PULSES PATTERNS PATHS: AUDITORY PROCESSING IN CRICKETS

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1 INSECTS AS MODEL SYSTEMS FOR THE STUDY OF AUDITORY PROCESSING

Finding food resources or a mate in a complex environment is a challenging task for animals like insects with small nervous systems. Different groups therefore have become specialists in foraging and mate attraction by modifying their behavioural strategies, adapting their sensory structures or specialising and tuning central neural processing. Among cicadas, grasshoppers and crickets the use of species-specific acoustic signals for intraspecific communication and mate attraction is widespread. As a fundamental requirement of acoustic communication these animals have to recognise and localize the sound pattern as they approach the signalling mate. Since insects allow detailed studies of their acoustic behaviour, the tuning of auditory behaviour can be characterised and important conclusions about its organisation can be drawn. At the neural level complex mechanisms are thought to underlie the processing of acoustic patterns even in insects with simple auditory pathways and central nervous systems. As the activity of single identified neurons can be recorded even in behaving animals, the neural basis of auditory pattern recognition and orientation can be studied and common neural principles underlying auditory processing may be revealed.[1,2]

2 ANALYSING CRICKET PHONOTAXIS

2.1 Acoustic Communication in Crickets

Among insects crickets have become a model system to study the neural basis of acoustic behaviour.[3,4,5] Males (*Gryllus bimaculatus*) produce a loud species-specific calling song by rubbing their front wings together. Each closing movement generates a sound pulse - also called syllable - of about 90 dB SPL, 20 ms duration and a carrier frequency of 4.8 kHz. Several of these pulses are grouped into chirps, which are repeated 2-3 times per second. Males have to be enduring singers, since the females are mute and do not respond to the males' calls. When females are ready to mate, they use the acoustic cues of the calling song to approach the singing male, a behaviour called phonotaxis. Like in other acoustically communicating species cricket phonotaxis is based on two fundamental processes: pattern recognition and orientation. The animals have to identify the temporal structure of the species-specific song pattern and have to reliably orient towards the sound source. From a neurobiological perspective we wish to understand the behaviour and the underlying neural mechanisms, their functional principles and the organisation of the neural networks involved.[6,7]

Complex hearing organs are located in the front legs of both sexes.[8] These function as pressure difference receivers. Sound acts on the tympanic membranes from the outside but also from the inside. At each side of the body a large acoustic trachea with an opening in the frontal thorax mediates the sound waves towards the ear in the ipsilateral leg. Moreover the left and right acoustic tracheas are mechanically coupled leading to a frequency dependent directionality of the ears, which is best at the carrier frequency of the calling song.[9] About 45-60 primary auditory afferents respond to the vibrations of the tympanic membrane. From a threshold of about 40 dB SPL any increase in sound intensity is coded by a larger number of action potentials and a shorter latency of the response. Action potentials travel along the axons into the first thoracic ganglion. Here local and intersegmental neurons form the first stage of auditory processing and increase the bilateral contrast. Auditory information is send up to the brain, where the pattern recognition networks are located. Pattern recognition and orientation require fundamentally different processing of the auditory information detected: whereas orientation is based on a comparison of the left and right auditory information, pattern recognition benefits from summing up the inputs of both ears for reliable processing. Therefore the networks for pattern recognition and auditory steering may be separated within the central nervous system.[10,11] Previous studies of cricket phonotaxis relied on slow, high inertia track ball systems,[12] but recently we were able to study cricket pattern recognition and orientation with a fast trackball as shown in Figure 1, that allows a detailed analysis of the animals' phonotactic walking and steering behaviour.[13,14]

In a dark sound proof chamber tethered crickets are placed on top of a lightweight trackball (Rohacell, 56 mm diameter, mass 3 g) floating within an air stream. Two speakers at 45 deg to the animal's length axis present different acoustic test patterns at 4.8 kHz, 75 dB SPL. During walking, the animals easily rotate and control the trackball. When a female is attracted to a test pattern it turns the trackball and intends to walk in the direction of the active speaker. An optical sensor at the bottom of the trackball captures the forward-backward and the left-right components of the ball's movements with a resolution of 127 μm and provides a precise and quantitative analysis of the animals walking behaviour. Since the animals are fixed, their alignment in the sound field cannot change and steering responses to sound patterns can even be averaged.

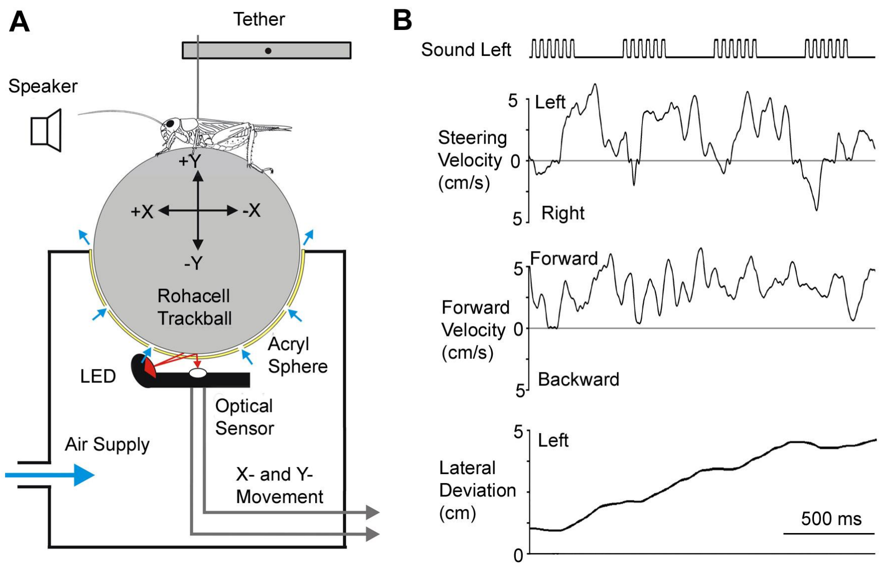

Figure 1. Analysing cricket phonotaxis. **A** A cricket is positioned on a trackball floating in an air stream. Sound patterns are presented by a speaker. When the animal walks and orients, it rotates the trackball and the movements of the trackball are captured with an optical sensor. **B** Sound pattern presented from the left, velocity components and lateral deviation as calculated for a short section of phonotactic walking. Modified from Refs. [13, 14].

2.1.1 The Temporal Tuning of Cricket Phonotaxis

The tuning of cricket phonotactic behaviour and the underlying pattern recognition process can be tested by systematic variation of the natural calling song in respect of pulse duration and pulse interval and measuring the animals' phonotactic response to these patterns. When a series of test patterns is presented from a set of 2 speakers the outcome as shown in Figure 2 demonstrates that phonotaxis is tuned to the natural repetition rate of sound pulses. The animals prefer sound patterns in the range of 34-42 ms pulse intervals corresponding to pulse repetition rates of 29-24 Hz. The animals do not respond to patterns with short pulses presented at a higher repetition rate or to longer pulses presented at a lower rate. [7] This led to the proposal, that pattern recognition is mainly based on the repetition rate of the sound pulses characteristic for the calling song[12] although the performance of phonotaxis may also be influenced by changes in the chirp pattern as well.[15,16] The neural processing underlying temporal filtering of the pulse rate are not yet fully established. It may involve a network of brain neuron acting as a band-pass filter for the pulse repetition rate [17] or a mechanism based on instantaneous discharge rate coding of sound pulses. [18]

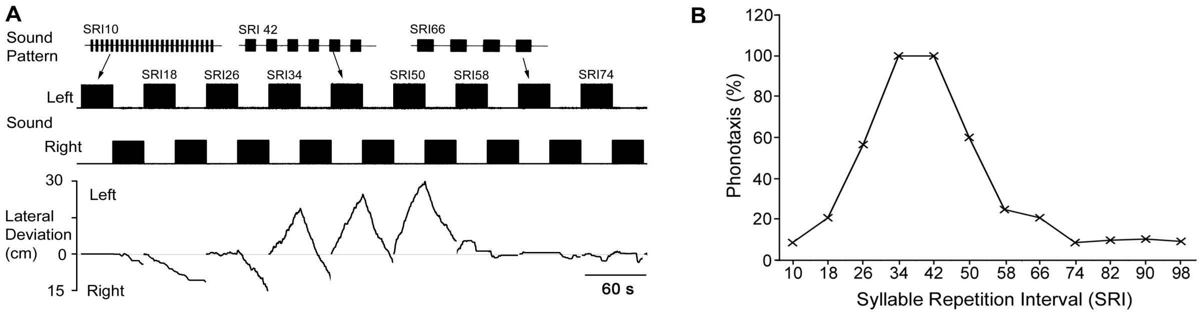

Figure 2: **A** Temporal tuning of cricket phonotaxis tested with different syllable repetition intervals (SRI) presented for 30 s from a left and right speaker. Only attractive patterns, e.g. SRI42 elicit a clear steering response to the side of acoustic stimulation. Lateral deviation is reset to zero at the beginning of each test pattern. **B** Quantitative analysis of the overall lateral deviation towards the different patterns reveals the tuning of the recognition system. The best phonotactic response occurs for syllable repetition intervals of 34-42 ms, n=10 crickets. Modified from Ref. [7].

2.1.2 Orientation and Pattern Recognition

Studies of cricket phonotaxis based on high inertia trackball systems indicated, that the animals turn to sound patterns presented from a new direction only after they had been listening to a whole

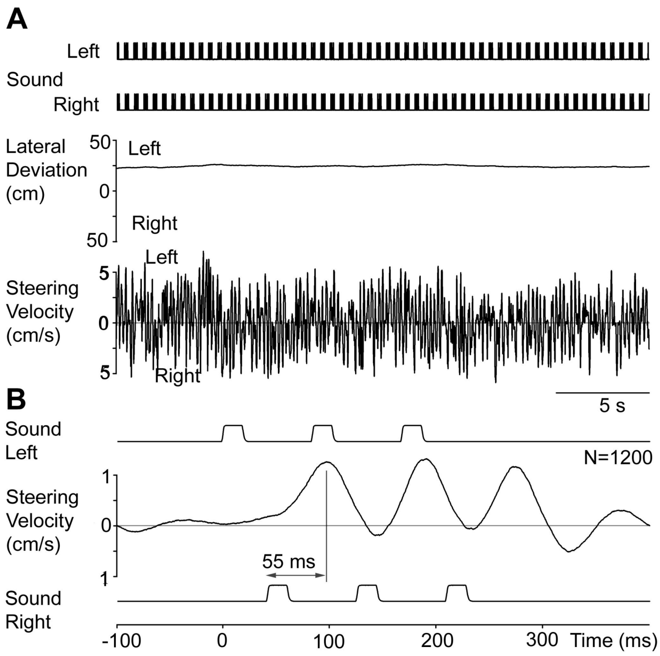

Figure 3: **A** Orientation and steering responses towards a split-song pattern with consecutive pulses presented from alternating sides. The animal walks straight ahead, but rapid steering responses occur to the left and right side. **B** Averaging the steering velocity together with the timing of the sound pulses demonstrates fast responses towards each sound pulse with a latency of 55 ms only. Modified from Ref. [14].

chirp. This indicated that pattern recognition and orientation are serial processes and that the decision to steer towards a new stimulus is only made after the pattern has been recognised.[10,11] However, analysing phonotaxis with the fast trackball system revealed much faster steering responses as shown in Figure 3A. In these experiments spilt-song patterns were used in which every other sound pulse of the chirp pattern is presented from a different speaker, located at 45 deg left and right to the animal's length axis. Although the animals walk straight ahead a closer inspection of the animal's steering behaviour reveals that the walking direction is the consequence of rapid steering events towards individual sound pulses presented from the left and right side. [13,14] Averaging the steering velocity as shown in Figure 3B demonstrates that the animals do not integrate over the whole duration of chirps, but that they rapidly steer towards individual sound pulses with a latency of 55-60 ms only. Since the same number of turns are made to the left and right side, a straight walking direction results. Any neural pattern recognition system analysing the temporal sequence of sound pulses needs at least 2 pulses for a reliable measurement of the pulse repetition rate. Since steering starts while the 2nd sound pulse of a chirp is still processed within the auditory pathway, steering rather appears to be a reactive response in which pattern recognition cannot directly be involved.

2.1.3 Orientation Emerges from Reactive Steering

Steering towards single sound pulses had not previously been observed in cricket phonotaxis. It indicates, that the animals' overall direction of walking, its orientation, may not be the result of calculating a direction for the walking course but may emerge from numerous consecutive steering responses. To test this possibility a split-song paradigm was designed in which the ratio of pulses presented from the left and right side was kept constant in different tests. However within this restriction it was randomly determined which pulse was to be presented from the left or right side. When all 6 pulses of the chirps were played from one side the animals clearly deviated to the pattern as shown in Figure 4A. At a ratio of 5:1 and 4:2 the animals steered increasingly less to the side presenting the larger number of pulses. When the ratio finally reached 3:3 the animals basically walked straight ahead. With the decrease in ratio steering towards the side with more sound pulses decreased in a reliably and predictable way as shown in Figure 4B. [13, 14] From this outcome it can be concluded, that the path of crickets during phonotactic walking emerges from consecutive steering events to individual single sound pulses.

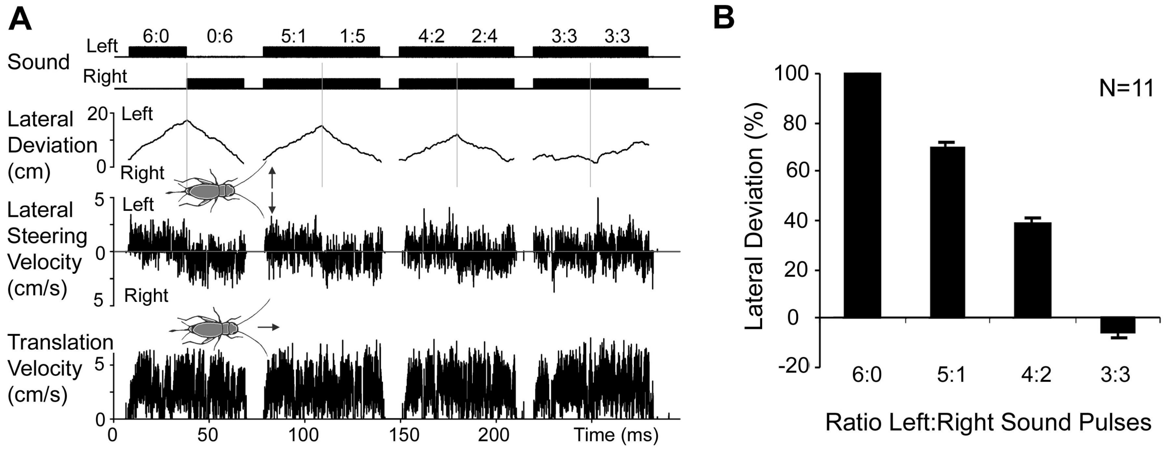

Figure 4: **A** Steering to randomly organised split-song patterns. Chirps with 6 sound pulses were randomly split and different numbers of pulses presented from each side. The animal steers towards the side with more sound pulses. **B** The lateral deviation of the animals within 30 s of sound presentation decreases in a predictable way with the ratio of sound pulses presented from both sides. Error bars indicate standard error of the mean. Modified from Ref. [13].

2.1.4 Pattern Recognition Modulates Auditory Steering

Cricket phonotaxis is tuned towards the species-specific pulse rate and the animals do not steer towards any arbitrary patterns. Steering responses therefore have to be under some control of pattern recognition. In order to analyse a possible modulatory impact of pattern recognition on auditory steering we again took advantage of the sensitivity of the track ball system. When we presented a series of non-attractive test pulses with 120 ms duration, which lack the pulse structure of normal chirps, females would not or only very weakly respond to these test pulses. We then replaced every 4th chirp of an attractive pattern (pulse repetition rate of 42 ms) with a test pulse and analysed the steering responses towards the pulses. If pattern recognition is robustly sensitive towards the temporal structure of the perceived pattern, the crickets should not respond to the test pulses. However, the inserted pulses elicited steering responses equal to the natural chirps. A systematic test with chirps of different pattern inserted into a natural calling song revealed a drastically altered tuning curve for the tested patterns with the females now responding to all pulse patterns with a lower than natural pulse rate.[19] From these results it became clear that steering responses are altered when pattern recognition was activated. By presenting a series of test pulses immediately after a sequence of calling song the time course of these changes became obvious. The responses towards the test pulses decayed within 5 sec. Thus a modulatory effect of pattern recognition on steering lasts for several seconds. As an advantage of such a modulation crickets walking in the field will continue to follow a sound signal, even when the signal is deteriorated due to environmental factors.

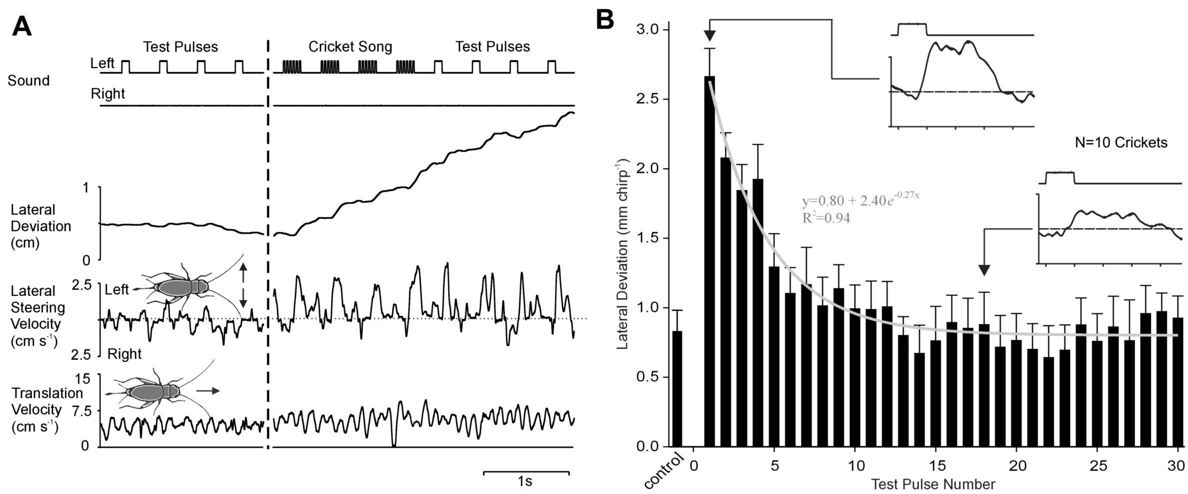

Figure 5: Pattern recognition modulates auditory steering. **A** Test pulses presented on their own evoke no or only minor steering responses. After a 10 s sequence of calling song the animals clearly steer towards the test pulses. **B** At the end of calling song the steering response towards the test pulses decays exponentially within 5 s to the control level. Modified from Poulet and Hedwig (2005).

2.1.5 Organisation of Pattern Recognition and Auditory Steering

How can these findings be incorporated in the organisation of pattern recognition and steering? One possible organisation of cricket auditory behaviour is given in Figure 6. Sound patterns perceived by the left and right ear are fed into separate recognition networks. (So far in crickets there is no neuroanatomical evidence that the information of the ascending neurons is summed up in the brain.) In parallel to the recognition pathway the acoustic signals impact on a steering pathway. Auditory steering responses are however minute as long as the pattern recognition network is not activated. Once a species-specific song is detected, a modulatory process with a time constant of several seconds is started, that changes the gain of the steering pathway. Acoustic signals now release significant steering responses. Since the auditory signals for steering are not passed through the recognition process, steering to non-attractive sound pulses can occur. Continuous presentation of non-attractive sound patterns however deactivates the recognition network, the modulatory process decays and phonotactic steering stops.[19] With mirror image systems at both sides of the central nervous system there is no need for a comparison of the bilateral acoustic signals to calculate and provide the magnitude of any steering commands. If the amplitude of the left and right motor commands correspond to the different amplitude of auditory afferent activation in both ears, respectively, the difference in the motor commands will automatically lead to directional walking of the animal. Two separate networks for reactive steering responses, controlled by a pattern recognition process, may be sufficient to allow a behaviour as complex as cricket phonotaxis.

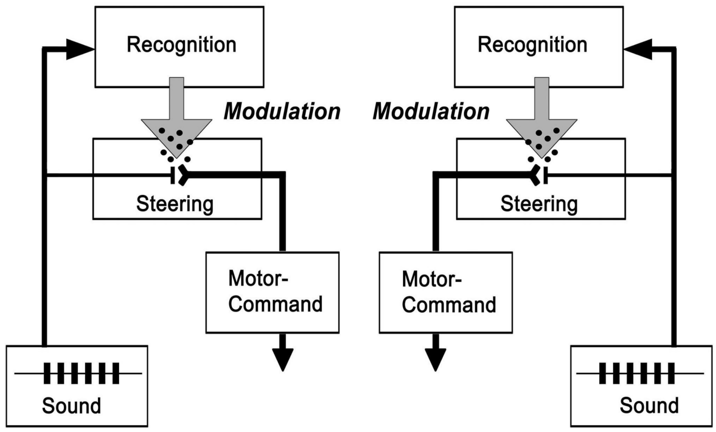

Figure 6: Proposed organisation of pattern recognition and steering in the central nervous system of a cricket. Pattern recognition and the auditory steering pathway work in parallel at both sides of the central nervous system. However only when the pattern recognition process is activated is the gain of the steering pathway increased and motor commands for auditory steering are generated. This organisation and the slow time course of the modulatory process transiently allow steering responses towards non-attractive sound patterns. Modified from Ref. [19].

3 DISCUSSION

Studying cricket (*G. bimaculatus*) phonotaxis with a highly sensitive trackball system allowed a detailed analysis of the walking and steering responses. It confirmed the temporal tuning of the behaviour to the species-specific syllable rate, which appears to be the crucial parameter for pattern recognition. The experiments also revealed rapid steering responses towards single sound pulses. Such responses cannot directly be mediated by a band-pass filtering process that had been proposed for pattern recognition,[17] since such a process requires at least two syllables to determine the actual pulse rate. Steering rather is a reactive auditory response that is not tuned to particular sound pattern.[19] As a consequence the direction of the animal's walking course emerges from numerous steering events to single sound pulses. The females do not calculate an overall walking direction but they rely on a continuous sequence of sound pulses produced by the male. Since the gain of auditory steering is modulated by pattern recognition on a time scale of seconds, this may maintain phonotaxis even when the signal is lost transiently. It also explains steering responses to non-attractive test pulses. These experiments, however, allow no conclusions on the nature of the recognition process underlying phonotaxis. Pattern recognition may be based on band-pass filtering,[17] cross correlation analysis [20] or spike rate evaluation.[18] Two open questions are studied in current experiments: 1. How is the modulation of the steering response mediated between the pattern recognition network and the steering pathway? Intracellular recordings and calcium imaging of networks of auditory brain neurons in actively behaving animals may provide further insights into the underlying neural processing. 2. How are the steering motor commands incorporated into the actual motor activity of the walking animals? Preliminary high speed video studies indicate that steering is mediate in particular by rapid movements of the front legs.

ACKNOWLEDGEMENTS

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