Classics is an occasional column, featuring historic publications from the literature. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work.

Classics

A SERENDIPITOUS MILESTONE IN RESPIRATORY NEUROBIOLOGY

Richard Kinkead discusses Edgar Adrian and Frederik Buytendijk's classic paper 'Potential changes in the isolated brain stem of the goldfish', published in *The Journal of Physiology* in 1931.

Breathing ensures the movement of oxygen and carbon dioxide between the environment and the organism. Besides its vital role in meeting the basic metabolic needs of each individual cell, breathing has a special, nearly mystical connotation to many. In man, prolonged cessation of breathing is a sign of death, and to some, it is possible to reach special states of enlightenment through breathing.

Throughout the history of science, explaining the origins of breathing has been one of the holy grails of animal physiology. During the antiquity, Galen of Pergamon noted that gladiators and animals injured below the neck continued to breathe whereas those injured in the neck stopped. With time, more precise transection experiments narrowed down the brain areas necessary for breathing and in 1858, the French biologist Pierre Flourens coined the evocative term 'noeud vital' to refer to the brainstem structures that regulate and generate breathing (Feldman and McCrimmon, 2003). Yet the specific anatomical and neurophysiological substrates for breathing remained unclear. As is often the case, the development of new experimental approaches allows giant leaps within a specific research area, and the work of Edgar Adrian and Frederik Buytendijk on isolated brainstem of goldfish reported in *The Journal of Physiology* in 1931 (Adrian and Buytendijk, 1931), unbeknownst to them at the time, represents such a milestone that ultimately led to significant advances in respiratory neurobiology.

For their study, Adrian and Buytendijk mainly aimed to investigate how changes in electrical potential recorded on the surface of the central nervous system can be localized and related to a basic activity. One must keep in mind that, historically, this work was performed shortly before Hodgkin and Huxley (Hodgkin and Huxley, 1939) explained how action potentials are produced and propagated by neurons. For their experiments, Adrian and Buytendijk chose the goldfish because 'of the large size of its brain stem compared with that of the frog, but there is also the outstanding advantage that the respiratory centres are far more active'. Following ablation of the forebrain (Fig. 1A), the brainstem was carefully removed from the animal and placed on a glass microscope slide moistened with Ringer's fluid. The preparation was then positioned on a stand carrying two electrodes ending in moist threads and their recording system consisted of a valve amplifier leading to a Matthews oscillograph and a loudspeaker. The description of the recording system is impressive as it makes us appreciate the challenges of recording biological signals at the time. Though their preparation and apparatus system were very basic, they were nonetheless able to address their research question as they reported small and rapid potential oscillations that varied with the position of the electrodes. They noted, for instance, that the signal waves were due to changes in the potential of the vagal lobes relative to the rest of the brainstem (Fig. 1B).

From a technical perspective, Adrian and Buytendijk commented on the viability of the preparation, which they estimated to 1 h; this is impressive considering that the brain tissue was not continuously superfused with an oxygenated solution as is the case nowadays. Furthermore, they underscored the importance of working with animals that 'breathe vigorously'. They also mentioned that the changes in potential produced by the goldfish brainstem were far more regular and frequent than those produced by preparations from frogs or tortoises, which, like many ectotherms, breathe episodically.

The fact that Adrian and Buytendijk ascribed this electrical activity to gill ventilation is one of the main reasons why this paper has a special place in the respiratory physiology literature and is still frequently cited. While their interpretation was limited to the knowledge that motor nerves activating respiratory muscles originate from the brainstem and that the regularity and frequency of the activity is similar to that of gill ventilation in an intact animal (Fig. 1C), the fact remains that this paper may well be the first direct evidence that the brainstem contains all the neural elements necessary to generate breathing. Whether this paper is truly the earliest description of using a reduced brainstem preparation to record respiratory-related activity is uncertain, as some of the comments and comparisons made by the authors suggest that similar experiments had been performed at the time. However, the limited citations and dates of the bibliography have made it difficult to confirm the originality of their experimental approach. Regardless, a closer look at this article reveals several gems of observations that have withstood the test of time. For

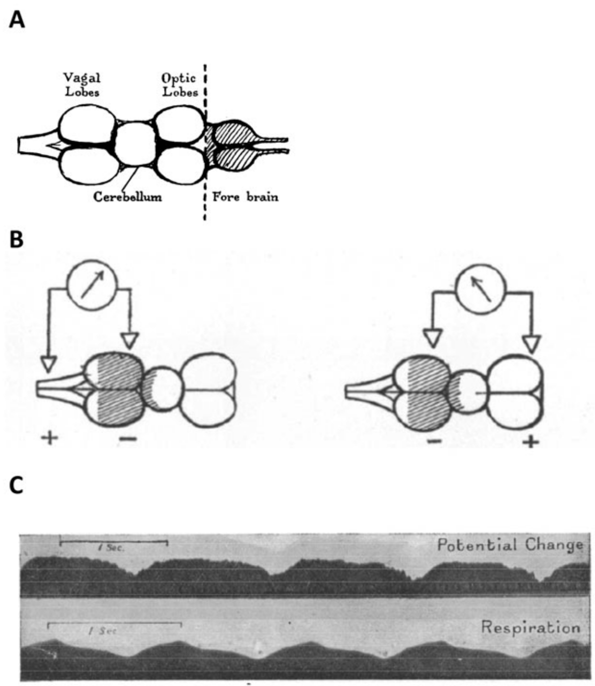

Fig. 1. (A) Diagram of the goldfish brain as published by Adrian and Buytendijk (Adrian and Buytendijk, 1931). The preparation consists of the brainstem; the forebrain (shaded) was ablated. (B) Diagram originally used by the authors to describe the localization of rhythmic potential change. In the left-hand panel, the earthed lead is on the lower part of the medulla and the input lead is on the vagal lobe. The deflection shows that the vagal lobe becomes negative to the medulla. In the right-hand panel, the earthed lead is on the vagal lobe and the input is on the optic lobe. The reversal of the deflection shows that the vagal lobe is also negative to the mid-brain. (C) Comparison of potential wave rhythm (top panel) and rhythm of opercular movements in normal respiration in intact fish (lower panel). Illustrations adapted with permission from *The Journal of Physiology*.

instance, they reported that the mid-brain can be extensively damaged without affecting the respiratory signal, and thus concluded that this area is not necessary for respiratory activity. Adrian and Buytendijk also cleverly pointed out that their ability to record respiratory-related activity in the complete absence of sensory afferent signals indicates that the respiratory centres are capable of spontaneous rhythmic activity, much like the heart muscle.

In the late 1990s, researchers identified the pre-Bötzinger complex as the kernel of the noeud vital for respiratory rhythm generation, and the use of a reduced brainstem preparation similar to the one used by Adrian and Buytendijk was instrumental to this discovery (Smith et al., 1991). Today, a broad variety of vertebrates ranging from newborn mammals to lampreys is commonly used in reduced brainstem preparations to investigate various aspects of respiratory control. In fact, rhythm generation has now become a sub-discipline of respiratory neurobiology. Recent technical advances have now made it possible to investigate breathing at the cellular and molecular levels, and we are progressively gaining substantial insight into the common principles that underlie the act of breathing across phylogenies.

This article by Adrian and Buytendijk is truly an inspiring ‘classic’. Besides its innovative aspect, it highlights the value of having an acute sense of observation. The authors’ sharp description of their results provided seminal knowledge to the field of respiratory physiology even though it was not their main interest. This is refreshing at a time when the growing pressure for publishing high-impact, ‘neat scientific stories’ leaves little room for orphan results and observations that are now frequently considered a distraction to the manuscript.

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