as many as eight different genes and with poorly understood function²⁷. Each of the major calcium channel subunits is known to undergo alternative splicing²⁸. Between the various splice isoforms and possible combinations of Cav α 1, Cav β and Cav α 2 δ subunits, a vast number of different calcium channels with a wide spectrum of functional and pharmacological properties (and potentially specific physiological functions) can be generated. This is a particularly important consideration during the development of calcium channel therapeutics, and it often relies on the heterologous expression of a specific combination of calcium channel subunits in a host cell line.

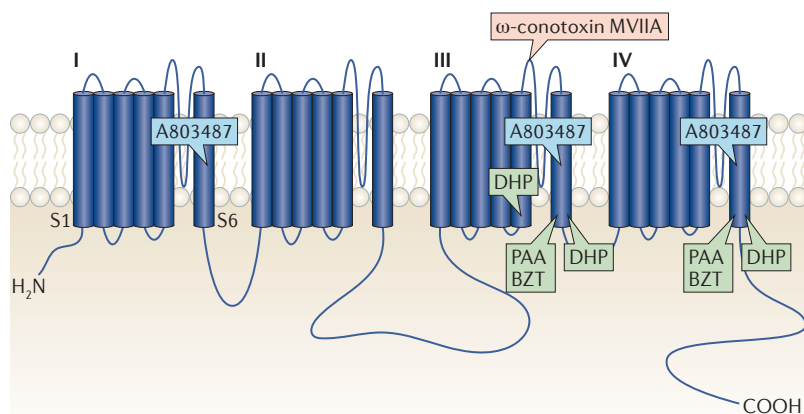


Figure 1 | Locations of drug interaction sites on voltage-gated calcium channels. The transmembrane topology of the pore-forming Cav1 subunit with its four major transmembrane domains (I–IV) is shown, each of which contains six membrane-spanning helices (termed S1–S6), as well as a re-entrant pore loop. Blockers of N-type, T-type and L-type channels are labelled, in red, blue, and green, respectively. Sites of drug interactions are shown. Note that the transmembrane domain III S6 and IV S6 regions are hotspots for drug interactions. BZT, benzothiazepine; DHP, dihydropyridine; PAA, phenylalkylamine.

Key nervous system functions of calcium channels

So why do vertebrates need so many types of calcium channels? Part of the answer may lie in the notion that different calcium channel subtypes fulfil specific physiological roles, and different variants of the same calcium channel subtype may serve as a fine-tuning mechanism not only because of their particular biophysical characteristics but also because of their differential regulation. The nervous system expresses nine of the ten major calcium channel isoforms, with the skeletal-muscle-specific Cav1.1 channels being the only isoforms that are absent²⁹. Different calcium channel isoforms often support multiple physiological roles, and those that are particularly pertinent are discussed here. Cav1.2 channels are expressed in most types of neurons. They are often localized on cell bodies and at dendritic regions³⁰, and are thought to be important for the activation of calcium-dependent enzymes³¹, the activation of calcium-activated potassium channels³² and the initiation of calcium-dependent gene transcription events⁴. Cav1.3 channels show a similar subcellular distribution to that observed with Cav1.2 (REF. 30). As well as contributing to postsynaptic activity, these channels are expressed at ribbon synapses of cochlear hair cells where they have a critical role in auditory transmission³³. Indeed, mice lacking Cav1.3 channels are deaf, as are human patients with null mutations in the Cav1.3-encoding gene *CACNA1D*^{34,35}. Cav1.4 channels are predominantly expressed in rod photoreceptors where they control glutamate release from ribbon synapses. Consequently, Cav1.4-null mice are blind³⁶ and loss of Cav1.4 expression in humans gives rise to night blindness³⁷.

Cav2.1 and Cav2.2 channels are expressed at pre-synaptic nerve terminals where they are closely associated with the neurotransmitter release machinery^{3,38}. Some synapses are designed to accommodate specific complements of each of these two channel subtypes³⁹, whereas

others such as primary afferent nerve terminals in the spinal dorsal horn almost exclusively express Cav2.2 channels. However, these two channel subtypes are not created equally. Whereas Cav2.2-null mice are viable and display hyposensitivity to pain⁴⁰, Cav2.1-null mice experience seizures and cerebellar ataxia⁴¹. This suggests that Cav2.1 channels may have a key role besides neurotransmitter release; indeed, Cav2.1 channels have been associated with gene transcription of synaptic proteins such as syntaxin 1A⁴². In humans, gain-of-function mutations in Cav2.1 channels can give rise to familial hemiplegic migraine⁴³, whereas loss-of-function mutations or polyglutamine expansions in the Cav2.1 C-terminal lead to conditions such as episodic ataxia type II⁴⁴ and spinocerebellar ataxia 6 (REF. 45), respectively. Conversely, mutations in Cav2.2 have been associated with myoclonus dystonia-like syndrome, which, interestingly, is accompanied by cardiac arrhythmias, perhaps owing to the role of N-type channels in the sympathetic nervous system⁴⁶. Cav2.3 channels are thought to contribute to neurotransmitter release at a subset of central nervous system (CNS) synapses^{47,48}, as well as helping to regulate neuronal excitability. Consequently, Cav2.3-null mice are resistant to certain types of pharmacologically induced seizures⁴⁹.

The three members of the Cav3 channel family have important roles in regulating neuronal firing, which is closely linked to their specific gating characteristics^{1,50}. Their low threshold of voltage activation, coupled with their tonic inactivation at typical resting membrane potential, underlies the rebound bursting phenomenon seen in many types of neurons⁵¹. In this context, it is important to note that different types of Cav3 channels differentially regulate neuronal firing behaviour⁵¹, and this diversity is further increased by the existence of different Cav3 splice variants⁵². Finally, there is recent evidence that Cav3 channels can partake in low-threshold exocytosis, perhaps owing to their interactions with synaptic proteins such as syntaxin 1A⁵³.

Discussed above are just some of the most important physiological roles of these various calcium channel subtypes, and this is by no means a comprehensive account of all calcium channel functions in the nervous system. A recent review⁷ on this topic provides a more in-depth discussion. This high-level summary does, however, provide appropriate context and background for the ensuing sections in this article, such as the potential of these channels as established and prospective drug targets.

Identifying new calcium channel therapeutics

Fifty years ago, research on the effects of verapamil and prenylamine on excitation–contraction coupling led to the discovery of several classes of calcium blockers (originally termed calcium antagonists)³⁴. Much of the initial work on these agents focused on L-type calcium channels and revealed that these channels have multiple drug interaction sites for compound classes such as dihydropyridines, phenylalkylamines and benzothiazepines⁵⁵. As these drug interaction sites are non-competitively coupled, this enabled the use of high-throughput radioligand displacement assays to identify new compounds

Dihydropyridines

A class of drug molecules that often act on calcium channels.

Phenylalkylamines

A specific class of small organic molecules that block calcium channels.

that interacted with these channels⁵⁶. These early studies involved native channel complexes from muscle or brain tissue⁵⁷. The cloning of various calcium channel isoforms allowed the generation of stable cell lines that expressed one specific calcium channel subtype and that could be loaded with a calcium indicator dye^{58,59}. Membrane depolarization induced by potassium chloride (KCl) leads to channel opening, and the effects of compounds on the associated rise in calcium fluorescence can be used to determine their ability to block the channels^{58,59}. Automation of this assay allows the high-throughput screening of large compound libraries in a short time frame. Moreover, unlike the early radioligand studies, this approach is applicable to all major calcium channel isoforms and does not require the availability of tritiated drug compounds⁶⁰.

There is a drawback, however, in the use of a single KCl depolarization, as this approach cannot easily identify compounds with strong use-dependence⁶¹, which is a desirable quality in compounds such as anti-arrhythmics and anticonvulsants. As many use-dependent compounds interact preferentially with inactivated channels, one way to use fluorescence assays to identify such types of blockers is to regulate the membrane potential of the cells by bathing them in slightly elevated KCl levels before applying a larger KCl depolarization that opens the channels^{62,63}. The development of high-throughput automated patch clamp systems has greatly facilitated the identification of drug compounds with specific kinetic properties^{61,64}; however, this additional information comes at the expense of throughput.

Techniques such as photoaffinity labelling⁶⁵, coupled with site-directed mutagenesis, have been used successfully to identify major drug interaction sites on L-type calcium channels at the single amino acid level (FIG. 1). In some cases, knowledge of drug interactions with other classes of ion channels (such as sodium channels) has been used to identify drug interaction sites on voltage-gated calcium channels, such as T-type channels. For example, key amino acid residues that comprise the local anaesthetic interaction site in sodium channels are conserved in T-type calcium channels, and mutagenesis of these residues reduces the interaction of these channels with local anaesthetic-like compounds¹⁹. Curiously, the S6 regions in domains III and IV of the Cava1 subunit appear to be hotspots for drug interaction sites in both L-type and T-type channels (FIG. 1), perhaps because these channel structures are linked to the inactivation-gating machinery and line the inner vestibule of the channel pore⁶⁶. When coupled with homology modelling⁶⁷, such knowledge of the molecular makeup of drug interaction sites could potentially provide important insights into drug structure requirements for calcium channel inhibition and thus enable a rational approach towards drug design⁶⁸.

One of the challenges in designing and developing small organic calcium channel blockers is the relatively high sequence conservation among different members of the calcium channel family and sequence similarities to other members of the voltage-gated ion channel superfamily⁸. This makes it very difficult to identify

compounds with high affinity and high selectivity for one particular calcium channel target. Indeed, several dihydropyridines (a class of compounds commonly thought of as being selective for L-type channels) have been shown to block other calcium channel subtypes such as T-type and N-type channels, in some cases even preferentially over L-type channels^{69–71}.

Despite decades of drug discovery efforts, only a few bona fide calcium channel therapeutics other than dihydropyridines have entered the clinic. This underscores the immense challenge in finding compounds that: have high affinity; have high target selectivity (especially over HERG channels); effectively cross the blood–brain barrier; have the appropriate physico-chemical properties; are not rapidly metabolized; and are non-toxic. The ensuing sections provide an overview of the therapeutic potential of calcium channel blockers in various nervous system disorders and a summary of existing drugs targeting calcium channels (TABLE 1), as well as compounds that are currently in the drug discovery pipeline.

Calcium channel inhibitors as pain therapeutics

Pain stimuli are detected by peripheral nociceptors that innervate the skin and organ tissues. Action potentials then propagate along the primary afferent fibre to synaptic nerve terminals in the spinal dorsal horn, where excitatory synaptic transmission then activates neurons that project to higher brain centres where pain is perceived⁷² (FIG. 2). Voltage-gated calcium channels are known to factor prominently in this afferent pathway in two principal ways⁷³. Cav3.2 T-type calcium channels are important regulators of afferent fibre excitability, whereas Cav2.2 and, to a lesser extent, Cav3.2 channels both contribute to neurotransmission at dorsal horn synapses⁷⁴ (FIG. 2). Both calcium channel subtypes are upregulated under chronic pain conditions^{75–77}; conversely, inhibiting Cav2.2 and/or Cav3.2 channel activity in rodents has been shown to mediate analgesia⁷⁴.

For therapeutic purposes, Cav2.2 channels can be targeted in multiple ways. First, Cav2.2 channels are under the powerful control of several G protein-coupled receptors, including GABA_B (γ -aminobutyric acid, type B) receptors and various members of the opioid receptor family⁷⁸. Indeed, the clinically used μ -opioid receptor agonist morphine inhibits Cav2.2 channel activity and thus neurotransmitter release from primary afferent neurons⁷⁹. However, although it is a potent analgesic, morphine has numerous side effects such as respiratory depression and constipation⁸⁰. Therefore, receptor-independent means of inhibiting Cav2.2 channels are desirable. The gabapentinoids gabapentin (Neurontin; Pfizer) and pregabalin (Lyrica; Pfizer) inhibit synaptic transmission mediated by the Cav2.2 channel through a very different mechanism that involves interactions with the Cava2 δ subunit. These subunits are upregulated in chronic pain states⁸¹, leading to increased Cav2.2 cell surface expression. Treatment with gabapentinoids interferes with Cava2 δ function, leading to a reduced Cav2.2 calcium channel density in the presynaptic plasma membrane^{82–84}. Despite their overall moderate

Use-dependence

A process by which an ion-channel-blocking drug becomes more effective during repetitive activation of the channel.

Opioid

A class of compounds that activates opioid receptors.

Gabapentinoids

A class of compounds that acts on the ancillary calcium channel Cava2 δ subunit. Gabapentinoids are used as analgesics.

Gabapentin

A compound that is used in the treatment of neuropathic pain.

Pregabalin

A compound that is used in the treatment of neuropathic pain.

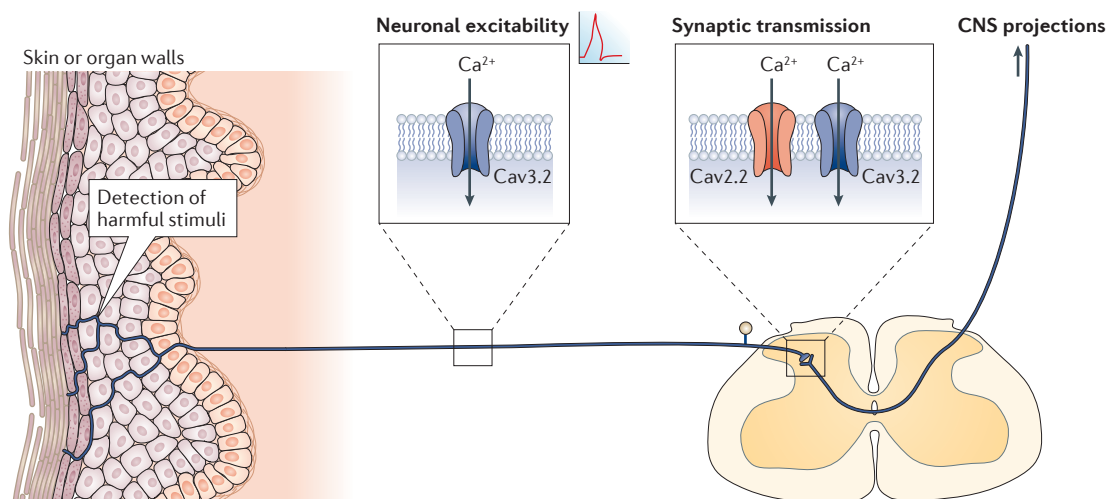


Figure 2 | Role of voltage-gated calcium channels in the primary afferent pain pathway. Harmful stimuli (such as pressure, heat and cold) are detected by nerve endings embedded in the skin or organs, leading to the generation of action potentials that travel along the afferent fibre to synaptic terminals in the spinal dorsal horn, where neurotransmitter release activates postsynaptic neurons that project to the brain. Cav3.2 calcium channels regulate afferent fibre excitability and contribute to calcium influx in synaptic nerve terminals. Cav2.2 channels are located presynaptically where their opening allows calcium entry and leads to neurotransmitter release. CNS, central nervous system.

efficacy, these compounds are important therapeutic choices for treating neuropathic pain and have become blockbuster drugs⁸⁵.

A direct inhibitor of Cav2.2 calcium channel activity is also currently used in the clinic. Ziconotide (Prialt; Elan) is a synthetic version of ω -conotoxin MVIIA, a 26-amino-acid peptide isolated from the venom of the *Conus magus* fish-hunting snail. Ziconotide is a highly selective pore blocker of Cav2.2 calcium channels, and when delivered intrathecally (this peptide does not cross the blood–brain barrier) it mediates analgesia^{86–88}. Besides the requirement for delivery via an implanted minipump and its limited indication for drug-resistant cancer pain, it also suffers from a narrow therapeutic window and thus has a variety of potential side effects that include memory loss and unruly behaviour^{87,89–91}. As a physical blocker of the Cav2.2 channel pore, ziconotide does not exhibit use-dependent blocking properties⁹² and therefore does not preferentially target Cav2.2 channels in hyperactive neurons. Several small organic Cav2.2 channel inhibitors, however, have been shown to mediate strong use-dependent inhibition of Cav2.2 channels in the nanomolar range. This includes Z160 (also known as NMED-160 or NP-118809; Epirus), a compound that mediates potent analgesia in several animal models of pain⁹³. Unfortunately (and very much surprisingly) this compound failed two different Phase II clinical trials for lack of efficacy, for reasons that are unclear. Other state-dependent Cav2.2 channel blockers that are currently in preclinical development include compounds such as TROX-1 (Merck)⁹⁴ and CNV2197944 (Convergence Pharmaceuticals, now held by its sister company Calchan Ltd), which has successfully completed Phase I clinical trials and is now in Phase II trials for postherpetic neuralgia and painful diabetic neuropathy (see the [Convergence Pharmaceuticals press](#)

[release](#) for further information). Several other classes of small organic Cav2.2 channel blockers with efficacy in animal models of pain have been reported in the literature (see REFS 95–99), some of which also target T-type calcium channels¹⁰⁰.

This latter point is pertinent, as T-type calcium channels are also emerging as suitable targets for analgesics. Following early studies showing that intrathecal delivery of the T-type channel inhibitor ethosuximide (Zarontin; Pfizer) mediates analgesia¹⁰¹, it was shown that intrathecal delivery of small interfering RNA (siRNA) against Cav3.2 channels, but not other T-type calcium channel isoforms, protected against inflammatory and neuropathic pain¹⁰². This led to the discovery of a number of different scaffolds for high affinity T-type calcium channel inhibitors with efficacy in a variety of pain models after either subdural or systemic (intraperitoneal) delivery^{103–108}. Notably, this included a novel class of dihydropyridine blockers that preferentially inhibited Cav3.2 channels over L-type channels, as well as several compounds that were derived from cannabinoid receptor ligands. This is in accordance with the observation that endocannabinoids such as anandamide have been found to potently block T-type channels^{109,110}. A mixed blocker of Cav3 and Nav1.7 sodium channels, Z123212, has been described as a potent analgesic in rodents¹¹¹. This compound interacts preferentially with the slow inactivated state of these channels, thereby giving rise to strong use-dependent inhibition. Although most of these compounds are still in preclinical development, Z944 (Epirus Pharmaceuticals)¹¹², a potent and state-dependent T-type calcium channel inhibitor, completed Phase I clinical trials for pain and is being advanced to Phase II trials. It remains to be determined whether the analgesic actions of T-type channel inhibitors occur via inhibition of synaptic activity in the dorsal horn, or

Neuropathic pain

A chronic pain condition arising from a peripheral nerve injury.

Ziconotide

A synthetic version of ω -conotoxin MIIA that is used for pain treatment.

Z160

A blocker of N-type calcium channels that was explored as an analgesic.

TROX-1

A blocker of N-type calcium channels that was developed by Merck.

Neuropathy

A disease or dysfunction of peripheral nerves.

Ethosuximide

A type of anti-epileptic drug that acts on T-type calcium channels.

Z944

A drug molecule that potently blocks T-type calcium channels.

by alterations of afferent fibre excitability⁷⁴. Interestingly, a small randomized trial evaluating the Cav3.2 channel blocker ABT-639 (AbbVie) as a therapeutic for diabetic pain revealed a lack of efficacy compared to placebo¹¹³. This does not, however, invalidate the potential of T-type calcium channel inhibitors as analgesics.

Finally, there is emerging evidence that Cav2.3 (R-type) calcium channels may be involved in pain signalling. Intrathecal delivery of the Cav2.3 inhibitor SNX-482 (a peptide derived from the venom of a Tarantula species) inhibits formalin-induced pain and neuropathic pain in rodents^{114,115}. This is consistent with the expression of these channels in dorsal root ganglion (DRG) neurons and in the spinal dorsal horn¹¹⁶, as well as with data showing that Cav2.3-null mice display pain hyposensitivity¹¹⁷. However, it should be noted that this compound also inhibits L-type calcium channels, albeit at higher concentrations than those needed for Cav2.3 channel block¹¹⁸. Polymorphisms in the gene encoding Cav2.3 have been linked to changes in fentanyl sensitivity in patients undergoing surgery¹¹⁹, altogether suggesting that Cav2.3 calcium channels could be explored as potential targets for pain. There is currently no selective small organic inhibitor of Cav2.3 channels, although it should be noted that the N-type channel blocker TROX-1 also mediates inhibition of Cav2.3 channels with about 50% lower affinity compared with Cav2.2 channels (REF. 94). This compound could thus perhaps form the starting point for the development of a preferential Cav2.3 inhibitor.

Calcium channels as targets for seizure disorders

Seizures occur as a result of a combination of hyperexcitability and abnormal synchrony of neurons. Focal seizures typically involve one hemisphere and can arise from injury to a specific brain structure or from a tumour¹²⁰. Conversely, idiopathic seizures are not accompanied by radiological abnormalities, typically involve both hemispheres and can be triggered by more diffuse insults to the brain, such as high fever (that is, febrile seizures), hypoxia, or even oxygen toxicity¹²¹. They may also arise from genetic abnormalities in a variety of ion channels and receptors, such as voltage-gated sodium channels and GABA receptors^{122–124}. Absence seizures are one of the hallmarks of idiopathic generalized epilepsy (IGE) and are characterized by brief periods of unresponsiveness and abnormal spike and wave discharges in EEG recordings that reflect hypersynchronous activity of thalamocortical structures¹²⁵. It is thought that the initiation of absence seizures is critically dependent on the activation of T-type calcium channels in thalamocortical neurons and reticular thalamic nucleus neurons (nRT neurons)¹²⁶, which express Cav3.1 and Cav3.2/Cav3.3 calcium channels, respectively (FIG. 3a). The firing of these neurons can be drastically altered by even small changes in T-type channel activity¹²⁷. Furthermore, in several genetic mouse models of absence epilepsy (such as Cav2.1-knockout mice, 'lethargic mice' that lack functional Cav β 4, and 'stargazer mice', which carry a loss-of-function mutation in the Cavy2 subunit), there is an increase in T-type calcium channel activity

in nRT neurons¹²⁸. Along these lines, in the GAERS rat model of absence epilepsy, there is an increase in thalamic T-type channel activity (which is accompanied by increased Cav3.2 mRNA levels) owing to a gain-of-function mutation in Cav3.2 (REF. 129). Interestingly, this gain-of-function mutation is only able to manifest functional changes in a specific Cav3.2 splice isoform that contains exon 25 (which is the predominant splice isoform expressed in the thalamus)¹²⁹.

The role of Cav3.2 channels in seizure genesis is underscored by genetic analysis of human patients with various forms of idiopathic generalized seizures¹²⁴. Mutations in the gene that encodes Cav3.2 (*CACNA1H*) have been associated with childhood absence epilepsy, juvenile absence epilepsy and juvenile myoclonic epilepsy, among several other types of epilepsy¹³⁰. In excess of thirty different *CACNA1H* mutations have been identified, and many of them have been introduced into recombinant Cav3.2 channels for electrophysiological analysis. A subset of these mutations was shown to mediate a gain of function in channel gating, with some of the mutations promoting plasma membrane trafficking of the channels^{131–133} (FIG. 3b). These gain-of-function effects may not only affect the electrical excitability of neurons but also alter gene transcription events¹³⁴. Given the findings with the GAERS model, it is important to consider the specific splice variant background that is used for such functional studies, such that they accurately reflect their physiological effects. Cav3.1-overexpressing mice show enhanced thalamocortical network activity and present with pure absence seizures¹³⁵.

Altogether, these findings indicate that enhanced activity or expression of T-type calcium channels in the thalamus increases seizure susceptibility. It thus stands to reason that decreasing the activity or expression of these channels should protect against seizures. Indeed, mice lacking Cav3.1 calcium channels are resistant to baclofen-induced seizures¹³⁶. Furthermore, when crossed with Cav3.1-knockout mice, the seizure abnormalities in several mouse models of absence epilepsy are normalized¹²⁸. Overall, these findings support the idea that targeting Cav3.1 and Cav3.2 channels could be a strategy for mitigating absence seizures.

The role of Cav3.3 channels in the generation of burst firing in nRT neurons is somewhat controversial. On the one hand, Cav3.3 channel activity can give rise to bursting behaviour that fits with that observed in nRT neurons¹³⁷, and this appears to be a critical factor in the generation of sleep spindles¹³⁸. On the other hand, mice that either globally lack Cav3.3 channels, or mice that are selectively deficient for Cav3.3 channels in nRT neurons, appear to show greater susceptibility to pharmacologically induced spike and wave discharges and increased inhibitory synaptic inputs onto thalamocortical neurons¹³⁹. It is currently not clear whether knockout of Cav3.3 could lead to compensatory increases in the expression of other ion channels or receptors that lead to such an increase in the excitability of nRT neurons. Furthermore, owing to the lack of selective Cav3.3 channel blockers, it is not clear whether acute inhibition of these channels mediates effects that

SNX-482

A blocker of R-type calcium channels that is isolated from tarantula venom.

Dorsal root ganglion

(DRG). A cluster of nerve cell bodies comprising primary afferent sensory fibres.

Polymorphisms

The presence of genetic variations in a given population.

nRT neurons

Reticular thalamic nucleus neurons; a specific group of neurons within the thalamus.

Thalamus

A specific brain region involved in functions such as sleep.

Trafficking

The process by which proteins are transported to specific loci in cells.

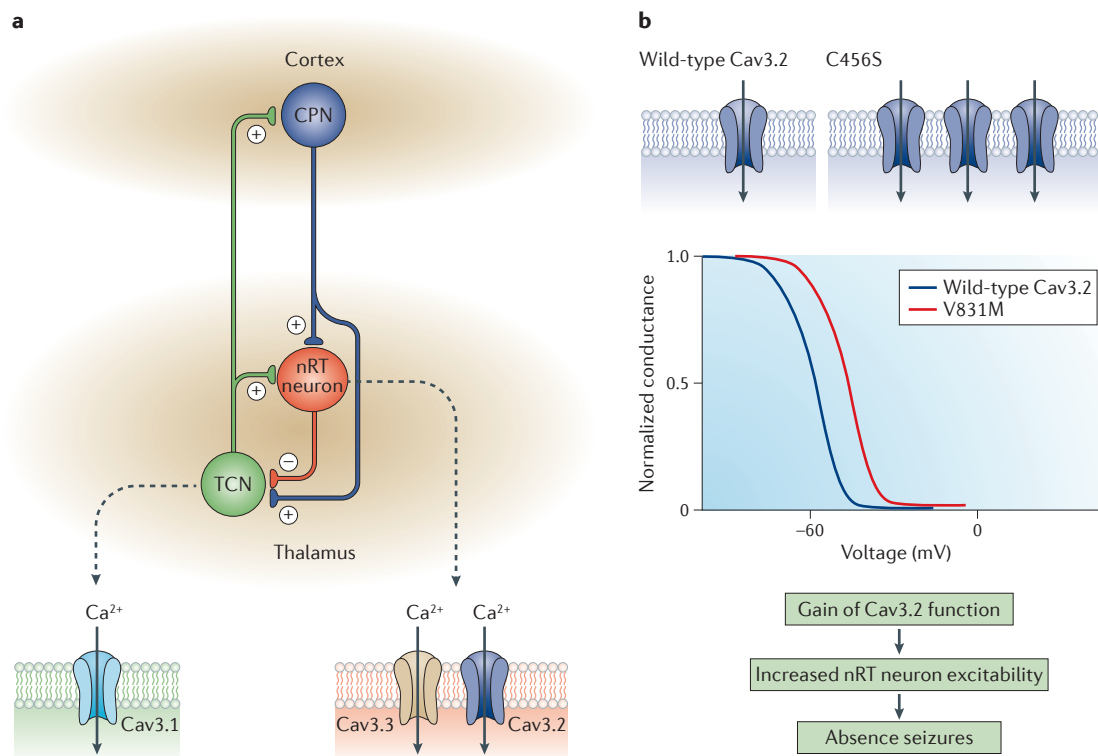


Figure 3 | Role of T-type calcium channels in the thalamocortical circuitry. **a** | Thalamocortical neurons (TCNs) have excitatory projections to both cortical pyramidal neurons (CPNs) and reticular thalamic nucleus (nRT) neurons. Descending excitatory projections innervate nRT neurons and TCNs, whereas nRT neurons have inhibitory inputs onto thalamocortical cells. The excitability of this network is strongly dependent on T-type calcium channels, with Cav3.1 channels being expressed in TCNs, and Cav3.2 and Cav3.3 channels being expressed in nRT neurons. **b** | Mutations in Cav3.2 that are found in patients with congenital forms of absence epilepsy increase Cav3.2 channel function. The C456S mutation increases cell surface expression of the channels, whereas the V831M mutation increases channel availability by mediating a depolarizing shift in the steady-state inactivation curve. Both cases result in increased T-type current amplitudes, leading to increased excitability of nRT neurons and thus absence seizures.

are equivalent to those observed upon deletion of the gene encoding Cav3.3. Nonetheless, when developing T-type calcium channel blockers for therapeutic intervention, this ambiguous role of Cav3.3 channels should be considered.

Ethosuximide is a T-type calcium channel blocker that has been used clinically to treat absence seizures¹⁴⁰. This compound is a low-affinity (in the submillimolar range) blocker of all three Cav3 channel isoforms, with a preference for inactivated channels, and it gives rise to use-dependent inhibition¹⁴¹. The anti-epileptic sodium valproate (Depakene/Convulex; Abbott) has also been described as a T-type channel inhibitor¹⁴². However, this compound is also known to inhibit other targets such as sodium channels¹⁴³ and histone deacetylases¹⁴⁴. Along these lines, the multitarget antiepileptic drug zonisamide (Zonegran; Eisai) has also been reported to inhibit T-type channels in the micromolar range, but curiously without any apparent state dependence¹⁴⁵. Interestingly, there is evidence that this compound also improves pain responses in rodents, further supporting the role of T-type channels in pain signalling (as discussed in the section above)¹⁴⁶. More recent drug discovery efforts by Zalicus (now Epirus Biopharmaceuticals) have identified

small organic molecules (including the aforementioned compound Z944 and a compound termed Z941) that potentially inhibit Cav3.2 T-type calcium channels and block seizure activity in the GAERS model¹⁴⁷. Z944 was also shown to inhibit burst firing in nRT neurons, which is consistent with the critical role of these channels in thalamic neuron excitability. As Z944 is already in clinical testing for pain, this may provide the opportunity for testing this compound in patients with absence seizures.

HVA calcium channels may also be targeted for the treatment of seizures. Gabapentin is used as an anticonvulsant to treat focal and partial seizures¹⁴⁸. However, given that this compound has the propensity to affect multiple types of HVA channels through its interaction with Cav α 2 δ subunits, it is not clear how this compound mediates its clinical effects at the cellular level. The anti-epileptic lamotrigine (Lamictal; GlaxoSmithKline) is also widely used for the treatment of focal and absence seizures, but its mode of action remains incompletely understood^{149,150}. This compound blocks transiently expressed R-type Cav2.3 channels in the low micromolar range, with a small effect on Cav3.1 channels¹⁵¹. A recent study revealed that the anti-seizure effects of lamotrigine observed in mice are dependent

Valproate

An anti-epileptic drug with multiple molecular targets.

Lamotrigine

An anti-epileptic drug with multiple molecular targets.

on the presence of Cav2.3 channels¹⁵². Specifically, lamotrigine protected from kainate-induced seizures in wild-type mice but not in Cav2.3-null mice, where this compound in fact mediated a paradoxical increase in seizure activity that remains to be explained¹⁵². Similar observations were made with topiramate (Topamax; Mylan)¹⁵², an anticonvulsant that targets multiple types of channels and receptors, including R-type channels that are blocked in a state-dependent manner, with an IC₅₀ (half-maximal inhibitory concentration) of around 50 mM¹⁵³. Although it is not clear to what extent the clinical actions of these compounds are mediated by Cav2.3 channel inhibition, the notion that Cav2.3 channels share overlapping biophysical properties with members of the Cav3 channel family fits with such a possibility. Finally, a recent study reported that Cav1.2 channels in pyramidal cells activate at relatively hyperpolarized potentials when the temperature is increased to 40 °C, thereby allowing these channels to drive intrinsic firing properties¹⁵⁴. The authors hypothesized that this effect could contribute to the development of febrile seizures. In support of this hypothesis, nimodipine (Nimotop; Bayer) blocked the development of temperature-induced seizures in rat pups¹⁵⁴, suggesting that dihydropyridines could be explored as a treatment option. However, nimodipine is known to weakly inhibit T-type calcium channels¹⁵⁵, and hence it remains to be determined whether this compound inhibited febrile seizures by blocking L-type channels, T-type channels, or perhaps both.

Overall, voltage-gated calcium channels provide potential targets for both idiopathic and focal seizures, with T-type calcium channels perhaps being the most promising candidates among the calcium channel family. However, given that there are already about two dozen medications on the market that can be used to control seizures¹⁵⁶, there has been a relative lack of new drug discovery efforts in the pharmaceutical sector.

Calcium channel blockers in Parkinson disease

Parkinson disease is one of the most common neurodegenerative disorders with a characteristic loss of dopaminergic neurons in the substantia nigra pars compacta; along with the loss of dopaminergic neurons in the striatum, which leads to a progressive impairment in motor skills, the occurrence of tremor¹⁵⁷ and other comorbidities such as the development of psychosis¹⁵⁸. Motor symptoms such as tremor are treated with a range of drugs, including anticholinergic agents, beta blockers and dopamine receptor agonists¹⁵⁹, but current options for preventing the loss of dopaminergic neurons remain insufficient. It is well known that neurons from patients with Parkinson disease exhibit inclusion bodies formed by α -synuclein¹⁶⁰, although it remains unclear whether these inclusion bodies are causal to neuronal degeneration. Several genes have been implicated in the development of familial Parkinson disease, including Parkin, α -synuclein, leucine-rich repeat serine/threonine protein kinase 2 (LRRK2) and PTEN-induced putative kinase 1 (PINK1)¹⁶¹, and many of these genes affect mitochondrial or lysosomal function¹⁶². Despite

many advances, the cellular and molecular basis of this selective neuronal loss remains to be fully understood, and this in turn has hampered the discovery of a cure for this disorder.

One possible mechanism that has been implicated in neuronal loss during Parkinson disease involves L-type Cav1.3 calcium channels. These channels can modulate pacemaking of substantia nigra neurons owing to their hyperpolarized range of voltage-dependent activation¹⁶³, although they do not appear to be critical for the pacemaking process per se¹⁶⁴. Indeed, earlier work showing that dihydropyridines could alter the pacemaking properties of substantia nigra neurons¹⁶⁵ may have been related to their off-target effects on other ion channels¹⁶⁴. Nonetheless, the repetitive opening of Cav1.3 channels (and possibly also Cav1.2 channels) during pacemaking may contribute to excessive calcium entry that in turn causes cytotoxicity through mitochondrial stress¹⁶⁶ (FIG. 4). It is also interesting to note that in human brains from patients with early-stage Parkinson disease, expression of Cav1.3 channels is increased, supporting a possible causal role of these channels in disease pathology¹⁶⁷.

A recent study identified an additional role of Cav1.3 channels in Parkinson disease¹⁶⁸. Calcium influx via Cav1.3 channels was shown to trigger a neuronal calcium sensor 1 (NCS1; also known as FLUP)-dependent enhancement of the activity of dopamine D2 auto-receptors (which are known to contribute to pathogenesis by virtue of their ability to regulate pacemaker activity) and loss of receptor desensitization^{168,169} (FIG. 4). This, in turn, would then be expected to contribute to a pathologically relevant dysregulation of dopamine neuron function. Hence, Cav1.3 channel activity may contribute to Parkinson disease pathology by multiple mechanisms, and therefore Cav1.3 channel-selective inhibitors could be a potential therapeutic strategy. Indeed, a Phase II clinical trial evaluating the safety of isradipine (Dynacirc; Reliant) as a potential Parkinson drug has been completed with promising results¹⁷⁰. This clinical study evaluated the safety, tolerability and efficacy of isradipine in patients with early symptoms of Parkinson disease that did not yet require treatment with dopamine receptor agonists. Results showed that the drug was tolerated in a dose-dependent manner, with an optimal dose determined at 10 mg daily. Side effects at higher doses included the development of oedema and dizziness. A large Phase III trial is currently underway at Northwestern University, Illinois, USA. It is also important to note that there is evidence that patients on dihydropyridine antihypertensives show a reduced risk of developing Parkinson disease when compared to either patients who are not on such regimens or patients who are treated with types of antihypertensives that do not cross the blood-brain barrier¹⁷¹.

A new class of compounds (pyrimidine-2,4,6-triones) was recently identified as a possible scaffold for Cav1.3-selective inhibitors. One of the derivatives (1-(3-chlorophenethyl)-3-cyclopentylpyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione) was shown to selectively inhibit Cav1.3 channels over Cav1.2 (REF. 172). A subsequent

Topiramate

An anti-epileptic drug with multiple cellular targets.

Nimodipine

A blocker of L-type calcium channels from the dihydropyridine class.

Neurodegenerative disorders

Disorders caused by the loss of nerve cells during disease.

Striatum

A specific subcortical part of the forebrain.

Tremor

Uncontrolled trembling and shaking motion of the limbs.

Dopamine

A neurotransmitter that acts on dopamine receptors.

Isradipine

A dihydropyridine that is used as an antihypertensive and currently being explored as a drug for Parkinson disease.

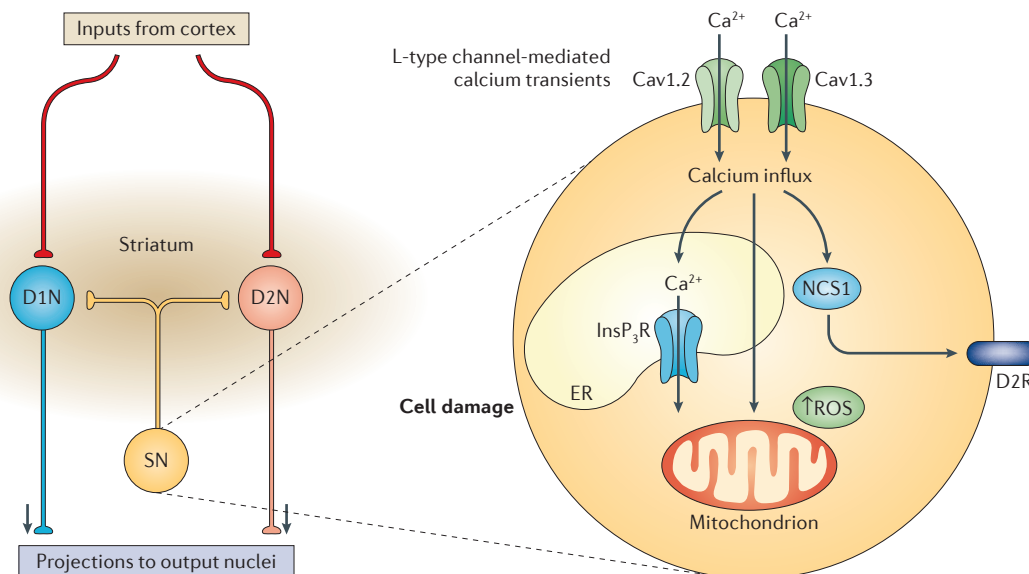


Figure 4 | Role of L-type calcium channels in the degeneration of dopaminergic neurons during Parkinson disease. Substantia nigra (SN) pars compacta neurons innervate dopamine D1 receptor-expressing neurons (D1Ns) and dopamine D2 receptor-expressing neurons (D2Ns) in the striatum, which in turn project to output nuclei. The pacemaker activity of SN neurons is modulated by Cav1.3 and possibly Cav1.2 calcium channels. The repetitive activation of Cav1.3 (and perhaps Cav1.2) channels leads to calcium influx and downstream calcium-dependent calcium release from the endoplasmic reticulum (ER). This leads to calcium elevation in the mitochondria and the generation of reactive oxygen species (ROS). This culminates in cell damage and the loss of dopaminergic neurons, and results in decreased dopaminergic input into the striatum. In addition, Cav1.3 channel activity has been linked to an increase in D2 receptor (D2R) expression via activation of neuronal calcium sensor 1 (NCS1), and this upregulation is thought to alter pacemaker activity. InsP₃R, inositol-1,4,5-trisphosphate.

study reported lower blocking affinities and weaker channel subtype selectivity of this compound compared to those reported earlier¹⁷³. However, the blocking effects were also shown to vary with the Cav β subunit that was co-expressed and with the particular Cav1.3 channel splice isoform that was used¹⁷³. Finally, yet another study reported that this compound in fact increased Cav1.3 channel activity by affecting channel gating in a complex manner, with inhibition only being observed when barium was used as a charge carrier¹⁷⁴. Although the reason for these discordant observations remains unclear, one possibility may be the use of different Cav1.3 channel splice isoforms in these pharmacological tests on transiently expressed channels. Irrespective of these discrepancies in the literature, the general premise of targeting Cav1.3 channels for preventing the dopaminergic neuron loss observed in Parkinson disease remains valid.

Finally, it should be noted that T-type calcium channels have recently been implicated as a potential target for treating motor abnormalities in Parkinson disease. In a rat model, T-type calcium channel inhibitors such as mibefradil (now withdrawn from the market) and NNC-55-0396 blocked burst firing activity in slices of the subthalamic nucleus, a brain structure that is known to exhibit increased bursting activity during Parkinson disease¹⁷⁵. Furthermore, locomotor deficits in a rat model of Parkinson disease were reduced by these compounds¹⁷⁵. Hence, it will be interesting to determine whether new T-type channel blockers such as Z944 may show efficacy in patients with Parkinson disease.

Role of calcium channels in drug dependency

Drug dependency can be considered a chronic disease in which affected individuals experience the compelling need to consume substances of abuse, and this is a major contributor to mortality^{176,177}. These substances include both legal drugs such as alcohol and nicotine, as well as prohibited substances such as cocaine. Dependency involves long-term changes to the brain architecture¹⁷⁸ that predispose individuals to relapse even after very long periods of abstinence, and these may differ from brain circuits that are involved in the immediate reward associated with psychostimulants^{179,180}. The cellular and molecular mechanisms underlying dependency are highly complex and multifaceted. The key brain structure that is involved in the development of dependency is the mesolimbic system¹⁸¹, in particular the dopaminergic projections from the ventral tegmental area to the nucleus accumbens and its outputs¹⁸² (FIG. 5). Even though different types of addictive substances may act at different receptors (for example, morphine activates μ -opioid receptors and nicotine activates nicotinic acetylcholine receptors), they all lead to an overall increase in dopamine levels in the mesolimbic system¹⁸³ and altered cAMP response element binding protein (CREB)-dependent gene expression in the ventral tegmental area and nucleus accumbens¹⁸⁴. For over two decades, L-type channels have been known to have a role in this process¹⁸⁵, as compounds such as nimodipine (Nimotop; Bayer) and diltiazem (Cardizem; Biovail) block behavioural sensitization to cocaine^{186,187} and attenuate alcohol intake¹⁸⁸.

Ventral tegmental area

A collection of specific neurons in the midbrain that is part of the reward system.

Nucleus accumbens

A specific brain region that is involved in reward behaviour.

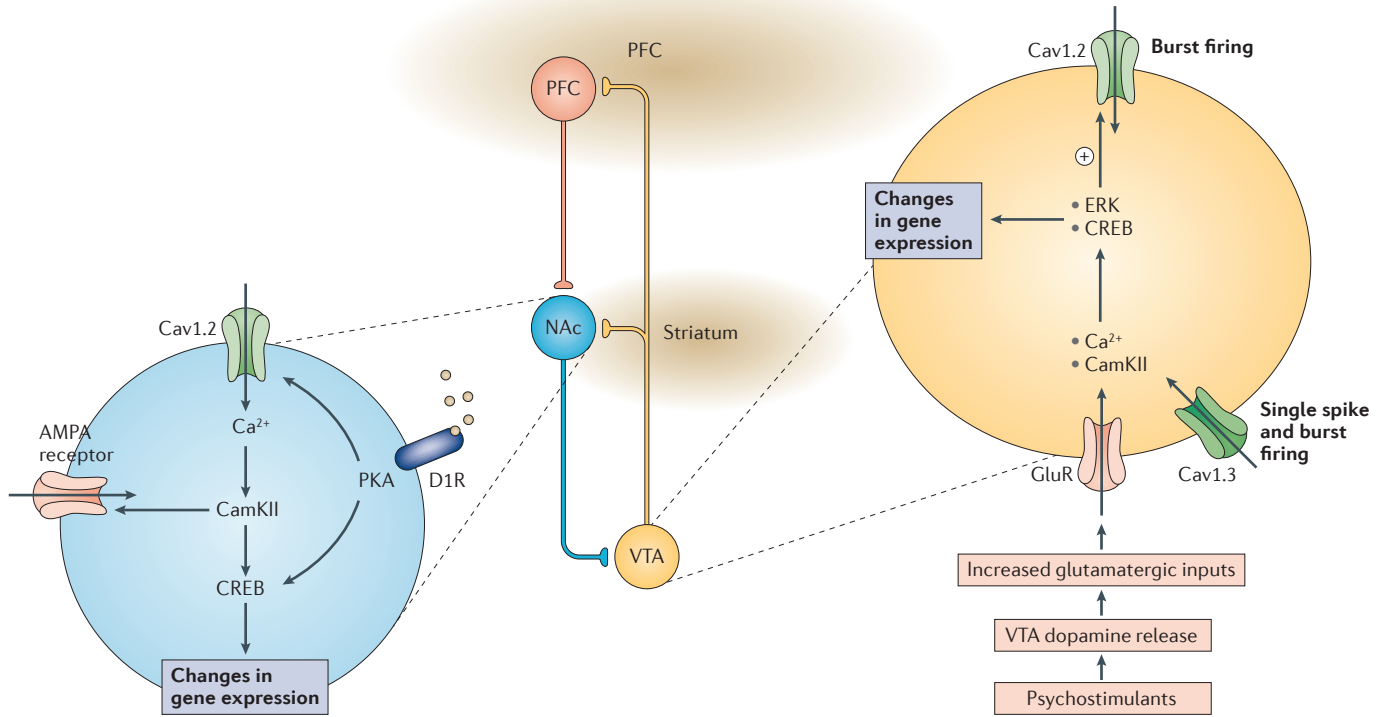


Figure 5 | Role of L-type calcium channels in drug addiction. Simplified neuronal circuitry involved in addiction is shown. Dopaminergic neurons in the ventral tegmental area (VTA) project to both the nucleus accumbens (NAc) and the prefrontal cortex (PFC). The NAc is a major output nucleus for reward-seeking behaviour and has both direct and indirect (not shown) projections back to the VTA. Chronic exposure to psychostimulants activates Cav1.3 channels and increases dopamine release from the VTA, which in turn stimulates glutamatergic inputs. Glutamate activates AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (*N*-methyl-D-aspartate) receptors (GluR) on VTA neurons, and together with Cav1.3 channel activity it leads to changes in gene expression that include the upregulation of Cav1.2 calcium channels. Cav1.2 and Cav1.3 channels differentially regulate the firing behaviour of VTA neurons, which in turn drives dopamine release in the NAc. There, activation of dopamine D1 receptors (D1Rs) activates protein kinase A (PKA), which, together with an upregulation of Cav1.2 channels, mediates long-term changes in gene expression and AMPA receptor insertion into the plasma membrane. CamKII, calcium/calmodulin-dependent protein kinase II; CREB, cAMP response element binding protein; ERK, extracellular signal-regulated kinase; GluR, glutamate receptor.

The Cav1.3 calcium channel subtype appears to contribute to the amphetamine-induced upregulation of dopamine receptor mRNA in the ventral tegmental area¹⁸⁹ and is essential for behavioural sensitization in response to cocaine and amphetamines¹⁹⁰. After pre-exposure to cocaine, Cav1.2 channels are upregulated in the nucleus accumbens, which in turn causes long-term changes in gene expression and a phosphorylation-dependent insertion of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors into the plasma membrane, which gives rise to long-term plasticity^{190,191} (FIG. 5). This process depends on the activation of Cav1.3 channels in the ventral tegmental area¹⁹², presumably via the release of dopamine and activation of D1 receptors. This plasticity is maintained after cocaine withdrawal. Conversely, in the dorsal striatum, long-term cocaine exposure causes a decrease in AMPA receptor phosphorylation or insertion that depends on the activation of D2 receptors and Cav1.3 channels, but not on those that are expressed in the ventral tegmental area¹⁹¹. Furthermore, during chronic methamphetamine treatment, there is an increase in Cav1.2

mRNA expression in ventral tegmental area neurons, which may also contribute to long-term changes in gene expression^{189,193}. A subsequent study revealed that Cav1.2 and Cav1.3 channels mediate distinct neuronal firing patterns in ventral tegmental area neurons, with Cav1.3 channels driving both single spike and burst firing, whereas Cav1.2 channels are mainly important for bursting behaviour¹⁹⁴ (FIG. 5). This, in turn, suggests that these two channel types differentially contribute to dopaminergic modulation of the nucleus accumbens.

Altogether, a picture emerges in which Cav1.3 channels are crucial mediators of plastic changes in the mesolimbic system in response to psychostimulants, which then lead to a longer form of plasticity in the nucleus accumbens that is dependent on the Cav1.2 channel. The latter may perhaps contribute to the long-term structural changes that predispose individuals to relapse after abstinence. This therefore suggests that L-type calcium channel blockers could prove beneficial in the treatment of addiction. Indeed, there is evidence from human studies that this may be the case^{195,196}. For example, L-type calcium channel inhibitors such as nifedipine (Procardia/Adalat; Pfizer/Bayer)

Nifedipine
A blocker of L-type calcium channels from the dihydropyridine class.

and verapamil (Calan; Pfizer) have been shown to reduce withdrawal symptoms from a number of addictive substances, including opiates and ethanol^{195,196}. It remains to be determined whether these effects in humans are dependent on the activity of Cav1.3, Cav1.2, or both types of channels. Such knowledge could potentially guide future therapeutic strategies in this setting.

In addition to L-type calcium channels, blockers of other types of calcium channels may also be a useful strategy. A mixed N-type/T-type calcium channel blocker (NMED-126; also known as NP078585) was shown to reduce alcohol intoxication in mice, as well as alcohol self-administration and reinstatement¹⁹⁷. The effects of this compound were absent in Cav2.2-knockout mice, indicating that it acted predominantly via N-type rather than T-type channel inhibition. Whether these effects occur at the network level in the mesolimbic system is not known; nonetheless, these data point to a potential strategy of using mixed N-type/L-type channel inhibitors to target drug and alcohol dependency. One such blocker that is used currently in the clinic is cilnidipine (Atelec/Cilacar; Fuji/Ajinomoto), an antihypertensive from the dihydropyridine class⁷⁰. Importantly, this compound suppresses ethanol-induced locomotor sensitization in rats¹⁹⁸.

Calcium channels and psychiatric disorders

Several calcium channel genes have been associated with the development of psychiatric symptoms. Single-nucleotide polymorphisms in intron sequences in the gene encoding Cav1.2 (in particular, a risk locus termed rs1006737) and in the Cav β 2 subunit have been associated with an increased risk of bipolar disorder and schizophrenia^{199,200}. Importantly, healthy individuals carrying the *CACNA1C* rs1006737 risk variant show compromised function of the anterior cingulate cortex, prefrontal cortex and hippocampus in functional magnetic resonance imaging (MRI) studies^{201–203}. A subsequent study identified Cav1.3 as another possible risk gene for bipolar disorder, with a lower degree of association for Cav2.2 and Cav3.1 (REF. 204). It was concluded that these channels and other types of ionic channels share a common role in regulating neuronal excitability; however, this may be an overly simplistic view as these channels have highly specialized roles that can differ across brain regions⁷. How polymorphisms in intronic sequences of Cav1.2 may contribute to an increased risk of schizophrenia is not yet understood. It is possible that they could lead to altered expression of Cav1.2 channels²⁰⁵, and this would be consistent with a recent report showing higher Cav1.2 activity in induced neurons from carriers of the polymorphism²⁰⁶. Nonetheless, the association of bipolar disorders with two different types of L-type calcium channels suggests that L-type calcium channel blockers are a possible treatment avenue. Indeed, a preliminary clinical study on the use of isradipine for the treatment of bipolar disorder yielded promising results²⁰⁷, but this needs to be followed up with larger cohort studies.

Gain-of-function mutations in Cav1.2 that interfere with the normal voltage-dependent inactivation mechanisms in these channels have been associated with Timothy syndrome, a severe condition that includes

fatal cardiac arrhythmias, developmental abnormalities and autism^{208,209}. This change in channel function not only affects the electrophysiological properties of excitable cells but, as evident from studies on neuronal progenitor cells from patients with Timothy syndrome, also leads to massive alterations in gene transcription²¹⁰ and enhanced dendritic retraction²¹¹. An autistic phenotype is also observed in human patients with gain-of-function mutations in Cav1.3 channels²¹². Furthermore, rare mutations in the Cav β 2 subunit in families with autism spectrum disorder lead to slowed L-type calcium channel inactivation kinetics²¹³. This raises the possibility that L-type calcium channel inhibitors or, more specifically, L-type channel inactivation enhancers, could be used as treatments for certain types of autism. However, so far there have been no clinical trials to test this hypothesis. It is also noteworthy that single-nucleotide polymorphisms in all three Cav3 channel isoforms have been associated with autism²¹⁴. In the case of Cav3.2, such mutations have been shown to cause a loss of function²¹⁵. To what extent Cav3 channels can be exploited as potential targets for autism remains to be determined.

Anxiety disorders are another group of highly prevalent psychiatric conditions that may involve voltage-gated calcium channels. Anxiety can be defined as an apprehensive reaction to a non-threatening stimulus, and it is thus not surprising that the neural circuitry that is involved in anxiety is intimately tied to that of fear^{216,217}, which encompasses the amygdala, the nucleus accumbens and the hippocampus²¹⁸. Optogenetics-based studies have revealed that inputs from the amygdala to the hippocampus are essential for the development of anxiety-related behaviours²¹⁹. A recent study reported an increase in the expression of the Cava2 δ 1 subunit in the amygdala in a rat model of chemically induced anxiety²²⁰. Notably, pregabalin reversed the anxiety phenotype, which fits with ample clinical data showing that both gabapentin and pregabalin are effective in treating anxiety disorders in humans²²¹. The upregulation of Cava δ subunits appears to be accompanied by an increase in the expression of Cav1.2 and Cav1.3 calcium channels²²². Along these lines, there is an increase in the expression of Cav1.2 calcium channels in fear-conditioned rats²²². In these experiments, nimodipine was shown to block startle responses²²². Although these findings raise the possibility of testing L-type calcium channel inhibitors as potential anxiolytics, deletion of Cav1.2 channels in the forebrain appears to give rise to an anxiety phenotype in mice²²³, as does haploinsufficiency of Cav1.2 channels in female mice²²⁴. Moreover, higher doses of nifedipine and verapamil have been shown to exert anxiogenic effects in mice²²⁵. Conversely, there are weak indications that Cav1.3 deficiency may have anxiolytic effects²²⁶. Therefore, it is currently unclear whether L-type channels may serve as targets for anxiety disorders and how they modulate the underlying neuronal circuitry.

It is important to note that N-type channels may also have a role in the development of anxiety disorders, as mice lacking Cav2.2 display reduced levels of anxiety²²⁷. It is conceivable that the aforementioned anxiolytic effects of gabapentin could be due to a Cava2 δ -dependent effect

Cilnidipine

A specific dihydropyridine that blocks both N-type and L-type calcium channels.

Bipolar disorder

A neuropsychiatric condition marked by alternating bouts of elation and depression.

Schizophrenia

A common neuropsychiatric disorder.

Amygdala

A specific brain region known to be involved in fear.

on Cav2.2 channels²²⁸. Finally, mice lacking the Cav β 3 subunit show reduced anxiety along with increased aggression²²⁹, and it is possible that this is due to reduced expression of Cav2.2 channels arising from the absence of this important auxiliary subunit.

Overall, there is evidence supporting the utility of voltage-gated calcium channel blockers in a variety of psychiatric disorders. What remains to be understood in greater depth is how individual calcium channel isoforms contribute to the development of such conditions at the neuronal network level.

Challenges and opportunities

The importance of voltage-gated calcium channels as drug targets for nervous system disorders has been clearly established. As there are already clinically approved inhibitors of N-, L- and T-type calcium channels, this may allow the rapid testing of these existing drugs in a wide range of neurological conditions, as exemplified by the use of the antihypertensive isradipine as a potential intervention for Parkinson disease¹⁷⁰. Furthermore, as calcium channels have important roles in many other physiological processes, such as the regulation of sleep patterns^{138,230} and food intake²³¹, the palette of possible therapeutic applications of calcium channel inhibitors may well increase to include conditions such as insomnia and eating disorders, among others. Nonetheless, there remains a pressing need for new calcium channel blockers, especially for conditions such as neuropathic and inflammatory pain, which are often refractory to treatment. In particular, to minimize side effects, it is important to develop new types of calcium channel inhibitors that specifically target the calcium channels that are involved in pathophysiological processes, while sparing those that contribute to normal physiological function. This requires an in-depth understanding of how calcium channels partake in the function of specific brain circuits that are implicated in pathophysiology and how these channels may be dysregulated in pathological states.

First, it is important to know the precise molecular architecture of the channels expressed in these neurons, which, as noted earlier in this article, may guide drug discovery strategies. For example, it has been shown that Cav2.2 calcium channels in pain-sensing neurons contain exon 37a rather than exon 37b, which is more widely expressed in the nervous system²³² and that the exon37a variant is critical for pain signalling²³³. If one were to develop an inhibitor that selectively targets exon37a-containing channels, then the potential of CNS side effects may be reduced. Along these lines, it is possible that calcium channels undergo age-dependent alternative splicing events (this has been demonstrated at least for P/Q-type channels)²³⁴, and this could potentially be relevant for the pathogenic role of Cav1.3 calcium channels in Parkinson disease. In the context of ageing, matters are further complicated by the fact that Cav1.2 channels undergo an age-dependent form of proteolysis in the plasma membrane that results in the generation of functional channels with altered biophysical characteristics²³⁵. Besides modification of the pore-forming channel subunit

per se, knowledge of the association of the calcium channel Cava1 subunits with specific ancillary subunits and the interactions of calcium channels with other regulatory elements, may be important considerations for drug development. For example, Cav1.3 channels in cochlear hair cells associate with calcium-binding proteins such as CaBP2 and CaBP4, which changes the biophysical and perhaps the pharmacological characteristics of these channels^{236,237}. When designing Cav1.3 channel inhibitors for the treatment of conditions such as Parkinson disease or addiction, it may thus prove advantageous to select compounds with lower affinity for channels that are complexed with CaBP2, in order to avoid the possibility of drug-induced hearing deficiencies. This could be accomplished by screening compounds against cell lines that stably co-express CaBP2 and Cav1.3 channels.

Second, it is important to understand the intrinsic firing properties of the neuronal circuits that are involved in pathological states. For example, hyperexcitability disorders such as epilepsy may call for calcium channel blockers that are strongly use-dependent. This strategy has been successfully exploited with anti-arrhythmic and anticonvulsant drugs and, as noted above, it is possible to identify these types of compounds with new drug screening technologies²³⁸. Indeed, several of the newer T-type calcium channel blockers such as Z944 exhibit this feature, as does the N-type channel inhibitor TROX-1. In other cases, it may prove advantageous to develop tonic blockers of a particular calcium channel subtype.

Third, it may be possible to target aberrant upregulation of calcium channels, as seen in pain-sensing neurons after injury. Indeed, such an approach has been demonstrated for the interactions between Cav2.2 channels and collapsin response mediator protein 2 (CRMP2; also known as DRP2), which promotes stability of the channel complex in the plasma membrane²³⁹. Uncoupling of CRMP2 from the channel via disruptor peptides reduces calcium currents and mediates analgesia²³⁹. Along these lines, interactions between Cav3.2 calcium channels and the deubiquitinase ubiquitin-specific processing protease 5 (USP5) have been shown to promote enhanced channel expression in the plasma membrane in a range of chronic pain conditions²⁴⁰. Disruptor peptides that interfered with the association of the two proteins prevented this aberrant upregulation, thereby mediating analgesia. Importantly, small organic mimetics of these peptides that were identified in an enzyme-linked immunosorbent assay (ELISA) screen prevented the USP5–Cav3.2 interaction *in vitro* and blocked the development of pain hypersensitivity in several different animal models²⁴¹. This is an example in which targeting a protein–protein interaction can be exploited towards therapeutic indications. Because such protein–protein interactions often occur in intracellular regions in which there is greater sequence divergence among various calcium channel isoforms, this approach has the potential to achieve greater target selectivity. Furthermore, by targeting an association that occurs in a pathophysiological state and not under normal physiological conditions, the potential of interfering with normal physiology (and thus the possibility of side effects) is minimized.

Channelopathies

A group of conditions in which mutations in specific ion channels give rise to disease.

Finally, knowledge of how mutations in calcium channel genes alter channel function can be exploited towards the development of therapeutics. This is exemplified by a recent study in which a gain-of-function mutation in Cav2.1 causing familial hemiplegic migraine was introduced into *Drosophila melanogaster* and shown to alter synaptic physiology at the neuromuscular junction²⁴². A compound (ter-butyl dihydroquinone) that is able to offset the enhancement of channel function to normalize the synaptic defects associated with the mutation was then applied, thus restoring normal synaptic physiology. Although such a targeted approach has not been described for treating calcium channelopathies in humans, it has been successfully applied to patients with erythromelalgia who have gain-of-function mutations

in Nav1.7 sodium channels²⁴³. It may thus be possible to adopt similar strategies for gain-of-function calcium channelopathies such as absence epilepsy, migraine or Timothy syndrome.

Altogether, there remains tremendous untapped potential towards the design of new calcium channel blockers for the precise (and perhaps personalized) targeting of a wide range of neurophysiological and psychiatric conditions. The above considerations underscore the need for fundamental insights into the roles of calcium channels in nervous system function at the cellular, molecular and network level. With many of the large pharmaceutical companies reducing in-house discovery efforts, this important task may need to fall on the academic community.

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Competing interests statement

The author declares no competing interests.

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