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EDITORIAL

El grillo è buon cantore: for Franz Huber on the occasion of his 90th birthday

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Let me start this little homage with a song by Josquin Desprez to congratulate Franz Huber on the occasion of his 90th birthday (https://www.youtube.com/watch?v=62-aBOZrqh8). This medieval madrigal praises the enduringly singing cricket, "el grillo è buon cantore". Its theme accompanied Franz Huber's entire career as a scientist and mentor, a theme that still challenges ethologists, neurobiologists and geneticists today in their quest to understand the very core of animal behaviour.

The explorations of Franz Huber (Fig. 1) as a PhD student in Tübingen included pioneering studies of the brain and the central nervous system of crickets aiming to link the function of neuropils to the insect's salient singing behaviour (Huber 1955). Were it reflexes or was it the brain that controlled singing? Were the mushroom bodies integrating sensory inputs? Was the central body timing the song pattern? What about the function of the thoracic ganglia and sensory inputs from the wings and the abdomen? These were challenging questions, yet methods were still coarse in those days: Iron Age tools from today's perspective, a metal pin and a blade. So who would dare to penetrate an insect's delicate brain with a needle? Franz Huber would, as the problem was clear: How is behaviour rooted in the function of the nervous system? Where in the nervous system are the centres programming and controlling cricket singing? Could he destroy or activate them? Not long after, the singing crickets provided the first answers. Once specific central brain regions were lesioned males sung to exhaustion. Franz Huber's pioneering questions and experiments (Huber 1960) provided good reasons to acknowledge him as one of the founders of the then new field of neuroethology.

His work did not go unnoticed. Taking over the Chair for Animal Physiology at the University of Cologne in 1963, the sensory side of the cricket communication system moved into the focus of attention. Franz Huber's students Dieter Möss, Harald Nocke, and Eckehard Eibl pushed research on the cricket peripheral auditory pathway forward, revealing the organisation of the cricket ears, the neuroanatomical projections of their sensory fibres to the central nervous system and their physiological and mechanical response properties. First, extracellular recordings of action potentials from the ascending neurons in the connectives together with John Stout gave insight into the physiological representations of song patterns in the central nervous system, while Dietmar Otto refined the method of electrical brain stimulation (Fig. 2) and together with Wolfram Kutsch provided deeper insight into the descending efferent control of calling, courtship and rivalry songs. These studies turned previous assumptions upside down: Remarkably, they demonstrated that crickets can even sing when the connectives to the brain are severed (Kutsch and Otto 1972). What did this mean? Was the original hypothesis all wrong? Was the central complex not the timer of the song pattern?

David Bentley then provided the first intracellular recordings from motoneurons in fictively singing crickets. At the same time, Norbert Elsner explored the acoustic behaviour of grasshoppers, refining the method of electromyogram recordings to perfection and demonstrating the richness of grasshopper motor control of courtship behaviour. These were important achievements and contributions to the field. I had the chance to attend Franz Huber's very last neurobiology seminar in Cologne, and for the first time encountered his sheer enthusiasm for the field, which was a

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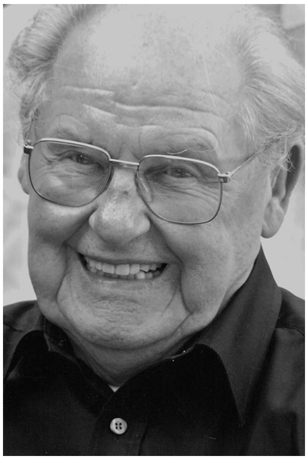

Fig. 1 Prof. Dr. Drs. h.c. mult. Franz Huber (2011)

crucial moment for me as a student. At the time when Franz Huber left Cologne, many of the biology students there dearly missed him.

Starting in 1973, the "Max Planck Institut für Verhaltensphysiologie" at Seewiesen provided the resources needed to develop Franz Huber's research further, while the international race was on for claims in the rapidly developing field of cricket neurobiology. The laboratories of Ron Hoy (Ithaca), Andreij Popov (St. Petersburg), and Gernot Wendler (Cologne) joined the field. To understand any communication system, behavioural studies are indispensable to reveal the tuning of sensory functions to the natural and biologically relevant stimuli. The Kramer treadmill, set up by Theo Weber, John Thorson and Hans-Ulrich Kleindienst allowed studying the phonotactic behaviour of freely walking crickets (Fig. 3). Thus, the temporal tuning of the female phonotaxis could be established. Clearly, the females preferred a pulse pattern that matched the male's song (Thorson et al. 1982). But how was the male song pattern processed in the female's auditory pathway? What were the specific filters that tuned the female's phonotactic behaviour to the male's song? Intracellular recording and labelling techniques allowed the exploration of the central auditory neurons. David Wohlers and Konrad Wiese identified the local and ascending auditory neurons in the prothoracic ganglion. Careful analysis demonstrated that the temporal filters certainly were not at this level, but needed to be sought for in the brain. Klaus Schildberger showed the first evidence for temporal processing by local brain neurons. These were moments of particular fascination for Franz Huber's "Abteilung" (Huber 1978; Huber and Thorson 1985). Phonotaxis works well for an intact female, but

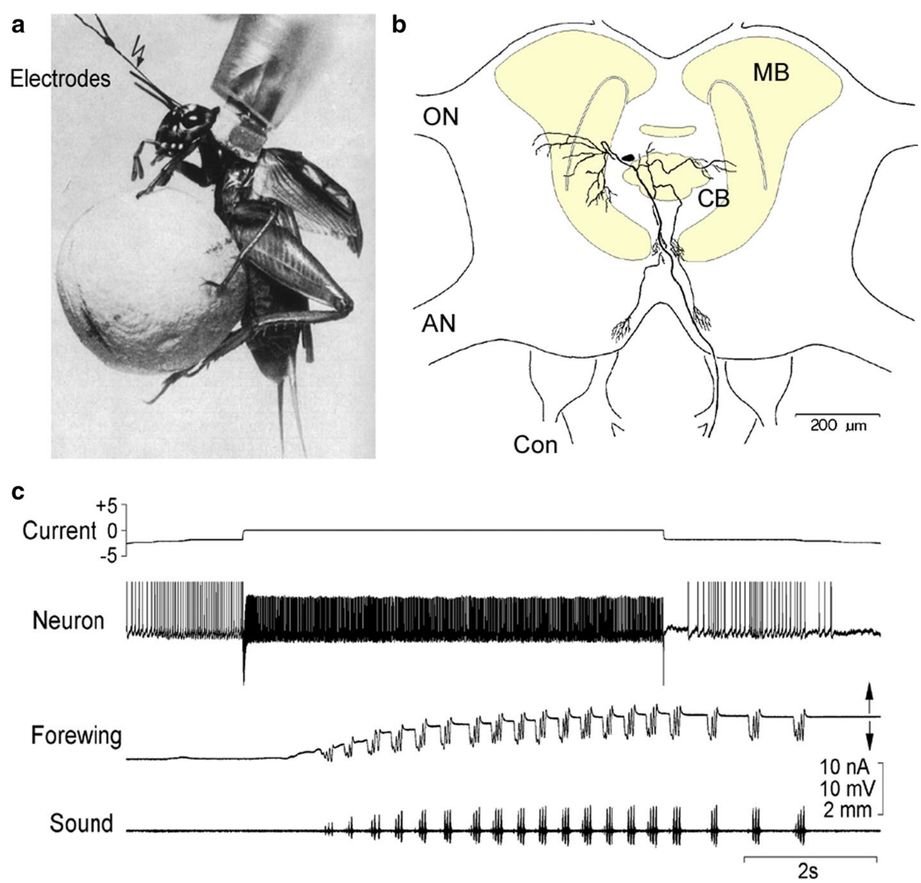

Fig. 2 **a** Male cricket with a wire electrode inserted in the brain sings upon electrical stimulation (Huber 1960). **b** The outcome of these experiments was crucial for localising the calling song command neuron with dendrites in the frontal protocerebrum, anterior to the mushroom bodies. **c** Intracellular current injection drives the spike activity of the command neuron and elicits a sequence of sonorous calling song (modified from Hedwig 2000). *MB* mushroom body, *CB* central body, *ON* Optic nerve, *AN* antennal nerve, *Con* connective

what happens when a cricket loses a front leg during development? Regeneration and plasticity assume an important role, afferents and interneurons sprout, find new specific targets in the auditory pathway and rewire the circuit. Thus even after such accidents, not all is lost; females still will have a good chance of approaching a singing mate (Huber 1987).

At each trip of the Elsner laboratory from Göttingen to Seewiesen, we students admired the steady experimental progress and the continuously deeper understanding of the cricket nervous system. However, more importantly, we always encountered an inspiring enthusiasm for neurobiology. Crickets by now had become a model for insect behaviour and the function of small nervous systems and small neuronal circuits in general. Such miniature circuits for information processing were shaped by evolution to perform what insects are best at: a highly species-specific task of sensory processing or motor control. It was Franz Huber's enthusiasm that attracted students and scientists from across the world to Seewiesen: P. Evans, M. Oshea, J. Horseman, J. Doherty, J. Altman, J. Kien, A. Popov, G. Pollack, A. Michelsen and A. Selverston to name just a few. Before retiring, friends F. Huber, T. Moore and W. Loher edited the handbook on "Cricket Behaviour and Neurobiology" (Huber et al. 1989). In a subsequent book, the common aspects of "Acoustic Communication in Insects and Anurans" (2002) were laid clear with C. Gerhardt. Until this day, these volumes are most useful references for any student of the field, guiding the new generation on how to think about the different aspects of acoustic communication.

So, where is the field of cricket studies now? Based on Franz Huber's initial experiments, a method of pharmacological brain stimulation has been successfully established. It now readily allows neurophysiological experiments in fictively singing crickets and is an invaluable tool for the neuroethologists. Using this technique in male crickets at the level of interneurons and synaptic connections, a remarkably clear case of a corollary discharge mechanism could be identified that modulates the sensitivity of the auditory pathway during singing (Poulet and Hedwig 2006). The descending command neurons for evoking the cricket's specific calling song behaviour have been identified in the brain (Fig. 2). However, we still do not know how courtship and rivalry songs are controlled at a cellular level. A deeper exploration of the brain's neuropils is required. The pharmacological stimulation technique has allowed exploring the singing central pattern generator. It is in the cricket's abdominal ganglia and controls the rhythmic pattern of its singing movements.

Franz Huber's original concepts on cricket singing have fundamentally changed by now: There is still no doubt that the decision about singing is made in the brain, but the actual motor pattern for singing is produced by a central pattern generator in the abdomen.

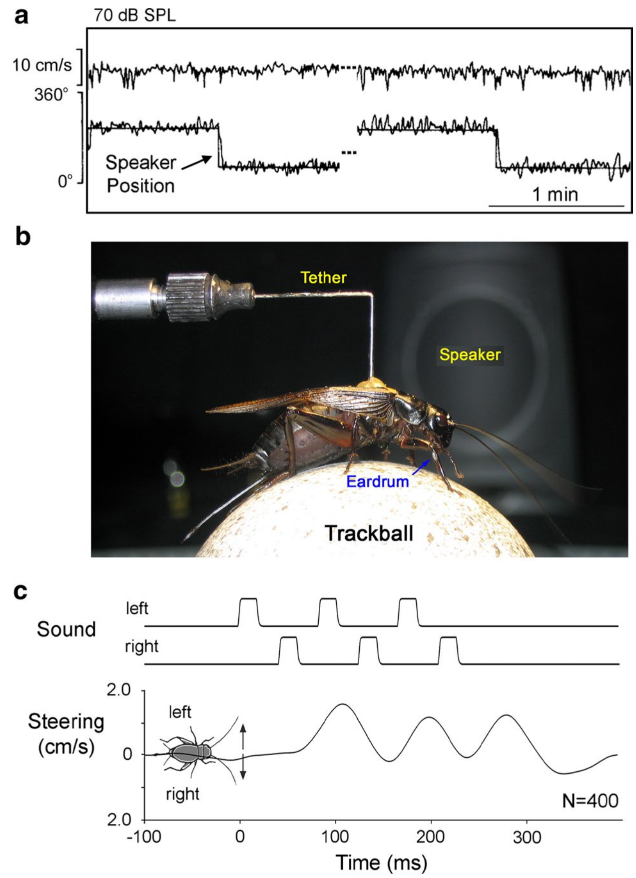

Fig. 3 **a** Direction and speed of a phonotactically walking female cricket as recorded with the Kramer treadmill; walking speed *top trace* and angular orientation *lower trace*. Systematic testing with different temporal patterns revealed the tuning of the female auditory behaviour (Weber et al. 1981). **b** A tethered cricket on a recently developed more sensitive trackball system. **c** The system even resolves the stepping cycle and reveals fast reactive auditory steering to individual sound pulses in split-song sound paradigm; averaged response to 400 chirps (Hedwig and Poulet 2004)

In the context of auditory pattern recognition, a neuroethological field of basic interest, a trackball system much smaller than the original one (https://www.youtube.com/watch?v=dAght57vEo8) has allowed to record unexpected fast steering responses to individual sound pulses (Fig. 3). Coupled with high speed video recordings, the leg trajectories of phototactically orienting crickets could be revealed. Once the species-specific pattern has been detected, the pattern recognition system has a modulatory effect on the steering behaviour. As a consequence, females do not wait to listen to a full chirp before they start orienting and transiently may even steer towards non-attractive chirps (Hedwig 2014).

Optical imaging can now be applied to study auditory processing in single neurons (https://www.youtube.

com/watch?v=0c-sGm9dE2Q) and neuropils. We know local auditory neurons in the brain that specifically match the tuning of female phonotaxis behaviour. Recently, a delay line and coincidence detection mechanism underlying pattern recognition have been identified, verifying a hypothesis already put forward by Weber and Thorson in the cricket handbook (Schöneich et al. 2015). Cricket rivalry behaviour was successfully explored to unravel the neurochemical basis of aggressive behaviour (Stevenson and Rillich 2015). I wish we had similar progress and insight for the neurochemical control of female phonotaxis. Crickets have indeed become a focus of behavioural ecologists. The rapid evolution of singing behaviour on the Hawaiian archipelago under the pressure of flies hearing the cricket song and preying on crickets is a fascinating example (Zuk et al. 2006). There are advances in molecular approaches as well; regeneration and plasticity in the cricket auditory system can now be studied at the molecular level (Horch et al. 2009) and in 2015 the first genetic knockout crickets have been developed with recent genome editing techniques (Awata et al. 2015). The fast developments in this field are promising. Sequencing the cricket genome may not be far away and should have a catalysing impact on the study of the nervous system.

The field of insect neurobiology and acoustic communication has changed, diversified and progressed since the early days of Franz Huber. What will neuroethologists say in 50 years' time, with their then again advanced perspective, about the intracellular analysis of auditory processing and singing motor pattern generation? Maybe: 'They just used sharper needles, now made of glass?' Exploring the principles of animal behaviour at the neurobiological and molecular level will always generate new questions, challenges and techniques. With every step of further understanding, our admiration for life and its evolution will be enhanced.

Dear Franz, I heard that you just finished your autobiography. Congratulations. I look forward to learning more about cricket research from your perspective. I also learned that age is starting to have a toll on your well-being. At the emperor's court in classical China, noble men kept crickets for fighting competitions; artistic tiny cages allowed them to carry their precious pets around. Kept warm under the mandarin's coat in wintertime, the charming songs of the little fellows reminded them of spring; *El grillo è buon cantore …*

With very best wishes,

Berthold Hedwig and a crowd of readers of the Journal of Comparative Physiology A.

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