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REVIEW

# Feeling the heat: source–sink mismatch as a mechanism underlying the failure of thermal tolerance

**Matti Vornanen***

## ABSTRACT

A mechanistic explanation for the tolerance limits of animals at high temperatures is still missing, but one potential target for thermal failure is the electrical signaling off cells and tissues. With this in mind, here I review the effects of high temperature on the electrical excitability of heart, muscle and nerves, and refine a hypothesis regarding high temperature-induced failure of electrical excitation and signal transfer [the temperature-dependent deterioration of electrical excitability (TDEE) hypothesis]. A central tenet of the hypothesis is temperature-dependent mismatch between the depolarizing ion current (i.e. source) of the signaling cell and the repolarizing ion current (i.e. sink) of the receiving cell, which prevents the generation of action potentials (APs) in the latter. A source–sink mismatch can develop in heart, muscles and nerves at high temperatures owing to opposite effects of temperature on source and sink currents. AP propagation is more likely to fail at the sites of structural discontinuities, including electrically coupled cells, synapses and branching points of nerves and muscle, which impose an increased demand of inward current. At these sites, temperature-induced source–sink mismatch can reduce AP frequency, resulting in low-pass filtering or a complete block of signal transmission. In principle, this hypothesis can explain a number of heat-induced effects, including reduced heart rate, reduced synaptic transmission between neurons and reduced impulse transfer from neurons to muscles. The hypothesis is equally valid for ectothermic and endothermic animals, and for both aquatic and terrestrial species. Importantly, the hypothesis is strictly mechanistic and lends itself to experimental falsification.

**KEY WORDS: Impulse transmission, Heart rate, Nerve and muscle function, Safety factor, Low-pass filtering**

## Introduction

Most animal life is limited to a temperature range between −2°C and +46°C (Clarke, 2014). This is the thermal window in which cellular macromolecules (e.g. nucleic acids, proteins and phospholipids) and body fluids allow the correct functioning of cellular life processes (Precht et al., 1973; Cossins and Bowler, 1987; Fields, 2001). Despite numerous studies on the temperature responses of animal bodies, organs, tissues, cells and macromolecules, the mechanistic basis of animal temperature tolerance is still unresolved. Although several ideas have been proposed as possible mechanisms for thermal failure, there are few formulated hypotheses that can guide research on the problem. The concept of oxygen- and capacity-limited thermal tolerance (OCLTT) has been the main formulated hypothesis on physiological thermal tolerance of ectotherms for approximately 20 years (Pörtner, 2001) (Box 1). This hypothesis proposes that the respiratory and cardiovascular systems are impaired at temperature extremes, thus limiting the availability of oxygen to tissues and cells. This results in hypoxemic deterioration of performance and fitness of the animal. The OCLTT hypothesis has had significant impact on research: its predictions have been tested experimentally in hundreds of studies. However, the OCLTT hypothesis is heavily disputed and it might be valid only for a limited number of (mainly) aquatic ectotherms (Clark et al., 2013; Lefevre, 2016). Clearly, alternative hypotheses are needed to guide and stimulate research on this fundamental and timely topic.

A scientific hypothesis should be simple and lead to clear-cut predictions, which can be tested experimentally. The results of such experiments should either conform to or refute the hypothesis, i.e. the hypothesis should, at least in principle, be falsifiable. A physiological hypothesis should also be truly mechanistic, explaining findings at all levels of biological organization, from molecules to cells, tissues, organs, individuals and the operation of animals in their natural habitats (Platt, 1964; Beard et al., 2005). Recently, I proposed a hypothesis of temperature-dependent deterioration of electrical excitability (TDEE) to explain the high temperature-induced depression of cardiac function in fish (Vornanen, 2016). Using a reductionist approach, TDEE starts from the molecular functions of a single cardiac myocyte *in vitro* and uses them to explain cardiac function in intact fish as it appears in the electrocardiogram (ECG) *in vivo*. In brief, temperature dependencies of the inward $Na^+$ current ($I_{Na}$) and the outward $K^+$ current ($I_{K1}$) – two antagonistic ion currents critical for the initiation of cardiac action potentials (APs) (Varghese, 2016) – are widely different. $I_{Na}$ is depressed at relatively low temperatures, whereas $I_{K1}$ is heat resistant and increases almost linearly with rising temperature (Vornanen et al., 2014; Badr et al., 2017b). The temperature-induced mismatch between $I_{Na}$ and $I_{K1}$ is considered to result in temperature-dependent depression of electrical excitability (EE) (Vornanen, 2016).

The objective of this Review is twofold: to further elaborate the TDEE hypothesis and to expand its scope of application from the heart to neuronal and muscular tissues. To this end, I first define EE, then describe how differential effects of temperature on inward and outward ion currents of fish cardiac myocytes could impair EE at high temperatures. I go on to collect data from the literature on the temperature responses of muscle and nerves and integrate them into the TDEE hypothesis. Finally, I propose that a source–sink mismatch between the inward current flow from the signaling (upstream) cell and the outward current flow (ion leak) of the resting receiving (downstream) cell is common for all electrically excitable cells and tissues at high temperatures. At critically high temperatures, source–sink mismatch compromises sensory, motor and cardiac processes, with consequent impairment of higher-level functions such as neuronal integration, behavior, locomotion, circulation and body homeostasis.

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**List of symbols and abbreviations**

| | |
|---|---|
| AP | action potential |
| AV | atrioventricular |
| CD | critical depolarization |
| $C_m$ | membrane capacitance |
| ECG | electrocardiogram |
| EE | electrical excitability |
| $f_H$ | heart rate |
| $I_{Ca}$ | inward $Ca^{2+}$ current |
| $I_{Cl}$ | outward $Cl^-$ current |
| $I_{Na}$ | inward $Na^+$ current |
| $I_{K1}$ | outward $K^+$ current |
| $[K^+]_o$ | extracellular $K^+$ concentration |
| OCLTT | oxygen- and capacity-limited thermal tolerance |
| $R_i$ | resistance to axial current flow |
| $R_{in}$ | input resistance of a cell |
| $R_m$ | resistance of the resting plasma membrane |
| $T_{BP}$ | break point temperature |
| TDEE | temperature-dependent deterioration of electrical excitability |
| $V_{rest}$ | resting membrane potential |
| $V_{th}$ | threshold potential |

**Glossary**

**Axial current flow**
The flow of current within the cell in the direction of action potential propagation.
**Bradycardia**
Slowing of heart rate.
**Breakpoint temperature**
The temperature at which the rate of a function starts to decrease.
**Cardiac output**
The volume of blood pumped by the heart in a unit of time.
**Charge transfer**
The number of charges transferred by an ion current, i.e. integral of current density.
**Conduction block**
Failure of impulse transmission along a nerve fiber or between electrically excitable cells.
**Critical depolarization**
The size of depolarization needed to trigger an action potential.
**Current density**
The size of ion current per unit membrane area.
**His bundle**
A component of the mammalian cardiac conduction system that extends from the atrioventricular node to the walls of the ventricles.
**Hyperthermia**
Abnormally high body temperature owing to the failure of thermoregulation.
**Input resistance**
Resistance of a cell membrane of the whole cell to current flow.
**Membrane capacitance**
The storage of electric charge by a lipid membrane.
**P wave**
A wave on an ECG that is generated by atrial depolarization.
**Resistance of the resting plasma membrane**
Resistance of cell membrane to current flow per unit membrane area.
**Resting membrane potential**
The negative membrane potential of a quiescent cell relative to external medium.
**QRS complex**
A waveform on an ECG that is generated by ventricular depolarization.
**Safety factor**
The excess charge delivered to a cell, beyond the amount required to trigger an action potential.
**T wave**
A wave of an ECG that is generated by ventricular repolarization.

## Electrical excitability

There is a rich literature on different aspects of EE, but the term itself is rarely defined. According to Boyett and Jewell (1980), 'Excitability of a cell can be defined loosely as the ease with which a response may be triggered. It is often expressed as the minimum current required to depolarize the membrane to the threshold potential' ($V_{th}$, see Glossary). $V_{th}$ is the value of membrane potential where the density (see Glossary) of voltage-activated inward currents exceeds the density of outward currents (membrane leak) and elicits an active membrane response, an all-or-none, self-propagating action potential (Fozzard, 1977; Koester and Siegelbaum, 2000) (Figs 1 and 2).

The electrical excitation of a cell requires that an inward current flows into the cell and depolarizes the resting membrane potential ($V_{rest}$; see Glossary) to $V_{th}$ (Spector, 2013; Ma and Huguenard, 2013; Varghese, 2016). In multicellular tissue, the current flow occurs between electrically coupled cells. The excited signaling cell functions as a 'source' of depolarizing current, which flows into the quiescent receiving cell, the latter forming the current 'sink' owing to the ion leak of the membrane. Each cell in a multicellular tissue functions both as a source and sink for the current flow. Robust propagation of impulses through a tissue requires that the source current is larger than the sink current at each location of the cell/tissue. Voltage- and time-dependent inward $Na^+$ ($I_{Na}$) and $Ca^{2+}$ ($I_{Ca}$) currents usually form the current source, which promotes EE. The passive electrical properties of the cell constitute the current sink. Thus, EE is dependent on both passive and active properties of the cell membrane, which are discussed in more detail below (Koester and Siegelbaum, 2000; Khodorov, 2012).

### Passive membrane responses

The 'ease' with which an electrical response is triggered in a cell is dependent on the passive electric properties of the excitable cell membrane at rest. It can be presented as a function of (i) resistance and (ii) capacitance in parallel at the plane of the plasma membrane, and (iii) the conductive/resistive intracellular solution, i.e. the cytoplasm of the cell (Fig. 1Ai) (Fozzard, 1977; Koester and Siegelbaum, 2000). Of these three passive membrane properties, the resistance of the resting plasma membrane ($R_m$; see Glossary) is most important for excitability, because it strongly influences the response of the cell to current flow from the neighboring cells. According to Ohm's law, $R_m$ determines how much current needs to be injected into the cell to increase $V_{rest}$ to the $V_{th}$ of an AP ($\Delta V = I \cdot R_m$): the higher the $R_m$, the less inward current is needed to change the membrane potential from $V_{rest}$ to $V_{th}$ ($\Delta V$) and to trigger an AP.

The magnitude of $R_m$ is dependent on ion flux through $Na^+$-, $Ca^{2+}$-, $K^+$- and $Cl^-$-specific ion channels in the cell membrane (Fig. 1Ai). If there are many open channels in the membrane of the quiescent cell, the ion leakage is large, and $R_m$ is low. The leakage current can be the flow of positive ions out of the cell (heart/nerve) or the flow of negative ions into the cell (muscle), both of which hyperpolarize the cell membrane. In resting cardiac myocytes, the ion leakage occurs mainly through Kir2 $K^+$ channels, which maintain the negative $V_{rest}$ (Hibino et al., 2010). If the leakage is large, a large inward current flow is needed to bring the membrane potential to $V_{th}$, because a greater part of the inward current is short-circuited by the leak (Ma and Huguenard, 2013). The second passive membrane property introduced above, membrane capacitance ($C_m$; see Glossary), is directly related to the surface area of the cell membrane, and it

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**Box 1. Oxygen- and capacity-limited thermal tolerance (OCLTT) and temperature-dependent deterioration of electrical excitability (TDEE)**

The OCLTT hypothesis attempts to explain ecologically relevant temperature tolerance limits of animals as an outcome of thermally induced failure of aerobic physical and metabolic performance (aerobic scope, defined as the difference between standard and maximum metabolic rates). According to the OCLTT hypothesis, depression of aerobic scope at low and high temperatures is due to insufficient oxygen availability resulting from a temperature-induced decrease in respiratory and circulatory capacities. Thus, vital functions, such as movement, growth and reproduction, and the fitness of the animal are impaired (Pörtner, 2001, 2002). The OCLTT hypothesis is built on the early concepts of temperature dependence of aerobic swimming performance in fishes (Fry and Hart, 1948; Brett, 1971). Revival and reformulation of these concepts have had a strong stimulating effect on research in the field of thermal physiology. Some of the studies support the OCLTT hypothesis, whereas others contradict it. Currently, the hypothesis is highly disputed, mainly because of the ambiguity about the central tenets of the most recent version of the hypothesis (Pörtner et al., 2017), and the absence of clear-cut mechanistic cause–effect explanations. In its current form, the hypothesis is not considered to provide clear and testable predictions (Clark et al., 2013; Jutfelt et al., 2018). The source–sink mismatch concept, which is a central feature of TDEE, does not contradict the OCLTT hypothesis. In fact, concerning the heat tolerance of fishes, it provides a well-defined mechanistic explanation for high temperature-induced failure of cardiovascular function (Haverinen and Vornanen, 2020 preprint). In the OCLTT hypothesis, thermal tolerance is examined at the level of an intact organism and explanations are given in relation to lower-level functions (e.g. mitochondrial respiration) that are determined experimentally. In contrast to the top-down approach of the OCLTT hypothesis, TDEE explains the higher-level functions of the animal from the fundamental electrical properties of cellular signaling and information transfer, i.e. in a bottom-up manner. TDEE is not limited in application to the physical and metabolic performance of the animal but is also relevant to mental and cognitive functions such as sensation, learning and memory.

indicates how much charge the membrane can store upon voltage change. Cell size is important for excitability, because a large cell has more cell surface area and therefore a larger number of leak channels than a smaller cell (Koester and Siegelbaum, 2000). Finally, resistivity of the cytoplasm determines the resistance to axial current flow ($R_i$; see Glossary) and affects the rate of AP conduction. Experimentally, the composite of the passive electrical properties of a cell is measured by injecting a small current into the cell and recording the corresponding change in the membrane potential: input resistance ($R_{in}$; see Glossary) is obtained from the Ohm's equation ($R_{in}=\Delta V/I$). $R_{in}$ takes into account all three passive membrane properties: it is proportional to $R_m$ and $R_i$ and inversely proportional to cell size ($C_m$). Because $R_m$ is much (typically about two orders of magnitude) larger than $R_i$, $R_{in}$ is a good measure for the membrane leak.

The passive electrical properties of a cell membrane are temperature dependent, and this has important implications for the function of electrically excitable cells. In this respect, $R_m$ is the most important passive electrical property of the cell. When temperature increases, $R_m$ decreases (because ion leakage increases) and $V_{rest}$ becomes more negative. Therefore, more depolarizing current is needed to excite the cell to the $V_{th}$ (Figs 1B, 2). This is a common property of the surface membrane of all excitable cells (Table 1). $R_i$ is suggested to slightly decrease with temperature and, in some preparations, $C_m$ may slightly increase with temperature (Adams, 1987, 1989b). Both changes will have a slight negative effect on excitability at high temperatures.

### Active membrane response

The 'response' of an electrically excitable cell to a suprathreshold stimulus current is an AP, which – once triggered – propagates through the cell with constant amplitude and velocity. A weaker subthreshold current elicits a smaller voltage change, which remains local and diminishes in amplitude with distance from the site of current injection (Fig. 1Aii).

EE is based on the asymmetric distribution of $Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$ ions across the cell membrane and passive flow of ions down their electrochemical gradients (Fozzard, 1977; Koester and Siegelbaum, 2000). The former is the result of energy-requiring ion transfer by ATP-driven pumps, and the latter is caused by the opening and closing of ion channels in the plasma membrane. A quiescent cell has a $V_{rest}$ of −60 to −90 mV, with the inside of the cell being negative relative to the external solution. In cardiac myocytes and nerve cells, $V_{rest}$ is largely due to diffusion of $K^+$ out of the cell through $K^+$-specific ion channels, whereas in skeletal muscle fibers, $Cl^-$ channels can make a significant contribution to $V_{rest}$ (Hutter and Noble, 1960; Adams, 1989b). $K^+$ efflux and/or $Cl^-$ influx maintain a value of $V_{rest}$ that is close to the Nernst equilibrium potential of $K^+$ and/or $Cl^-$ ions. The initiation and conduction of an AP is a function of voltage- and time-dependent opening and closing of $Na^+$-, $Ca^{2+}$-, $K^+$- and $Cl^-$-specific ion channels (Fozzard, 1977; Koester and Siegelbaum, 2000).

### Safety factor

The robustness of AP propagation under varying physiological conditions and stresses is based on the excess supply of the inward charge of the active signaling cell relative to the charge demand of the receiving cell (Hodgkin, 1948; Spector, 2013). When this ratio is ≥1.0, impulse propagation will occur; if the ratio is <1.0, impulse propagation will fail (Fig. 2). The reserve capacity of the inward charge of the active cell is called the 'safety factor' (see Glossary; Rasminsky, 1973; Targ and Kocsis, 1985; Wood and Slater, 2001). Most estimates of the safety factor for the vertebrate heart vary between 1.5 and 3.5, whereas the value is placed at between 2 and 5 for the vertebrate neuromuscular junction (Harris and Ribchester, 1979; Wang and Rudy, 2000; Wood and Slater, 2001; Ruff, 2011). Therefore, in healthy tissues, small decreases in inward current or increases in outward leak current do not endanger the propagation of APs. However, the safety factor is not uniform throughout an excitable cell/tissue, and it may be lost under heat stress, as indicated by conduction block (see Glossary) in neurons, muscles and heart (Hodgkin and Katz, 1949; Rasminsky, 1973; Westerfield et al., 1978; Vornanen et al., 2014; Badr et al., 2016). The sites where impulse conduction is most vulnerable to failure are found at functional and structural discontinuities of cells and tissues, such as synapses, branching points of neurons and muscle fibers and coupled cardiac cells (Fig. 3). Such discontinuities can create a local source–sink mismatch – an imbalance between the current provided by a smaller mass of tissue (source) and the current necessary to bring to threshold an adjacent larger mass of tissue (sink). Source–sink mismatch can induce a local slowing of conduction, therefore resulting in reduced frequency of response, i.e. low-pass filtering or complete conduction block (Rasminsky, 1973; Aslanidi et al., 2009) (see 'Functional consequences of impaired excitability', below).

## Failure of excitation of fish heart at high temperatures

When temperature rises, the metabolic rate and oxygen demand of the tissues of ectothermic animals increases. It has been suggested that, in fish, oxygen convection of the circulatory system cannot

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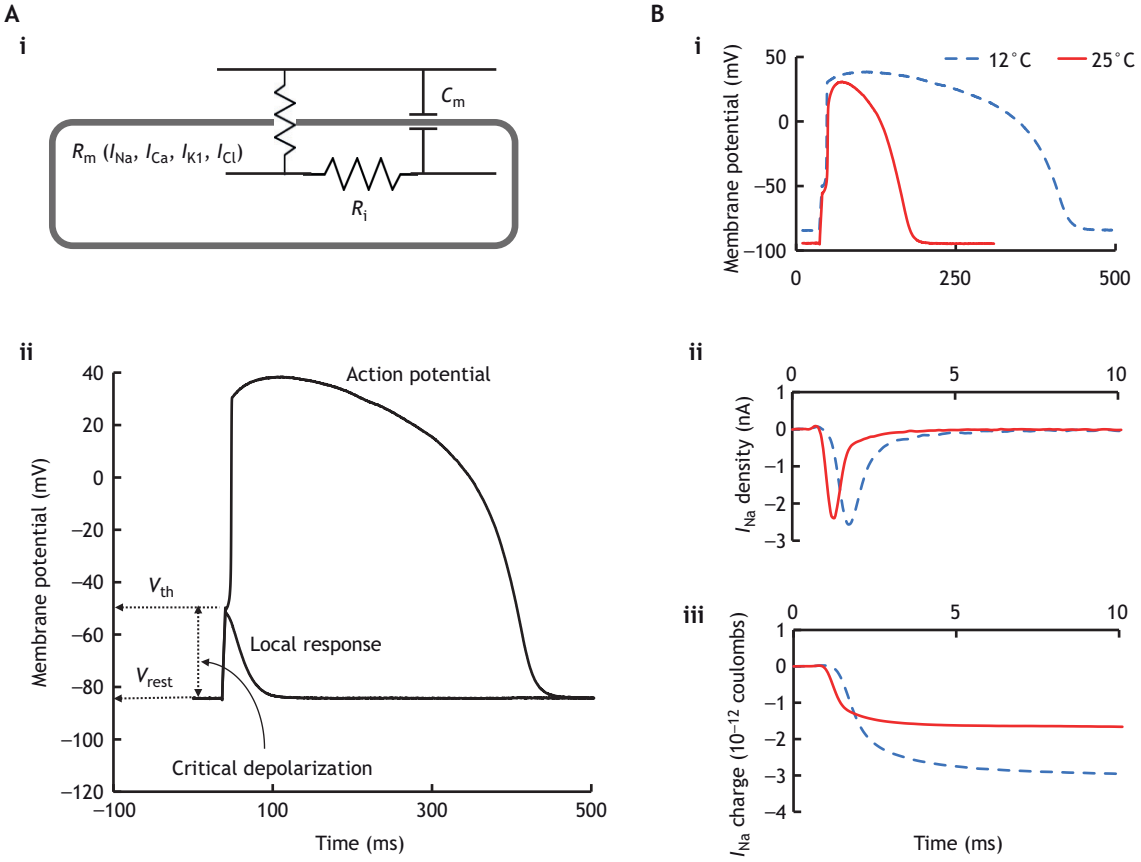


**Fig. 1. Effect of warming on electrical excitability (EE) of fish ventricular myocytes.** (Ai) EE of a cell is dependent on passive [resistance of the resting plasma membrane ($R_m$), membrane capacitance ($C_m$), resistance to axial current flow ($R_i$)] and active [inward $Na^+$ current ($I_{Na}$), ($I_{Ca}$), outward $K^+$ current ($I_{K1}$), ($I_{Cl}$)] properties of the cell membrane. (Aii) An action potential (AP) is initiated when the charge transfer by $I_{Na}$ depolarizes the membrane from the resting membrane potential ($V_{rest}$) to the threshold potential ($V_{th}$). At $V_{th}$, charge transfer of $I_{Na}$ exceeds that of $I_{K1}$. A subthreshold stimulus causes a local non-propagating voltage change, which exponentially decays to $V_{rest}$. (Bi) At high temperature, the outward leak of $K^+$ via $I_{K1}$ hyperpolarizes $V_{rest}$: the critical depolarization (CD=$V_{th}$−$V_{rest}$) becomes bigger and more inward current/charge is needed to depolarize the membrane to $V_{rest}$. (Bii,iii) When temperature rises, the charge transfer by $I_{Na}$ becomes smaller owing to the fast inactivation of $I_{Na}$ and because the increased $K^+$ current density makes the AP shorter. If the reduction is large enough to depress the safety factor below 1.0 excitation will fail.

keep pace with the increased oxygen demand, and thus the aerobic performance of the animal decreases (Farrell, 2009). Below, I describe how high temperature may cause heart failure as a result of the collapse of EE.

### Cardiac output in fish is frequency-modulated under heat stress

Cardiac output (see Glossary) is the product of heart rate ($f_H$) and stroke volume. When temperature is raised acutely, $f_H$ increases in fish up to a point called the breakpoint temperature ($T_{BP}$; see Glossary), above which $f_H$ declines and finally ceases. In contrast to $f_H$, stroke volume is largely independent of temperature (Stevens et al., 1972; Steinhausen et al., 2008; Mendonca and Gamperl, 2010). This means that cardiac output under acute temperature changes is closely correlated with $f_H$ (i.e. it is frequency modulated).

The rate and rhythm of the fish heart are determined by the primary pacemaker, the site of origin of cardiac APs at the border between the sinus venosus and the atrium (Yamauchi and Burnstock, 1968; Haverinen and Vornanen, 2007). APs propagate from the primary pacemaker to the atrium and, from there, via the atrioventricular (AV) canal to the ventricle, thus triggering sequential contractions of the atrium and ventricle. To understand the mechanism of temperature limitation of heart function, it is necessary to know the location(s) in the heart where temperature impairs AP generation/conduction. This can be achieved using electrocardiography, which produces electrocardiograms (ECGs) that show the familiar patterns of P waves, QRS complexes and T waves (see Glossary). Fig. 4A shows the beating rate of brown trout (*Salmo trutta*) cardiomyocytes as a function of temperature at the level of single pacemaker cells *in vitro* and in the intact animal *in vivo* (Haverinen et al., 2017). In single pacemaker cells, the frequency of APs increases almost exponentially in response to increases in temperature, reaching a rate of 191.4 beats $min^{-1}$ at 24°C. By contrast, in the intact fish, $f_{HV}$ (calculated from QRS complexes) reaches its peak value of 76.5 beats $min^{-1}$ at the $T_{BP}$ of 15.7°C. This strongly suggests that the warming-induced depression of $f_H$ is not caused by the failure of the primary pacemaker mechanism. ECGs taken from roach (*Rutilus rutilus*), rainbow trout (*Oncorhynchus mykiss*) and brown trout at temperatures above $T_{BP}$ show that atrial P waves occur at regular intervals, whereas several of the ventricular QRS complexes are missing (Vornanen et al., 2014; Badr et al., 2016). Indeed, there is often a regular 2:1, 3:1 or 4:1 P wave:QRS complex pattern, indicating various extents of AV block (Fig. 4C,D). This indicates that the atrium can follow the pacemaker rate, but that excitation of the ventricle fails, resulting in dissociation of atrial and ventricular beating rates: the temperature-induced bradycardia (see Glossary) is specific for the ventricle.

Journal of Experimental Biology (2020) 223, jeb225680. doi:10.1242/jeb.225680

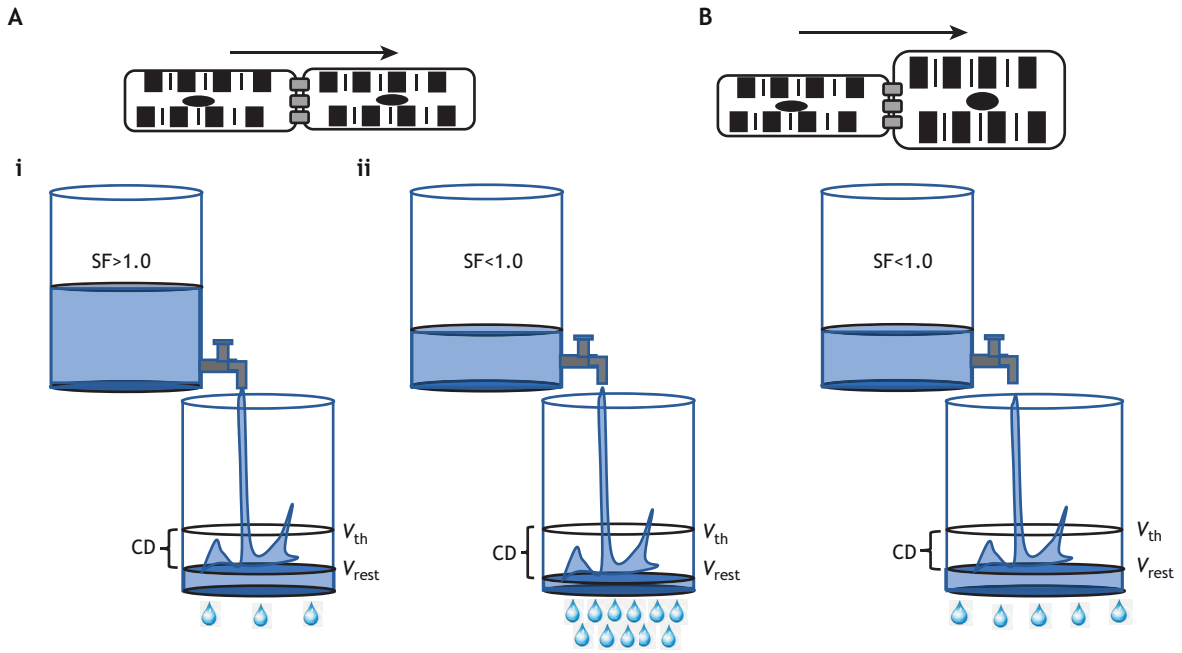


**Fig. 2. Effects of warming and increased capacitive load on safety factor (SF) and impulse transmission between ventricular myocytes.** (Ai) At physiological body temperature, the upstream cell (source; upper bucket) provides more depolarizing charge into the downstream cell (sink; lower bucket) than is needed for electrical excitation, because ion leak of the resting cell is low. (Aii) At high temperature, ion leak becomes larger; thus, $V_{rest}$ is more negative and critical depolarization (CD=$V_{th}$–$V_{rest}$) becomes larger. Simultaneously, charge transfer by the upstream cell via $I_{Na}$ reduces (shown as less liquid in the upper bucket), and therefore $V_{rest}$ of the downstream cell cannot be depolarized to $V_{th}$. (B) When a small cell is connected to a larger cell, there is an increased capacitive load (a larger area of leaky membrane): the small cell cannot provide enough depolarizing charge to bring $V_{rest}$ of the downstream cell to $V_{th}$.

What causes the AV block? It could be caused either by the failure of impulse conduction in the AV canal or by an inability of the ventricular tissue to respond to APs arriving from the AV canal. In mammalian hearts, there are three main categories of AV block: first-, second- and third-degree blocks (Merideth and Pruitt, 1973). In the first-degree AV block, AP conduction in the AV node is delayed but there are no dropped ventricular beats (thus, the P wave is always associated with a QRS complex). The two types (type I and type II) of the second-degree AV block are both associated with the normal rhythm of P waves but with intermittent absence of QRS complexes. The type-I block is characterized by a delay in AV node conduction, which manifests in the ECG as a prolonged PQ interval. The high temperature-induced AV block in roach and trout is not associated with prolongation of the PQ interval, suggesting that the block is not analogous to the first-degree block or type I of the second-degree block of the mammalian heart. In mammals, the type-II second-degree block is also characterized by regular P waves with the intermittent absence of QRS complexes but does not involve prolongation of the PQ interval. This kind of ECG pattern is explained by the failure of impulse propagation in the conduction pathways of the ventricles. If the block is in the branch of the His bundle (see Glossary), the type-II AV block is characterized by widening of the QRS complex. In the third-degree AV block, impulse transmission to the ventricles is completely prevented. Interestingly, the temperature-induced AV block of the trout and roach hearts resembles the second-degree type-II AV block of the mammalian heart with skipped ventricular beats and widening of the QRS complex (Vornanen et al., 2014; Badr et al., 2016). This suggests that the temperature-induced AV block is not due to the failure of impulse conduction in the AV canal, but is somehow related to the depression of ventricular excitation. If temperature is high enough, it will cause a complete standstill of the ventricle (third-degree block). I discuss how this might occur in more detail below.

#### Heat-induced ventricular failure by source–sink mismatch

The excitability of atrial and ventricular myocytes is governed by two antagonistic ion currents: the inwardly rectifying $K^+$ current ($I_{K1}$) and the fast inward $I_{Na}$ (Varghese, 2016). $I_{Na}$ is the source current, which provides the necessary inward charge movement for depolarization of $V_{rest}$ to $V_{th}$ and determines the rate of AP propagation in the atrium and ventricle. $I_{K1}$ forms the current sink by allowing the outward leak of $K^+$ in quiescent myocytes. (Within the voltage range of an AP, $I_{K1}$ is always an outward current, but the current decreases when membrane voltage becomes more positive than approximately −50 mV, i.e. it rectifies in inward direction.) At physiological temperatures, $I_{Na}$ is large enough to enable normal excitation of the ventricle (Fozzard, 1977; Shaw and Rudy, 1997). However, at critically high temperatures, the safety factor reduces and may be totally lost owing to opposing effects of high temperature on $I_{Na}$ and $I_{K1}$ (Box 2). Initially, moderate increases in temperature increase the density of both $I_{Na}$ and $I_{K1}$, and the excitation of ventricular myocytes is ensured (Vornanen et al., 2014; Badr et al., 2017b; Badr et al., 2018). However, the charge transfer (see Glossary) by $I_{Na}$ starts to decline immediately when temperature rises, because the fast inactivation of the channels allows less time for $Na^+$ influx (Fig. 1Bii,iii). This means that the surplus of $I_{Na}$ that was present at the acclimation temperature is lost when temperature approaches the upper thermal tolerance limit of the fish. The charge transferred by $I_{Na}$ declines so much that it cannot exceed the leak by $I_{K1}$; consequently, a mismatch between source and sink develops and excitation fails.

The significance of the temperature-dependent kinetics of $I_{Na}$ in the reduction of safety factor has been studied in rainbow trout

**Table 1. Temperature-induced changes in passive membrane properties and action potentials (APs) when temperature is acutely increased**

| Species | Preparation | Resting membrane potential | Input resistance | AP amplitude | AP duration | Reference |
|---|---|---|---|---|---|---|
| Earthworm, *Lumbricus terrestris* | Nerve | Hyperpolarization 5–20°C | Decrease 5–20°C | No change 5–20°C | Decrease 5–20°C | Dierolf and McDonald (1969) |
| Locust, *Schistocerca gregaria* | Muscle | Hyperpolarization 15.5–27°C | Decrease 15.5–27°C | | | Kornhuber and Walther (1987) |
| Locust, *Schistocerca gregaria* | Motoneuron | | Decrease 10–40°C | Decrease 10–40°C | Decrease 10–40°C | Burrows (1989) |
| Locust, *Locusta migratoria* | Visual interneuron | Hyperpolarization 25–40°C | Decrease 25–35°C | Decrease 25–40°C | Decrease 25–40°C | Money et al. (2005) |
| Crayfish, *Astacus astacus* | Giant axon | Hyperpolarization 2–32°C | | Decrease >26°C | | Kivivuori and Lagerspetz (1982) |
| Crayfish, *Astacus astacus* | Stretch receptor neuron | Hyperpolarization 5–20°C | | | | Kivivuori et al. (1990) |
| Crayfish, *Astacus leptodactylus* | Muscle | Hyperpolarization 6–32°C | | | | Harri and Florey (1977) |
| Crayfish, *Austropotamobius pallipes* | Muscle | Depolarization 0–45°C | Decrease 0–32°C | | | Gladwell et al. (1976) |
| Crayfish, *Procambarus clarkii* | Leg muscle | Hyperpolarization 10–35°C | Decrease 20°C versus 36°C | | | White (1983) |
| Crab, *Portunus depurator* | Leg muscle | Hyperpolarization 2–17°C | Decrease 2–17°C | Decrease –0.4–12.8°C | Decrease –0.4–12.8°C | Fatt and Katz (1953) |
| Crab, *Pachygraphus crassipes* | Motor axon | Hyperpolarization 16–32°C | Decrease[a] 16–32°C | Decrease 12–26°C | Decrease 12–26°C | Stephens et al. (1983); Stephens (1988) |
| Crab, *Cancer borealis* | Lateral gastric neuron | Hyperpolarization 0–13°C | Decrease 10–13°C | | | Städele et al. (2015) |
| Lobster, *Panulirus interruptus* | Somatogastric nerves | Hyperpolarization 11.3–20.4°C | Decrease[b] 11.3–20.4°C | Decrease 11.3–20.4°C | | Johnson et al. (1991) |
| Lobster, *Homarus americanus* | Giant axon | Hyperpolarization 20–36°C | | Decrease 18–36°C | Decrease 4–36°C | Dalton and Hendrix (1962) |
| Lobster, *Homarus americanus* | Muscle | Hyperpolarization 3.3–18°C | Decrease 3.3–18°C | | | Colton and Freeman (1975) |
| Squid, *Loligo forbesi* | Giant axon | Depolarization 20–35°C | | Decrease 3–35°C | Decrease 3–35°C | Hodgkin and Katz (1949) |
| Fish, *Lepomis cyanellus* | Muscle | Hyperpolarization 7–25°C | Decrease 2–31°C | | | Klein and Prosser (1985) |
| Fish, *Salmo trutta* | Heart ventricle | Hyperpolarization 4–28°C | | Increase 4–26.4°C; decrease >26.4°C | Decrease 4–28°C | Vornanen et al. (2014) |
| Fish, *Oncorhynchus mykiss* | Heart ventricle | Hyperpolarization 12–25°C | Decrease 12–25°C | Decrease 12–25°C | Decrease 12–25°C | Haverinen and Vornanen (2020) |
| Frog, *Rana pipiens* | Peroneal motoneuron | | | Decrease 8–25°C | Decrease; 8–25°C | Schoepfle and Erlanger (1941) |
| Frog, *Xenopus laevis* | Muscle | Hyperpolarization 2.5–30°C | Decrease[c] 2.5–30°C | | | Adams (1989a) |
| Frog, *Rana temporaria* | Muscle | Hyperpolarization 2.5–18.5°C | Decrease 2.5–18.5°C | | | Hodgkin and Nakajima (1972) |
| Lizard, *Diplosaurus dorsalis* | Muscle | Hyperpolarization 15–45°C | Decrease[c] 15–45°C | | | Adams (1989b) |
| Lizard<br>*Sceloporus occidentalis*<br>*Anolis cristatellus*<br>*Dipsosaurus dorsalis* | Muscle | | Decrease<br>15–35°C<br>15–40°C<br>15–45°C | | Decrease<br>15–35°C<br>15–40°C<br>15–45°C | Adams (1987) |
| Turtle, *Trachemys scripta* | Heart ventricle | Hyperpolarization 5°C versus 21°C | | 5°C versus 21°C decrease | 5°C versus 21°C decrease | |
| Rat, *Rattus norvegicus* | Visual cortex | Hyperpolarization 8–35°C | Decrease 8–35°C | Decrease 8–35°C | Decrease 8–35°C | Volgushev et al. (2000) |
| Cat, *Felis catus* | Spinal motoneuron | Hyperpolarization 30–41°C | Decrease 30–41°C | Decrease 30–41°C | Decrease 30–41°C | Klee et al. (1974) |
| Rat, *Rattus norvegicus* | Visual cortex cells | Hyperpolarization 7–35°C | Decrease 7–35°C | Decrease 7–35°C | Decrease 7–35°C | Volgushev et al. (2000) |

[a]Spike threshold hyperpolarized, and critical depolarization (see Glossary) increased.
[b]Decrease in amplitude of antidromic APs.
[c]Threshold current and critical depolarization increased.

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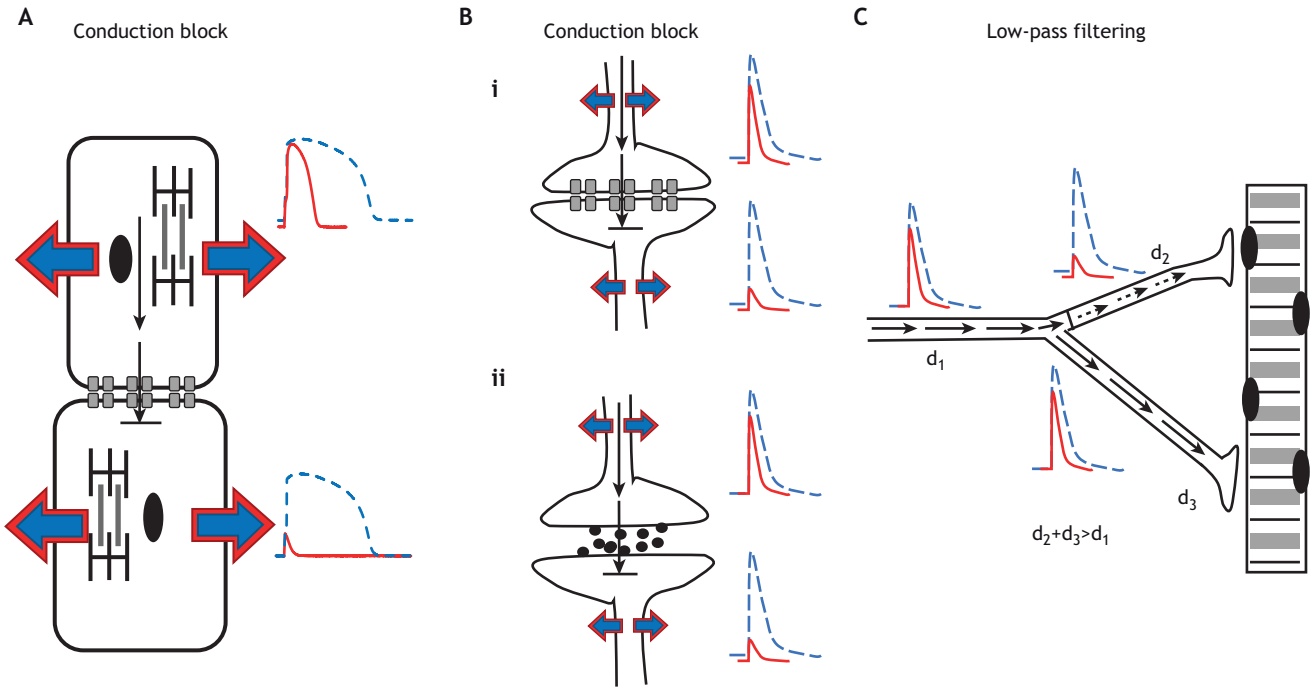


**Fig. 3. Effect of warming on impulse transmission.** Transmission between cardiac myocytes (A), at electrical and chemical synapses (Bi,ii) and in a branching neuron (C). Line tracings show membrane potential during an AP at high (red) and low (blue) temperatures. AP transmission is impaired by a mismatch between inward and outward current/charge flow across the plasma membrane. Warming increases passive ion leak of the resting cell. Simultaneously, the inward charge transfer is decreased, owing to the reductions in duration and amplitude of AP. At high temperatures, the safety factor falls below 1.0, and excitation of the downstream cell fails. Branching points of axons represent sites of reduced safety factor due to a higher total capacitive load of the branches. Outward leak current is shown by blue and red arrows for low and high temperature, respectively. The inward charge during the upstroke of AP is reduced by warming owing to reduction in the duration and amplitude of AP. Functionally, the source–sink mismatch of ion current/charge flow may appear as a low-pass filter, resulting in reduced frequency of heartbeat and neuronal APs. $d_1$, $d_2$ and $d_3$ are diameters of axon branches. Arrows show the direction of AP propagation; dashed arrows in C indicate the blocked propagation of AP.

ventricular myocytes. In these cells, the open $Na^+$ channels quickly inactivate, and this happens faster at high temperatures, thereby reducing the integral of $I_{Na}$ (Fig. 1Bii,iii). In rainbow trout ventricular myocytes, the peak density of $I_{Na}$ at 25°C is approximately 10% larger than at 12°C, whereas the charge transfer by $I_{Na}$ is 56% smaller at 25°C than 12°C (Haverinen and Vornanen, 2020). Collectively, experimental findings suggest that ventricular bradycardia is caused by high temperature-induced mismatch between source ($I_{Na}$) and sink ($I_{K1}$). It is notable, however, that in enzymatically isolated ventricular myocytes, APs can be elicited much above the $T_{BP}$ of the *in vivo* $f_H$ (Vornanen et al., 2014). This indicates that when the stimulus – applied from an amplifier – is sufficiently large, ventricular myocytes can generate APs at temperatures considerably above the upper critical temperature of the fish. However, in the intact heart, the source current is finite and becomes a limiting factor.

### Why are the nodal tissues and atrium resistant to thermal deterioration?

The ionic mechanisms underlying AP generation by the fish nodal tissues (the sinoatrial pacemaker and AV canal) are poorly understood, and therefore we must largely rely on data from other vertebrates in our attempts to understand why increases in temperature do not affect the nodal tissues and atrium (Efimov et al., 2004; Monfredi et al., 2010; George et al., 2017). In vertebrate hearts, the cells of the primary pacemaker and AV node do not have $I_{Na}$ and $I_{K1}$, or if they are expressed the currents are small. In the absence of $I_{K1}$, the nodal myocytes do not have a stable $V_{rest}$ but are self-excitatory, i.e. spontaneously active. The AP upstroke of nodal cells is based on the L-type $Ca^{2+}$ current ($I_{CaL}$), which is resistant to high temperatures in fish (Vornanen et al., 2014; Badr et al., 2017b). Thus, nodal cells do not experience the antagonism between $I_{Na}$ and $I_{K1}$, at least not to the same extent as ventricular myocytes.

In contrast to the nodal cells, fish atrial myocytes have a stable negative $V_{rest}$ and they express $I_{K1}$. However, the density of the atrial $I_{K1}$ is small and therefore the $R_{in}$ of atrial myocytes is about an order of magnitude higher than in ventricular myocytes (Vornanen et al., 2002; Haverinen and Vornanen, 2009; Abramochkin and Vornanen, 2015; Badr et al., 2017a). In contrast, the density of atrial $I_{Na}$ is similar to or higher than that of ventricular myocytes (Haverinen and Vornanen, 2006; Badr et al., 2017b). The favorable $I_{Na}$:$I_{K1}$ ratio (large safety factor) makes the atrial myocytes easily excitable and protects them against heat-dependent deterioration of excitability. It is noteworthy that EE of atrial myocytes can be depressed by inducing an outward $K^+$ current by acetylcholine ($I_{KAch}$) (Abramochkin and Vornanen, 2017). However, the temperature-induced increase in $I_{KAch}$ is not large enough to prevent atrial excitability *in vivo*, as shown by the persistence of P waves in the ECG (Fig. 4C,D).

Although the pacemaker rate is not suppressed by high temperatures, there are marked changes in the shape of the pacemaker AP as temperatures increase. Both the duration and amplitude of the AP decrease when temperature is acutely increased (Haverinen et al., 2017). Although not yet documented, similar effects are expected to occur in the AV nodal cells under warming. Because of their smaller and shorter AP, the AV nodal cells may be less able to provide depolarizing $I_{Na}$ to trigger ventricular APs at higher temperatures.

Journal of Experimental Biology (2020) 223, jeb225680. doi:10.1242/jeb.225680

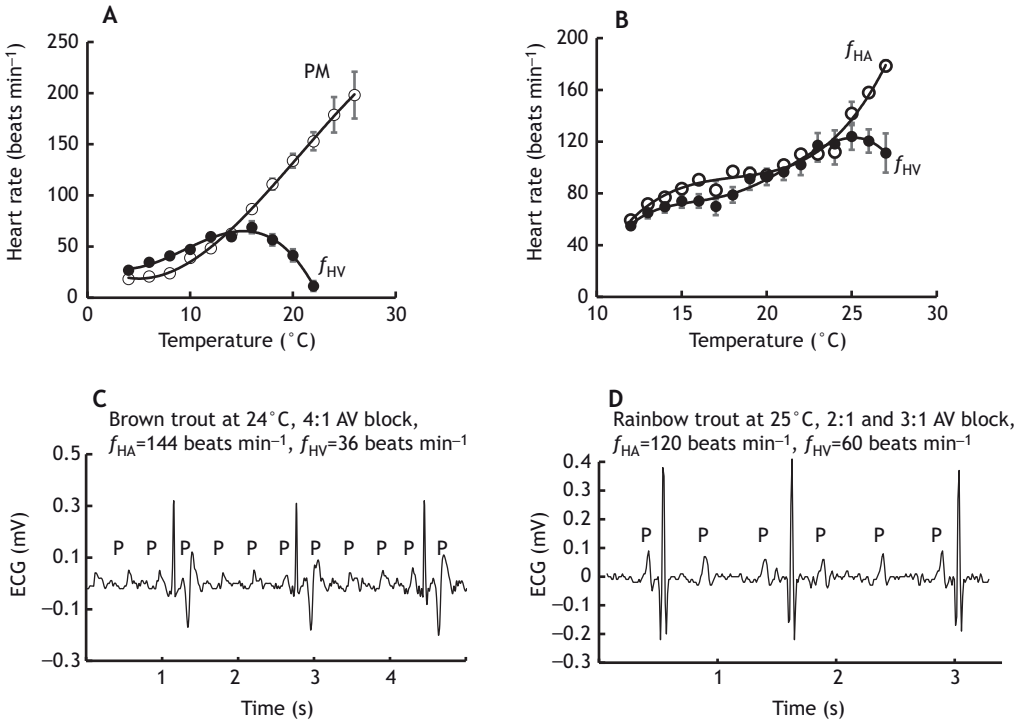


**Fig. 4. Warming-induced dissociation of sinoatrial and ventricular beating in fish hearts.** (A) Action potential rate of sinoatrial pacemaker (PM) cells and ventricular rate ($f_{HV}$) *in vivo* of 4°C-acclimated brown trout (Haverinen et al., 2017). (B) *In vivo* atrial and ventricular heart rates ($f_{HA}$ and $f_{HV}$, respectively) of rainbow trout (acclimated at 12°C). Atrial and ventricular heart rates dissociate at approximately 25°C (Haverinen and Vornanen, 2020). (C,D) Original ECG tracings of brown trout acclimated at 4°C (C) and rainbow trout acclimated at 12°C (D) showing 2:1, 3:1 and 4:1 AV block. P, atrial depolarization.

## Responses of neuronal and muscle tissues to high temperature

Of course, AP propagation is not only important for cardiac function; for example, the transmission of different forms of sensory information and the activation and coordination of motor functions are mediated by trains of APs. Changes in the strength and quality of sensory information and in the force of muscle contraction are achieved by differences in the frequency of APs (i.e. sensory and motor information is largely frequency coded; Gerstner et al., 1997). Therefore, the same principles and mechanisms that apply to the thermal responses of the fish heart could also operate in nervous and muscular tissues under heat stress (Fig. 5).

### Effects of high temperature on the current sink of neurons and muscle cells

High temperatures compromise the EE of neurons and muscle cells in both ectotherms and endotherms (White, 1983; Rutkove et al., 1997; Racinais and Oksa, 2010). Considering the potential importance of sarcolemmal ion leak in the source–sink mismatch of fish ventricular myocytes, the temperature dependence of the passive membrane properties of neurons and muscle cells is of interest. Fortunately, passive membrane properties have been extensively studied in nervous and muscular tissues, both in invertebrates and in ectothermic and endothermic vertebrates. A coherent picture is emerging from these studies. In general, high temperatures reduce $R_{in}$ via increases in $K^+$ or $Cl^-$ leak across the cell membrane. Because the diffusion of $K^+$ and/or $Cl^-$ maintain $V_{rest}$ in nerve and muscle cells, $V_{rest}$ is regularly hyperpolarized at high temperatures (Table 1). Although the ion channels that mediate the resting leak may differ at least partly in heart, muscles and neurons, all tissues show increased outward current leak in response to high temperatures. Consequently, the current sink of neurons and muscle cells is larger at higher temperatures.

### Effect of temperature on current source in neurons and muscle cells

$I_{Na}$ is the main depolarizing current in most neurons, skeletal muscle fibers and atrial and ventricular myocytes, and it is also present in smooth muscle cells (Catterall, 2012; Ulyanova and Shirokov, 2018). Temperature-induced increases in the outward leak reduce the safety factor for AP generation/conduction unless the inward current is simultaneously increased (Adams, 1987). Several indirect findings indicate that rising temperatures have a greater ($Q_{10}$) effect on outward $K^+$ than inward $Na^+$ currents, with a consequent reduction in the source-to-sink ratio (Hodgkin and Katz, 1949; Westerfield et al., 1978; Volgushev et al., 2000). Extracellular recordings of APs indicate that the amplitude and duration of APs are reduced in the myelinated axons of the frog (*Rana pipiens*) when temperature is raised from 8°C to 26°C (Schoepfle and Erlanger, 1941). Notably, the effect of temperature is much stronger on the descent ($K^+$ current) than the ascent ($Na^+$ current) of the AP. Similarly, Hodgkin and Katz (1949) found that higher temperatures (2–40°C) have a much greater effect on the duration of the falling than the rising phase of the AP in the giant axons of the squid (*Loligo forbesi*). This results in complete block of conduction at temperatures between 35°C and 40°C. Later, similar results were reported for the giant nerve of *Loligo pealeii* and several other preparations from different species (Westerfield et al., 1978) (Table 1). Humans are no exceptions in this respect; electromyograph recordings of APs have shown that an increase in muscle temperature of a few degrees diminishes the $Na^+$ current of muscle fibers (Rutkove et al., 1997; Racinais et al., 2008).

With the advent of the voltage-clamp method it was possible to directly demonstrate that the thermal responses of APs are caused by

Journal of Experimental Biology (2020) 223, jeb225680. doi:10.1242/jeb.225680

**Box 2. Temperature dependence of ion channel function**

Ion channels form water-filled pores in the plasma membrane that allow ion flux into and out of the cell. Opening and closing of the pore result from conformational changes of the channel: the pore becomes conducting only when both the activation and inactivation gate move to the open position. Ion flux stops when the inactivation gate closes. Notably, the inactivation gate may sometimes close before the activation gate opens, rendering the channel non-conducting (Armstrong, 2006). This is termed 'closed-state inactivation'.

Activation energy for the voltage-gating of ion channels comes from changes in the membrane potential. However, the kinetic energy of temperature also contributes to activation energy: temperature affects the function of ion channels in the same way that it affects the function of enzyme proteins (Liang et al., 2009; Fields et al., 2015). Warming increases the rate of opening (activation) and closing (inactivation) of the gates and the size of the ion current (Collins and Rojas, 1982; Cavalié et al., 1985; Liang et al., 2009). The temperature dependence of ion channels consists of two components: (i) the effect of temperature on the movement of the gates of the channel and (ii) the effect of temperature on the passage of ions through the pore. The latter is relatively weakly dependent on temperature, with a 1.2- to 1.6-fold increase in rate for each 10°C increase in temperature ($Q_{10}$ value). In contrast, conformational changes of the channel are strongly dependent on temperature, with typical $Q_{10}$ values between 2 and 6.

Temperature sensitivity of ion channel function is also affected by the general physical properties and specific components of the lipid membrane and the cytoskeleton of the cell to which the channel may be attached (Maltsev and Undrovinas, 1997; Tillman and Cascio, 2003; Duncan et al., 2020). If the attached cytoskeletal protein and/or the surrounding lipid membrane are rigid, the gating is slow. If the temperature exceeds the optimum of the ion channel, then either the components of the channel assembly start to dissociate, or the conformational changes may become disorganized, with a consequent decrease in ion current (Charalambous et al., 2009).

differential effects of temperature on $Na^+$ and $K^+$ currents (Huxley, 1959). Still, relatively few studies have examined the effects of acute temperature changes on the size of neuron and muscle $I_{Na}$ over a wide temperature range (most of this work has been done in fish cardiac myocytes). In mammals, the peak amplitude of the neuronal $I_{Na}$ increases with rising temperature up to the normal body temperature (<36.5–37.5°C). However, at 37–43°C, the peak $I_{Na}$ stops increasing or decreases (Volgushev et al., 2000; Touska et al., 2018). Relatively little is known about temperature effects on $I_{Na}$ density; however, the temperature dependence of $I_{Na}$ kinetics is better understood (Schwarz, 1979; Collins and Rojas, 1982; Schmidtmayer, 1989). In fact, the inactivation kinetics of $Na^+$ channels are more important than the peak $I_{Na}$ amplitude for excitability, because the amount of transferred charge (integral of $I_{Na}$) is decisive for excitation (Hodgkin, 1951). $Na^+$ channels open very rapidly and spontaneously inactivate from the open state a few milliseconds later (Yue et al., 1989; Catterall, 2012). Because the opening of $Na^+$ channels is almost instantaneous, changes in the rate of activation do not affect charge transfer as much as the rate of inactivation (Fig. 1Bii,iii). Owing to the accelerated inactivation of $I_{Na}$ at higher temperatures, the charge transfer through $Na^+$ channels decreases throughout the rising temperature range (Volgushev et al., 2000). Furthermore, at high temperatures, a larger fraction of $Na^+$ channels could inactivate from the closed state (Box 2), which reduces the availability of $Na^+$ channels for opening and therefore decreases the peak density of $I_{Na}$ (Irvine et al., 1999). Collectively, a significant body of data indicates that charge transfer by $I_{Na}$ decreases at rising temperatures and thereby reduces the safety factor in various excitable cells and tissues (Volgushev et al., 2000) (Table 1).

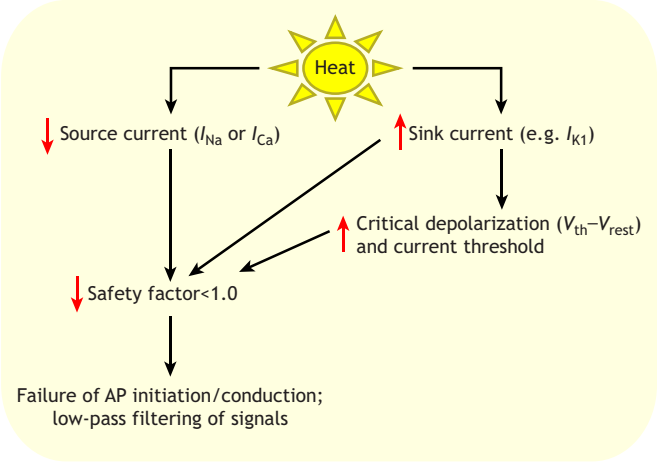


**Fig. 5. Basic principles of the source–sink mismatch hypothesis.** Warming causes mismatch between the depolarizing current of the active cell (source) and repolarizing currents of the resting (sink) cell, which increases the voltage and current threshold for excitation. When mismatch is so large that the safety factor drops below 1.0, excitation of the downstream cell will fail. In the heart, this causes ventricular bradycardia, whereas in nervous and muscle tissues it causes reduced frequency of conducted APs (i.e. low-pass filtering of information coding).

## Functional consequences of impaired excitability

Functionally competent neuronal and neuromuscular connections are necessary for sensory, motor and behavioral responses of animals (Friedlander et al., 1976; Janssen, 1992). As long as neuronal, sensory and motor functions remain intact, normal body functions are maintained under acute and chronic temperature changes. Conversely, impairment of the nervous system is suggested to play a critical role in determining the upper and lower thermal limits of behavior and survival (Hamby, 1975; Prosser and Nelson, 1981; Cossins and Bowler, 1987). Complex polysynaptic behaviors are considered to be more susceptible to high temperature than simple reflexes (Prosser and Nelson, 1981; Montgomery and Macdonald, 1990). Thermal failure of excitability often appears as conduction block, in particular at branching points of cells and at synaptic connections between cells.

### Conduction block

The normal functioning of the nervous system involves several processes that may be susceptible to thermal failure (i.e. initiation and conduction of APs, synaptic transmission) (Fig. 3B). Owing to the fast inactivation of $I_{Na}$ and increase in the amplitude of $K^+$ currents at high temperatures, acute temperature increases result in the reduction of AP duration and amplitude (Table 1). Small, short APs carry less inward current and are therefore weak triggers for AP initiation/conduction, eventually resulting in conduction block. If high temperature increases membrane leak, the small and short APs cannot provide enough depolarizing source current to trigger an AP in the receiving cell. Demyelinated axons are especially vulnerable to high temperature, because removal of the resistive myelin layer increases ion leakage of the axolemma. In fact, in the demyelinated axons of the rat spinal ventral root preparation, conduction block can appear at normal body temperature (Bostock et al., 1978). Notably, AP conduction can be restored by reducing temperature to below the body temperature or by exposing the axons to venom from the scorpion *Leiurus quinquestriatus*; both of these measures delay the

inactivation of $I_{Na}$ and prolong AP duration, and therefore increase source current (Bostock et al., 1978).

Another example of temperature-sensitive cardiac conduction failure appears in mice that are deficient in an intracellular $Na^+$ channel modulator, the fibroblast growth factor homologous factor (FHF2) (Park et al., 2016). In mice missing this protein, $I_{Na}$ inactivates faster and is therefore unable to provide enough depolarizing charge for excitation at high temperature (+40°C). In addition, temperature-dependent conduction block can also be observed in invertebrates: in the fruit fly (*Drosophila*), functional defects in neuronal $Na^+$ channels result in temperature-sensitive phenotypes. In flies carrying a single gene mutation, *para*$^{ts}$, heating from 22–25°C to 29–38°C causes an almost instant but reversible paralysis of the skeletal muscles. This is due to temperature-induced failure of motoneuron function (Suzuki et al., 1971; Siddiqi and Benzer, 1976; Wu et al., 1978; Benshalom and Dagan, 1981). The number of functional $Na^+$ channels is too low to raise $V_{rest}$ to $V_{th}$ (Loughney et al., 1989) because $K^+$ currents overwhelm the small $I_{Na}$ (O'Dowd et al., 1989), a clear source–sink mismatch. It is noteworthy that the flight and leg muscles of the fly remain fully functional, because depolarization of the muscle fiber is based on $I_{Ca}$ (Ganetzky and Wu, 1986; Peron et al., 2009). Although the above examples are from animals with defective $Na^+$ channels, a conduction block can be induced by high temperature in healthy animals and intact preparations (Hodgkin and Katz, 1949).

### Branching

Once initiated, AP conduction is not particularly sensitive to high temperature when the AP is conducted along a structurally homogeneous plasma membrane (see 'Conduction block', above) (Li and Gouras, 1958; Jensen, 1972; Macdonald and Montgomery, 1982). However, nerves and muscle are geometrically complex tissues, with extensive branching and variations in cell diameter. In particular, the branching points of axons and muscle fibers are sensitive to conduction block when temperature rises (Spira et al., 1976; Westerfield et al., 1978; Grossman et al., 1979; Smith, 1980) (Figs 2B, 3). After the branching point, the total membrane area and therefore the summed membrane leak are bigger than those of the main axon (Fig. 3). Owing to this geometry, the branches have a low total $R_{in}$, and the safety factor is smaller at the branching point than before the branching point. This means that more inward charge is needed to drive $V_{rest}$ of the branches to $V_{th}$ (Swadlow et al., 1980). When the temperature rises, increased ion leak may depress the safety factor below 1.0 and prevent AP conduction to the branches (Fig. 3) (Westerfield et al., 1978; Smith, 1980). In the branching axon of the squid *L. pealeii*, a 2°C increase in temperature from 5°C to 7°C causes a conduction block. Mathematical modeling suggests that the failure occurs because the shortened AP is unable to provide enough inward charge to exceed the capacitive load (sink) of the branches (Westerfield et al., 1978). Similarly, in the crayfish *Orconectes viris*, motor axon conduction failure at the branching point is preceded by a reduction in $I_{Na}$, i.e. not only is the leak increased but the source current is also depressed (Smith, 1980). Clearly, the low safety factor of branching points makes these sites prone to conduction failure. Functionally, this appears as a reduction in the firing frequency of APs (Fig. 3C). This low-pass filtering of signals may set constraints on the motor and behavioral performance of animals under rising temperatures.

### Synaptic transmission

Besides the branching points, synapses (both chemical and electrical) are thermally the weakest sites of nervous and motor systems (Krnjević and Miledi, 1959; Swadlow et al., 1980; White, 1983; Robertson and Money, 2012) (Fig. 3B). High temperatures have been shown to depress or cause complete failure of synaptic transmission in several invertebrates and vertebrates, including locusts (*Locusta migratoria*, *Schistocerca gregaria*), fruit fly (*Drosophila melanogaster*), crayfish (*Procambarus clarkii*), goldfish (*Carassius auratus*), frog (*Xenopus laevis*) and humans (Eusebi and Miledi, 1983; Burrows, 1989; Adams, 1989a; Dawson-Scully and Robertson, 1998; Heitler and Edwards, 1998; Barclay and Robertson, 2000). Depression of synaptic transmission is known to impair neuromuscular function in several invertebrate species, including locust (*L. migratoria*), fruit fly (*D. melanogaster*) and crayfish (*P. clarkii*); this is also observed in vertebrate ectotherms (e.g. *C. auratus*) and in humans (Friedlander et al., 1976; White, 1983; Karunanithi et al., 1999; Barclay and Robertson, 2000). Similar to all excitable membranes, ion leak of the postsynaptic membrane increases with warming (Heitler and Edwards, 1998). This may be one of the reasons why the amplitude of postsynaptic potentials decreases at high temperatures (Jensen, 1972; Macdonald and Montgomery, 1982). Even if the inward currents of the postsynaptic membrane were increased, they could be offset by the increased leak.

In the electrical synapses of invertebrates, the situation is analogous to the signal transmission between ventricular myocytes of the vertebrate heart. Charge is transferred directly, without the involvement of neurotransmitters, from the presynaptic neuron to the postsynaptic neuron or muscle. Experiments on the crayfish (*P. clarkii* and *Pacifastacus leniusculus*) neuromotor system have shown that synaptic transmission between the command nerve and the giant motoneuron fails at high temperature. In this system, the shortened presynaptic AP of the command nerve cannot provide enough depolarizing charge to the postsynaptic giant motoneuron, for which $R_{in}$ is lowered by high temperature (Heitler and Edwards, 1998). Consequently, the excitatory postsynaptic potential remains too small to depolarize the membrane to the $V_{th}$.

Results on human subjects indicate that a 2°C increase in core temperature or local heating of the leg muscles reduces voluntary muscle activity (Dewhurst et al., 2005; Racinais et al., 2008). The decrement of activity is explained by failures in synaptic transmission at the neuromuscular junction owing to shortening of the depolarization time and therefore reduced $Na^+$ entry through the muscle membrane, i.e. shortage of the source current (Rutkove, 2001; Racinais et al., 2008). Similar failures of synaptic transmission have been reported for *Drosophila* neuromuscular junction and mouse brain stem slices (Karunanithi et al., 1999; Kelty et al., 2002). Interestingly, the 2°C increase in body temperature in humans is associated with decreased memory capacity, which can be prevented by brain cooling (Racinais et al., 2008). These findings indicate that relatively mild hyperthermia (see Glossary) can weaken locomotor and learning skills at peripheral and central levels (i.e. thermal effects can appear as relatively subtle decrements in human/animal performance).

Collectively, these findings suggest that high temperature-induced increase in the sink current and/or decrease in the source current of either presynaptic or postsynaptic membrane can cause the failure of synaptic transmission.

### Significance of hyperkalemia

Warming may also reduce EE via increases in extracellular $K^+$ concentration ($[K^+]_o$, hyperkalemia), which can be substantial (2- to 3-fold) (Bowler, 1963; O'Sullivan et al., 2017). Hyperkalemia

reduces source current and increases sink current. Increased $[K^+]_o$ depolarizes $V_{rest}$ with a consequent reduction in the number of available $Na^+$ channels for opening (by steady-state inactivation); this depresses $I_{Na}$, reducing the rate of AP upstroke and conduction velocity (Nielsen and Gesser, 2001; Badr et al., 2018; Abramochkin et al., 2019). Increased $[K^+]_o$ may occur when APs are generated at high frequency, because of the $K^+$ efflux from the cell via the repolarizing $K^+$ currents (Frankenhaeuser and Hodgkin, 1956; Spira et al., 1976). Tight diffusion-restricted extracellular space between cardiac myocytes and between the axolemma and myelin sheath around axons promotes increases in $[K^+]_o$ (Frankenhaeuser and Hodgkin, 1956; Castel et al., 1976; Kline and Morad, 1978). Temperature- and frequency-dependent hyperkalemia stimulates outward $K^+$ currents, further aggravating the situation (Badr et al., 2018). Reductions in $I_{Na}$ and increases in outward $K^+$ current reduce the amplitude and duration of APs, which lowers the safety factor and may result in frequency limitation (low-pass filtering) or a complete conduction block of APs (Parnas et al., 1976). Indeed, depression of the rate of AP upstroke and shortening of AP duration by high $[K^+]_o$ has been documented for fish ventricular myocytes (Badr et al., 2018; Abramochkin et al., 2019).

## Conclusions and perspectives

The TDEE hypothesis is based on experiments on fish hearts (Vornanen, 2016). Here, the hypothesis is generalized to all excitable cells and tissues, including neurons, skeletal muscle fibers, cardiac myocytes and smooth muscle cells. The basic idea remains the same: high temperature affects inward currents ($I_{Na}$ and $I_{Ca}$) and outward $K^+$ currents ($I_K$ and $I_{Cl}$) disproportionally, and therefore $V_{rest}$ cannot be depolarized to $V_{th}$ at high temperatures, and excitation/conduction will fail (Vornanen, 2016). Because inward and outward currents of excitation may differ between cells and tissues, the central tenet of the hypothesis – the relationship between inward and outward currents – is now more generally expressed as a temperature-dependent mismatch between the depolarizing source current and the repolarizing sink current. Therefore, the hypothesis could be called the source–sink mismatch hypothesis (Fig. 5). Furthermore, in its current form, the hypothesis includes the concept of safety factor and indicates the thermally vulnerable tissue morphometries where the safety factor is low and may fall below the critical value of 1.0. According to the source–sink mismatch hypothesis, the function of electrically excitable cell membranes can be impaired at critically high temperatures. This agrees with Ken Bowler's notion that thermal limitation of animal life is a cellular membrane event (Bowler, 1981, 2018). However, the membrane malfunction may not require any cellular or molecular damage in order to compromise excitability: denaturation of ion channel proteins or damage to the lipid membrane need not occur. Instead, the impairment of EE occurs as an outcome of its molecular properties, which evolved for fast impulse transmission. High-frequency impulse transmission requires a large and fast inward charge transfer and rapid recovery of ion conductance after excitation. To this end, the voltage-gated $Na^+$ channels evolved from the $Ca^{2+}$ channels (Liebeskind et al., 2011). They provide a large, non-toxic and transient surge of inward charge without interfering with intracellular $Ca^{2+}$ signaling (Nishino and Okamura, 2017). $Na^+$ channels respond to membrane depolarization by almost instantaneous opening, followed by fast inactivation that terminates the $Na^+$ influx (Mangold et al., 2017). This on–off behavior of voltage-gated $Na^+$ channels underlies the coding of neuronal information and muscle function. Although the fast gating process is necessary for high-frequency neural coding, this ultimately results in temperature-dependent failure of excitation, as charge influx decreases with increasing temperature. Indeed, experiments on mouse pain receptors have shown that $Na^+$ channels can operate at high temperatures (>46°C), if their kinetics are sufficiently slow. In the pain receptors this is possible because the relevant $Na^+$ channel isoforms, $Na_V1.8$ and $Na_V1.9$, have very slow activation and inactivation kinetics (Touska et al., 2018).

The function of neuronal networks and smooth/skeletal/cardiac muscles are based on electrically excitable cells. If excitability of these tissues is threatened by high temperatures, is one of the tissues more likely to fail earlier than the other, or will they concurrently lose their function? Experiments on fish show that the functionally specialized parts of the heart are not equally sensitive to temperature (see 'Heat-induced ventricular failure by source–sink mismatch'). A similar principle also applies to different parts of the neuromuscular system (Hodgkin, 1951; Orr, 1955b; Grainger, 1973). According to the source–sink mismatch hypothesis, the sensitivity of tissues to heat depends on the ion channel composition (source/sink channels), the structural complexity of tissues (branching) and the magnitude of the safety factor (surplus of the inward current). In addition, animals can defend tissues against high temperature by triggering various defenses (e.g. neuromodulation, heat shock response, differential gene expression) at different time scales (Ramirez et al., 1999; Haverinen and Vornanen, 2004; Money et al., 2005; Robertson and Money, 2012; Städele et al., 2015). Finally, in the light of this hypothesis, it is easy to see why the effects of temperature on vital functions and animal performance are gradual. At lower temperatures, dysfunction may be mild, and onset may be slow and difficult to detect (e.g. impairment of memory, learning and motor functions). At higher temperatures, the effects are faster and more intense, and they can quickly lead to death (e.g. cessation of heartbeat, loss of consciousness/equilibrium).

The source–sink mismatch hypothesis does not exclude any alternative hypotheses. Indeed, it is unlikely that there would be a single body function or process that limits the thermal tolerance of an animal. The limiting mechanism may differ depending on stage of ontogenetic development, phase of the life cycle, time scale of the temperature change or plasticity of the species' genomics (Orr, 1955a,b; Hollingsworth and Bowler, 1966; Davison, 1969; Irvin, 1974; Gracey et al., 2004; Vornanen et al., 2005). Practically all organs, tissues, cells and macromolecules are adapted to some extent to function properly in the thermal habitat of the animal: energy production by mitochondria, the structure of membrane lipids, the activity of enzymes and the oxidative stress response of cells may all be compromised under thermal stress. However, if an integrative model of thermal tolerance/failure, consisting of several body processes, is to be constructed (MacMillan, 2019), temperature dependence of EE should be an integral part of it.

The principles of the source–sink mismatch hypothesis should be valid for all multicellular animals, only excluding sponges (phylum Porifera) and placozoans (phylum Placozoa), which do not have excitable cells similar to the neurons and muscle fibers of other metazoans (Liebeskind, 2011). The hypothesis does not make any distinction between ectotherms and endotherms or between aquatic and terrestrial animals. Excitable cells are vital constituents of most tissues and organs and are needed for practically all basic functions of life, including sensation, locomotion, circulation, digestion and reproduction. Body homeostasis, learning and behavior are critically dependent on the function of excitable cells. In principle, each of these functions could be impaired by high temperature owing to the source–sink mismatch. The objective of outlining the source–sink hypothesis here is to stimulate research on this

fundamental and timely topic of thermal physiology; in the future I hope to see more studies of thermal physiology from the perspective of EE. This would likely deepen our understanding of the effects of climate warming on animal life and motivate measures to mitigate its negative effects.

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