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EDITORIAL

Neurocardiology: a neurobiologist's perspective

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Pre- and postganglionic neurones of the autonomic nervous system are functionally differentiated according to their target tissues. Individual sympathetic pre- and postganglionic neurones can be activated or inhibited reflexly by appropriate physiological stimuli as has been shown in anaesthetized animals (mainly cats and rats) for neurones of the lumbar sympathetic outflow to skeletal muscle, skin and pelvic viscera and for neurones of the thoracic sympathetic outflow to the head and neck (Jänig, 2006; Jänig & McLachlan, 2013) and in humans for postganglionic neurones projecting in muscle or skin nerves (Jänig & Häbler, 2003; Wallin, 2013). The reflexes correspond to the effector responses that are induced by changes in activity of these neurones. The reflex patterns are characteristic and therefore represent the physiological 'fingerprints' for each type of sympathetic pathway. They are the functional expression of the neural circuits in spinal cord, brain stem and hypothalamus connected to the peripheral sympathetic pathways.

The same types of reflex patterns have been observed in both preganglionic and postganglionic neurones. The neurones in most of these pathways (e.g. the vasoconstrictor, sudomotor, motility-regulating pathways, and other pathways) have ongoing activity whereas the neurones in other pathways are normally silent in anaesthetized animals (e.g. pilomotor and vasodilator pathways, pathways to sexual organs). It is likely that other target cells are similarly innervated by functionally distinct groups of sympathetic neurones that have not been systematically studied so far. These include heart (pacemaker, myocytes, coronary arteries), kidney (blood vessels, juxtaglomerular cells, tubules), urogenital tract, hindgut, spleen, and brown and white adipose tissue (Verberne & Sartor, 2010; Morrison, 2011, 2013).

Several systematic studies have been made on the functional properties of parasympathetic pre- and postganglionic neurones. The principle of organization into functionally discrete pathways is likely to be the same as in the sympathetic nervous system, the main difference being that some targets of the sympathetic system are widely distributed throughout the body (e.g. blood vessels, sweat glands, erector pili muscles, fat tissue) whereas the targets of the parasympathetic pathways are more restricted (Jänig, 2006; Jänig & McLachlan, 2013).

The centrally generated signals are faithfully transmitted from the preganglionic neurones to the postganglionic neurones in the autonomic ganglia and from the postganglionic neurones to the effector tissues at the neuroeffector junctions or at the sites where the varicosities of the postganglionic axons are in close proximity with the tissue (e.g. non-excitable tissues). This signal transmission is function-specific and the basis for the precise regulation of autonomic effector tissues by the brain.

A few effector tissues also contain intrinsic neuronal networks that include the components for reflex control within the periphery. The most prominent is the enteric nervous system, which contains afferent neurones, interneurones and motoneurones that regulate various target tissues (smooth musculature, secretory epithelia, endocrine cells) and even modulate postganglionic sympathetic neurones in prevertebral ganglia. The preganglionic parasympathetic and sympathetic pathways regulate the activity of these intrinsic pathways in an integrative manner to determine organ function. Some peripheral afferents from the visceral organs project as far as prevertebral sympathetic ganglia and amplify postganglionic activity (e.g. Szurszewski, 1981; Jänig & McLachlan, 1987; McLachlan & Meckler, 1989). Peripheral neuronal reflex systems have been identified in the heart and pancreas, and possibly other organs. However, other than the important control of peristalsis and secretion in the gut (Furness, 2006; Furness *et al.* 2014), the operation of these peripheral reflex pathways in determining organ function remains largely unknown and needs further investigation.

The conclusions to be drawn from these neurophysiological studies are important for the discussion of the neural regulation of the heart (Jänig, 2006; Jänig & McLachlan, 2013):

(1) The peripheral autonomic (parasympathetic and sympathetic) systems consist of several separate neuronal channels transmitting the central messages to the autonomic target tissues. This conclusion is supported by morphological studies using tracers and by studies of neuropeptides co-localized in postganglionic and preganglionic neurones with the classical transmitters acetylcholine or noradrenaline (Gibbins, 1995, 2004). Peripheral circuits may also modulate the central activity patterns related to some visceral organs.
(2) The distinct reflex patterns generated in autonomic neurones by physiological stimulation of afferent neurones innervating visceral, skin or deep somatic tissues indicate that each autonomic pathway is connected to specific neural circuits in spinal cord, brain stem and hypothalamus that are involved in autonomic regulation.
(3) We have some knowledge about the central circuits involved in cardiovascular regulation and thermoregulation. However, the central circuits, including the spinal ones, are largely unknown for most peripheral final autonomic pathways (see Jänig, 2006; Llewellyn-Smith & Verberne, 2011; Paton & Spyer, 2013).

The brain–heart axis

The brain–heart axis is involved in the neural regulation of the heart via the autonomic cardiomotor (CM) pathways. This axis was already present in vertebrates some 500 million years ago in elasmobranch and teleost fishes. The heart of elasmobranchs is under parasympathetic (vagal) CM control and the heart of teleosts under both parasympathetic and sympathetic CM control (Nilsson, 2011; Jänig, 2013). This shows that the regulation of the heart by the brain is phylogenetically old, as is the case for the foregut, but probably went through various changes in the central and peripheral nervous system, although

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the basic principles of the organization of the peripheral CM pathways remained the same throughout the vertebrate kingdom (transmitters and their receptors, excitatory effects, inhibitory effects, etc.). This shows that the brain–heart axis is biologically rather important. However, our knowledge about the mechanisms underlying the functioning of this axis under biological conditions remains still amazingly poor in view of the fact that the pathophysiology of the neural regulation of the heart and its consequences for therapy has a high representation in medicine. Knowledge about these peripheral and central mechanisms is the gate to understanding the pathophysiology underlying various diseases related to the heart in humans (Floras, 2012; Golombek, 2012; Robertson & Sato, 2012; Bajpai & Camm, 2013; Francis & Cohn, 2013; Hainsworth & Claydon, 2013; Samuels, 2013). Thus, research in neurocardiology should primarily focus on the neurobiology of the regulation of the heart, covering the field from neuroeffector transmission to telencephalic control of the heart.

Here I will discuss research questions related to the brain–heart axis. This discussion is based on my personal opinion and does not imply any priority of the type of basic research involved and of the level of integration at which the neural control of the heart occurs. However, it should be kept in mind that the 'neural machineries' of the peripheral CM pathways (neuroeffector transmission, transmission and integration of impulse activity in cardiac and possibly stellate ganglia) are fully integrated in the central integrative processes, so that the peripheral neural machineries are 'used' by the brain as tools to regulate the functioning of the heart.

Neuroeffector transmission (1 in Fig. 1)

To unravel the transmission of the neural signals in the postganglionic cardiomotor (CM) neurones to the effector cells (cells of the sinoatrial node (SAN) and the atrioventricular node (AVN), myocytes, coronary vascular cells) is of utmost importance. This transmission of neural signals occurs at rather small areas of the surface of the target cell syncytia (<0.5%). The idea that exogenous application of transmitter (acetylcholine, noradrenaline, neuropeptides) to a preparation (e.g. blood vessels, heart) mimics the effect of neurally released transmitter is a misconception. Therefore, it is not far-fetched to assume that the intracellular pathways connecting junctional and extrajunctional membrane receptors for acetylcholine or noradrenaline with the cellular effector mechanisms

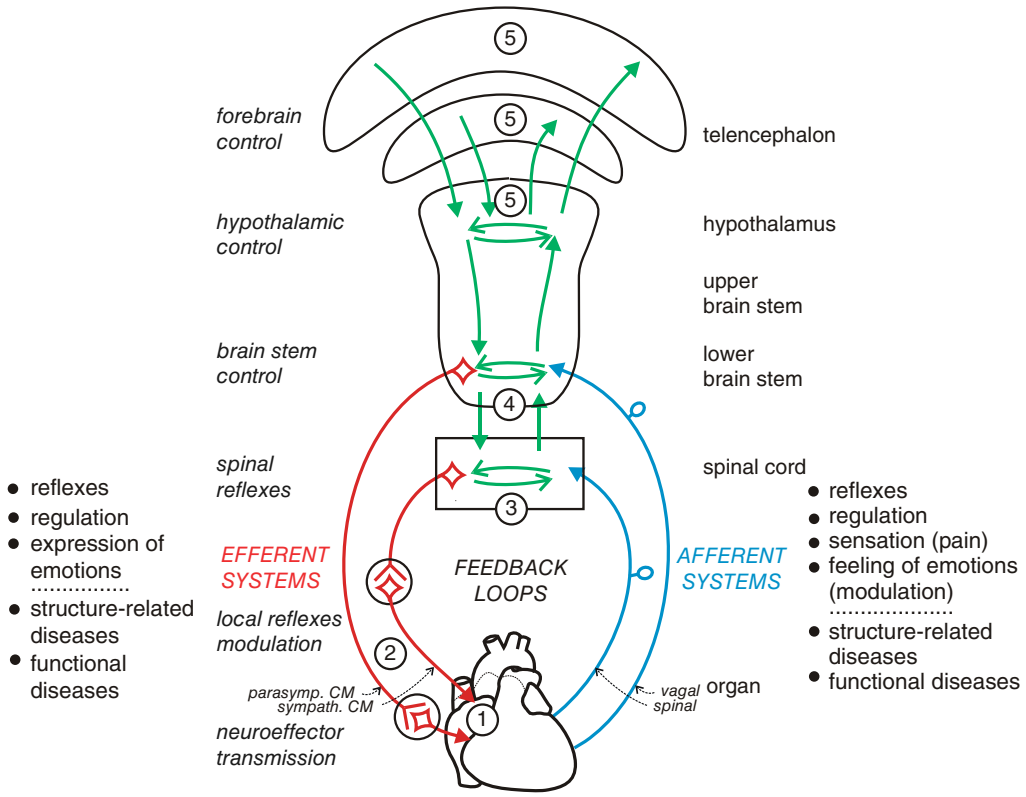

Figure 1. Schematic figure showing the neural regulation of the heart: levels of integration and afferent feedback (spinal, vagal)
Possible extracentral feedback loops between intrinsic primary afferent neurones and local circuit neurones of the intrinsic cardiac ganglionated plexus or extrinsic spinal cardiac afferent neurones and postganglionic neurones in the stellate ganglion have not been included (see Ardell *et al*. 2016; Rajendran *et al*. 2016). 1, neuroeffector transmission. 2, transmission and integration in autonomic ganglia. 3, integration in spinal cord. 4, integration in lower brain stem. 5, integration in hypothalamus and telencephalon. Pathophysiological changes in the heart result in structure-related cardiac diseases, including functional and structural neural remodelling. Changes in neural regulation of the heart by the brain may produce functional cardiac diseases mediated by the autonomic innervation. CM, cardiomotor.

(ionic channels, contractile machinery) are different for the two groups of receptors. The function(s) of the extrajunctional receptors under physiological conditions is (are) largely unclear. However, this may radically change under pathophysiological conditions, as far as the functions of the extrajunctional receptors are concerned (Jänig, 2011). The experimental work of Hirst and co-workers (Bywater *et al.* 1989, 1990; Campbell *et al.* 1989; Bramich *et al.* 1990; Klemm *et al.* 1992; Choate *et al.* 1993; Edwards *et al.* 1993, 1995; Hirst *et al.* 1991, 1996) and others has to be reproduced and extended. Clarification of the neurobiology will lead to a better understanding of the pathophysiology (e.g. after myocardial infarction (Rajendran *et al.* 2016), atrial and ventricular fibrillation, myocardial ischaemia, sudden cardiac death, ventricular tachycardia, in diabetic neuropathy) and to new mechanism-based therapeutical strategies. Neurobiological research (e.g. *in vitro* experimentation as well as quasi *in vivo* experimentation using the working heart–brain stem preparation (Paton, 1996) and associated techniques) should concentrate on the following topics:

- Neuroeffector transmission to the SAN cell syncytium (parasympathetic, sympathetic) and to cardiac myocytes (sympathetic).
- Role of junctional and extrajunctional cholinergic muscarinic receptors and adrenoreceptors and their connections via intracellular pathways to the cellular effectors (Ca^{2+}, Na^{+}, K^{+} channels, contractile mechanism).
- Role of neuropeptides and their receptors in neuroeffector transmission to the heart.
- Postganglionic parasympathetic CM neurones do not only innervate atria, SAN and AVN but also the ventricles. The parasympathetic innervation of the cardiac ventricles may act on the myocytes either directly or via the sympathetic innervation or via interaction with neurones of the intrinsic cardiac plexus (Coote, 2013). How dense is this parasympathetic innervation of the ventricles compared to the atria? Quantitative immune histochemical studies of atria and ventricles have to be done on the same sections using tyrosine hydroxylase (as a noradrenergic marker) and vesicular acetylcholine transporter (as a cholinergic marker).
- The long-held story about the physiological interaction between sympathetic postganglionic CM axons and parasympathetic postganglionic CM axons, based on pharmacological investigations, is largely open and unclear (Levy, 1984; Manabe *et al.* 1991). This interaction involves acetylcholine and prejunctional muscarinic receptors in sympathetic terminals and possibly neuropeptides. A better understanding of such interactions could provide novel targets for treatment in postinfarct ventricular tachycardia, such as pharmacological stimulation of the parasympathetic axons (Coote, 2013).

Transmission of activity in cardiac ganglia (2 in Fig. 1)

The cardiac ganglia have not been studied extensively, the main reason being that these studies are technically demanding. It is silently assumed [with some exceptions, Edwards *et al.* 1995; Armour, 2008; Ardell *et al.* 2016 (in this issue)] that transmission of activity through these ganglia follows the simple rules of relay and distribution of activity. However, this may not be true.

- What are the functions of 60% of neurones in parasympathetic ganglia that cannot be activated by preganglionic CM neurones and are suspected to be local afferent neurones or local motoneurones without preganglionic synaptic input (based on *in vivo* and *in vitro* experimentation and on experiments using the working heart–brain stem preparation (WHBP); Edwards *et al.* 1995; Jänig, 2006, 2011; McAllen *et al.* 2011)? Are these neurones functionally unimportant under normal conditions but play a role in the preganglionically denervated heart? Based on the available data, we plainly cannot know whether integrative processes in the parasympathetic ganglia are important under physiological or pathophysiological conditions. Would activity in the parasympathetic ganglia be expected to counter-regulate the influence of sympathetic nerves on the injured heart? Could a dysregulation in parasympathetic ganglia play a role in the detrimental effect of sympathetic excitation on the injured heart (Ardell *et al.* 2016; Rajendran *et al.* 2016)? One postganglionic parasympathetic CM neurone is innervated and driven by *one* strong preganglionic parasympathetic input. Can this safe synaptic transmission be modulated by intraganglionic synaptic inputs (McAllen *et al.* 2011)?
- What is the nature of synaptic transmission from sympathetic preganglionic CM neurones to sympathetic postganglionic CM neurones in the stellate ganglion and in the superior cervical ganglion? The activity of the postganglionic CM neurones seem to be dominated by one or a few strong synaptic inputs (E. M. McLachlan & L. V. Melnitchenko, unpublished observations) as is the case in ganglia of the sympathetic chain (Lichtman *et al.* 1980; McLachlan *et al.* 1997, 1998; Bratton *et al.* 2010). In the stellate ganglion cardiac and non-cardiac neurones are not different in morphology or electrophysiological properties (Mo *et al.* 1994).
- How are synaptic connectivity and strength within cardiac ganglia set and adjusted under physiological and pathophysiological situations (Rajendran *et al.* 2016)? What is the feedback that is used in this process, and where and how is this feedback registered?
- What is the nature of putative local afferent neurones or interneurones in the stellate ganglion? It is important to show whether integration based on local excitatory neurones principally exists (Bosnjak & Kampine, 1982, 1989), whether it is functionally important during normal neural regulation of the heart, or whether it is some sort of developmental left-over. Do peptidergic spinal afferent neurones innervating the heart form synapses by collaterals with sympathetic postganglionic neurones innervating the heart? Under which physiological or pathophysiological conditions is this peptidergic synaptic transmission functioning?
- Which data support the interesting concept of the 'intrinsic cardiac nervous system' (ICNS) ('little brain of the heart') as propagated by Armour and co-workers (Armour, 2008; Beaumont *et al.* 2013; Rajendran *et al.* 2016; Ardell *et al.* 2016) and

others? Is the ICNS functioning in a similar manner to the enteric nervous system (ENS) (Furness, 2006; Furness *et al.* 2014)? Is there evidence for intrinsic reflexes mediated by the ICNS in analogy to the ENS? Do intrinsic afferent neurones form peripheral feedback loops with postganglionic cardiomotor neurones?

- Clinical and experimental evidence suggest that neurones of the ICNS undergo plastic changes (described by the concept of 'structural and functional remodelling') under pathophysiological conditions (Rajendran *et al.* 2016). Changes of the cardiac target tissue of the cardiomotor neurones under pathophysiological conditions may lead to plastic changes of the autonomic innervation of the heart. Thus, the intrinsic afferent, the intrinsic efferent and the preganglionic neurones may start to sprout and form new synapses (Kepper & Keast, 1998; Keast, 2004).

Sympathetic preganglionic CM neurones, spinal afferent feedback and spinal integration (3 in Fig. 1)

Do functionally separate sympathetic CM pathways exist, e.g. to the SAN or AVN and to the myocytes? Is the sympathetic pathway to the coronary arteries (cardiovasoconstrictor neurones) separate from the sympathetic pathway(s) to the SAN, AVN or myocytes? If functionally separate sympathetic pathways to the heart do exist this should be reflected in functionally characteristic discharge patterns as functional markers for different populations of sympathetic neurones supplying the heart (Jänig, 2006; Jänig & McLachlan, 2013). This neurophysiological work should be combined with morphological work (intracellular labelling of functionally identified postganglionic neurones with reconstruction of their dendrites and axons). Intracellular recording from postganglionic neurones using the WHBP with attached spinal cord (McAllen *et al.* 2011) and *in vitro* experimentation using the SAN and other parts of the heart with attached nerves should be done.

Which sympathetic neuronal subtype mediates the detrimental effects on the heart in pathophysiological conditions such as atrial or ventricular fibrillation, myocardial ischaemia, myocardial infarction, sudden cardiac death and the like? Is it possible that the changes in the coronary innervation regulating blood flow is important in these pathological conditions?

The heart is innervated by spinal visceral afferent neurones which are involved in cardiac nociception and pain as well in the regulation of cardiac output during body exercise. It is hypothesized that sympathetic cardiomotor neurones, spinal visceral afferent cardiac neurones and spinal cord circuits, including excitatory and inhibitory interneurones, form cardio-cardiac reflexes. These reflex circuits are normally involved in adaptation of cardiac output during exercise but may form, under pathophysiologically deleterious conditions, a positive feedback loop with the heart. Which types of sympathetic CM neurones are involved in these reflexes? Which classes of spinal interneurones are involved (Deuchars, 2011)?

- Muscle vasoconstrictor neurones are inhibited by stimulation of muscle nociceptors of the same extremity but not by stimulation of cutaneous nociceptors. Cutaneous vasoconstrictor neurones are inhibited by stimulation of cutaneous nociceptors of the same extremity but not by stimulation of muscle nociceptors (Kirillova-Woytke *et al.* 2014). These protective inhibitory nociceptive reflexes are most likely organized at the level of the spinal cord and under powerful supraspinal control. Do these types of protective nociceptive reflexes exist for the heart (e.g. in the vasoconstrictor neurones innervating the coronary blood vessels)?
- What are the functions and underlying mechanisms of the descending control of spinal cardiac circuits involving brain stem, hypothalamus and telencephalon? This important question should also be studied in humans using brain imaging of grey and white matter changes under resting state activity and under cardiac load.
- What are the mechanisms underlying the referral of cardiac diseases (e.g. coronary disease) to deep somatic tissues (myotomes, sclerotomes), superficial somatic tissues (skin, subcutaneous zone, dermatomes) and other visceral organs (oesophagus, upper gastrointestinal tract (GIT), airways) involving spinal circuits and their supraspinal control (King *et al.* 2011)?
- Can functional cardiac diseases be diagnosed by manual palpation (and other procedures) of the deep somatic and superficial referred zone of the heart and discriminated from functional diseases of other visceral organs innervated by the same spinal (thoracic) segments (e.g. lungs and airways, lower oesophagus, stomach and duodenum, pancreas and liver) as propagated in the field of osteopathic medicine (Cox *et al.* 1983; Beal, 1985; Nicholas *et al.* 1985)? Brain imaging approaches should be used to identify abnormal central processing in functional heart diseases. Data obtained in this way should be correlated with quantitative data describing the changes in the referred body zones (changes of blood flow, sweating, tissue consistency (trophic changes)) (Jänig *et al.* 2015).
- Can functional diseases of the heart be treated by manual interventions and other interventions in the referred superficial and deep somatic zone of the heart as propagated in osteopathic medicine and other subdisciplines of manual medicine? This idea is based on the concept (philosophy) that there exist special somato-visceral and viscero-somatic neural relations between visceral organs and somatic tissues (deep somatic, body surface) involving spinal circuits and their control by supraspinal centres (Jänig, 2006; King *et al.* 2011).
- What are the interactions between different visceral organs (heart, lungs, airways, GIT) via the spinal circuits, leading to referred viscero-visceral hyperalgesia and referred functional changes involving spinal circuits, sympathetic outflows and afferent feedback (Giamberardino *et al.* 2010; Jänig, 2010)?

Parasympathetic preganglionic cardiomotor neurones (4 in Fig. 1)

- Are the parasympathetic preganglionic CM neurones in the nucleus ambiguus functionally uniform?
- What are the types and organization of reflex arcs and their modulation by supramedullary centres connected to the parasympathetic preganglionic CM neurones?
- What are the functions of the parasympathetic preganglionic CM neurones with unmyelinated fibres located in the lateral part of the dorsal motor nucleus of the vagus? These preganglionic neurones are normally silent or exhibit irregular non-respiration-related discharges. They appear to be unaffected by stimulation of arterial baro- or chemoreceptors (Jones, 2001). Recent experiments on rats demonstrate that these preganglionic neurones may be involved in the ventricular excitability via a nitrergic mechanism (Machhada *et al.* 2015). However, evidence that these parasympathetic neurones innervate the cardiac ventricles is lacking (but see Coote, 2013).
- What is the evidence for parasympathetic vasodilator neurones supplying the coronary arteries of the heart? Investigation of the vasculature of the rat heart using immunohistochemical techniques shows a much denser innervation of atrial than ventricular coronary arteries by noradrenergic and cholinergic efferent nerve fibres, whereas nitrergic fibres are sparse. Peptidergic sensory nerve fibres are uniformly distributed to atrial and ventricular vessels (Schäfer *et al.* 1998; Sequeira *et al.* 2005).
- How do reflex arcs in the lower brain stem connect to parasympathetic CM pathways, to preganglionic neurones innervating airways (Canning, 2011), to preganglionic neurones innervating the proximal GIT (including liver; Jänig, 2006; Travagli *et al.* 2006) interact? These reflex arcs include the second-order neurones in the nucleus of the solitary tract (NTS), the nucleus ambiguus and the dorsal motor nucleus of the vagus, and their multiple supramedullary controls (Jänig, 2006; Andresen & Paton, 2011; Jordan, 2011), and are closely associated with the respiratory ponto-medullary network (Spyer & Gourine, 2009; Guyenet, 2014; Guyenet & Bayliss, 2015).

Cardio-respiratory integration and its control (4 in Fig. 1)

Neural regulation of the cardiovascular system (blood vessels, heart) and of respiration are closely integrated in the lower brain stem (pons, medulla oblongata) (Jänig, 2006; Guyenet, 2011; Paton & Pickering, 2012).

- Knowledge of neural cardio-respiratory integration is still in its infancy. Unravelling this integration in the lower brain stem (medulla oblongata and pons) is essential for understanding the neural regulation of both systems under various functional conditions (Dampney, 1994; Jänig, 2006; Spyer & Gourine, 2009; Guyenet, 2011, 2014) and may give us a better lead to the so-called cardioprotective effects of the activity in parasympathetic cardiomotor neurones.
- What are the mechanisms underlying the integration between the respiratory neural network and the preganglionic CM neurones (Jänig, 2006; Paton & Pickering, 2012)?
- How are the reflex circuits related to parasympathetic CM neurones (baroreceptor reflexes, peripheral chemoreceptor reflexes, central chemoreceptor reflexes, trigeminal reflexes, nociceptor reflexes, other) and the respiratory network integrated under various functional conditions (e.g. diving, exercise, mental activity) (Guyenet, 2011, 2014; Paton & Spyer, 2013)? Are these reflex circuits affected by cardiac pathology?
- Neuroanatomical and neurophysiological experiments show that centres in the lower brain stem that are involved in cardiovascular regulation, such as the NTS, the rostral ventrolateral medulla and the caudal ventrolateral medulla, are also influenced by the vestibular system (in particular the vestibular otolith organs). This influence serves to adjust arterial blood pressure and organ blood flow during movement and changes in posture (Yates *et al.* 2014). Mechanisms underlying the regulation of the heart via parasympathetic and sympathetic CM neurones during these adjustments are almost unknown.
- Regulation of heart, blood flow through resistance vessels of skeletal muscle and viscera, and blood flow through skin are under powerful cortical control, as shown during diving, exercise and other behavioural conditions (Elsner *et al.* 1966; Goodwin *et al.* 1972; Casson & Ronald, 1975; Gandevia *et al.* 1993). Where in the ponto-medullary cardio-vascular-respiratory network do the cortical command signals interfere to adjust the cardiovascular system during these behaviours? Knowing the neural mechanisms underlying this coupling would give us a better lead to the neural control of the heart by the forebrain under pathophysiological conditions.

Heart, interoception and emotions (5 in Fig. 1)

In humans and higher vertebrates, expression of emotions signals the state of behaviour to conspecifics, being involved in the regulation of social behaviour, whereas the feeling of emotions is involved in internal signalling and regulation of their own behaviour. The generation and modulation of the emotions are believed to be dependent on the state of the body tissues, in particular visceral organs and deep somatic tissues (e.g. skeletal muscle), and therefore on the functions and on the spatio-temporal patterns of the activity in the afferent feedback from these body tissues to their central representations. The afferent neurones have small-diameter myelinated or unmyelinated axons and encode in their activity the mechanical, thermal, metabolic and inflammatory states of the body tissues. They project to lamina I of the spinal or trigeminal dorsal horn (spinal/trigeminal afferent neurones, Craig, 2003*b*) or to the NTS (vagal afferent neurones). Both lamina I and NTS are the interoceptive interface between body tissues and brain. Their tract neurones project, in addition to various nuclei in brain stem and hypothalamus, via specific thalamic nuclei (the posterior part of the ventromedial nucleus (VMpo) for the lamina I tract neurones and the basal part of the ventromedial nucleus (VMb) for the tract neurones in the NTS) to the dorsal posterior

insular cortex. This cortex is according to Craig the primary interoceptive cortex or limbic sensory cortex (Craig, 2003*a*,*b*, 2015, 2016).

Activity in these afferent feedback systems is also or mainly dependent on the activity in the efferent (autonomic, somatomotor) systems innervating the body tissues. Thus, efferent systems, body tissues and afferent interoceptive feedback form body loops which are hypothesized to be essential in the development (after birth), generation, maintenance and modulation of emotional expression as well as emotional feelings (James, 1884; Damasio, 1999, 2003). What is fact and what fiction?

The heart is traditionally believed to play an important role in body interoception [see Shivkumar *et al.* 2016 (in this issue)] as well as in the generation or shaping of emotions. The efferent limbs of the body loop involving the heart are formed by the parasympathetic and sympathetic cardiomotor neurones. The afferent limbs are formed by arterial baroreceptor afferent neurones and cardiac vagal afferent neurones, both projecting to the NTS, as well as spinal cardiac afferent neurones projecting to the dorsal horn of the thoracic spinal cord.

- How important is the efferent–afferent body loop involving the heart to shape and/or generate emotional feelings in comparison to potential loops involving other visceral organs or somatic tissue (e.g. gastrointestinal tract, pelvic organs, skeletal musculature)? This question should be explored carefully using imaging methods focusing on spinal cord, brain stem and forebrain combined with psychophysics and other methods. After all it should be kept in mind (1) that the afferent feedback from different tissues is functionally different, (2) that the peripheral target tissues are innervated by many functionally different peripheral autonomic pathways, which is reflected in the distinct discharge patterns of their neurones, and dependent on the distinct central organization of the autonomic systems, and (3) that the activity in the afferent feedback is also or mainly dependent on the activity in the peripheral autonomic pathways.
- Various cortical and subcortical areas of the forebrain (e.g. insular cortex, anterior cingulate cortex, medial and ventral prefrontal cortex, amygdala) are involved, via autonomic 'centres' in hypothalamus and brain stem, in autonomic regulation of the heart and in the processing of the afferent feedback from the heart. What are the mechanisms underlying this forebrain control?
- Experimental work addressing the role of the heart in shaping and generating emotions will be conducted on the basis of the James–Lange–Damasio theory of emotions (James, 1884; Damasio, 1999, 2003). This theory suggests that the efferent–afferent body loop via the heart, as well as the corresponding centrally organized 'as if' loop that is vicarious for the real body loop, to the central representations of the body tissues (Damasio, 1996) are essential to generate emotional feelings. Based on their experimental investigations of various groups of human subjects Ekman and co-workers have shown that the basic emotions are characterized by distinct autonomic patterns (expressed as change in heart rate, change in skin conductance, change in skin temperature). Thus, subjective emotional feelings, expression of emotions mediated by the somatomotor system (facial expression) and activation of autonomic systems are highly correlated. They conclude that 'there is an innate affect program for each emotion that once activated directs for each emotion changes in the organism's biological state by providing instructions to multiple response systems, including facial skeletal muscles, other skeletal muscles and autonomic nervous system [to various effectors, one being the heart]' (Levenson *et al.* 1990, 1991; Ekman, 1992).
- Research on the autonomic body loop (real and 'as if') in human subjects should be performed using modern imaging techniques combined with measurements of autonomic parameters, psychophysical parameters, etc. Independent of the biological importance of this basic research (we learn who we are), this experimental approach will suggest leads to various functional diseases of the heart (e.g. related to the effect of bodily and psychological stress on the heart). Therefore experimental work on this topic should have a high priority (Emani & Binkley, 2010; Taggart *et al.* 2011; Garfinkel & Critchley, 2016).

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Additional information

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