

Introduction

Brainstem: neural networks vital for life

The brainstem is critically important for the survival of an animal. It lies between higher centres and the spinal cord and constitutes one of the oldest parts of our brains. Respiratory and cardiovascular systems fail and death ensues after circumscribed injury to the brainstem. Its neurons create the rhythm of inspiration and expiration, which starts *in utero*, and continues until the moment of death. How do brainstem circuits provide commands that are so inexorable that you cannot decide to commit suicide by holding your breath (although you can do so by fasting)? The brainstem also regulates blood pressure, second by second, to ensure that the blood supply to the cerebral cortex is always adequate, for example, in the face of gravity, as you get up from bed, or during strenuous exercise, when much of the blood flow is diverted to skeletal muscles. In spite of its role in these and many other essential bodily functions, the study of the brainstem has been neglected compared with research made on structures such as the cortex, hippocampus, cerebellum, retina, basal ganglia and spinal cord.

1. WHY HAS THE BRAINSTEM BEEN NEGLECTED?

What we mean by saying that the brainstem has been ‘neglected’ until recently is shown by the following example. The standard textbook of neuroscience comprehensively and elegantly covers functions of the nervous system from the molecular level to perception, memory and linguistics. Yet in all the 1568 pages, there is not one sentence about how the respiratory rhythm is generated and controlled by the brainstem. The relatively low profile of brainstem studies can be attributed to three main factors.

First, higher functions of the brain attract incoming students as a prime goal for scientific study. It is quite natural for a curious person to be drawn to the study of memory and learning, perception and motor coordination. How fascinating, for example, to unravel messages that travel from eye to cortex, from cortex to muscles during a voluntary movement, such as hitting a tennis ball. How does the brain generate and integrate signals that enable the player to calculate exactly where the ball, coming over the net at over 100 km h^{-1} , will hit the ground, how high it will bounce and which muscles must contract or relax to drive the ball into the far corner, all this in a few milliseconds? At the same time, however, it is the brainstem that must

provide continuous unflinching, second-by-second modulation of respiration, heart rate and blood pressure. If the brainstem fails, the player will fall to the ground, unconscious. Surely the way in which this rapid response to different bodily needs is achieved is also a fascinating subject for study.

A second reason for neglect is that, until recently, the brainstem was hard to approach experimentally. It lies in a relatively inaccessible region of the nervous system; worse still, the structure, consisting of an apparently diffuse, reticular network, is devoid of useful landmarks such as layers, columns or distinct cell types in recognizable groupings. Inevitably, the complex circuits that regulate respiration, heartbeat and blood pressure are extremely hard to unravel.

A third reason arises from the history of ‘Neurobiology’ as a discipline. In the early 1960s, Stephen Kuffler, who headed a group of neurobiologists in the Department of Pharmacology at Harvard, decided that it was an anachronism that neurophysiologists should be working in physiology departments, separated from neuroanatomists in anatomy departments, and neurochemists in biochemistry departments (and later, molecular biologists and computational neuroscientists). All of them were interested in how the nervous system works but they never, or extremely rarely, worked together. Much better, Kuffler thought, would be to bring people from different disciplines together in a single new department so that problems rather than techniques would define the scope of the research. Accordingly, he created the first Department of Neurobiology, and this example was then followed by the formation of new departments and neuroscience training courses in most medical schools and universities throughout the USA and Europe. A predictable result was that physiology departments lost most of their scientists concerned with the physiology of the nervous system. In many departments of physiology, the neurobiologists who remained were concerned with respiratory and cardiovascular systems (which they had to teach), rather than somatic or higher cognitive functions. While the separation was not complete, control of circulation and respiration tended to be represented at meetings of physiological societies and in physiology textbooks rather than at neuroscience societies.

2. WHAT MADE THE PRESENT TIME PARTICULARLY APPROPRIATE FOR THIS ROYAL SOCIETY DISCUSSION MEETING?

In the past few years, knowledge of brainstem circuitry has advanced greatly and it has become possible to

One contribution of 17 to a Discussion Meeting Issue ‘Brainstem neural networks vital for life’.

investigate problems that previously seemed unapproachable, thanks to major technical advances. These include transgenic and molecular biological approaches, electrical and optical recording techniques and computational methods. Accordingly, the papers in this issue of *Philosophical Transactions of the Royal Society* describe new types of experiments made to understand how the brainstem performs its functions under normal conditions and also in conditions that give rise to devastating but common clinical disorders.

3. AIMS OF THIS DISCUSSION MEETING

One major objective of this Royal Society Discussion Meeting was to correct an obvious imbalance in the way in which people, especially students entering the field, think about the nervous system. Vast numbers of nerve cells in the brain are *not* concerned with learning, memory or perception: they regulate the gut, the circulation, body temperature, airway resistance, respiration and other homeostatic tasks necessary for survival and adaptation to the environment. Disorders of these systems, such as sleep apnoea or essential hypertension, are among the commonest reasons for patients to seek medical help. Today, it is clear that the brainstem is becoming a field of real interest to neurobiologists, physiologists, molecular and developmental biologists and clinicians. One reason is that, in spite of the difficulties outlined above, the brainstem does offer certain advantages for the study of integrative mechanisms. Thus, afferent inputs to brainstem circuits can be activated precisely to provide a quantitative assessment of input, while output, in the form of motor activity and target organ responses, can be measured directly. This is not practicable in many other higher centres of the brain. A second aim of this meeting was to bring together neurobiologists who work on problems of cardiovascular and respiratory regulation by the brainstem and who, strangely enough, seldom meet. To this end, experts from different disciplines came together to discuss how the brainstem controls housekeeping functions of the body so as to make all our higher functions possible.

4. PROBLEMS TACKLED BY PAPERS IN THIS ISSUE OF *PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY*

Four major groups of problems were dealt with in the papers of this issue: the *circuitry* of the brainstem, the *interactions of circuits involved in cardiovascular and respiratory activity*, *brainstem development* and the role of the brainstem in *clinical disorders*.

(a) *Brainstem circuitry for respiration*

The papers in this section were devoted to identifying groups of neurons in the brainstem that generate and regulate the rhythm of respiration. All exemplify and make use of novel techniques and new preparations. Thus, for the first time, it has now become possible to record the activity of large numbers of neurons simultaneously by multi-electrode arrays (Nuding

et al. 2009) and by optical methods, without electrodes (Champagnat *et al.* 2009; Homma *et al.* 2009; Muller *et al.* 2009; Smith *et al.* 2009). Inevitably, when one records from vast numbers of cells at once, complexities arise in both analysis and interpretation that require sophisticated computational programmes (Nuding *et al.* 2009; Smith *et al.* 2009). In addition, several new preparations have been devised in which one can make use of these techniques, including slices, isolated brainstems, intrarterially perfused *in situ* and intact anaesthetized animals. State-of-the-art high-speed cameras are needed to measure optical signals produced by dyes when neurons fire in suitable preparations. Dyes differ greatly in their response times and signal amplitude and the type of microscope, for example, two photon or conventional, will determine the depths of cells beneath the surface that can be observed (Homma *et al.* 2009).

A major problem discussed with regard to circuitry concerns the interconnections of brainstem respiratory neurons. Does the rhythm originate from a discrete group of pacemaker neurons that display inherent rhythmicity of firing (as, for example, in cardiac muscle) (Smith *et al.* 2009; St John 2009). Or does it result from the integrated activity of diffuse networks of inspiratory and expiratory neurons that excite and inhibit each other? Or from a combination of both mechanisms (Muller *et al.* 2009)? For analyses of such problems, one needs to know more than just the areas in which nerve cells are situated. Information is required about their membrane properties and the characteristics of their synapses. How prevalent are chemically and electrically mediated synaptic transmission and what transmitters are used (Cifra *et al.* 2009)? Related problems concern whether multiple sites for rhythm generation exist, and if so how many there are and where they are situated in the pons and/or medulla (Nuding *et al.* 2009; Smith *et al.* 2009; St John 2009).

The talks, as well as discussion from the floor, showed that many open questions remain. For example, as in other systems, problems arise in interpreting and drawing firm conclusions about the function of a brainstem region from experiments made with lesions: loss of function after removal of an area does not in itself define with certainty the role of those cells, and the loss may be temporary rather than permanent (St John 2009). A further problem concerns the nature of the rhythmical activity that is recorded from isolated preparations in which it is often difficult to distinguish eupneic respiration from gasping or retching. Moreover, while it has been established which groups of neurons produce and regulate the rhythm of respiration, the precise arrangement of inspiratory and expiratory neurons, their synaptic connections and intrinsic mechanisms are still not known in anything like the same detail as for circuits in the cortex, hippocampus or the cerebellum.

Together, the papers in this section show that, thanks in part to the development of sophisticated computational models, testable hypotheses can now be formulated to explain how brainstem respiratory networks react under different physiological and pathophysiological states.

(b) Coordination of cardiovascular and respiratory regulation

That the brainstem should regulate both the respiratory and cardiovascular systems together is a necessary condition for adaptation of the body as a whole to different needs and circumstances. Thus, cardiac output is matched with minute ventilation at rest, during exercise and under stress, such as 'fight' or 'flight'. Papers dealing with this issue address questions such as: how are the cells for respiratory and cardiovascular functions distributed in the brainstem? Do the same neurons participate and do the same transmitters influence both the respiratory and the cardiovascular systems and how do sensory inputs from the periphery act on cardiovascular and respiratory motor outflows? How are respiratory and circulatory systems regulated during sleep?

A review of the organization of visceral afferents within the nucleus tractus solitarius shows the organization to be based on the targets of its motor outputs, rather than on the topography of simple sensory terminal maps (Spyer & Gourine 2009). Moreover, brainstem regions controlling cardiovascular and respiratory function are juxtaposed and intermingled. This facilitates interactions by synaptic transmission and paracrine mediators. Hence, neuromodulators within a particular region of the brainstem rarely affect the cardiovascular system without a concomitant change in breathing or vice versa (Coddou *et al.* 2009; Pilowsky *et al.* 2009). The large numbers of transmitters found in the brainstem enable reflex inputs to regulate motor outputs selectively while maintaining background autonomic motor tone: in addition to acetylcholine, glutamate and gamma-aminobutyric acid (GABA), ionotropic and metabotropic modulators include nicotine, galanin, somatostatin and pituitary adenylate cyclase activating polypeptide (PCAP). Under various conditions, such as flight or fight, modulatory transmitters in the nucleus tractus solitarius cause inhibition of reflexes that normally lower blood pressure (e.g. through baroreceptors). By contrast, reflexes that induce increases in breathing and blood pressure (e.g. peripheral chemoreceptors) are accentuated (Spyer & Gourine 2009). Particularly important for respiratory control is serotonin (Manzke *et al.* 2009), which has also been implicated in central chemosensitivity. Knowledge of receptor subtype specificity and neuron-type distribution has been shown to be key for understanding the varied actions of serotonin, as well as its role in reversing opiate-mediated respiratory depression without affecting analgesia. Similarly, brainstem cardiovascular control is heavily depressed by opiates but rescued by activating a specific serotonergic receptor subtype localized to medullary and pontine neurons (Dutschmann *et al.* 2009). Indeed, the development of chronic recording of arterial pressure and respiration combined with somatic gene transfer (see Kasparov & Teschemacher 2009) in freely moving unrestrained conscious animals could make possible detailed analyses of central cardiorespiratory coupling and how this changes in disease states such as essential hypertension (Dutschmann *et al.* 2009).

Sleep is a condition in which there is a shift in balance from excitatory to an inhibitory influence on

hypoglossal respiratory modulation. Tonic drives are mediated by excitatory noradrenergic and glutamatergic inputs and inhibited by GABA. Tonic excitatory drives, mediated by noradrenalin and glutamate, are active in wakefulness, but withdrawn in sleep when GABA-mediated inhibition predominates. The pathways for arousal are separate from those that generate the respiratory rhythm; they contribute to the muscle relaxation that occurs in sleep, which has consequences for upper airway patency (Horner 2009).

(c) Developmental and genetic approaches to brainstem circuitry

By the use of molecular techniques, one can determine which genes are responsible for creating the rhombomeric segments of the brainstem, each of which has a separate identity, properties and physiological functions (Champagnat *et al.* 2009). This in turn clarifies the organization of the reticular formation, which is so devoid of clear boundaries or divisions in the adult. Thus, a single gene can account for the migration and final position of brainstem neurons. Moreover, the embryonic activity of certain transcription factors is essential for defining the post-natal phenotype of neurons, their connectivity and their role in motor functions.

One essential gene is *Phox2b*. This homeotic gene provides an essential 'drive to breathe' and is required for the generation of respiratory rhythm at birth (Dubreuil *et al.* 2009). The *Phox2b* transcription factor is also required for the embryological development of the cardiorespiratory system and central chemosensitivity. Mutations of this transcription factor in man at birth cause respiratory disturbances and eliminate sensitivity to carbon dioxide. Its absence results in the loss of neurons in the retrofacial nucleus, a key region mediating central chemosensitivity. Moreover, neurons in this region in the embryo appear to be autorhythmic at an earlier stage of development, which was suggested as key to the normal development of the central pattern generator for breathing (Champagnat *et al.* 2009). After birth, neurotrophic factors play a major role in the development of neurons, synaptic activity and connectivity at the level of the Kolliker-Fuse nucleus in the pons (Mörschel & Dutschmann 2009). Clearly, this region is essential for the normal pattern of breathing, its reflex control and viewed as a master of ceremonies for coordinating spinal and cranial respiratory motor outflows.

A problem that remains to be resolved is the embryonic development of 'cardiovascular' neurons. A related problem concerns the cellular and molecular mechanisms by which visceral afferents controlling the cardiovascular and respiratory systems find their central neuronal targets within the nucleus tractus solitarius, for example.

It has become possible to examine the role of specific types of cells in the brainstem by genetic manipulation, using specific promoters for neurons and glial cells (Kasparov & Teschemacher 2009). Thus, one can visualize brain cell phenotypes such as glia or neurons containing either catecholamines or serotonin *in vitro* and assess the consequences of

silencing them *in vivo* by viral-mediated gene transfer. For example, genetically induced silencing of catecholaminergic neurons (A2 group) causes hypertension. Similarly, targeting of glial cells shows that they are involved in the central chemoreceptor response to hypercapnia (Spyer & Gourine 2009).

(d) *Clinical disorders*

The shift in sleep from excitatory to inhibitory modulation of hypoglossal respiratory modulation has consequences for upper airway patency (Horner 2009). Central and obstructive sleep apnoea might be explained by either poor excitatory and/or excessive inhibitory drive to motoneurons regulating upper airway muscles, for example.

Particularly important for disorders in cardio-respiratory control is serotonin. Moreover, serotonin receptor subtype specificity is a key to understanding its actions and as well as its ability to rescue opiate-mediated respiratory and cardiovascular depression while sparing analgesia (Manzke *et al.* 2009). Human victims of Rett syndrome, a critical condition of prolonged inspiratory effort (apneusis), have already been successfully treated with serotonin agonists (Manzke *et al.* 2009).

Alterations in the neurochemistry of transmitters and modulators within the brainstem can distort the intricate coupling between respiration and cardiovascular control and thereby produce an imbalance in autonomic and respiratory motor drives. This may be a consequence of their embryonic development and abnormal expression of transcription factors such as *Phox2b*. Thus, conditions such as sleep apnoea, sudden infant death, cardiac arrhythmias and sudden cardiac death may not be owing to failure of rhythm generating circuits, but rather to defective cardio-respiratory coupling or inability to generate adequate sympathetic tone to maintain blood pressure.

One can expect that modern molecular tools, such as the use of transgenic mice and viral vector-mediated targeting using specific promoters of distinct brain cell types, will help in the elucidation of brainstem mechanisms in health and disease and in the design of new therapies.

5. CONCLUDING REMARKS

The topics dealt with in the papers in this issue are diverse yet coherent and make use of new techniques, ranging from molecular biology to neuronal network imaging. Four principal conclusions stand out from advances that have been presented.

First, one can hope that the brainstem will in the future no longer be considered simply as a black box containing an assembly of secondary black boxes. It seems realistic to predict that detailed descriptions of cellular wiring and properties will reveal how the rhythm of respiration is produced by brainstem neurons.

Second, cardiovascular and respiratory functions of the brainstem are regulated together to meet the needs of the body. The neural networks regulating respiratory and cardiovascular function are so interwoven

within the pons and medulla that it might be best to consider them as a single system.

Third, techniques are now being devised to identify cardiovascular and respiratory neurons on the basis of their transcriptional factor activity and embryological origins. Such advances will facilitate our understanding of the role of different brainstem cell phenotypes in cardiorespiratory control.

Finally, from an understanding of basic mechanisms, new therapies may be on the horizon for treating and preventing conditions such as sleep apnoea, sudden infant death syndrome and hypertension. Perhaps, in the future, students of the nervous system will discover the fascination of trying to understand how the brainstem performs essential housekeeping activities that underlie all the higher functions of our brain.

John G. Nicholls^{1,2} and Julian F. R. Paton^{3,*}

¹*Department of Neurobiology, and* ²*Department of Cognitive Neuroscience, SISSA, Trieste, Italy*
(nicholls@sissa.it)

³*Department of Physiology and Pharmacology, Bristol Heart Institute, School of Medical Sciences, University of Bristol, Bristol, UK*
(julian.f.r.paton@bristol.ac.uk)

*Author for correspondence.

REFERENCES

- Champagnat, J., Morin-Surun, M. P., Fortin, G. & Thoby-Brisson, M. 2009 Developmental basis of the rostral-caudal organization of the brainstem respiratory rhythm generator. *Phil. Trans. R. Soc. B* **364**, 2469–2476. (doi:10.1098/rstb.2009.0090)
- Cifra, A., Nani, F., Sharifullina, E. & Nistri, A. 2009 A repertoire of rhythmic bursting produced by hypoglossal motoneurons in physiological and pathological conditions. *Phil. Trans. R. Soc. B* **364**, 2493–2500. (doi:10.1098/rstb.2009.0071)
- Coddou, C., Bravo, E. & Eugenin, J. 2009 Alterations in cholinergic sensitivity of respiratory neurons induced by pre-natal nicotine: a mechanism for respiratory dysfunction in neonatal mice. *Phil. Trans. R. Soc. B* **364**, 2527–2535. (doi:10.1098/rstb.2009.0078)
- Dubreuil, V., Barhanin, J., Goridis, C. & Brunet, J.-F. 2009 Breathing with *Phox2b*. *Phil. Trans. R. Soc. B* **364**, 2477–2483. (doi:10.1098/rstb.2009.0085)
- Dutschmann, M., Waki, H., Manzke, T., Simms, A. E., Pickering, A. E., Richter, D. W. & Paton, J. F. R. 2009 The potency of different serotonergic agonists in counteracting opioid evoked cardiorespiratory disturbances. *Phil. Trans. R. Soc. B* **364**, 2611–2623. (doi:10.1098/rstb.2009.0076)
- Homma, R., Baker, B. J., Jin, L., Garaschuk, O., Konnerth, A., Cohen, L. B. & Zecevic, D. 2009 Wide-field and two-photon imaging of brain activity with voltage- and calcium-sensitive dyes. *Phil. Trans. R. Soc. B* **364**, 2453–2467. (doi:10.1098/rstb.2009.0084)
- Horner, R. L. 2009 Emerging principles and neural substrates underlying tonic sleep-state-dependent influences on respiratory motor activity. *Phil. Trans. R. Soc. B* **364**, 2553–2564. (doi:10.1098/rstb.2009.0065)
- Kasparov, S. & Teschemacher, A. G. 2009 The use of viral gene transfer in studies of brainstem noradrenergic and

- serotonergic neurons. *Phil. Trans. R. Soc. B* **364**, 2565–2576. (doi:10.1098/rstb.2009.0073)
- Manzke, T., Dutschmann, M., Schlaf, G., Mörschel, M., Koch, U. R., Ponimaskin, E., Bidon, O., Lalley, P. M. & Richter, D. W. 2009 Serotonin targets inhibitory synapses to induce modulation of network functions. *Phil. Trans. R. Soc. B* **364**, 2589–2602. (doi:10.1098/rstb.2009.0068)
- Mörschel, M. & Dutschmann, M. 2009 Pontine respiratory activity involved in inspiratory/expiratory phase transition. *Phil. Trans. R. Soc. B* **364**, 2517–2526. (doi:10.1098/rstb.2009.0074)
- Muller, K. J., Tschepnakis, G., Homma, R., Nicholls, J. G., Cohen, L. B. & Eugenin, J. 2009 Optical analysis of circuitry for respiratory rhythm in isolated brainstem of foetal mice. *Phil. Trans. R. Soc. B* **364**, 2485–2491. (doi:10.1098/rstb.2009.0070)
- Nuding, S. C., Segers, L. S., Shannon, R., O'Connor, R., Morris, K. F. & Lindsey, B. G. 2009 Central and peripheral chemoreceptors evoke distinct responses in simultaneously recorded neurons of the raphé-pontomedullary respiratory network. *Phil. Trans. R. Soc. B* **364**, 2501–2516. (doi:10.1098/rstb.2009.0075)
- Pilowsky, P. M., Lung, M. S. Y., Spirovski, D. & McMullan, S. 2009 Differential regulation of the central neural cardiorespiratory system by metabotropic neurotransmitters. *Phil. Trans. R. Soc. B* **364**, 2537–2552. (doi:10.1098/rstb.2009.0092)
- Smith, J. C., Abdala, A. P. L., Rybak, I. A. & Paton, J. F. R. 2009 Structural and functional architecture of respiratory networks in the mammalian brainstem. *Phil. Trans. R. Soc. B* **364**, 2577–2587. (doi:10.1098/rstb.2009.0081)
- Spyer, K. M. & Gourine, A. V. 2009 Chemosensory pathways in the brainstem controlling cardiorespiratory activity. *Phil. Trans. R. Soc. B* **364**, 2603–2610. (doi:10.1098/rstb.2009.0082)
- St John, W. M. 2009 Noeud vital for breathing in the brainstem: gasping—yes, eupnoea—doubtful. *Phil. Trans. R. Soc. B* **364**, 2625–2633. (doi:10.1098/rstb.2009.0080)