REVIEW

The proarrhythmogenic role of autonomics and emerging neuromodulation approaches to prevent sudden death in cardiac ion channelopathies

Johanna B. Tonko ⓘ [1]* and Pier D. Lambiase[1,2]

[1]Institute of Cardiovascular Science, University College London, 5 University Street, London WC1E 6JF, London, UK; and [2]Department for Cardiology, Bart's Heart Centre, West Smithfield EC1A 7BE, London, UK

Abstract

Ventricular arrhythmias in cardiac channelopathies are linked to autonomic triggers, which are sub-optimally targeted in current management strategies. Improved molecular understanding of cardiac channelopathies and cellular autonomic signalling could refine autonomic therapies to target the specific signalling pathways relevant to the specific aetiologies as well as the central nervous system centres involved in the cardiac autonomic regulation.

This review summarizes key anatomical and physiological aspects of the cardiac autonomic nervous system and its impact on ventricular arrhythmias in primary inherited arrhythmia syndromes. Proarrhythmogenic autonomic effects and potential therapeutic targets in defined conditions including the Brugada syndrome, early repolarization syndrome, long QT syndrome, and catecholaminergic polymorphic ventricular tachycardia will be examined. Pharmacological and interventional neuromodulation options for these cardiac channelopathies are discussed.

Promising new targets for cardiac neuromodulation include inhibitory and excitatory G-protein coupled receptors, neuropeptides, chemorepellents/attractants as well as the vagal and sympathetic nuclei in the central nervous system. Novel therapeutic strategies utilizing invasive and non-invasive deep brain/brain stem stimulation as well as the rapidly growing field of chemo-, opto-, or sonogenetics allowing cell-specific targeting to reduce ventricular arrhythmias are presented.

Keywords

Sympathetic • Parasympathetic • Autonomic imbalance • Ventricular arrhythmias • Sudden cardiac death • Brugada syndrome • Early repolarization syndrome • Catecholaminergic polymorphic ventricular tachycardia • Long QT syndrome • Short QT syndrome • Short coupled torsade de pointes

1. Introduction

Sudden cardiac death (SCD) secondary to ventricular arrhythmias (VAs) can affect patients in all age groups, with primary inherited arrhythmia syndromes accounting for up to a third of these events in the young.[1] Sympathetic arousal as well as abnormal parasympathetic tone or an imbalance of the two limbs of the autonomic nervous system (ANS) have been described as an important trigger for VAs in channelopathies. Neuromodulation is a promising yet underutilized treatment modality to prevent arrhythmogenic events in these conditions. Current clinical approaches are limited to general anti-adrenergic therapies including pharmacological betablockade or, in high risk cases, cardiac sympathectomy for patients with long QT or catecholaminergic polymorphic ventricular tachycardia (CPVT). There are no clinically available neuro-modulatory options for patients with vagally induced arrhythmic events, e.g. in Brugada, long QT 3, or early repolarization syndromes.

A number of innovative therapeutic modalities have been proposed to offer tailored modulation of the proarrhythmogenic adrenergic and vagal influences in cardiac channelopathies.

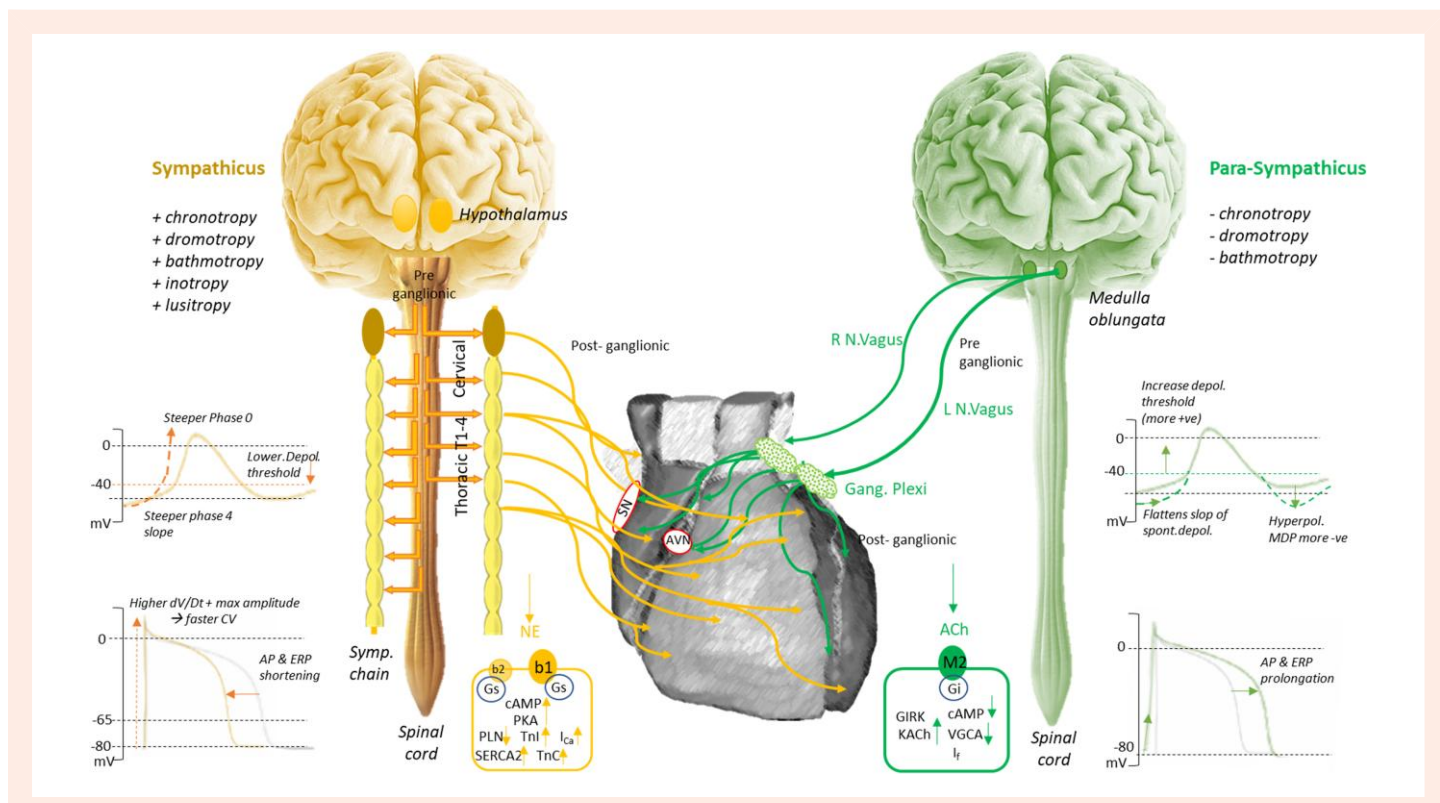## 2. Autonomic influences on cardiac electrophysiology

2.1 Anatomy

The ANS is regulated over a complex hierarchical network organized within numerous centres in the cortex, amygdala, hypothalamus, brainstem, spinal level, intrathoracic ganglia, and peripheral target organ with multiple feedback and reflex loops at each level (Figure 1).[4] The vagal nuclei providing preganglionic fibres to the heart are sited in the nucleus ambiguous and dorsal motor nucleus of the medulla oblongata. Key components of the cardiac sympathetic

Figure 1 Autonomous innervation of the heart: left (yellow): the cell bodies of the post-ganglionic sympathetic neurons are located in the ganglia adjacent to the cervical and thoracic spinal cord, which receive inputs from the dorsal root ganglia of the spinal cord and central nervous system. Their axons travel along the epicardial vascular structures of the heart and penetrate through into the myocardium. The highest density in innervation is located in the subepicardial structures in the atria and base of the ventricles and surrounding the specialized conduction system. There are distinct afferent and efferent parasympathetic and sympathetic neural projections. The afferent nerve endings have numerous functions including their roles as mechano-, chemo-, or nociceptors. Among others, this allows that the afferent sympathetic fibres can provide beat-to-beat information to the central nervous system.[2] Right (green): parasympathetic neurons are preganglionic fibres arising from the left and right vagus nerve and synapsing with a dense multiplexed network of ganglia located in the epicardial fat pats. Post-ganglionic fibres cross the AV groove and are thought to then dive intramurally to the subendocardium. Parasympathetic innervation is more heterogenous and was long thought to have little effect on the electrical activity of the ventricle, yet *in vivo* vagal influence on the ventricle has been highlighted and direct effects of acetylcholine on the ventricular myocardium demonstrated.[3] A schematic representation of the effect of sympathetic and parasympathetic inputs on the action potential (AP) configuration of impulse forming cells and working myocytes is shown: left bottom—key changes following sympathetic stimulation include a steeper phase 4 slope in impulse forming cells, an increase in dV/dt in phase 0 with higher AP amplitude and thus faster conduction velocity, a shortening of the action potential and effective refractory period. Electrophysiological changes are mediated via G_s-protein coupled beta-adreno-receptors inducing intracellular signal cascades via protein kinase A (PKA) interacting with numerous proteins, including key calcium-handling proteins. Right bottom—vagal stimulation antagonizes sympathetic effects and via muscarinic G_i-protein coupled receptors induces changes in intracellular cAMP level and ion channel availability/opening probability. A more detailed description is provided in Supplementary material online, *Table S1*.

pathways include the dorsomedial hypothalamus (DMH), periaqueductal grey (PAG) in the midbrain, and rostral ventrolateral medulla (RVLM) in the brainstem, the intermediolateral column in the spinal cord to the cervical and thoracic sympathetic ganglia. This *extrinsic* cardiac nervous system connecting the brain and the heart is complemented by the *intrinsic* cardiac nervous system. The latter is composed primarily of autonomic nervous fibres forming an interconnected neural system organized in ganglionated plexi (GP) concentrated within epicardial fat pads on the cardiac surface and intramural micro-ganglia containing autonomic efferents and afferents[5]

2.2 Physiology

Sympathetic and parasympathetic activity is continuously modulating cardiac electrophysiological properties, electromechanical coupling, and contractile performance. This is complemented by their links to the neurohumoral system (e.g. over the renin angiotensin aldosterone system[6]) and cardiovascular reflexes (e.g. via mechano-receptors in the baroreceptor reflex).[7] Their relationship has been described as 'accentuated antagonism' (e.g. enhanced effect of vagal stimulation in presence of background sympathetic stimulation) and 'reciprocal excitation' (a peripheral component of one division is activated as a consequence of activation of the other).[8–10] Both are subject to agonist-promoted desensitization and down-regulation, a regulatory process that diminishes receptor response to continuous or repeated agonist stimulation.[11]

The dominant effects of the ANS on cardiac electrophysiological properties are summarized in Supplementary material online, *Table S1*. There is significant anatomical regional heterogeneity and lateralization in innervation as well as intrinsic spatial differences in expression and activity of the autonomically modulated ion channels[3,12] translating into regionally distinct action potential characteristics (see *Figure 2*). Due to these physiological differences in action potential duration (APD) and configuration, the wave of repolarization moves in the opposite direction of depolarization from epicardium to endocardium causing a natural dispersion of repolarization across the ventricular wall, as well as apex to base and right to left.[16–18]

On a cellular level, the sympathetic effects act via noradrenaline binding to G_s-protein coupled beta-adreno-receptors. Activation of G_s stimulates
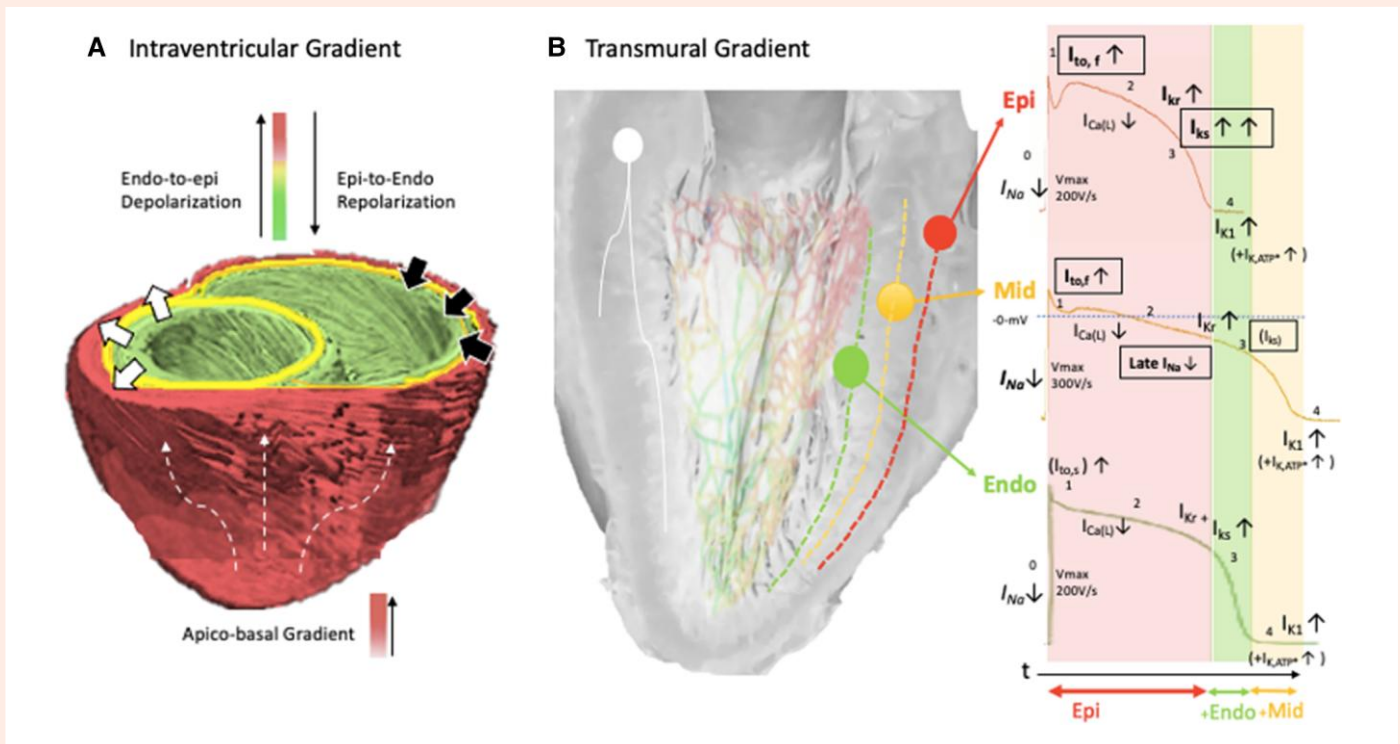
Figure 2 Intraventricular and transmural activation and repolarization gradients: (*A*) cardiac activation sequence spreads rapidly through the Purkinje network from apex to base and from endo- to epicardium (white arrows). Due to shorter APDs, repolarization occurs earliest in the subepicardial layer causing repolarization to move in the opposite direction (black arrows). (*B*) Transmural ventricular AP configurations: the prominent phase 1 notch of epicardial and midmyocardial APs is attributed to a high density of I_{to} channels. Subepicardial APs have a shorter phase 3 due to stronger I_{Ks} currents, whereas the midmyocardial cells take longest to repolarize due to weaker I_{Ks} but stronger late I_{Na} and Na^+–Ca^{2+} exchanger currents.[13–15]

positive chrono-, dromo-, bathmo-, ino-, and lusitropic effects over a complex cascade of intercellular signalling. The parasympathetic influences are transmitted via acetylcholine binding to the muscarinic inhibitory G-protein coupled receptors counterbalancing the sympathetic influences via negative chrono- and dromotropic effects and a tonic inhibitory inotropic effect.[19]

2.3 Pathophysiology of the ANS in SCD

Increased sympathetic and reduced vagal activity, but in certain inherited arrhythmia syndromes also *increased parasympathetic* activation, has been associated with a propensity of lethal ventricular arrhythmias in a number of cardiovascular diseases.[20–22] Although disease-specific mechanisms may dominate in different disorders, in general, the proarrhythmogenic effect of sympathetic stimulation may be attributed to: (i) changes in calcium handling that facilitate delayed after-depolarizations and trigger sustained ventricular arrhythmias,[23] (ii) modulation of the APD and repolarization gradients due to heterogeneity in cardiac innervation, and (iii) pathological remodelling of neuronal structures.[24,25] More detailed discussion regarding adrenergically driven arrhythmias in inherited arrhythmia syndromes is provided below. Mechanisms of vagally driven arrhythmias associated with LQT3, Brugada syndrome (BrS), and early repolarization syndrome are diverse and discussed below in the respective sections.

3. Management of cardiac channelopathies with focus on neuromodulation

Diagnosis and genetics of CPVT, long QT syndrome (LQTS), BrS, early repolarization syndrome, short QT syndrome (SQT), and idiopathic ventricular fibrillation (VF)[26,27] are summarized in Supplementary material online,

Table S2. Detailed reviews discussing genetic and clinical background have been published for each channelopathy.[28–31] Characteristic ECG features, action potential configurations, and key ion channel alterations associated with these channelopathies are illustrated in *Figure 3*.

Existing clinically employed treatment options for interventional autonomic neuromodulation are summarized in *Figure 4* and include (bilateral or left) cardiac sympathetic denervation, low level vagus nerve stimulation, auricular branch vagus nerve stimulation (VNS) ('Tragus Stimulation'), baro-reflex activation therapy, renal denervation, and spinal cord stimulation. They have been reviewed for treatment of heart failure, cardiomyopathies, ischaemic heart disease, and atrial fibrillation.[34,35] Cardiac sympathectomy and to a lesser degree renal denervation remain the only two clinically employed interventional neuromodulation options in channelopathies.

3.1 Catecholaminergic polymorphic VT

VAs occur secondary to inappropriate cytosolic calcium overload generating delayed after-depolarization. Several mechanisms of the inappropriate cytosolic calcium release, particularly in the more susceptible Purkinje cells,[36,37] and proarrhythmogenic adrenergic changes have been discussed[38] and are summarized in *Figure 5*.

In brief, one hypothesis relates to an enhanced basal activity and increased Ca^{2+} sensitivity decreasing the amount of calcium required for RYR2 activation.[39,40] Others suggest a disrupted interaction with the binding protein that stabilizes the receptor in its closed state under physiological circumstances and promotes more frequent RyR2 channel opening.[47] A third hypothesized mechanism may be a defective inter-domain folding and pathological interaction between certain domains, which may result in high sensitivity of RyR2 channel agonists and decrease the threshold for opening.[42]

Figure 3 Characteristic ECG pattern, action potential configurations, and key ion channel alterations associated with ion channel alterations. Proposed treatment as per ESC guidelines for the Managements of Patients with Ventricular Arrhythmias excluding ICD indications and lifestyle recommendations.

3.1.1 Pharmacological neuromodulation

To suppress the adrenergic influences, betablockers are recommended for all patients, including for silent carrier of pathogenic mutations. Type and dose of betablocker are important due to differences in pharmacodynamics and pharmacokinetics (including cardioselectivity, half-life, and lipophilicity), concomitant cardiac ion channel modulation, but also ease of dosing regimen (once to three times daily) that may affect compliance.[48] Lower event rates have been reported with nadolol[49] when
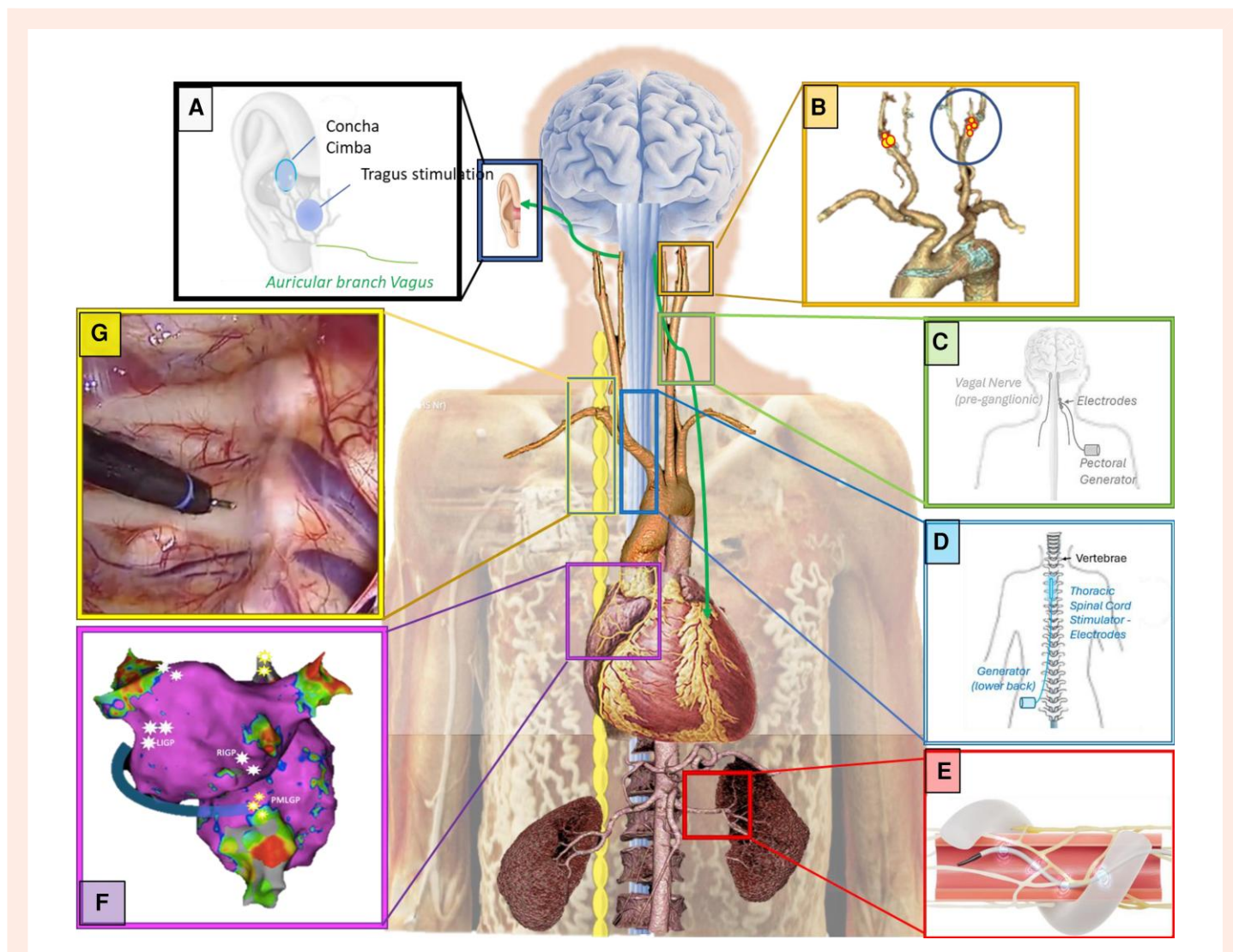
Figure 4 Overview of existing autonomic neuromodulation therapies as relevant for the treatment of cardiac arrhythmias: green arrow = vagus nerve and branches, yellow chain = paravertebral sympathetic chain. (*A*) Non-invasive auricular branch VNS stimulation (as described in Dumoulin *et al.*[32]). (*B*) Baroreceptor activation therapy. (*C*) Schematic representation of low level invasive vagus nerve stimulation. (*D*) Schematic representation of spinal cord stimulation (as described in Harned *et al.*[33]) (*E*) Renal sympathetic denervation (reprinted with permission of Medtronic). (*F*) Ganglionated plexi ablation. (*G*) Cardiac sympathetic denervation.

compared to β1 selective betablockers in equivalent doses[50,51] and should therefore be given preference. The superiority may be due to the low inter-individual pharmacokinetic variability and longer half-life with overall stronger negative chronotropic effect and lower maximum heart rate. If unavailable, most authors recommend propranolol, yet higher inter-individual variability in pharmacokinetics as well as more central nervous system side effects (due to its lipophilic properties) are to be taken into consideration.[52] Dose titration and efficacy testing should be assessed with exercise testing. Both nadolol and propranolol also exhibit peak sodium current blocking properties (and late current by propranolol) that may further reduce the risk of delayed after-depolarizations via driving the sodium-calcium exchange causing an inward sodium flux to remove calcium overload secondary to the spontaneous calcium release from the SR. The role of carvedilol and nebivolol, which have been associated with suppression of calcium leakage of the cardiac ryanodine receptor,[53,54] are not well explored. Lastly, preclinical studies suggest that additional alpha-adrenergic blockade may be beneficial in CPVT.[55] In patients with breakthrough arrhythmias while on betablockers, flecainide has been shown to reduce cardiac events regardless of genotype[56–58] including in

a small prospective randomized trial.[59] There is an ongoing debate whether its preventive effect is mediated by a reduction in sodium channel availability[60] or its ryanodine receptor blocking properties suppressing sarcoplasmic reticulum calcium release.[57,61]

3.1.2 Interventional neuromodulation

For uncontrolled arrhythmias despite maximal medical therapy, cardiac sympathetic denervation (CSD) can be considered to reduce cardiac events and ICD shocks[62–64] The anti-arrhythmic mechanism of CSD is thought to not only be due to a reduction in cardiac sympathetic stimulation and release of norepinephrine but also to a reflex increase in vagal efferent activity. CSD largely interrupts the centrally projecting cardiac sympathetic afferents, which 'normally' may have an inhibitory effect on the vagal outflow directed to the heart. The resulting vagotonic effect of CSD is likely an important contributor to the anti-arrhythmic mechanism of action.[65] Evidence supporting other interventional neuromodulation approaches is sparse. Isolated case reports of percutaneous renal sympathetic denervation for CPVT[66] as well as ablation of discrete origins of bidirectional ventricular premature ectopics triggering VF in CPVT[67]
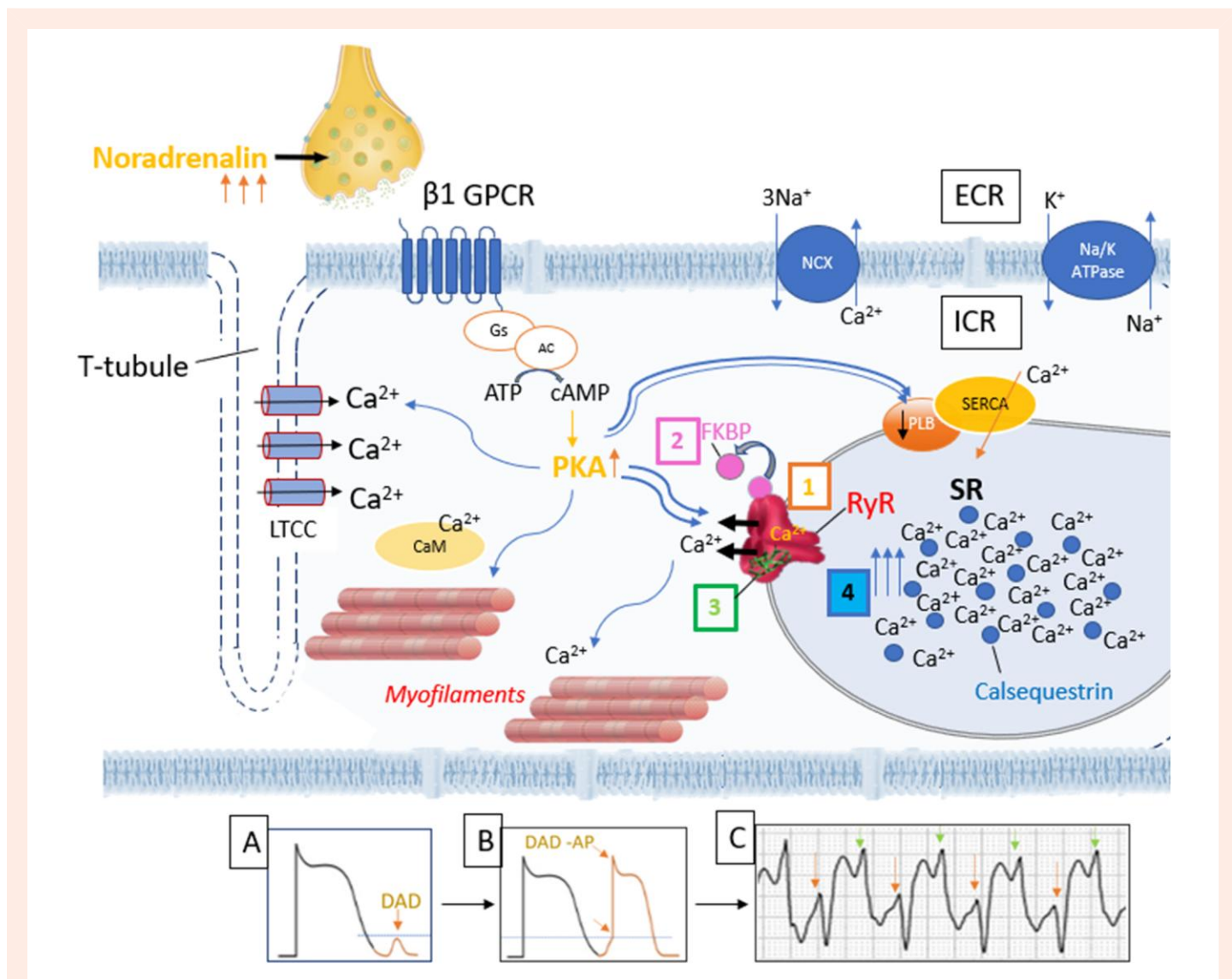
Figure 5 Arrhythmogenesis in CPVT: proposed mechanism of the spontaneous calcium release of the mutant ryanodine receptor (in red, large 'mushroom' shaped homo-tetramer) under adrenergic stimulation are: [1 orange] an enhanced basal activity and increased Ca^{2+} sensitivity decreasing the amount of calcium required for RYR2 activation[39,40]; [2 purple] a disrupted interaction with the binding protein (FKBP), which stabilizes the receptor in its closed form under physiological circumstances, leads to untimely opening[41]; [3 green] a defective inter-domain folding and pathological interaction between certain domains, which result in high sensitivity of RyR2 channel agonists and decrease the threshold for activation[42]; and [4 blue] calcium overload in the sarcoplasmic reticulum (SR) has been associated with increased triggered activity even in absence of adrenergic drive (store overload induced Ca^{2+} release).[43] The resulting inappropriate diastolic calcium release from the sarco-plasmatic reticulum causes intracellular calcium overload and delayed after-polarization (*A*) that result in triggered activity (*B*), most prominently in the more susceptible Purkinje cells than the ventricular myocardium.[36,37] The ensuing VT with beat-to-beat alternating QRS morphology (*C*) and cycle length is thought to be the result of alternating foci (termed 'reciprocating bigeminy') developing at different heart rate thresholds, as found in a mouse model with CPVT[44] and suggested in computer models.[45] If more than three foci fire, the bidirectional VT may transition into polymorphic VT. Other studies suggest that spiral wave re-entry may be an important mechanism in CPVT.[46]

have been published but are not investigated in a larger population. Programming of ICDs needs to focus on minimizing unnecessary shocks with high heart rate detection zones and long delays for shock delivery due to the known increase in adrenergic stress and self-perpetuating electrical storm following ICD shocks.

3.2 Long QT syndrome

The LQTS has numerous different subtypes according to the ion channels affected promoting prolonged ventricular repolarization times and accentuate transmural dispersion. Long QT subtypes 1 and 2 are particularly prone to sympathetic stimuli triggering VAs. The heterogeneous sympathetic innervation reported in nuclear imaging studies in LQTS likely further amplifies the arrhythmogenic risk during adrenergic stimulation.[68] LQT1 is associated with a loss of function mutation in I_{Ks} (slowly activating delayed inward rectifier potassium channel) preventing rapid repolarization and action potential shortening to adapt to the faster heart rates in the context of adrenergic drive. This facilitates the occurrence of early after-depolarizations triggering torsade de pointes that can be maintained by re-entry mechanisms and predispose to exercise or stress related cardiac events.[69] The increase spontaneous inward current of calcium over I_{CaL} under adrenergic stress further increases the likelihood of triggered

activity. The relationship between adrenergic stress and VAs is less prominent in LQTS2 (characterized by an abnormal I_{Kr}, the rapidly activating delayed inward rectifier potassium channel). The pathognomonic genotype specific trigger is generally considered to be loud noise while at rest or asleep,[70,71] which is associated with a sudden acceleration in heart rate and slower adaptation of repolarization in LQT2.[72] Also, an excessive risk in the postpartum period[73] is thought to be secondary to the rapid reversal of changes in cardiac haemodynamics, output, and contractility, but importantly also alterations in adrenergic activity in the peripartum period and disrupted sleep patterns.[74,75]

General management recommendations include avoidance of QT prolonging medication, electrolyte imbalances, and genotype specific triggers.

3.2.1 Pharmacological neuromodulation

Betablockers are the main stay in all LQTS patients. Initial large studies grouping all genotypes together showed no relevant difference between types of betablockers[76] yet later investigations highlighted that similarly to CPVT, not all betablocker are equally effective—non-selective betablockers like nadolol (1–1.5 mg/kg/day) and propranolol were more effective than metoprolol[77,78] (see Supplementary material online, Table S3).

3.2.2 Interventional neuromodulation

CSD increases the ventricular fibrillation threshold[79] and ventricular refractory period.[80] It has been repeatedly demonstrated and highlighted as an efficient anti-adrenergic therapy to reduce syncope and ICD shocks in LQTS.[81–83] Anti-adrenergic therapy is particularly effective in LQT1 and more individualized and refined patient selection incorporating clinical and genetic features have been proposed to maximize procedural success.[84] Early consideration of device and surgical therapies has been suggested in high risk patients with malignant LQTS genotypes but also in case of betablocker intolerance or non-compliance.[85] The use of renal denervation has been described as a successful neuromodulation strategy in LQT animal models.[86,87] Experience in humans is currently limited to isolated case reports.[88,89] There is an ongoing debate as to whether renal sympathetic denervation is indirectly beneficial by modulating sympathetic inputs or directly shortening the QTc.[90]

3.3 Long QT syndrome 3

Unlike the above, in LQT3 cardiac events have been associated with increased vagal tone. At lower heart rates, the action potential duration prolongs due to a more pronounced late sodium current at low stimulation rates. In turn, during tachycardia, there appears to be a protective role of elevated intracellular Ca^{2+}, which suppresses the late sodium current.[91]

3.3.1 Pharmacological modulation

Betablockers have been discussed controversially for long QT 3 patients due to the association with bradycardia, conduction disturbances as well as increased cardiac events at rest.[92] Yet, there is evidence that indeed betablockers were not proarrhythmic but protective in females and neutral in males.[93] Propranolol may theoretically be preferred due to its additional use dependent peak and late sodium channel blocking properties,[94] which may in selected cases contribute to QT interval shortening. Functional heterogeneity of the underlying mutation may play an important role on the therapeutic effect of sodium channel blockers such as mexiletine that is a second line therapy (see Supplementary material online, Table S3).

3.3.2 Interventional therapy

A limited number of LQT3 patients have been included in studies of CSD and found to benefit from the reduction in sympatho-excitation.

3.4 Brugada syndrome

A large body of literature has been published about the possible underlying mechanism of BrS.[95,96] On a cellular level, two principal hypotheses have been proposed: the repolarization hypothesis and the depolarization hypothesis, which are likely not exclusive and a combination of de- and repolarization abnormalities has been described.[97] The original repolarization hypothesis[98] suggests that an outward shift in the balance of currents in the right ventricular epicardium (via reduction in inward sodium and accentuation of outward currents) can result in repolarization abnormalities and facilitate the development of phase 2 re-entry generating closely coupled premature beats that can precipitate VF. The depolarization hypothesis[99] suggests that the ST elevation is caused by slow conduction/delay in the RVOT that in turn creates a potential difference and thereby facilitates VAs. Autonomic influences play an important but complex role in arrhythmogenesis of BrS.[100,101] There is a recognized association of cardiac events with proarrhythmogenic vagal influences and/or decreased sympathetic input supported by circumstantial evidence of cardiac events,[100,102,103] findings in Holter ECG[104] and nuclear imaging (MIBG SPECT[105–109] and PET CT[110]). The mechanism of vagally driven arrhythmias may be caused by a reduction in I_{ca-L} during the action potential plateau and indirectly via a decrease in heart rate that in turn decreases intracellular calcium, reduces myofilament calcium sensitivity, and is associated with elevated ST-segments.[96] In turn, β-adrenergic activation with isoproterenol is effective in suppressing arrhythmias by enhancing inward calcium current.

However, in a small subset of Brugada patients, malignant arrhythmias were observed under increased adrenergic stress. The latter has been associated with a specific SCN5A mutation involving the C-terminal portion of the sodium channel that augments slow inactivation and delays recovery of sodium channel availability.[111,112]

3.4.1 Pharmacological neuromodulation

In case of acute electrical instability and/or recurrent ICD discharges, intravenous isoproterenol is considered the first-line treatment to suppress VAs. The anti-arrhythmic effect of isoproterenol is thought to be secondary to its augmentation of L-type calcium channel current that contribute to restoring the action potential dome and thereby prevention of phase 2 re-entry.[113] The requirement for intravenous access precludes its use in the long-term. Options for oral anti-arrhythmic treatment are sparse and essentially limited to quinidine. Quinidine acts as a transient outward potassium current (I_{to}) inhibitor with additional anticholinergic properties contributing to its anti-arrhythmic effect in Brugada patients.[114] Its benefits are off set by the frequent adverse effects leading to poor patient compliance as well as often restricted availability. Alternative oral drugs targeting proarrhythmogenic autonomic influences have been explored, although only anecdotal evidence is available for their beneficial effects. Among these are orciprenaline[115] and denopamine, acting as oral beta-adrenergic agonists, as well as bepridil, a calcium channel block with I_{to} inhibiting properties.[116] Yet, more evidence is needed to define their role in the clinical management of Brugada patients.

3.4.2 Interventional treatments

In case of recurrent ventricular arrhythmias and ICD shocks, ablation of both VF triggers and epicardial abnormal electrograms was found to prevent arrhythmic recurrences.[117] There are no clinical recommendations for interventional neuromodulation to reduce VAs in BrS. Investigational approaches to reduce cardiac vagal influences via central or peripheral neuromodulation are described below and may provide possible therapeutic options to minimize the proarrhythmogenic vagal influences associated with arrhythmic events.

3.5 Early repolarization pattern/syndrome (ERS)

The electrical substrate of early repolarization pattern/syndrome (ERS) is thought to arise from the current imbalances between the epi- and endocardial layers, predominantly in the infero(lateral) wall. The accentuation of the phase 1 action potential notch results in steep transmural action potential duration and repolarization gradients.[118] The loss of the action potential dome predisposes to phase 2 re-entry and VF.[119] Both, the more

prominent early repolarization pattern and cardiac events have been associated with periods of high vagal tone.[120–125] In experimental settings, acetylcholine caused loss of epicardial dome at some sites but not others resulting in epicardial dispersion and precipitating repeated episodes of phase 2 re-entry and polymorphic VT/VF, whereas quinidine and isoproterenol restored epicardial AP dome and supressed VT/VF.[126]

3.5.1 Pharmacological modulation

Similar to the above BrS, intravenous isoproterenol has been found to be effective in acute suppression of VAs and ICD discharges. The augmentation of inward calcium current counterbalances the excess net outward potassium current and restores the epicardial action potential dome explaining the anti-arrhythmic properties in ERS.[126] So does the transient outward potassium current inhibition by quinidine, which reduced the magnitude of the J wave and remains the only available oral drug therapy. Alternative drugs, including betablockers, lidocaine, mexiletine, and verapamil, have not been found to be beneficial.[127] Case reports of oral Phosphodiesterase III inhibitors Cilostazol have been described to reduce the occurrence of phase 2 re-entry in ERS. The latter is thought to be secondary to an increase in I_{Ca2+} and reduction in I_{to} currents.[128] No larger studies are available to support their use.

3.5.2 Interventional treatment

In case of recurrent ventricular arrhythmias triggered by a consistent PVC morphology, ablation of such trigger should be attempted. Targeted interventional autonomic modulation has not been reported for ERS.

4. Emerging therapeutic targets and novel concepts for precision neuromodulation

Available neuromodulation strategies are comparatively blunt with only limited consideration of the pathophysiological specificities and anatomical distribution of functionally critical areas of the underlying channelopathy pathology. Yet, the improved molecular understanding of cardiac channelopathies and cellular autonomic signalling have allowed to identify more precise targets for autonomic therapies. These include receptors mediating adrenergic and parasympathetic inputs to the heart, encompassing the large group of G-protein couple receptors and the still sparsely investigated sigma-1 receptors, as well as neurotransmitters, neural chemorepellents and attractants, and metabolic ligands like adiponectin.

4.1 Precision medicine in cardiac neuromodulation: molecular and central nervous system targets

4.1.1 Modulation of G-protein coupled receptors

These mediate important cardiac transmembrane and intracellular signalling pathways. They are divided into three main categories: stimulatory (G_s) and inhibitory subunits ($G_{i/0}$) affecting cAMP (adenosine monophosphate) and $G_{q/11}$ involved in the phospholipase C-β and inositol-1,4,5-triphosphate (IP$_3$) and 2-diacylglycerol (DAG) signalling pathway. Adrenergic receptors, including β1, primarily signal through heterotrimeric G_s proteins.[11] In turn, binding of acetylcholine to the muscarinic M2 receptors leads to activation and dissociation of inhibitory G-protein (G_i) heterotrimers. In pacemaker cells, this directly activates the inward rectifying potassium (GIRK) channel causing hyperpolarization and slowing sinus rate.[129] $G_{q/11}$ also has an important impact on electrophysiological signalling and is involved in impulse generation and propagation by modulating myocytes Ca^{2+} handling and intercellular communication.[130,131] As such, G-protein coupled receptors have been identified as promising targets for autonomic precision medicine to modulate their activity and intracellular downstream effectors.

Preclinical *in vivo* studies associated loss of G$_{i2}$ with sinus tachycardia and loss of high frequency power in heart rate variability (HRV) assessment.[132] This could represent a possible target for vagally induced VA e.g. BrS or LQT3 by increasing baseline heart rate and reduce vagal inputs. Alternatively, in Brugada patients, ^{11}C-PET-CT studies demonstrated increased norepinephrine recycling with no change in post-synaptic beta-adrenoreceptor density. The latter may suggest a possible altered signal transduction by G$_s$ proteins and resulting lower cAMP and PKA activity and calcium current. Targeting and enhancing beta-adrenergic G$_s$-coupled receptors may therefore represent another approach to mitigate proarrhythmogenic autonomic influences. Lastly, G$_q$ mediates the response to hormonal (angiotensin-II and endothelin-1) and neural (noradrenalin/adrenaline over alpha-adrenergic receptors) inputs. It has been associated with proarrhythmogenic properties via contributing over multiple pathways to intracellular calcium overload associated with delayed afterdepolarizations, as well as reducing conduction velocity by PKC mediated phosphorylation of CX43.[133] Selective cardiac inhibition of the G$_q$ pathway could represent a target to suppress VAs e.g. in CPVT.

4.1.2 Neuropeptides

Neurotransmitter-mediated autonomic modulation other than by noradrenaline and acetylcholine are complementary promising targets for neuromodulation to address sympathetic hyperactivity.[134] Neuropeptide Y, galanin, and dopamine have been investigated in this regard:

- Neuropeptide Y is released by sympathetic nerves and known for its parasympathetic inhibition,[135] stimulating calcium release in myocytes[136] and cardiac sympathetic electrical modulation including shortening of action potential duration in the context of betablocker therapy.[137] High levels of neuropeptide Y in the coronary circulation have been associated with increased adverse events in heart failure and higher recurrent ventricular arrhythmias after percutaneous coronary angioplasty. NPY1 receptor antagonist has been found to mitigate and reduce sympathetic effects and may complement existing anti-adrenergic therapies.[137,138]
- Galanin, a slowly diffusing sympathetic co-transmitter, reduces vagal acetylcholine release via a receptor-mediated protein kinase dependent pathway. It may contribute to regeneration of sympathetic nerves following myocardial infarction and contribute to VAs.[139] In preclinical studies, galanin receptor antagonists abolished the effect of galanin on vagal bradycardia as did protein kinase C inhibitors.[140]
- Dopamine is well known and in clinical use for its inotropic and natriuretic effect. Yet, it has also been associated with VAs secondary to overexpression of cardiac D1 receptors.[141] It is thought that this is associated with hyperactivation of RyR2 receptors that in turn represent a driver for dysregulated calcium handling.[142] Whether dopamine and dopamine receptors could represent possible targets for anti-arrhythmic therapies remains to be investigated.

Most neuropeptide research relates to heart failure or myocardial infarction, with a distinct lack of data in autonomic mediated arrhythmias in channelopathies. Yet, they may offer important additional treatment options. For example CPVT or LQTS1 and 2 with breakthrough cardiac events during betablocker therapy may possible benefit from complementary NPY receptor antagonists.

4.1.3 Sigma-1 receptors (S1R)

These ligand-regulated chaperone proteins are sensitive to a wide number of ligands including neuroactive steroids. Their role and interaction is complex and many aspects still controversial. They are thought to be involved in modulation of cardiac contractility, voltage gated potassium, sodium and calcium ionic channels in cardiomyocytes but also sympathetic and parasympathetic neurons.[143] In preclinical studies, sigma 1-receptor activation reversibly inhibited voltage gated sodium currents,[144] depresses neuronal excitability of intracardiac neurons,[145] and reduced Ca^{2+} leakage into the cytosol via modulating calcium channels.[146] Chronic activation of S1R

was shown to beneficially influence ventricular remodelling and decreases susceptibility to VAs after myocardial infarction in animal studies.[147]

With a better understanding, targeting specific sigma-1 receptor ligands may achieve precise ion channel modulation to oppose disease-specific gain- or loss of function mutations in channelopathies. Nuclear imaging with SPECT (I-125iodophenyl-piperidino-cyclopentanol, 125I-0I5V) may be used to guide and titrate neuromodulating therapies.[148]

4.1.4 Semaphorines

Semaphorines are a family of secreted and membrane anchored glycoproteins that are important during neuro-embryogenesis and reinnervation of peripheral organs following neural injury. Sema3a in particular acts as a potent neural chemorepellent, inhibiting neural growth and thereby regulating axon growth and neuronal migration.[149] Dysfunction, over- or underexpression of Sema3a may, among others, result in pathological innervation patterns of sympathetic nerves.

In preclinical studies, overexpression of Sema3a in the heart and the left stellate ganglion was associated with a reduced inducibility of VAs associated with sympathetic hyperinnervation e.g. in the post-infarction remodelling.[150,151] The anti-arrhythmic effect of Sema3a is thought to be secondary to the combination of decreased sympathetic hyperinnervation, decrease in norepinephrine content and restoration of dephosphorylated CX43 in the infarct border zone.

In clinical studies, certain polymorphisms of Sem3A have been associated with suspected abnormal sympathetic innervation pattern with otherwise unexplained ventricular fibrillation.[152] Tailored stimulation or inhibition of Sema3A and/or nerve growth factors could potentially allow to modulate innervation pattern on a tissue-based level to counterbalance pathological proarrhythmogenic autonomic influences.

4.1.5 Adiponectin

Adiponectin is secreted by adipocytes and known for a multitude of metabolic effects including prevention of inflammation, oxidative stress, regulating energy metabolism, and improving insulin sensitive. Yet, it has also important regulatory effects on the ANS. It is involved in the regulation of neuronal excitability of the paraventricular nucleus in the hypothalamus,[153] reduce peripheral sympathetic tone via signalling in the locus coeruleus,[154] and inhibit excessive activation in the GP of the heart thereby preventing atrial fibrillation.[155] Injection in the adipose tissue surrounding the left stellate ganglion using a chemogenetic approach has been shown to decrease neural activity and cardiac effective refractory period. In turn, this has been associated with a reduction in VAs in a preclinical infarct model.[156]

Adiponectin is not the only promising target with other metabolic targets involved in connexin and gap junction signalling already highlighted.[157] Yet, overall, the highly complex metabolic–immunological–autonomic cardiac interactions and their contribution to ventricular arrhythmogenesis are still poorly understood.

4.1.6 Central cardiac autonomic centres

Genetic neuronal targeting and functional neuroanatomical mapping has allowed to identify several key centres involved in central cardiac autonomic regulation (see *Figure 6*).

Their complex interaction has been reviewed.[159,160] The improved understanding of the effect on electrophysiological properties of each of these central structures in combination with the technological advances in invasive and non-invasive brain stimulation plus molecular based therapies opens new possibilities for central cardiac neuromodulation.

In brief, central targets on the vagal side include the preganglionic parasympathetic neurons of the nucleus ambiguous and vagal dorsal motor nucleus, which have been reported to have preferential control over pacemaker and ventricular tissue. The cardiovagal neurons of the nucleus ambiguous innervate the cardiac nodal tissues, activate cholinergic cardiac

ganglion neurons, and inhibit the automaticity of the sinus (and AV) node with beat-to-beat modification by the baroreflex.[161,162] They are also modulated by respiration with a rhythmic respiratory related pattern of discharge (inspiration inhibiting and expiration stimulating the cardiovagal neurons) causing the respiratory associated sinus arrhythmia.[163] In turn, ventricular contractility is affected by the tonic inhibitory muscarinic influence via the preganglionic neurons of the caudal regions of dorsal motor nucleus. Silencing of the neurons of the dorsal motor nucleus has been found to increase left ventricular contractility.[19,164] Yet, any form of intervention targeting the central and/or peripheral extra cardiac nervous system with the aim to modulate electrophysiological properties specifically at a distinct cardiac site only, will need to take the complex interconnectivity of the intrinsic cardiac nervous system into account.[165] The latter likely mitigates the lateralization of the extrinsic cardiac nervous system to a substantial degree.

Sympathetic tone is largely mediated by the discharge of the sympatho-excitatory glutamatergic neurons in the RVLM oblongata. Silencing the RVLM neurons has been shown to eliminate the central sympathetic outflow.[166] Yet, there remains an ongoing debate about the functional organization of the RVLM neurons. Some evidence suggests that pre-sympathetic neurons are organized organotrophic in that distinct populations regulate defined peripheral targets, for example the heart.[167] This has been supported by micro-stimulation of specific sites in the RVLM activating specific sympathetic nerves e.g. in the heart.[168] In turn, other studies found that pre-sympathetic neurons in the RVLM may serve multiple organs[169] to allow the brain to recruit a molecularly defined subset of RVLM neurons to produce a suitable autonomic outflow for a particular stimulus. Clarification of the functional organization and molecular features of these pre-sympathetic neurons will be key to allow tailored cardio selective neuromodulation without inadvertent non-cardiac side effects. Furthermore, the PAG in the midbrain has been identified as an important contributor to central autonomic regulation with efferent pathways to cardiac-related sympathetic premotor neurons[170] as well as projecting to vagal preganglionic neurons.[171,172] Its complex interactions and feedback loops still require a more detailed understanding prior to suggesting strategies for anti-arrhythmic modulation. Lastly, also the upper cervical spinal cord has been identified as a potential treatment target for cardiovascular disease due to its role in controlling the sympathetic outflow to thoracic (as well as visceral) organs. Spinal cord stimulation at the upper cervical spinal segments (C1–2) has been shown to increase cerebral blood flow, which in turn was associated with a decrease in sympathetic activity and increase in vasomotor centre.[173]

4.1.7 Glia cell modulation

There is substantial interest in different types of glia cells due to their vital role in the development and protection of neurons.[174] These include not only the central macroglia (e.g. astrocytes and oligodendrocytes) and microglia, the resident immune cells that may be targeted to suppress pathological inflammation and modulate neuronal activity, but also satellite glia cells (SGC) in the peripheral nervous system. SGC influence cholinergic transmission and synaptic activity in sympathetic ganglia, where they promote synapse formation, neuronal activity and survival,[175] and become enlarged and reactive in the context of VAs.[176] Also, activation of peripheral satellite glia cells in the stellate ganglia has been shown to increase sympathetic outflow to the heart, probably via activation of G_q-protein couple receptors leading to an increase in norepinephrine release and beta-1 adrenergic activation.[177] In turn, genetic ablation inducing loss of satellite glia results in reduced expression of noradrenergic enzymes, soma atrophy, and enhanced apoptosis of adult sympathetic neurons, although persisting neurons compensated with elevated activity causing resulting in a net increase in heart rates.[178] This underlines the importance of peripheral glia in sympathetic ganglia and their potential role as a target for neuromodulation including in cardiovascular disease.
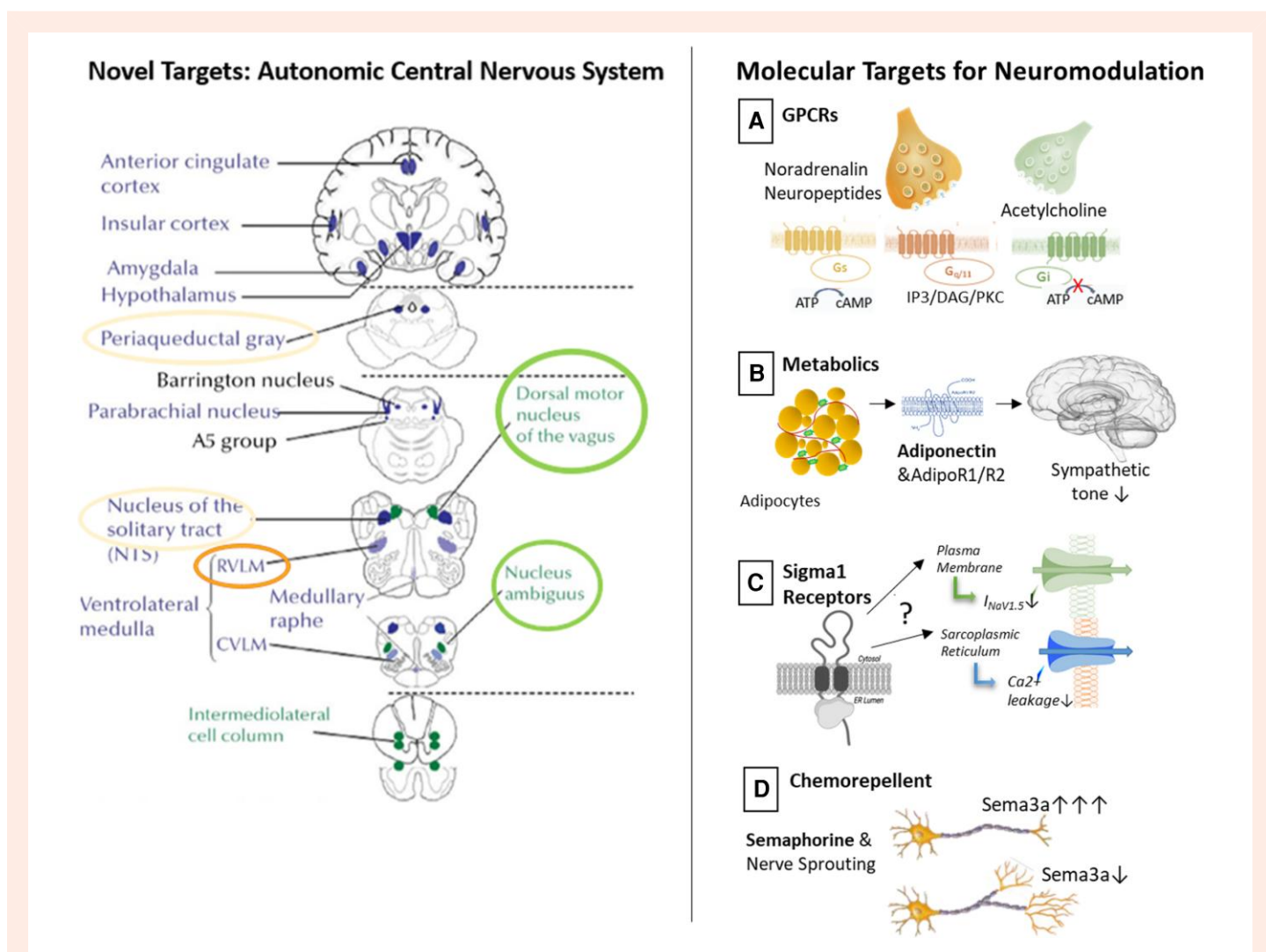
Figure 6 Overview novel molecular and central nervous system targets. Left: central autonomic nervous system centres, key targets for cardiac neuromodulation highlighted in green (vagal) or orange (sympathetic) (adapted from Benarroch et al.[158]) Right: overview of novel molecular targets including (*A*) inhibitory and excitatory G-protein coupled receptors including the cardiac receptors of norepinephrine, acetylcholine, and other neuropeptides. (*B*) Metabolic targets including adiponectin released from adipocytes. (*C*) Sigma-1 receptors, ligand specific cellular signalling pathways are still under investigation, inhibition of sodium channel currents, and reduction in calcium leakages have been described as possible anti-arrhythmic mechanism via sigma-1 receptors. (*D*) Chemorepellents/attractants, e.g. Semaphorin3A suppressing sympathetic nerve sprouting in the heart.

4.2 The future of neuromodulation: deep brain stimulation, chemogenetics, optogenetics?

Several promising therapeutic approaches (see *Figure 7*) have been suggested in addition to the existing autonomic therapies outlined in *Figure 4*. Broadly, these potential candidates for cardiac neuromodulation can be divided in two categories: physical stimulation technologies take advantage of the *in vivo* synaptic plasticity, which allows to adapt the strength of synaptic signalling in the neural network following a defined stimulatory or inhibitory stimulus. This allows to provoke long-term effects beyond the duration of the stimulus (long-term 'potentiation' or 'depression').[182] The exact underlying molecular mechanism of such long-term potentiation and depression is a major area of interest in modern neuroscience and the complex processes suggested to be involved have been reviewed.[183,184]

In turn, molecular precision medicine approaches like chemo- and optogenetics have raised hopes to target very precisely inter- and intracellular signalling pathways based on the underlying aetiology while minimizing inadvertent side effects.

4.2.1 Physical central and peripheral neuronal stimulation technologies

4.2.1.1 Deep brain stimulation (DBS)

Deep brain stimulation (DBS) is an established neurosurgical procedure for selected neurological conditions (e.g. movement disorders, essential tremor, dystonia, and chronic pain). It involves implantation of a device ('neurostimulator') connecting a lead positioned in the specific area of the brain to a generator placed under the collarbone or abdomen. Via delivery of electrical impulses of modifiable frequency, intensity, and location, the activity of the neural circuits can be modulated. DBS of the brainstem has been employed as well and found to elicited autonomic (cardiovascular and cardiorespiratory) effects.[185] Despite the high complexity of the area, several studies have demonstrated feasibility of targeting discrete structures, including the PAG and nucleus of the solitary tract involved in co-ordination of autonomic response. The potential of deep brain stimulation for treatment of dysautonomia including modulation of cardiovascular functions has been highlighted.[186] Yet, its use for targeted cardiac electrophysiological modulation has not yet been explored. The invasiveness of
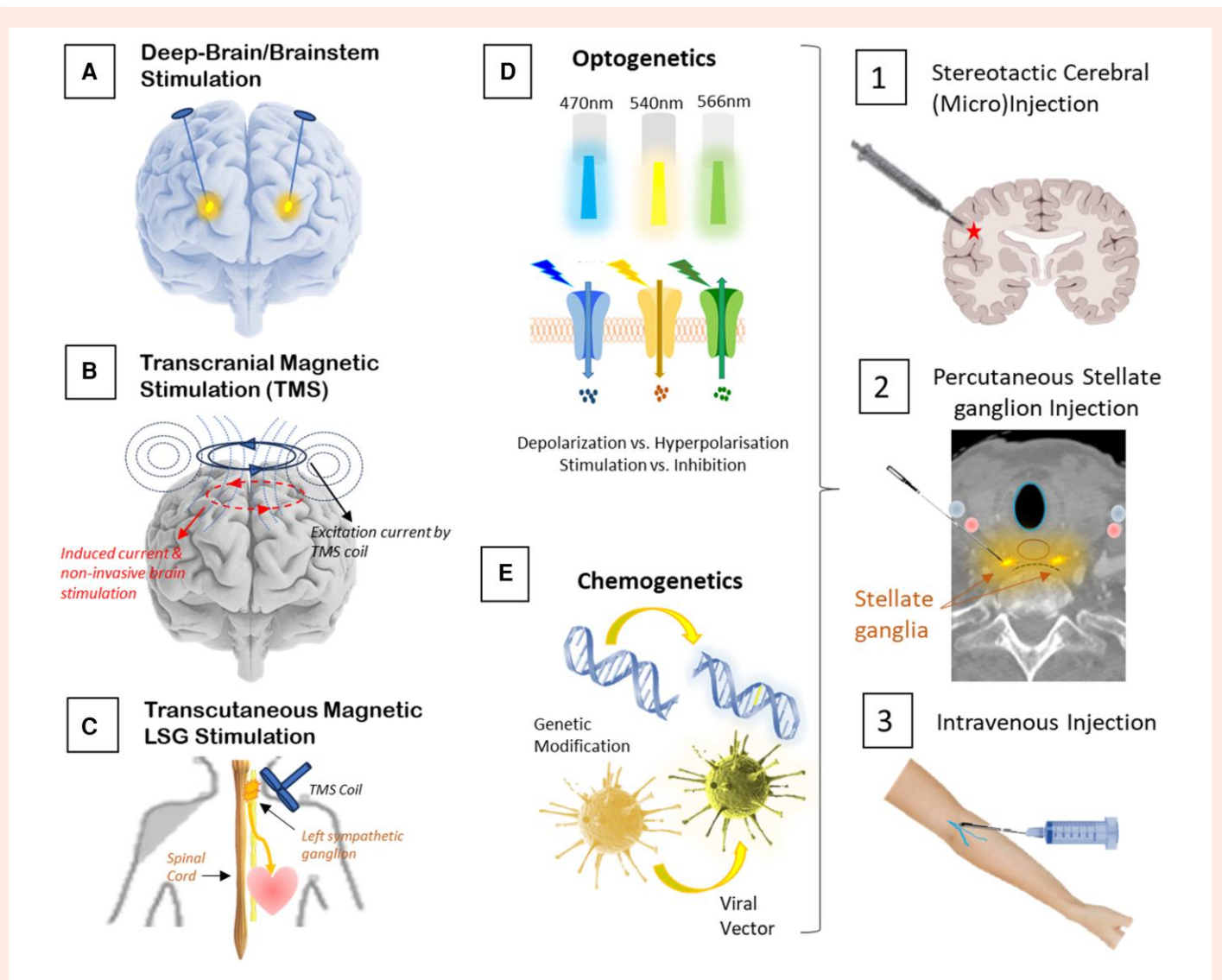
Figure 7 Investigational neuromodulation techniques: left: (*A*) Schematic representation of deep brain stimulation with implantable stimulator. (*B*) Schematic representation of non-invasive transcranial magnetic stimulation (as described in Holvoet *et al.*[179]). (*C*) Schematic representation of transcutaneous magnetic stimulation of the left stellate ganglion (as described in Markman *et al.*[180]). Middle: (*D*) Optogenetics: commonly used opsins in neuroscience are channelrhodopsin activated by blue light facilitating combined sodium and calcium current causing depolarization of the cell, halorhodopsin activated by yellow light and allowing chlorid currents causing hyperpolarisation and cell 'silencing', and bacteriorhodopsin a proton pump gated by green light. (*E*) Chemogenetics: genetically modified receptors that respond exclusively to specific ligands are generated and induced in a viral vector. Right: schematic illustration of possible delivery modalities of opto- or chemogenetical therapies over (i) direct stereotactic injection to central autonomic centres, (ii) stellate ganglion (as described in Goel *et al.*[181]), or (iii) directly intravenous.

this approach will require a very strong level of evidence for efficacy and safety for the treatment of VAs, particular if less invasive options (outlined below) are available.

4.2.1.2 Transcranial electromagnetic stimulation (TMS)
Transcranial electromagnetic stimulation (TMS) is a non-invasive neurological therapy modality that applies a changing magnetic field to induce an electric current at specific areas of the brain through electromagnetic induction. This results in depolarization or hyperpolarization of the neurons in the target area and may be delivered up to several centimetres deep into the brain tissue.[187] A variety of stimulation methods have been reported.[188] Effects can be modified by adjusting frequency and intensity of the magnetic impulse, number of repetitions and length of treatment causing long-term potentiation or depression in synaptic efficacy. Cardiac rhythm and HRV have

been observed during TMS for neurological diseases. Small clinical feasibility studies investigating the effect on cardiac rhythm indeed demonstrated that significant changes in heart rate and heart rate variability parameters (including LF/HF ratio) can be achieved by repetitive TMS.[189] Improved neurofunctional imaging and understanding of target areas may open possibilities of non-invasively targeting central cardiac autonomic areas to modify pathological autonomic remodelling and responses by tailored stimulation or inhibition of synaptic efficacy via TMS.

4.2.1.3 (Left) stellate ganglion transcutaneous magnetic stimulation (TcMS)
A peripheral application via transcutaneous magnetic stimulation (TcMS) of the (left) stellate ganglion has already been employed in clinical studies. A small feasibility study demonstrated an impressive reduction of ventricular

arrhythmia burden (99 to 5 episodes within 48 h) in a small case series of five patients with low frequency stimulation of the left stellate ganglion (LSG).[180] A subsequent double-blind sham controlled RCT including 26 patients with VT storm further confirmed the beneficial use of TcMS to suppress VAs safely and non-invasively with a single session, including in patients with cardiac devices.[190] Further research will need to focus on refining stimulation sequences and pattern and exploring the long-term effect.

A similar concept employing low intensity (1–2 W) ultrasound stimulation as opposed to magnetic stimulation has been proposed. In a sham controlled animal study, this successfully modified sympathetic neural activity in the LSG and reduced VAs in a canine infarct model.[191]

4.2.1.4 Selective fascicular vagal nerve stimulation for organ specific neuromodulation

It is recognized that there are closed loop vagal reflexes in the brainstem and peripherally that modulate sympathetic activity and vice versa. Vagal up-regulation could counterbalance sympathetic overdrive but may need to be targeted to specific fascicles supplying certain cardiac sites and ganglia. This requires detailed knowledge of functional anatomical organization of the nerve as well as sophisticated lead designs and cuffs to be applied. Precision medicine with spatially selective stimulation of specific fibres is currently under active investigation to maximize therapeutic benefit while mitigating the traditional wide ranging side effects of non-selective vagal nerve stimulation.[192]

One important example of vagal neuromodulation is the auricular nerve stimulation. Afferent auricular vagus nerve (aVN) stimulation recruits sensory afferent vagus nerve (aVN fibres) and thus mimics/projects sensory input to the brainstem in terms of neuromodulation, forming the so-called auriculo-vagal afferent pathway.[193] Since auricular vagus nerve stimulation (aVNS) projects directly to nucleus tractus solitarius, both the peripheral and central nervous systems can be modulated by aVN stimulation. Selective targeting of specific fibres of the aVN may allow precise modulation of systemic parameters of cardiovascular and respiratory functions by a sympatho-inhibitory effect.[194] Clinical research has been so far focused on employing aVNS for atrial arrhythmias and blood pressure management, but preclinical studies indicate a protective anti-fibrillatory effect also in VAs.[195]

4.2.2 Molecular precision medicine

4.2.2.1 Chemogenetics

One of the most exciting advances in recent years has been the progress in using a combined genetic and chemical approach to generate exogenous 'designer receptors' that respond to a specific synthetic ligand to modify cellular signalling and activity.

Types of chemogenetics

Initial chemogenetic receptors were termed 'RASSLs' (receptors activated solely by synthetic ligands) but were found to exhibit off-target effects and high levels of constitutive activity.[196] Further improvements let to the development of the current two main types of chemogenetic receptors as follows:

(1) DREADD ('designer receptors exclusively activated by designer drugs'), which are monomeric proteins with low constitutive activity and few off-target effects. They have become the most widely employed type of chemogenetic receptors to target G-protein coupled receptor signalling to replicate the signalling output of the naturally occurring receptors. They are classified in excitatory and inhibitory types: G_q DREADDS signal through the $G_{q/11}$ protein, G_s and G_i DREADD activate respectively inhibit neuronal signalling by increasing intracellular cAMP concentrations. Detailed reviews about their use in neuronal research have been published.[197]

(2) 'PSAM' (pharmacologically selective actuator modules) are modified ligand binding domains, which respond to specific small molecules termed PSEM ('pharmacologically selective effector molecules'). PSAMs may be coupled to an ion pore domain (forming a chimeric protein) to form ligand gated ion channels that can directly excite or inhibit neuronal signalling.[198] Initial challenges of PSEM have been short clearance times and low micromolar potency preventing *in vivo* use, though ultrapotent chemogenetics have been developed to overcome these limitations.[199]

Delivery

To achieve *in vivo* expression in the target cells, chemogenetic receptors are traditionally delivered through viral injections, most commonly using a replicant deficient adeno-associated virus. Cell specificity, e.g. selectively targeting cardiomyocytes, can be achieved by choosing AAV serotypes with cardio-tropism and adding cell type specific promotors in the chemogenetic plasmids[200–202] that may be complemented by enhancers. This has allowed to achieve highly precise spatiotemporal targeting of cardiomyocyte and cardiac autonomic specific effects.

Examples of chemogenetics for autonomic modulation of cardiac electrophysiology

In a transgenic mouse model with G_q coupled DREADD, G_q, *in vivo* activation over designer ligands caused a measurable increase in generation of cAMP and transient positive inotropy. This was likely by activation of the ryanodine receptors and increase in cellular Ca^{2+} as well as PKC activation and phosphorylation of calcium-handling proteins. Secondly, changes in impulse propagation in the AV node as well as spatiotemporal dispersion of ventricular excitation were observed and thought to be secondary to a direct effect on connexin assembly, function, and ion conductivity.[131] These findings suggest that with further refinement of chemogenetic engineering very specific G-protein cascades at the level of the sinus node, AV node, Purkinje or working myocardium may be targeted to allow highly precise modulation of impulse propagation and contractility.[133]

In BrS, chemogenetic targeting of the protein trafficking regulator MOG1 (a chaperone that binds to the voltage gated sodium channel and traffics it to the cell surface) has allowed an increase cell surface expression of $NaV_{1.5}$ and ventricular I_{Na} and thereby normalize action potential abnormalities and abolished J waves.[203]

Chemogenetic modulation of central autonomic neuronal circuit activity has also been shown to be feasible.[204] Silencing the dorsal motor nucleus of the vagus nerve achieved successful modulation of ventricular excitability by shortening the ventricular ERP and elevating the threshold for VT induction.[164]

Challenges

Whereas the initial concerns in regards to the effect of high levels of engineered protein expression in the absence of chemical activation and constitutive activity may have been overcome, questions of desensitization and down-regulation remain. Also, protein interactions need to be considered as they dictate the degree of viral spread within the injected tissue and may impact gene expression. Lastly, agonists may activate more than one downstream effector pathway inducing effects other than just silencing or enhancing neural activity. Further research is required to confirm safety and absence of inadvertent side effects. Lastly durability of chemogenetic modulating effects is still an open question.

4.2.2.2 Optogenetics

Genetic information coding for light sensitive proteins ('opsins', ion channels or pumps) is introduced into cellular DNA of the target cells. Once the target protein is expressed, exposure to a defined activation wavelength allows for reversible modulation of its activity via the opsins, which may then result in depolarization or hyperpolarization of the cell membrane causing cellular excitation or silencing.[205]

Types of opsins and delivery

The original opsin used was channelrhodopsin-2, a membrane channel that opens in response to blue light and allows cation flow to depolarize the cell. Subsequent variants have been engineered to increase the spectral and kinetic properties and improve expression and membrane targeting in the cells. The use of different activation wavelength also allowed to combine multiple opsins to be expressed on the same cell. The light sensitive opsins are induce by e.g. using a viral vector injected in the area of interest and activated via photo stimulation devices ('photo switch'). This offers not only high spatial but also temporal reversible control of protein activity.

Examples of optogenetics for autonomic modulation of cardiac electrophysiology

Optogenetic approaches have been successfully employed to modulate cardiac autonomics in preclinical studies. In animal infarct model, an inhibitory light sensitive opsin was introduced to the left stellate ganglion and reversible neural silencing achieved via neuronal hyperpolarization during transient light emitting diode illumination. The optogenetic inhibitions significantly but reversibly suppressed LSG neural activity, sympathetic heart rate variability indices and ischaemia induced arrhythmias. It let to prolonged left ventricular effective refractory periods and action potential duration with all values turning back to baseline within 2 h after illumination was turned off.[206] Long-term optogenetic neuromodulation of the LSG over a self-powered optogenetic system in an infarct model equally reported successful suppression of LSG hyperactivity and prolongation of effective refractory period, action potential duration and reduction in VA inducibility.[207] The findings highlight the potential of optogenetic neuromodulation to suppress adrenergically driven ventricular arrhythmias, not only in ICM but also potentially CPVT and LQTS.

In turn, optogenetics have also been successfully employed to modulate pacemaker activity of cardiomyocytes via G_q signalling,[208] target the vagus nerve to change heart rate and blood pressure regulation[209] as well as stimulate the dorsal brainstem vagal preganglionic neurons associated with improvement in left ventricular function in an animal infarct model.[210]

Challenges

The most challenging consideration for the use of opsins as well as preservation of gene expression are the practicality of delivery (the opsin needs to be expressed and the area then sufficiently illuminated), which requires an invasive approach. Yet, novel developments of wireless self-powered optogenetic systems may overcome this limitation and also allow to study the anti-arrhythmic effect in awake and moving subjects over longer time. In canine studies, a wireless LED lead was placed over the LSG and connected to a power receiver under the skin. An external wireless charging module was then applied to the chest wall of the dog to harvest the mechanical energy from the body motion and convert into electricity and achieve optical illumination off the LSG.[207]

4.2.2.3 Sonogenetics

To overcome the challenges of optogenetics for *in vivo* application, the related concept of sonogenetics has been proposed. This may allow to maintain the benefits—cell type selective stimulation—but facilitate non-invasive application and stimulation. Analogue to optogenetics, sonogenetics utilizes genetically encoded, ultrasound responsive mediators for non-invasive and selective control of neural activity via the mechanical wave.[211] Feasibility of activating specific regions and cell types in the brain by sensitizing them to ultrasound using such mechanosensitive ion channels has been demonstrate in animal studies.[212] Spatial resolution is defined by the spatial resolution of ultrasound focusing as well as the spatial distribution of sonogenetic mediators and has been reported as high as 0.59 mm[213] The majority of published research has been focused around its application in brain tissue, whereas its benefit and use as anti-arrhythmic treatment have only been reported in an open-access *in silico* experiment.[214]

Overall, sonogenetics is another promising modality, although substantially under investigated in cardiovascular diseases. More generally, it has also been highlighted that many *in vivo* performance metrics, including *in vivo* spatiotemporal resolution, selectivity and specificity as well as safety are still under investigation and important challenges for translating it into clinically efficient and safe therapy options remain.[215]

5. Conclusion

Contemporary diagnostic and therapeutic means to efficiently and safely address proarrhythmic autonomic inputs to the heart remain unsatisfactory. Improving methods to assess autonomic influences on the cardiac substrate to refined patient selection and delivery of neuromodulating techniques as well as better definition of acute and longer therapeutic effects of each modality are required. Also, uncertainties of long-term tolerance to stimulation and/or inhibitions, dynamic changes in the responses of autonomic targets as well as inadvertent side effects caused by the complex autonomic interactions remain and need further addressing.

Yet, multiple new molecular and central nervous system targets involved in proarrhythmogenic autonomic signalling pathways in cardiac channelopathies have been identified. Complemented by a number of promising emerging neuromodulating technologies, this may open a new era in the treatment and prevention of VAs. Non-invasive electromagnetic stimulation, deep brain stimulation, as well as the exciting field of chemo-, opto-, or sonogenetics all may contribute to fill that important gap in contemporary anti-arrhythmic therapies. These new developments could address the heterogenous pathophysiology in patients with cardiac channelopathies and VAs, by offering precise, individualized and, if desired, reversible cardiac neuromodulation to successfully prevent SCD.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Conflict of interest: none declared.

Data availability

No new data were generated or analysed in support of this manuscript.

References

1. Puranik R, Chow CK, Duflou JA, Kilborn MJ, McGuire MA. Sudden death in the young. *Heart Rhythm* 2005;**2**:1277–1282.
2. Dusi V, de Ferrari GM, Mann DL. Cardiac sympathetic parasympathetic interaction: the endless story of yin and yang. *JACC Basic Transl Sci* 2020;**5**(8):811–814.
3. Litovsky SH, Antzelevitch C. Differences in the electrophysiological response of canine ventricular subendocardium and subepicardium to acetylcholine and isoproterenol. A direct effect of acetylcholine in ventricular myocardium. *Circ Res* 1990; **67**(3):615–627.
4. Benarroch E. Central autonomic network. In: Benarroch E (ed.), *Autonomic Neurology.* Online Ed. New York: Oxford University Press; 2014. p3–14.
5. Armour JA. Cardiac neuronal hierarchy in health and disease. *Am J Physiol Regul Integr Comp Physiol* 2004;**287**:R262–R271.
6. Goldsmith SR. Interactions between the sympathetic nervous system and the RAAS in heart failure. *Curr Heart Fail Rep* 2004;**1**:45–50.
7. Benarroch EE. The arterial baroreflex: functional organization and involvement in neurologic disease. *Neurology* 2008;**71**:1733–1738.
8. Levy MN. Sympathetic–parasympathetic interactions in the heart. *Circ Res* 1971;**29**: 437–445.
9. Stramba-Badiale M, Vanoli E, De Ferrari GM, Cerati D, Foreman RD, Schwartz PJ. Sympathetic–parasympathetic interaction and accentuated antagonism in conscious dogs. *Am J Physiol* 1991;**260**:H335–H340.
10. Shen MJ, Shinohara T, Park HW, Frick K, Ice DS, Choi E-K, Han S, Maruyama M, Sharma R, Shen C, Fishbein MC, Chen LS, Lopshire JC, Zipes DP, Lin S-F, Chen P-S. Continuous low-level vagus nerve stimulation reduces stellate ganglion nerve activity and paroxysmal atrial tachyarrhythmias in ambulatory canines. *Circulation* 2011;**123**:2204–2212.
11. Lymperopoulos A, Rengo G, Koch WJ. Adrenergic nervous system in heart failure: pathophysiology and therapy. *Circ Res* 2013;**113**(6):739–7753.
12. Fontes MA, Filho ML, Santos Machado NL, de Paula CA, Souza Cordeiro LM, Xavier CH, Marins FR, Henderson L, Macefield VG. Asymmetric sympathetic output: the dorsomedial

hypothalamus as a potential link between emotional stress and cardiac arrhythmias. *Auton Neurosci* 2017;**207**:22–27.

13. Antzelevitch C, Fish J. Electrical heterogeneity within the ventricular wall. *Basic Res Cardiol* 2001;**96**:517–527.

14. Boukens BJ, Christoffels VM, Coronel R, Moorman AFM. Developmental basis for electro-physiological heterogeneity in the ventricular and outflow tract myocardium as a substrate for life threatening ventricular arrhythmias. *Circ Res* 2009;**104**:19–31.

15. Liu DW, Gintant GA, Antzelevitch C. Ionic bases for electrophysiological distinctions among epicardial midmyocardial and endocardial myocytes from the free wall of the canine left ventricle. *Circ Res* 1993;**72**(3):671–687.

16. Molina CE, Heijman J, Dobrev D. Differences in left versus right ventricular electrophysiological properties in cardiac dysfunction and arrhythmogenesis. *Arrhythm Electrophysiol Rev.* 2016;**5**(1):14–19.

17. Sekiya S, Ichikawa S, Tsutsumi T, Harumi K. Nonuniform action potential durations at different sites in canine left ventricle. *Jpn Heart J* 1983;**24**:935–945.

18. Zheng Y, Wei D, Zhu X, Chen W, Fukuda K, Shimokawa H. Transmural, interventricular, apicobasal and anteroposterior action potential duration gradients are all essential to the genesis of the concordant and realistic T wave: a whole-heart model study. *J Electrocardiol* 2016;**49**:569–578.

19. Machhada A, Marina N, Korsak A, Stuckey DJ, Lythgoe MF, Gourine AV. Origins of the vagal drive controlling left ventricular contractility. *J Physiol* 2016;**594**(14):4017–4030.

20. Huang W, Boyle N, Vaseghi M. Cardiac innervation and the autonomic nervous system in SCD. *Cardiology Electrophysiology Clin* 2017;**9**(4):665–679.

21. Goldberger JJ, Arora R, Buckley U, Shivkumar K. Autonomic nervous system dysfunction. *J Am Coll Cardiol* 2019;**73**(10):1189–1206.

22. Zhu C, Hanna P, Rajendran PS, Shivkumar K. Neuromodulation for ventricular tachycardia and atrial fibrillation: a clinical scenario-based review. *JACC Clin Electrophysiol* 2020;**5**(8):881–896.

23. Myles RC, Wang L, Kang C, Bers DM, Ripplinger CM. Local beta-adrenergic stimulation overcomes source-sink mismatch to generate focal arrhythmia. *Circ Res* 2012;**110**(11):1454–1464.

24. Ajijola OA, Yagishita D, Patel KJ, Vaseghi M, Zhou W, Yamakawa K, So E, Lux RL, Mahajan A, Shivkumar K. Focal myocardial infarction induces global remodeling of cardiac sympathetic innervation: neural remodeling in a spatial context. *Am J Physiol Heart Circ Physiol* 2013;**305**(7):H1031–H1040.

25. Vaseghi M, Lux RL, Mahajan A, Shivkumar K. Sympathetic stimulation increases dispersion of repolarization in humans with myocardial infarction. *Am J Physiol Heart Circ Physiol* 2012;**302**(9):H1838–H1846.

26. Zeppenfeld K, Tfelt-Hansen J, de Riva M, Winkel BG, Behr ER, Blom NA, Charron P, Corrado D, Dagres N, de Chillou C, Eckardt L, Friede T, Haugaa KH, Hocini M, Lambiase PD, Marijon E, Merino JL, Peichl P, Priori SG, Reichlin T, Schulz-Menger J, Sticherling C, Tzeis S, Verstrael A, Volterrani M; ESC Scientific Document Group. 2022 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Eur Heart J* 2022;**43**:3997–4126.

27. Wilde AA, Semsarian C, Marquez MF, Shamloo AS, Ackerman MJ, Ashley EA, Sternick EB, Barajas-Martinez H, Behr ER, Bezzina CR, Breckpot J, Charron P, Chockalingam P, Crotti L, Gollob MH, Lubitz S, Makita N, Ohno S, Ortiz-Genga M, Sacilotto L, Schulze-Bahr E, Shimizu W, Sotoodehnia N, Tadros R, Ware JS, Winlaw DS, Kaufman ES, Aiba T, Bollmann A, Choi JI, Dalal A, Darrieux F, Giudicessi J, Guerchicoff M, Hong K, Krahn AD, MacIntyre C, Mackall JA, Mont L, Napolitano C, Ochoa JP, Peichl P, Pereira AC, Schwartz PJ, Skinner J, Stellbrink C, Tfelt-Hansen J, Deneke T. EHRA/HRS/APHRS/LAHRS expert consensus statement on the state of genetic testing for cardiac disease. *Europace* 2022;**24**:1307–1367.

28. Brugada J, Campuzano O, Arbelo E, Sarquella-Brugada G, Brugada R. Present status of Brugada syndrome: JACC state-of-the-art review. *J Am Coll Cardiol* 2018;**72**(9):1046–1059.

29. Wilde AA, Amin AS, Postema PG. Diagnosis, management and therapeutic strategies for congenital long QT syndrome. *Heart* 2022;**108**:332–338.

30. Dewi IV, Dharmadjati BB. Short QT syndrome: the current evidences of diagnosis and management. *J Arrhythm* 2020;**36**(6):962–966.

31. Abbas M, Miles C, Behr E. Catecholaminergic polymorphic ventricular tachycardia. *Arrhythm Electrophysiol Rev* 2022;**11**:e20.

32. Dumoulin M, Liberati G, Mouraux A, Santos SF, El Tahry R. Transcutaneous auricular VNS applied to experimental pain: a paired behavioral and EEG study using thermonociceptive CO2 laser. *PLOS One* 2021;**16**:e0254480.

33. Harned ME, Gish B, Zuelzer A, Hessel EA. Anesthetic considerations and perioperative management of spinal cord stimulators: literature review and initial recommendations. *Pain Physician* 2017;**20**(4):319–329.

34. Zhu C, Hanna P, Rajendran PS, Shivkumar K. Neuromodulation for ventricular tachycardia and atrial fibrillation: a clinical scenario-based review. *JACC Clin Electrophys* 2020;**5**(8):881–896.

35. Duncker D, Bauersachs J. Current and future use of neuromodulation in heart failure. *Eur Heart J Suppl* 2022;**24**(Suppl_E):E28–E34.

36. Liu N, Colombi B, Memmi M, Zissimopoulos S, Rizzi N, Negri S, Imbriani M, Napolitano C, Lai FA, Priori SG. Arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia: insights from a RyR2 R496C knock-in mouse model. *Circulation Res* 2006;**99**:292–298.

37. Herron TJ, Milstein ML, Anumonwo J, Priori SG, Jalife J. Purkinje cell calcium dysregulation is the cellular mechanism that underlies catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm* 2010;**7**:1122–1128.

38. Leenhardt A, Denjoy I, Gulcheney P. Catecholaminergic polymorphic ventricular tachycardia. *Circulation Arrhythm Electrophysiol* 2012;**5**:1044–1052.

39. Jiang D, Wang R, Xiao B, Kong H, Hunt DJ, Choi P, Zhang L, Chen SRW. Enhanced store overload-induced Ca^{2+} release and channel sensitivity to luminal Ca^{2+} activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death. *Circ Res* 2005;**97**:1173–1181.

40. Fernandez-Velasco M, Rueda A, Rizzi N. Increased Ca^{2+} sensitivity of the ryanodine receptor mutant RyR2R4496C underlies CPVT. *Circ Res* 2009;**104**:201–212.

41. Wehrens XH, Lehnart SE, Reiken SR, Deng SX, Vest JA, Cervantes D, Coromilas J, Landry DW, Marks AR. Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin 2. *Science* 2004;**304**:292–296.

42. Uchinoumi H, Yano M, Suetomi T, Ono M, Xu X, Tateishi H, Oda T, Okuda S, Doi M, Kobayashi S, Yamamoto T, Ikeda Y, Ohkusa T, Ikemoto N, Matsuzaki M. Catecholaminergic polymorphic ventricular tachycardia is caused by mutation linked defective conformational regulation of the ryanodine receptor. *Circ Res* 2010;**106**:1413–1424.

43. Sedej S, Heinzel FR, Walther S, Dybkova N, Wakula P, Groborz J, Gronau P, Maier LS, Vos MA, Lai FA, Napolitano C, Priori SG, Kockskämper J, Pieske B. Na-dependent SR Ca^{2+} overload induces arrhythmogenic events in mouse cardiomyocytes with a human CPVT mutation. *Cardiovasc Res* 2010;**87**(1):50–59.

44. Cerrone M, Noujaim SF, Tolkacheva EG, Talkachou A, O'Connell R, Berenfeld O, Anumonwo J, Pandit SV, Vikstrom K, Napolitano C, Priori SG, Jalife J. Arrhythmogenic mechanisms in a mouse model of catecholaminergic polymorphic ventricular tachycardia. *Circ Res* 2007;**101**:1039–1048.

45. Baher A, Uy MJ, Qu Z, Garfinkel A, Weiss JN. Reciprocating bigeminy: a possible mechanism of bidirectional ventricular tachycardia. *Circulation* 2010;**122**:A18446.

46. Park SJ, Zhang D, Qi Y, Li Y, Lee KY, Bezzerides VJ, Yang P, Xia S, Kim SL, Liu X, Lu F, Pasqualini FS, Campbell PH, Geva J, Roberts AE, Kleber AG, Abrams DJ, Pu WT, Parker KK. Insights into the pathogenesis of catecholaminergic polymorphic ventricular tachycardia from engineered human heart tissue. *Circulation* 2019;**140**(5):390–404.

47. George CH, Higgs GC, Lai FA. Ryanodine receptor mutations associated with stress induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes. *Circ Res* 2003;**93**(6):531–540.

48. Van der Werf C, Lieve KV. Betablockers in the treatment of catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm* 2016;**13**(2):441–442.

49. Hayashi M, Denjoy I, Extramiana A, Maltret A, Buisson NR, Lupoglazoff J-M, Klug D, Hayashi M, Takatsuki S, Villain E, Kamblock J, Messali A, Guicheney P, Lunardi J, Leenhardt A. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2009;**119**:2426–2434.

50. Leren IS, Saberniak J, Majid E, Haland TF, Edvardsen T, Haugaa KH. Nadolol decreases the incidence and severity of ventricular arrhythmias during exercise stress testing compared with b1 selective b-blockers in patients with catecholaminergic polymorphic ventricular tachycardia. *Heart rhythm* 2016;**13**:433–440.

51. Peltenburg PJ, Kallas D, Bos JM, Lieve KVV, Franciosi S, Roston TM, Denjoy I, Sorensen KB, Ohno S, Roses-Noguer F, Aiba T, Maltret A, LaPage MJ, Atallah J, Giudicessi JR, Clur S-AB, Blom NA, Tanck M, Extramiana F, Kato K, Barc J, Borggrefe M, Behr ER, Sarquella-Brugada G, Tfelt-Hansen J, Zorio E, Swan H, Kammeraad JAE, Krahn AD, Davis A, Sacher F, Schwartz PJ, Roberts JD, Skinner JR, van den Berg MP, Kannankeril PJ, Drago F, Robyns T, Haugaa K, Tavacova T, Semsarian C, Till J, Probst V, Brugada R, Shimizu W, Horie M, Leenhardt A, Ackerman MJ, Sanatani S, van der Werf C, Wilde AAM. An international multicentre cohort study on b-blockers for the treatment of symptomatic children with catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2022;**145**:333–344.

52. Westerlund A. Central nervous system side-effects with hydrophilic and lipophilic beta-blockers. *Eur J Clin Pharmacol* 1985;**28**(suppl):73–76.

53. Zhou Q, Xiao J, Jiang D, Wang R, Vembaiyan K, Wang A, Smith CD, Xie C, Chen W, Zhang J, Tian X, Jones PP, Zhong X, Guo A, Chen H, Zhang L, Zhu W, Yang D, Li X, Chen J, Gillis AM, Duff HJ, Cheng H, Feldman AM, Song L-S, Fill M, Back TG, Chen SRW. Carvedilol and its new analogues suppress arrhythmogenic store overload induced Ca^{2+} release. *Nat Med* 2011;**17**:1003–1009.

54. Tan Z, Xiao Z, Wei J, Zhang J, Zhou Q, Smith CD, Nani A, Wu G, Song L-S, Back TG, Fill M, Chen SRW. Nebivolol suppresses cardiac ryanodine receptor-mediated spontaneous Ca^{2+} release and catecholaminergic polymorphic ventricular tachycardia. *Biochem J* 2016;**473**:4159–4172.

55. Kurtzwald-Josefson E, Hochhauser E, Bogachenko K, Harun-Khun S, Katz G, Aravot D, Seidman JG, Seidman CE, Eldar M, Shainberg A, Arad M. Alpha blockade potentiates CPVT therapy in calsequestrin-mutant mice. *Heart Rhythm* 2014;**11**:1471–1479.

56. Van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, Leenhardt A, Shimizu W, Sumitomo N, Fish FA, Bhuiyan ZA, Willems AR, van der Veen MJ., Watanabe H, Laborderie J, Haïssaguerre M, Knollmann BC, Wilde AAM. Flecainide therapy reduces exercise induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. *J Am Coll Cardiol* 2011;**57**(22):2244–2254.

57. Watanabe H, van der Wert C, Roses Noguer F, Adler A, Sumitomo N, Veltmann C, Rosso R, Bhuiyan ZA, Bikker H, Kannankeril PJ, Horie M, Minamino T, Viskin S, Knollmann BC, TillJ, Wilde AAM. Effects of flecainide on exercise induced ventricular arrhythmias and recurrences in genotype negative patients CPVT. *Heart Rhythm* 2013;**10**:542–547.

58. Khoury A, Marai I, Suleiman M, Blich M, Lorber A, Gepstein L, Boulos M. Flecainide therapy suppresses exercise-induced ventricular arrhythmias in patients with CASQ2 associated CPVT. *Heart Rhythm* 2013;**10**(11):1671–1675.

59. Kannankeril PJ, Moore JP, Cerrone M, Priori SG, Kertesz NJ, Ro PS, Batra AS, Kaufman ES, Fairbrother DL, Saarel EV, Etheridge SP, Kanter RJ, Carboni MP, Dzurik MV, Fountain D, Chen H, Ely EW, Roden DM, Knollmann BC. Efficacy of flecainide in the treatment of CPVT: a randomized clinical trial. *JAMA Cardiol* 2017;**2**(7):759–766.

60. Liu N, Denegri M, Ruan Y, Avelino-Cruz JE, Perissi A, Negri S, Napolitano C, Coetzee WA, Boyden PA, Priori SG. Short communication: flecainide exerts an antiarrhythmic effect in a mouse model of catecholaminergic polymorphic ventricular tachycardia by increasing the threshold for triggered activity. *Circ Res* 2011;**109**:291–295.

61. Kryshtal D, Blackwell D, Egly CL, Smith AN, Batiste SM, Johnston JN, Laver DR, Knollmann BC. RYR2 channel inhibition is the principal mechanism of flecainide action in CPVT. *Circ Res* 2021;**128**(39):321–331.

62. Collura CA, Johnson JN, Moir C, Ackerman MJ. Left cardiac sympathetic denervation for the treatment of long QT syndrome and catecholaminergic polymorphic ventricular tachycardia using video-assisted thoracic surgery. *Heart Rhythm* 2009;**6**:752–759.

63. Wilde AA, Bhuiyan ZA, Crotti L, Facchini M, De Ferrari GM, Paul T, Ferrandi C, Koolbergen DR, Odero A, Schwartz PJ. Left cardiac sympathetic denervation for catecholaminergic polymorphic ventricular tachycardia. *N Engl J Med* 2008;**358**:2024–2029.

64. De Ferrari G, Dusi V, Spazzolini C, Bos JM, Abrams DJ, Berul CI, Crotti L, Davis AM, Eldar M, Kharlap M, Khoury A, Krahn AD, Leenhardt A, Moir CR, Odero A, Olde Nordkamp L, Paul T, Rosés i Noguer F, Shkolnikova M, Till J, Wilde AAM, Ackerman MJ, Schwartz PJ. Clinical management of catecholaminergic polymorphic ventricular tachycardia. The role of left cardiac sympathetic denervation. *Circulation* 2015;**131**:2185–2193.

65. Dusi V, De Ferrari GM, Pugliese L, Schwarty PJ. Cardiac sympathetic denervation in channelopathies. *Front Cardiovasc Med* 2019;**6**:27.

66. Aksu T, Guler E. Percutaneous renal sympathetic denervation in catecholaminergic polymorphic ventricular tachycardia. *J Arrhythm* 2017;**33**:245.

67. Kaneshiro T, Naruse Y, Nogami A. Successful catheter ablation of bidirectional ventricular premature contractions triggering ventricular fibrillation in catecholaminergic polymorphic ventricular tachycardia with RyR2 mutation. *Circ Arrhythm Electrophysiol* 2012;**5**:e14–e17.

68. Mazzadi AN, Andre Fouet X, Duisit J, Gebuhrer V, Costes N, Chevalier P, Rodriguez C, Schott J-J, Le Marec H, Guicheney P, Le Bars D, Janier M. Cardiac retention of 11C-HED in genotyped long QT patients: a potential amplifier role for severity of the disease. *Am J Physiol Hear Circ Physiol* 2003;**285**:1286–1293.

69. Goldenberg I, Thottathil P, Lopes CM, Moss AJ, McNitt S, O-Uchi J, Robinson JL, Zareba W, Ackerman MJ, Kaufman ES, Towbin JA, Vincent M, Barsheshet A. Trigger specific ion-channel mechanisms, risk factors and response to therapy in type 1 long QT syndrome. *Heart Rhythm* 2012;**9**(1):49–56.

70. Wilde AA, Jongbloed RJE, Doevendans PA, Düren DR, Hauer RNW, van Langen IM, van Tintelen JP, Smeets HJM, Meyer H, Geelen JLMC. Auditory stimuli as a trigger for arrhythmic events differentiate HERG-related (LQTS2) patients from KVLQT1-related patients (LQTS1). *J Am Coll Cardiol* 1999;**33**:327–332.

71. Viskin S, Postema PG, Bhuiyan ZA, Rosso R, Kalman JM, Vohra JK, Guevara-Valdivia ME, Marquez MF., Kogan E, Belhassen B, Glikson M, Strasberg B, Antzelevitch C, Wilde AAM. The response of the QT interval to the brief tachycardia provoked by standing: a bedside test for diagnosing long QT syndrome. *J Am Coll Cardiol* 2010;**55**:1955–1961.

72. Marstrand P, Almatlouh K, Kanters JK, Graff C, Christensen AH, Bundgaard H, Theilade J. Long QT syndrome type 1 and 2 patients respond differently to arrhythmic triggers: the TriQarr in vivo study. *Heart Rhythm* 2021;**18**(2):241–249.

73. Seth R, Moss AJ, McNitt S, Zareba W, Andrews ML, Qi M, Robinson JL, Goldenberg I, Ackerman MJ, Benhorin J, Kaufman ES, Locati EH, Napolitano C, Priori SG, Schwartz PJ, Towbin JA, Vincent GM, Zhang L. Long QT and pregnancy. *JACC* 2007;**49**(10):1092–1098.

74. Rashba EJ, Zareba W, Moss AJ, Hall WJ, Robinson J, Locati EH, Schwartz PJ, Andrews M. Influence of pregnancy on the risk for cardiac events in patients with hereditary long QT syndrome: LQTS investigators. *Circulation* 1998;**97**:451–456.

75. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, Towbin JA, Beggs AH, Brink P, Wilde AAM, Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH, Schwartz K, Coumel P, Bloise R. Genotype–phenotype correlation in the long QT syndrome: gene-specific triggers for life threatening arrhythmias. *Circulation* 2001;**103**:89–95.

76. Moss AJ, Zareba W, Hall WJ, Schwartz PJ, Crampton RS, Benhorin J, Vincent GM, Locati EH, Priori SG, Napolitano C, Medina A, Zhang L, Robinson JL, Timothy K, Towbin JA, Andrews ML. Effectiveness and limitations of beta-blocker therapy in congenital long-QT syndrome. *Circulation* 2000;**101**:616–623.

77. Ahn J, Kim HJ, Choi JI, Lee KN, Shim J, Ahn HS, Kim Y-H. Effectiveness of beta blockers depending on the genotype of congenital long QT syndrome: a meta-analysis. *PLoS One* 2017;**12**(10):e0185680.

78. Chockalingam P, Crotti L, Girardengo G, Johnson JN, Harris KM, van der Heijden JF, Hauer RNW, Beckmann BM, Spazzolini C, Rordorf R, Rydberg A, Clur S-AB, Fischer M, van den Heuvel F, Kääb S, Blom NA, Ackerman MJ, Schwartz PJ, Wilde AAM. Not all beta-blockers are equal in the management of long QT syndrome types 1 and 2: higher recurrence of events under metoprolol. *J Am Coll Cardiol* 2012;**60**:2092–2099.

79. Schwartz PJ, Snebold NG, Brown AM. Effects of unilateral cardiac sympathetic denervation on the ventricular fibrillation threshold. *Am J Cardiol* 1976;**37**:1034–1040.

80. Schwartz PJ, Verrier RL, Lown B. Effect of stellectomy and vagotomy on ventricular refractoriness in dogs. *Circ Res* 1977;**40**:536–540.

81. Antonopoulos A, Lawrence D, Patrini D, Scarci M, George R, Hayward M, Mitsos S, Panagiotopoulos N. The role of sympathectomy in long QT syndrome. *J Thorac Dis* 2017;**9**(9):3394–3397.

82. Schwartz PJ, Priori SG, Cerrone M, Spazzolini C, Odero A, Napolitano C, Bloise R, De Ferrari GM, Klersy C, Moss AJ, Zareba W, Robinson JL, Hall WJ, Brink PA, Toivonen L, Epstein AE, Li C, Hu D. Left cardiac sympathetic denervation in the management of high-risk patients affected by the long-QT syndrome. *Circulation* 2004;**109**:1826–1833.

83. Niaz T, Bos JM, Sorensen KB, Moir C, Ackerman MJ. Left cardiac sympathetic denervation monotherapy in patients with congenital long QT syndrome. *Circ Arrhythm Electrophysiol* 2020;**13**:e008830.

84. Schwartz PJ, Ackerman MJ. Cardiac sympathetic denervation in the prevention of genetically mediated life threatening ventricular arrhythmias. *Eur Heart J* 2022;**43**(22):2096–2102.

85. Giudicessi JR, Ackermann MJ, Fatkin D, Kovacic JC. Precision medicine approaches to cardiac arrhythmias: JACC focus seminar 4/5. *JACC* 2021;**77**(20):2573–2591.

86. Yu L, Huang B, Zhou X, Wang S, Wang Z, Wang M, Li X, Zhou L, Meng G, Yuan S, Wang Y, Jiang H. Renal sympathetic stimulation and ablation affect ventricular arrhythmia by modulating autonomic activity in a cesium-induced long QT canine model. *Heart Rhythm* 2017;**14**:912–919.

87. Ton N, Liu SH, Lo LW, Khac TC-N, Chou Y-H, Cheng W-H, Lin W-L, Peng T-Y, Lin P-Y, Chang S-L, Chen S-A. Renal artery denervation prevents ventricular arrhythmias in long QT rabbit models. *Sci Rep* 2022;**12**:2904.

88. Mingyang X, Weijie C, Yuehui Y. Congenital long QT syndrome treated by renal sympathetic denervation. *Europace* 2019;**21**:1741.

89. Dusi V, Pugliese L, De Ferrari GM, Odero A, Crotti L, Dagradi F, Castelletti S, Vicentini A, Rordorf R, Li C, Shkolnikova M, Spazzolini C, Schwartz PJ. Left cardiac sympathetic denervation for the long QT syndrome: 50 years' experience provides guidance for management. *JACC Clin Electrophysiol* 2022;**8**:281–294.

90. Kiuchi MG, Chen S, Carnagarin R, Matthews VB, Schlaich MP. Renal denervation for treating long QT syndrome: shortening the QT interval or modulating sympathetic tone? *EP Europace* 2019;**21**(11):1755–1756.

91. Malan D, Zhang M, Stallmeyer B, Muller J, Fleischmann BK, Schulze-Bahr E. Human iPS cell model of type 3 long QT syndrome recapitulates drug-based phenotype correction. *Basic Res Cardiol* 2016;**111**:14.

92. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ., Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating M, Towbin J, Beggs AH, Brink P, Wilde AAM., Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH., Schwartz K, Coumel P, Bloise R. Genotype–phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation* 2001;**103**:89–95.

93. Wilde AA, Moss AJ, Kaufman ES, Shimizu W, Peterson DR, Benhorin J, Lopes C, Towbin JA, Spazzolini C, Crotti L, Zareba W, Goldenberg I, Kanters JK, Robinson JL, Qi M, Hofman N, Tester DJ, Bezzina CR, Alders M, Aiba T, Kamakura S, Miyamoto Y, Andrews ML, McNitt S, Polonsky B, Schwartz PJ, Ackerman MJ. Clinical aspects of type 3 long QT syndrome. An international multicenter study. *Circulation* 2016;**134**(12):872–882.

94. Bankston JR, Kass RS. Molecular determinants of local anaesthetic action of beta-blocking drugs: implications for therapeutic management of long QT syndrome variant 3. *J Mol Cell Cardiol* 2010;**48**:246–253.

95. Antzelevitch C, Yan GX, Ackerman MJ, Borggrefe M, Corrado D, Guo J, Gussak I, Hasdemir C, Horie M, Huikuri H, Ma C, Morita H, Nam G-B, Sacher F, Shimizu W, Viskin S, Wilde AAM. J-wave syndromes expert consensus conference report: emerging concepts and gaps in knowledge. *Heart Rhythm* 2016;**13**:e295–e324.

96. Monasky M, Pappone C, Piccoli M, Ghiroldi A, Micaglio E, Anastasia L. Calcium in Brugada syndrome: questions for future research. *Front Physiol* 2018;**9**:1088.

97. Lambiase P, Ahmed AK, Ciaccio EJ, Brugada R., Lizotte E., Chaubey S., Ben-Simon R, Chow AW, Lowe MD, McKenna WJ. High-density substrate mapping in Brugada syndrome: combined role of conduction and repolarization heterogeneities in arrhythmogenesis. *Circulation* 2009;**120**:106–117, 1–4.

98. Antzelevitch C. In vivo human demonstration of phase 2 reentry. *Heart Rhythm* 2005;**2**:804–806.

99. Coronel R, Casini S, Koopmann TT, Wilms-Schopman FJG, Verkerk AO, de Groot JR, Bhuiyan Z, Bezzina CR, Veldkamp MW, Linnenbank AC, van der Wal AC, Tan HL, Brugada P, Wilde AAM, de Bakker JMT. Right ventricular fibrosis and conduction delay in a patient with clinical signs of Brugada syndrome: a combined electrophysiological, genetic, histopathologic and computational study. *Circulation* 2005;**112**:2769–2777.

100. Nakazawa K, Sakurai T, Takagi A, Kishi R, Osada K, Nanke T, Miyake F, Matsumoto N, Kobayashi S. Autonomic imbalance as a property of symptomatic Brugada syndrome. *Circ J* 2003;**67**:511–514.

101. Teodorovich N, Kogan Y, Swiss M. Vagally mediated ventricular arrhythmia in Brugada syndrome. *Heart Rhythm Case Rep* 2016;**2**(6):530–535.

102. Takigawa M, Noda T, Shimizu W, Miyamoto K, Okamura H, Satomi K, Suyama K, Aihara N, Kamakura S, Kurita T. Seasonal and circadian distributions of ventricular fibrillation in patients with Brugada syndrome. *Heart Rhythm* 2008;**5**:1523–1527.

103. Lieve V, Wilde A. Inherited ion channel diseases: a brief review. *Europace* 2015;**17**(Suppl. 2): ii1–ii6.

104. Kasanuki H, Ohnishi S, Ohtuka M, Matsuda N, Nirei T, Isogai R, Shoda M, Toyoshima Y, Hosoda S. Idiopathic ventricular fibrillation induced with vagal activity in patients without obvious heart disease. *Circulation* 1997;**95**:2277–2285.

105. Nomura M, Nada T, Endo J, Kondo Y, Yukinaka M, Saito K, Ito S, Mori H, Nakaya Y, Shinomiya H. Brugada syndrome associated with an autonomic disorder. *Heart* 1998;**80**: 194–196.

106. Agostini D, Scanu P, Loiselet P, Babatasi G, Darlas Y, Grollier G, Potier JC, Bouvard G. I 123-MIBG SPECT of regional cardiac adrenergic denervation in Brugada syndrome. *J Nucl Med* 1998;**39**:1129–1132.

107. Oyama NO, Oyama NO, Yokoshiki H, Satoh K, Katoh N, Hayashi T, Miyasaka K, Kitabatake A. I-123 MIBG scintigraphy of total cardiac adrenergic denervation in Brugada syndrome. *Jpn Heart J* 2002;**43**:183–186.

108. Kawaguchi T, Nomura M, Tujikawa T, Nakaya Y, Ito S. 123I-MIBG myocardial scintigraphy in the Brugada type ECG. *J Med Investig* 2006;**53**:95–102.

109. Wichter T, Matheja P, Eckardt L, Kies P, Schäfers K, Schulze-Bahr E, Haverkamp W, Borggrefe M, Schober O, Breithardt G, Schäfers M. Cardiac autonomic dysfunction in Brugada syndrome. *Circulation* 2002;**105**(6):702–706.

110. Kies P, Wichter T, Schafter M, Paul M, Schäfers KP, Eckardt L, Stegger L, Schulze-Bahr E, Rimoldi O, Breithardt G, Schober O, Camici PG. Abnormal myocardial presynaptic nor-epinephrine recycling in patients with Brugada syndrome. *Circulation* 2004;**110**:3017–3022.

111. Masrur S, Memon S, Thompson P. Brugada syndrome, exercise, and exercise stress testing. *Clin Cardiol* 2015;**38**:323–326.

112. Aboyme A, Coromilas J, Scheinman M, Kassotis J. Exercise induced Brugada syndrome type 1 pattern. *Heart Rhythm Case Reports* 2022;**8**(4):288–291.

113. Watanabe A, Fukushima Kusano K, Morita H, Miura D, Sumida W, Hiramatsu S, Banba K, Nishii N, Nagase S, Nakamura K, Sakuragi S, Ohe T. Low-dose isoproterenol for repetitive ventricular arrhythmia in patients with Brugada syndrome. *Eur Heart J* 2006;**27**:1579–1583.

114. Marquez MF, Salica G, Hermosillo AG, Pastelín G, Gómez-Flores J, Nava S, Cárdenas M. Ionic basis of pharmacological therapy in Brugada syndrome. *J Cardiovasc Electrophysiol* 2007;**18**:234–240.

115. Schweizer RA, Becker R, Katus HA, Thomas D. Successful acute and long-term management of electrical storm in Brugada syndrome using orciprenaline and quinine/quinidine. *Clin Res Cardiol* 2010;**99**:467–470.

116. Aizawa Y, Yamakawa H, Takatsuki S, Katsumata Y, Nishiyama T, Kimura T, Nishiyama N, Fukumoto K, Tanimoto Y, Tanimoto K, Mitamura H, Ogawa S, Fukuda K. Efficacy and safety of bepridil for prevention of ICD shocks in patients with Brugada syndrome and idiopathic ventricular fibrillation. *Int J Cardiology* 2013;**168**(5):5083–5085.

117. Pappone C, Brugada J, Vicedomini G, Ciconte G, Manguso F, Saviano M, Vitale R, Cuko A, Giannelli L, Calovic Z, Conti M, Pozzi P, Natalizia A, Crisà S, Borrelli V, Brugada R, Sarquella-Brugada G, Guazzi M, Frigiola A, Menicanti L, Santinelli V. Electrical substrate elimination in 135 consecutive patients with Brugada syndrome. *Circ Arrhythm Electrophysiol* 2017;**10**:e005053.

118. Bourier F, Denis A, Cheniti G, Lam A, Vlachos K, Takigawa M, Kitamura T, Frontera A, Duchateau J, Pambrun T, Klotz N, Derval N, Sacher F, Jais P, Haissaguerre M, Hocini M. Early repolarization syndrome: diagnostic and therapeutic approach. *Front Cardiovasc Med* 2018;**5**:169.

119. Antzelevitch C, Yan GX. J wave syndromes. *Heart Rhythm* 2010;**7**:549–558.

120. Kawata H, Noda T, Yamada Y, Okamura H, Satomi K, Aiba T, Takaki H, Aihara N, Isobe M, Kamakura S, Shimizu W. Effect of sodium-channel blockade on early repolarization in inferior/lateral leads in patients with idiopathic ventricular fibrillation and Brugada syndrome. *Heart Rhythm* 2012;**9**:77–83.

121. Abe A, Ikeda T, Tsukada T, Ishiguro H, Miwa Y, Miyakoshi M, Mera H, Yusu S, Yoshino H. Circadian variation of late potentials in idiopathic ventricular fibrillation associated with J waves: insights into alternative pathophysiology and risk stratification. *Heart Rhythm* 2010;**7**:675–682.

122. Mizumaki K, Nishida K, Iwamoto J, Nakatani Y, Yamaguchi Y, Sakamoto T, Tsuneda T, Kataoka N, Inoue H. Vagal activity modulates spontaneous augmentation of J-wave elevation in patients with idiopathic ventricular fibrillation. *Heart Rhythm* 2012;**9**:249–255.

123. Koutbi L, Roussel M, Haïssaguerre M, Deharo JC. Hyperpnea test triggering malignant ventricular arrhythmia in a child with early repolarization. *Heart Rhythm* 2012;**9**:1153–1156.

124. Gourraud JB, Le Scrouarnec S, Sacher F, Chatel S, Derval N, Portero V, Chavernac P, Sandoval JE, Mabo P, Redon R, Schott J-J, Le Marec H, Haïssaguerre M, Probst V. Identification of large families in early repolarization syndrome. *JACC* 2013;**61**(2):164–172.

125. Gross GJ. Early repolarization and ventricular fibrillation: vagally familiar? *Heart Rhythm* 2010;**7**:653–654.

126. Koncz I, Gurabi Z, Patocskai B, Panama BK, Szél T, Hu D, Barajas-Martínez H, Antzelevitch C. Mechanism underlying the development of the electrocardiographic and arrhythmic manifestations of early repolarization syndrome. *J Mol Cell Cardiol* 2014;**68**:20–28.

127. Haissaguerre M, Sacher F, Nogami A, Komiya N, Bernard A, Probst V, Yli-Mayry S, Defaye P, Aizawa Y, Frank R, Mantovan R, Cappato R, Wolpert C, Leenhardt A, de Roy L, Heidbuchel H, Deisenhofer I, Arentz T, Pasquié J-L, Weerasooriya R, Hocini M, Jais P, Derval N, Bordachar P, Clémenty J. Characteristics of recurrent ventricular fibrillation associated with inferolateral early repolarization role of drug therapy. *J Am Coll Cardiol* 2009; **53**:612–619.

128. Iguchi K, Noda T, Kamakura S, Shimizu W. Beneficial effects of cilostazol in a patient with recurrent ventricular fibrillation associated with early repolarization syndrome. *Heart Rhythm* 2013;**10**:604–606.

129. Ang R, Opel A, Tinker A. The role of inhibitory G proteins and regulators of G protein signalling in the in vivo control of heart rate and predisposition to cardiac arrhythmias. *Front Phys* 2012;**3**:96.

130. Capote LA, Mendez Perez R, Lymperopoulos A. GPCR signalling and cardiac function. *Eur J Pharmacol* 2015;**763**:143–148.

131. Kaiser E, Tian Q, Wagner M, Barth M, Xian W, Schröder L, Ruppenthal S, Kaestner L, Boehm U, Wartenberg P, Lu H, McMillin SM, Bone DBJ, Wess J, Lipp P. DREADD technology reveals major impact of Gq signalling on cardiac electrophysiology. *Cardiovasc Res* 2019; **115**(6):1052–1066.

132. Sebastian S, Ang R, Abramowitz J, Weinstein LS, Chen M, Ludwig A, Birnbaumer L, Tinker A. The in vivo regulation of heart rate in the murine sinoatrial node by stimulatory and inhibitory heterotrimeric G proteins. *Am J Physiol Regul Integr Comp Physiol* 2013;**305**(4): R435–R442.

133. Ferrantini C, Coppini R, Sacconi L. Cardiomyocyte-specific Gq signalling and arrhythmias: novel insights from DREADD technology. *Cardiovasc Res* 2019;**115**(6):992–994.

134. Shanks J, Herring N. Peripheral cardiac sympathetic hyperactivity in cardiovascular disease: role of neuropeptides. *Am J Physiol Regul Integr Comp Physiol* 2013;**305**(12):R1411–R1420.

135. Herring N, Lokale MN, Danson EJ, Heaton DA, Paterson DJ. Neuropeptide Y reduces acetylcholine release and vagal bradycardia via a Y2 receptor-mediated, protein kinase C-dependent pathway. *J Mol Cell Cardiol* 2008;**44**:477–485.

136. Heredia Mdel P, Delgado C, Pereira L, Perrier R, Richard S, Vassort G, Benitah J, Gomez A. Neuropeptide Y rapidly enhances [Ca^{2+}]i transients and Ca^{2+} sparks in adult rat ventricular myocytes through Y1 receptor and PLC activation. *J Mol Cell Cardiol* 2005;**38**:205–212.

137. Hoang JD, Salavatian S, Yamaguchi N, Swid MA, Vaseghi M. Cardiac sympathetic activation circumvents high-dose beta blocker therapy in part through release of neuropeptide Y. *JCI Insight* 2020;**5**(11):e135519.

138. Kalla M, Hao G, Tapoulal N, Tomek J, Liu K, Woodward L, Dall'Armellina E, Banning AP, Choudhury RP, Neubauer S, Kharbanda RK, Channon KM, Ajijola OA, Shivkumar K, Paterson DJ, Herring N. The cardiac sympathetic co-transmitter neuropeptide Y is pro-arrhythmic following ST-elevation myocardial infarction despite beta-blockade. *Eur Heart J* 2020;**41**:2168–2179.

139. Ewert TJ, Gritman KR, Bader M, Habecker BA. Post infarct cardiac sympathetic hyperactivity regulates galanin expression. *Neurosci Lett* 2008;**436**(2):163–166.

140. Herring N, Cranley J, Lokale MN, Li D, Shanks J, Alston EN, Girard BM, Carter E, Parsons RL, Habecker BA, Paterson DJ. The cardiac sympathetic co-transmitter galanin reduces acetylcholine release and vagal bradycardia: implications for neural control of cardiac excitability. *J Mol Cell Cardiol* 2012;**52**:667–676.

141. Nakamura S, Numata G, Yamaguchi T, Tokiwa H, Higashikuni Y, Nomura S, Sasano T, Takimoto E, Komuro I. Endoplasmic reticulum stress-activated nuclear factor-kappa B signaling pathway induces the upregulation of cardiomyocyte dopamine D1 receptor in heart failure. *Biochemi Biophys Res Commun* 2022;**637**:247–253.

142. Yamaguchi T, Sumida TS, Nomura S, Satoh M, Higo T, Ito M, Ko T, Fujita K, Sweet ME, Sanbe A, Yoshimi K, Manabe I, Sasaoka T, Taylor MRG., Toko H, Takimoto E, Naito AT, Komuro I. Cardiac dopamine D1 receptor triggers ventricular arrhythmia in chronic heart failure. *Nat Commun* 2020;**11**(1):4364.

143. Bhuiyan MS, Fukunaga K. Targeting sigma-1 receptor signaling by endogenous ligands for cardioprotection. *Expert opinion on Therapeutic Targets* 2011;**15**(2):145–155.

144. Johannessen M, Ramachandran S, Riemer L, Ramos-Serrano A, Ruoho AE, Jackson MB. Voltage-gated sodium channel modulation by sigma-receptors in cardiac myocytes and heterologous systems. *Am J Physiol Cell Physiol* 2009;**296**:C1049–C1057.

145. Zhang H, Cuevas J. Sigma receptor activation blocks potassium channels and depresses neuroexcitability in rat intracardiac neurons. *J Pharmacol Exp Ther* 2005;**313**:1387–1396.

146. Lewis R, Li J, McCormick PJ, L-H Huang C, Jeevaratnam K. Is the sigma-1 receptor a potential pharmacological target for cardiac pathologies? A systematic review. *Int J Cardiol Heart Vasc* 2020;**26**:100449.

147. Fo Y, Zhang C, Chen X, Liu X, Ye T, Guo Y, Qu C, Shi S, Yang B. Chronic sigma-1 receptor activation ameliorates ventricular remodeling and decreases susceptibility to ventricular arrhythmias after myocardial infarction in rats. *Eur J Pharmacol* 2020;**889**:173614.

148. Wakabayashi H, Mori H, Hiromasa T, Akatani N, Inaki A, Kozaka T, Kitamura Y, Ogawa K, Kinuya S, Taki J. ^{125}I-labeled 2-[4-(2-iodophenyl) piperidino]cyclopentanol imaging visualised augmented sigma-1-receptor expression according to the severity of myocardial ischemia. *J Nucl Cardiol* 2023;**30**(2):653–661.

149. Goshima Y, Sasaki Y, Yamashita N, Nakamura F. Class 3 semaphorins as a therapeutic target. *Expert Opin Ther Targets* 2012;**16**:933–944.

150. Chen RH, Li YG, Jiao KL, Zhang P-P, Sun Y, Zhang L-P, Fong X-F, Li W, Yu Y. Overexpression of Sema3a in myocardial infarction border zone decreases vulnerability of ventricular tachycardia post myocardial infarction in rats. *J Cell Molecular Medicine* 2013;**17**(5):608–616.

151. Yang LC, Zhang PP, Chen XM, Li C-Y, Sun J, Hou J-W, Chen R-H, Wang Y-P, Li Y-G. Semaphorin 3a transfection into the left stellate ganglion reduces susceptibility to ventricular arrhythmias after myocardial infarction in rats. *Europace* 2016;**18**(12):1886–1896.

152. Nakano Y, Chayama K, Ochi H, Toshishige M, Hayashida Y, Miki D, Hayes CN, Suzuki H, Tokuyama T, Oda N, Suenari K, Uchimura-Makita Y, Kajihara K, Sairaku A, Motoda C, Fujiwara M, Watanabe Y, Yoshida K, Ohkubo K, Watanabe I, Nogami A, Hasegawa K, Watanabe H, Endo N, Aiba T, Shimizu W, Ohno S, Horie M, Arihiro K, Tashiro S, Makita N, Kihara Y. A non-synonymous polymorphism in semaphorin 3A as a risk factor for human unexplained cardiac arrest with documented ventricular fibrillation. *PLoS Genet* 2013;**9**:e1003364.

153. Hoyda TD, Ferguson AV. Adiponectin modulates excitability of rat paraventricular nucleus neurons by differential modulation of potassium currents. *Endocrinology* 2010;**151**: 3154–3162.

154. Kajimura D, Lee HW, Riley KJ, Arteaga-Solis E, Ferron M, Zhou B, Clarke CJ, Hannun YA, DePinho RA, Guo XE, Mann JJ, Karsenty G. Adiponectin regulates bone mass via opposite central and peripheral mechanisms through FoxO1. *Cell Metab* 2013;**17**: 901–915.

155. Zhou Z, Li S, Sheng X, Liu Z, Lai Y, Wang M, Wang Z, Zhou L, Meng G, Chen H, Zhou H, Zhou X, Jiang H. Interactions between metabolism regulator adiponectin and intrinsic cardiac autonomic nervous system: a potential treatment target for atrial fibrillation. *Int J Cardiol* 2020;**302**:59–66.

156. Zhou Z, Liu C, Xu S, Wang J, Guo F, Duan S, Deng Q, Sun J, Yu F, Zhou Y, Wang M, Wang Y, Zhou L, Jiang H, Yu L. Metabolism regulator adiponectin prevents cardiac remodelling and ventricular arrhythmias via sympathetic modulation in myocardial infarction model. *Basic Res Cardiol* 2022;**117**(1):34.

157. Gonzalez-Casanova JE, Duran-Aguero S, Caro Fuentes NJ, Gamboa-Arancibia ME, Bruna T, Bermúdez V, Rojas-Gómez DM. New insights on the role of connexions and gap junctions channels in adipose tissue and obesity. *Int J Mol Sci* 2021;**22**(22): 12145.

158. Benarroch EE. 'Central autonomic network'. In: Benarroch E (ed.), *Autonomic Neurology, Contemporary Neurology Series*. online edn. New York: Oxford Academic; 2014. p3–14.

159. Benarroch EE. Brainstem integration of arousal, sleep, cardiovascular and respiratory control. *Neurology* 2018;**91**(21):958–966.

160. Dampney RA. Central neural control of the cardiovascular system: current perspectives. *Adv Physiol Educ* 2016;**40**:283–296.

161. Gourine AV, Machhada A, Trapp S, Spyer KM. Cardiac vagal preganglionic neurones: an update. *Auton Neurosci* 2016;**199**:24–28.

162. McAllen RM, Spyer KM. Two types of vagal preganglionic motoneurones projecting to the heart and lungs. *J Physiol* 1978;**282**:353–364.

163. Farmer DG, Dutschmann M, Paton JF, Pickering AE, McAllen RM. Brainstem sources of cardiac vagal tone and respiratory sinus arrhythmia. *J Physiol* 2016;**594**:7249–7265.

164. Machhada A, Ang R, Ackland GL, Ninkina N, Buchman VL, Lythgoe MF, Trapp S, Tinker A, Marina N, Gourine AV. Control of ventricular excitability by neurons of the dorsal motor nucleus of the vagus nerve. *Heart rhythm* 2015;**12**(11):2285–2293.

165. Fedele L, Brand T. The intrinsic cardiac nervous system and its role in cardiac pacemaking and conduction. *J Cardiovasc Dev Dis* 2020;**7**(4):54.

166. Dampney RAL, Horiuchi J, Tagawa T, Fontes MAP, Potts PD, Polson JW. Medullary and supramedullary mechanisms regulating sympathetic vasomotor tone. *Acta Physiol Scand* 2003;**177**:209–218.

167. Guyenet PG, Stornetta RL. Rostral ventrolateral medulla, retropontine region and autonomic regulations. *Auton Neurosci* 2022;**237**:102922.

168. Ootsuka Y, Terui N. Functionally different neurons are organized topographically in the rostral ventrolateral medulla of rabbits. *J Auton Nerv Syst* 1997;**67**:67–78.

169. Jansen ASP, Nguyen XV, Karpitskiy V, Mettenleiter TC, Loewy AD. Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. *Science* 1995;**270**:644–646.

170. Farkas E, Jansen ASP, Loewy AD. Periaqueductal gray matter input to cardiac-related sympathetic premotor neurons. *Brain Res* 1998;**792**(2):179–192.

171. Ennis M, Xu SJ, Rizvi TA. Discrete subregions of the rat midbrain peraqueductal gray project to nucleus ambiguus and the periambigual region. *Neuroscience* 1997;**80**:829–845.

172. Farkas E, Jansen ASP, Loewy AD. Periaqueductal gray matter projection to vagal preganglionic neurons and the nucleus tractus solitarius. *Brain Res* 1997;**764**:257–261.

173. Wu M, Linderoth B, Foreman FD. Putative mechanisms behind effects of spinal cord stimulation on vascular disease: a review of experimental studies. *Auton Neurosci* 2008;**138**(1–2): 9–23.

174. Hanani M, Spray DC. Emerging importance of satellite glia in nervous system function and dysfunction. *Nat Rev Neurosci* 2020;**21**(9):485–498.

175. Enes J, Haburcak M, Sona S, Gerard N, Mitchell AC, Fu W, Birren SJ. Satellite glial cells modulate cholinergic transmission between sympathetic neurons. *PLoS* 2020;**15**(2): e0218643.

176. Van Weperen VY, Vos MA, Ajijola OA. Autonomic modulation of ventricular electrical activity: recent developments and clinical implications. *Clin Auton Res* 2021;**31**(6):659–676.

177. Xie AX, Lee JJ, McCarthy KD. Ganglionic GFAP+ glial Gq-GPCR signalling enhances heart functions in vivo. *JCI Insight* 2017;**2**:e90565.

178. Mapps AA, Boehm E, Beier C, Keenan WT, Langel J, Liu M, Thomsen MB, Hattar S, Zhao H, Tampakakis E, Kuruvilla R. Satellite glia modulate sympathetic neuron survival activity and autonomic function. *Elife* 2022;**11**:e74295.

179. Holvoet L, De Geeter N, Dupre L, Crevecoeur G. A DTI based tractography research exploiting the brain network for object recognition and comparison with a TMS Speech Mapping experiment. University Gent Thesis defence 2015, accessed online on researchgate.net/publication/277583502 on 06.06.2023.

180. Markman TM, Hamilton RH, Marchlinski FE, Nazarian S. Case series of transcutaneous magnetic stimulation for ventricular tachycardia storm. *JAMA* 2020;**323**:2200–2202.

181. Goel V, Patwardhan AM, Ibrahim M, Howe CL, Schultz DM, Shankar H. Complications associated with stellate ganglion nerve block: a systematic review. *Regional Anesthesia & Pain Medicine* 2019;**44**:669–678.

182. Ashton JL, Burton RAB, Bub G, Smaill BH, Montgomery JM. Synaptic plasticity in cardiac innervation and its potential role in atrial fibrillation. *Front Physiol* 2018;**9**:240.

183. Cooke SF, Bliss TVP. Plasticity in the human central nervous system. *Brain* 2006;**129**(7): 1659–1673.

184. Lisman J, Cooper K, Sehgal M, Silva AJ. Memory formation depends on both synapse-specific modifications of synaptic strength and cell specific increase in excitability. *Nat Neurosci* 2018;**21**(3):309–314

185. Elias GJ, Loh A, Gwun D, Pancholi A, Boutet A, Neudorfer C, Germann J, Namasivayam A, Gramer R, Paff M, Lozano AM. Deep brain stimulation of the brainstem. *Brain* 2021;**144**(3): 712–723.

186. Hyam JA, Kringelback ML, Siburn PA, Aziz TZ, Green AL. The autonomic effects of deep brain stimulation—a therapeutic opportunity. *Nature Reviews Neurology* 2012;**8**:391–400.

187. Zangen A, Roth Y, Voller B, Hallett M. Transcranial magnetic stimulation of deep brain regions: evidence for efficacy of the H coil. *Clin Neurophysiol* 2005;**116**(4):775–779.

188. Huang YZ, Sommer M, Thickbroom G, Hamada M, Pascual-Leonne A, Paulus W, Classen J, Peterchev AV, Zangen A, Ugawa Y. Consensus: new methodologies for brain stimulation. *Brain Stimul* 2009;**2**(1):2–13.

189. Cabrerizo M, Cabrera A, Perez JO, de la Rua J, Rojas N, Zhou Q, Pinzon-Ardila A, Gonzalez-Arias SM, Adjouadi M. Induced effects of transcranial magnetic stimulation on the autonomic nervous system and the cardiac rhythm. *The Scientific World Journal* 2014; **2014**:349718.

190. Markman TM, Pothineni NV, Zghaib T, Smietana J, McBride D, Amankwah NA, Linn KA, Kumareswaran R, Hyman M, Arkles J, Santangeli P, Schaller RD, Supple GE, Frankel DS, Deo R, Lin D, Riley MP, Epstein AE, Callans DJ, Marchlinski FE, Hamilton R, Nazarian S. Effects of transcutanes magnetic stimulation in patients with ventricular tachycardia storm: a randomoized clinical trial. *JAMA Cardiol* 2022;**7**(4):445–449.

191. Wang S, Li B, Li X, Wu L, Jiang H. Low intensity ultrasound stimulation might reduce ventricular arrhythmia by modulating symoatehtic neural activity in myocardial infarction canine model. *JACC* 2019;**73**(9_Suppl_1):531.

192. Fitchett A, Mastitskaya S, Aristovich K. Selective neuromodulation of the vagus nerve. *Front Neurosci* 2021;**15**:685872.

193. He W, Jing X-H, Zhu B, Zhu XL, Li L, Bai W-Z, Ben H. The auriculo-vagal afferent pathway and its role in seizure suppression in rats. *BMC Neurosci* 2013;**14**:85.

194. Kaniusas E, Kampusch S, Tittgemeyer M, Panetsos F, Gines RF, Papa M, Kiss A, Podesser B, Cassara AM, Tanghe E, Samoudi AM, Tarnaud T, Joseph W, Marozas V, Lukosevicius A, Ištuk N, Šarolić A, Lechner S, Klonowski W, Varoneckas G, Széles JC. Current directions in the auricular vagus nerve stimulation I—a physiological perspective. *Front Neurosci* 2019; **13**:854.

195. Brack KE, Patel VH, Coote JH, Ng GA. Nitric oxide mediates the vagal protective effect on ventricular fibrillation via effects on action potential duration restitution in the rabbit heart. *J Physiol* 2007;**583**:695–704.

196. Conklin BR, Hsiao EC, Claeysen S, Dumuis A, Srinivasan S, Forsayeth JR, Guettier J-M, Chang WC, Pei Y, McCarthy KD, Nissenson RA, Wess J, Bockaert J, Roth BL. Engineering GPCR signaling pathways with RASSLs. *Nat Methods* 2008;**5**:673–678.

197. Roth BL. DREADDs for neuroscientists. *Neuron* 2016;**89**(4):683–694.

198. Magnus J, Lee PH, Atasoy D, Su HH, Looger LL, Sternson SM. Chemical and genetic engineering of selective ion channel ligand interactions. *Science* 2011;**333**(6047):1292–1296.

199. Magnus CJ, Lee PH, Bonaventura J, Zemla R, Gomez JL, Ramirez MH, Hu X, Galvan A, Basu J, Michaelides M, Sternson SM. Ultrapotent chemogenetics for research and potential clinical applications. *Science* 2019;**364**(6436):eaav5282.

200. Glover CP, Bienemann AS, Heywood DJ, Cosgrave AS, Uney JB. Adenoviral mediated high level cell specific transgene expression: a SYN1-WPRE cassette mediates increased transgene expression with no loss of neuron specificity. *Mol Ther* 2002;**5**(5 Pt 1): 509–516.

201. Mayford M, Baranes D, Podsypanina K, Kandel ER. The 3'-untranslated region of CaMKIIa is a cis-acting signal for the localization and translation of mRNA in dendrites. *Proc Natl Acad Sci U S A* 1996;**93**(23):1320–13255.

202. Lee Y, Messing A, Su M, Brenner M. GFAP promoter elements required for region specific and astrocyte-specific expression. *Glia* 2008;**56**(5):481–493.

203. Yu G, Chakrabarti S, Tischenko M, Chen A-L, Wang Z, Cho H, French BA, Naga Prasad SV, Chen Q, Wang QK. Gene therapy targeting protein trafficking regulator MOG1 in mouse models of Brugada syndrome, arrhythmias and mild cardiomyopathy. *Sci Trans Med* 2022; **14**(648):eabf3136.

204. Ikrar T, Shi Y, Velasquez T, Goulding M, Xu X. Cell type specific regulation of cortical excitability through the allatostatin receptor system. *Front Neural Circuits* 2012;**6**:2.

205. Ferenczi EA, Tan X, Huang CL-H. Principles of optogenetic methods and their application to cardiac experimental systems. *Frontiers Physiology* 2019;**10**:1096.

206. Yu L, Zhou L, Cao G, Po SS, Huang B, Zhou X, Wang M, Yuan S, Wang Z, Wang S, Jiang H. Optogenetic modulation of cardiac sympathetic nerve activity to prevent ventricular arrhythmias. *J Am Coll Cardiol* 2017;**70**(22):2778–2790.

207. Zhou L, Zhang Y, Cao G, Zhang C, Zheng C, Meng G, Lai Y, Zhou Z, Liu Z, Liu Z, Guo F, Dong X, Liang Z, Wang Y, Guo S, Zhou X, Jiang H, Yu L. Wireless self-powered optogenetic system for long term cardiac neuromodulation to improve post MI cardiac remodeling and malignant arrhythmias. *Adv Sci (Weinh)* 2023;**10**(9):e2205551.

208. Beiert T, Guegmann T, Sasse P. Optogenetic activation of Gq signalling modulates pacemaker activity of cardiomyocytes. *Cardiovasc Res* 2014;**102**(3):507–516.

209. Okonogi T, Sasaki T. Optogenetic manipulation of the vagus nerve. In: Yawo H, Kandori H, Koizumi A, Kageyama R (eds.), *Optogenetics. Advances in Experimental Medicine and Biology, Vol 1293*. Singapore: Springer; 2021. p459–469.

210. Machhada A, Hosford PS, Dyson A, Ackland GL, Mastitskaya S, Gourine AV. Optogenetic stimulation of vagal efferent activity preserves left ventricular function in experimental heart failure. *JACC Basic Trans Science* 2020;**5**(8):799–810.

211. Duque M, Lee Kubli CA, Tufail Y, Magaram U, Patel J, Chakraborty A, Mendoza Lopez J, Edsinger E, Vasan A, Shiao R, Weiss C, Friend J, Chalasani SH. Sonogenetics control of mammalian cells using exogenous transient receptor potential A1 channels. *Nat Commun* 2022;**13**:600.

212. Qiu Z, Kala S, Guo J, Xian Q, Zhu J, Zhu T, Hou X, Wong KF, Yang M, Wang H, Sun L. Targeted neurostimulation in mouse brains with non-invasive ultrasound. *Cell Rep* 2020; **32**:108033.

213. Vasan A, Allein F, Duque M, Magaram U, Boechler N, Chalasani SH, Friend J. Microscale concert Hall acoustics to prodcue uniform ultrasound stimulation for targeted sonogenetics in hsTRPA1 transfected cells. *Advanced Nanobiomed Research* 2022;**2**(5): 2100135.

214. Li Y, Wang X, Guo J, Wang Y, Zykov V, Bodenschatz E, Gao X. Sonogenetics is a novel antiarrhythmic treatment. arXiv: 2109.13613. https://arxiv.org/abs/2109.13613. Accessed online 17/09/2023.

215. Liu T, Choi MH, Zhu J, Zhu T, Yang J, Li N, Chen Z, Xian Q, Hou X, He D, Guo J, Fei C, Sun L, Qiu Z. Sonogenetics: recent advances and future directions. *Brain Stimul* 2022;**15**(5): 1308–1317.