

Neural evolution in the bat-free habitat of Tahiti: partial regression in an anti-predator auditory system

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Noctuid moths endemic to the mountains of Tahiti have evolved in an environment without bats and these insects have lost a defensive behaviour against these predators, the acoustic startle response (ASR). The ASR in noctuid moths is presumed to be activated by a single auditory receptor neuron (A2 cell) and we report that while this cell still exists in endemic species and possesses similar sensitivity thresholds compared to the A2 cell of recently introduced species, it exhibits reduced firing activity to ASR-evoking sounds. This partial neural regression suggests that the evolutionary disappearance of the ASR in these insects is incomplete and that sensoribehavioural integration decays gradually following the removal of stabilizing selective forces.

Keywords: moths; evolution; neuroethology; regression; auditory ecology; predator release

1. INTRODUCTION

While the evolutionary degeneration of sensory systems continues to receive much attention and debate (Romero & Green 2005), much less is known of the impact of such a loss on the behaviours normally controlled by these senses (i.e. their sensoribehavioural integration). Examining sensoribehavioural regression is complicated by the fact that a particular behaviour will not necessarily be affected by a lost sensory structure if another compensates for its disappearance (e.g. non-visual control of swimming in blind cavefish; Teyke & Schaerer 1994).

Many insects use ears to detect the echolocation calls of hunting bats and evoke evasive flight behaviours (Miller & Surlykke 2001). One such behaviour is the acoustic startle response (ASR), the cessation of flight when confronted by a nearby bat (Roeder 1967; Skals & Surlykke 2000). Of all the bat-listening insects, the simple ears of the noctuid moth have been the most studied with most species using them solely to detect bats. The moth ASR therefore offers the opportunity to study the effects of one selection pressure (bats) on the integration of one

sense (hearing) with an adaptive behaviour (ASR) and how this network will change if not maintained by stabilizing selection.

The island of Tahiti provides an ideal opportunity to test these questions because this isolated habitat has been continually bat free since it first appeared 0.25–1.75 Myr ago and is now home to endemic moth species that have evolved in the absence of this potent selection pressure. These moths both possess ears that are less sensitive than those of adventive species (i.e. recently arrived; Fullard 1994) and do not exhibit an ASR to simulated bat calls (Fullard *et al.* 2004), suggesting that the ear/ASR pathway of these moths has disintegrated as a result of the absence of stabilizing selection pressure. Noctuid moth ears send their auditory responses via two receptor nerve cells (A1 and A2) of which the A2 cell fires only when a bat is close. Roeder (1974) proposed that the anti-bat flight defence of noctuid moths is bimodal with the most sensitive auditory cell (A1) initially evoking controlled flight away from an approaching bat and the less sensitive cell (A2) then activating the ASR. In this study, we test the prediction that the A2 cell of endemic Tahitian moths has either disappeared or is physiologically regressed compared to adventive species.

2. MATERIAL AND METHODS

We conducted our study from January to February 2005 in French Polynesia using the facilities of the Gump South Pacific Research Station, Moorea. We collected endemic and adventive moths (Family: Noctuidae) on Mount Marau (elevation: 1413 m) on the island of Tahiti using incandescent mercury vapour and fluorescent ultraviolet lights. We identified moths according to Orhant (2002, 2003).

For electrophysiological recordings, the tympanic nerve (IIIN1b; Nüesch 1957) of the moths was exposed and auditory receptor action potentials were recorded with a stainless steel hook electrode referenced to another in the moth's abdomen. Neural responses were amplified with a Grass Instruments P-15 preamplifier and stored on a PC laptop using a 50 MHz sampling board (Pico Technology ADC 212/3) run by an oscilloscope-emulating program (PicoSCOPE v. 5.10.7). The ears were exposed to pulsed synthetic sounds generated by a customized MATLAB application and run in a PC laptop, amplified (Model 70101, Avisoft Bioacoustics, Berlin) and broadcast from a speaker (Technics EAS-10TH400B) mounted 20 cm from the moths. The auditory preparation and speaker were mounted in a sound-absorbing Faraday cage. Intensities were recorded as peak-to-peak voltages delivered to the speaker and then converted to peak equivalent sound pressure levels (dB peSPL; re 20 µPa r.m.s.) from equal amplitude continual tones as measured with a Brüel and Kjær (B&K) type 4135 6.35 mm microphone and Type 2610 B&K measuring amplifier. The system was calibrated before and after the study with a B&K Type 4228 pistonphone.

We derived auditory threshold curves (audiograms) using 20 ms, 0.2 rise/fall time, 2 s^{-1} sound pulses at 5 kHz frequency increments randomly chosen from 5 to 100 kHz. The A1 cell threshold was determined as the stimulus intensity that evoked two receptor spikes per stimulus pulse, while A2 threshold was determined as that stimulus intensity first evoking the (usually) smaller A2 cell spike. Intensity–response relationships were derived using 10 ms pulses of 25 kHz (the stimulus used to examine flight responses in Fullard *et al.* 2004) delivered at intensities subthreshold to A1 threshold to those approximately 15 dB suprathreshold to the A2 cell.

We choose endemic versus adventive species pairs within the same noctuid subfamilies where possible for pairwise comparisons (Tella 2002). We performed comparisons of the following matched pairs of endemic versus adventive species that were tested in Fullard *et al.* (2004): *Dysgonia insularum* (*Bastilia insularum* in Fullard *et al.* 2004) versus *Mocis trifasciata*; *Callopietria alticola* versus *Chasmina tibialis*; and *Trichoplusia collardi* (*Chrysodeixis collardi* in Fullard *et al.* 2004) versus *Callopietria eriosoma* (taxonomic revisions following Orhant 2003). All pairs except the last were from the same noctuid subfamilies. Significant differences were determined ($p < 0.05$) using either Wilcoxon signed-rank or paired-sample *t*-tests, depending upon the normality and variance of the data.

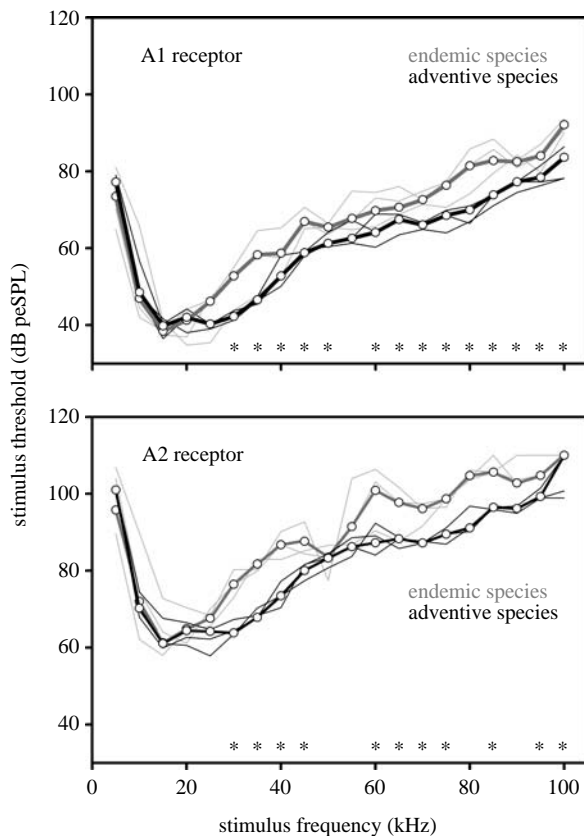


Figure 1. Frequency sensitivity curves of the A1 and A2 receptor cells in endemic versus adventive moth species. Species medians ($n=5$ individuals) are indicated by fine lines and residency class medians ($n=3$ species) are indicated by bold lines. In both graphs, significant differences (asterisks) are determined from matched-pairs analyses.

3. RESULTS

The ears of all the moths tested, endemic and adventive, exhibited both A1 and A2 cell activities when stimulated by sound. Figure 1 compares median A1 and A2 audiograms for the endemic versus adventive moth species ($n=3$ species, 5 individuals for each residency class). The differences between the audiograms were determined from matched-pairs analyses and demonstrate that endemic A1 and A2 cells exhibit significantly reduced sensitivities at frequencies above 30 kHz.

Figure 2a illustrates a typical intensity–response curve of the A1 and A2 receptor cells to a 10 ms burst of 25 kHz sound. The first receptor to fire is the A1 cell which increases its firing rate as the sound intensity rises, levelling off at approximately 70 dB. The second, smaller amplitude A2 cell begins its response at thresholds typically 20–30 dB higher than those of the A1 cell and also increases its firing to more intense sounds but does not level off to the highest stimulus decibel applied. Figure 2b shows the comparisons of adventive versus endemic spiking responses at four stimulus intensities. Matched-pair analyses reveal that while there were no significant differences in the A1 spikes per stimulus pulse of adventives versus endemics, the A2 cell of endemics revealed significantly fewer spikes per 80 dB stimulus pulse compared with adventives.

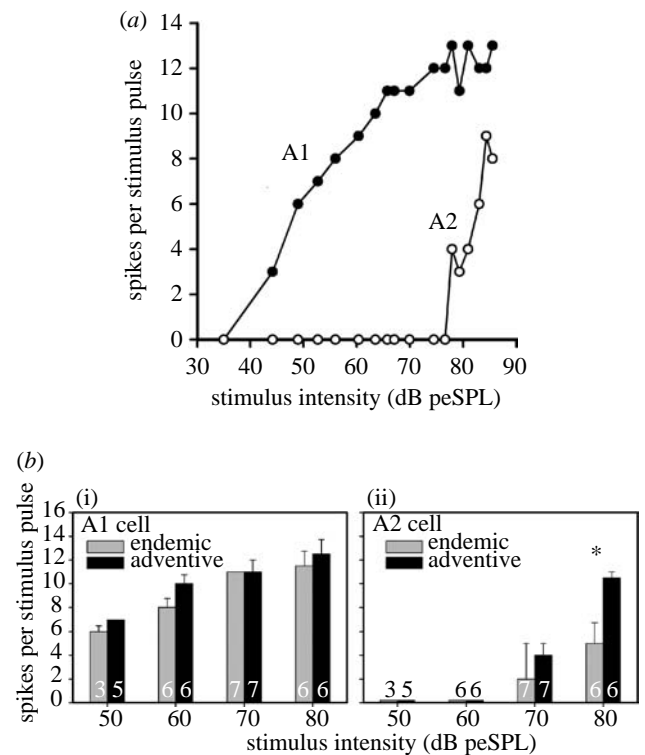


Figure 2. (a) Single stimulus intensity–response curves of the A1 and A2 receptor cells from which the spike numbers per stimulus pulse were taken. (b) Comparisons of median (+75th percentile) A1 and A2 cell spiking responses for endemic versus adventive moths to 25 kHz, 10 ms pulses at four stimulus intensities, sample sizes superimposed upon bars. Significant differences (asterisks) are determined from matched-pairs analyses. Values for A2 responses at 50 and 60 dB are zero.

4. DISCUSSION

Based on Roeder's (1974) theory of neural bimodal control of noctuid defensive flight, our prediction that the ASR-evoking A2 cell in bat-released endemic moths has regressed was supported by our findings but not to the point of the complete disappearance of this neuron. Endemic moth ears exhibit increased A2 thresholds to ultrasound above 30 kHz reflecting differences in the A1 cell as reported by Fullard (1994) and reduced A2 firing responses to intense pulses of 25 kHz sound, the stimulus that fails to evoke ASR in these moths (Fullard *et al.* 2004). We therefore suggest that the partial regression of the A2 cell has resulted in the disappearance of the ASR, but the fact that responsiveness persists in this neuron implies that this process is not yet complete. As with the audiograms, the differences in A2 firing, although significant, reveal overlap between the two classes of moths, suggesting that some endemics will still exhibit an ASR. Fullard *et al.* (2004) demonstrated that five out of six endemic species exhibited no ASR when exposed to 25 kHz pulses when these sounds were presented at intensities simulating that of approaching bats. However, one of the endemics, *Mythimna mouai*, exhibited adventive-like flight responses suggesting that this species still possesses normal ASR thresholds. The reduced firing response of the A2 cell in endemic species suggests that it is possible to evoke ASRs in regressed Tahitian noctuids by simply

increasing the ultrasonic stimulus to levels above those normally experienced by moths sympatric with insectivorous bats. If true, this would imply that the circuitry for the ASR in endemics remains intact but requires a supernormal stimulus to activate it.

Our results suggest that neural regression in the form of sensoribehavioural disintegration without stabilizing selection is a gradual process favouring the theory of character degeneration arising from random or neutral mutation (Leys *et al.* 2005) as opposed to an adaptive loss of characters (e.g. cavefish eyes; Jeffery 2005). While the ears of Tahitian endemic noctuids appear to be only partially regressed, total ear loss exists in other bat-released moths (e.g. island moths (Clarke 1971), wingless moths (Sattler 1991)), presumably following a longer time without this selection pressure. Other studies of regressed behaviours in insects also report subtle degenerative changes to associated neural circuitry (e.g. wing proprioceptors in flightless grasshoppers (Arbas 1983), auditory receptors in silent grasshoppers (Riede *et al.* 1990)). Although gross cellular structure persists in the neurons studied in these reports, all exhibit reductions in their terminal regions predicting a decrease in their synaptic output with interneurons. If the same is true for the A2 cell of endemic Tahitian noctuids, this compromised efficacy would add to the observed effects of reduced firing in failing to evoke the ASR.

The noctuid moth anti-bat ASR offers a unique opportunity to study the details of sensoribehavioural integration and its regression since it is a highly adaptive behaviour that is evoked by extremely simple neural pathways. Furthermore, since it appears to be the auditory trigger of evoking the ASR in Tahitian moths that has degenerated rather than the behaviour of flight cessation itself (Fullard *et al.* 2004), the search for the underlying neural regression can be focussed to one sensory pathway and, perhaps, to a single synaptic level. Another resident of bat-released Tahiti, the Polynesian field cricket (*Teleogryllus oceanicus*) whose anti-bat behaviour appears to be evoked by critical firing rates in a single interneuron (Nolen & Hoy 1984) offers the opportunity to study the sensoribehavioural regression at the central nervous system and we are currently pursuing this avenue of research.

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