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Auditory change detection by a single neuron in an insect

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Abstract

The detection of novel signals in the auditory scene is an elementary task of any hearing system. In *Neoconocephalus* katydids, a primary auditory interneuron (TN-1) with broad spectral sensitivity, responded preferentially to rare deviant pulses (7 pulses/s repetition rate) embedded among common standard pulses (140 pulses/s repetition rate). Eliminating inhibitory input did not affect the detection of the deviant pulses. Detection thresholds for deviant pulses increased significantly with increasing amplitude of standard pulses. Responses to deviant pulses occurred when the carrier frequencies of deviant and standard were sufficiently different, both when the deviant had a higher or lower carrier frequency than the standard. Recordings from receptor neurons revealed that TN-1 responses to the deviant pulses did not depend on the population response strength of the receptors, but on the distribution of the receptor cell activity. TN-1 responses to the deviant pulse occurred only when the standard and deviant pulses were transmitted by different groups of receptor cells. TN-1 responses parallel stimulus specific adaptation (SSA) described in mammalian auditory system. The results support the hypothesis that the mechanisms underlying SSA and change-detection are located in the TN-1 dendrite, rather than the receptor cells.

Keywords

Hearing; Stimulus-specific adaptation; Auditory scene analysis; Novelty detection;
Neoconocephalus

Introduction

Animals that use acoustic signals to perceive their environment depend on their ability to detect relevant sound signals among an abundance of irrelevant sounds (Alain and Arnott 2000; Fay and Popper 2000). Auditory scene analysis is generally described as a two-stage process in which selective attention ('schema-based' process) operates on the output of a pre-attentive analysis ('primitive' process) that forms auditory objects from the sound mixture reaching the ears (Alain and Arnott 2000; Cooke and Ellis 2001).

One fundamental task of auditory scene analysis is the detection of change. Sudden changes in the auditory scene, e.g., the appearance of sound made by a predator, may indicate events that are highly relevant for an individual's fitness. Change detection also plays important roles in signal recognition; for example, sudden amplitude modulation (AM) changes correspond to phonemes in speech recognition (Viemeister 1979; Bacon and Viemeister 1985).

Reduced activity of single neurons or networks to repeated stimulation is generally considered to underlie change detection. Such stimulus-specific reduction of neuronal activity is commonly referred to as stimulus-specific adaptation (SSA, Ulanovsky et al. 2003) or repetition suppression (RS) (Grill-Spector et al. 2006). It occurs at various levels in the mammalian auditory pathway, including the auditory cortex (Ulanovsky et al. 2003), midbrain (Malmierca et al. 2009) and thalamus (Antunes et al. 2010). The underlying neuronal mechanisms, especially the effects of repetitive stimulation on individual neurons, however, are not well understood (Antunes and Malmierca 2011). Recent findings in an insect system (Schul and Sheridan 2006) provide a model system to study the detection of change in auditory scenes in an identified neuron.

In the katydid genus *Neoconocephalus* (Orthoptera, Tettigoniidae), the auditory system functions in two contexts, interspecific communication and the detection of predators, namely echolocating bats (Greenfield 1990; Libersat and Hoy 1991). The two signal types differ in their amplitude modulation rate (male calls typically >100 Hz, bat cries <10 Hz) and carrier frequency (male calls 10–15 kHz, bat cries 30–50+ kHz) (Greenfield 1990; Fenton et al. 1998).

Bat-like signals are extracted from the background of the auditory scene early in the auditory pathway by a first-order interneuron (TN-1). In the presence of standard pulses presented at high repetition rates (equivalent to male calls), TN-1 responded selectively to deviant pulses with low repetition rates (equivalent to bat signals), if the deviants differed in carrier frequency from the standard (Schul and Sheridan 2006). This process resembles SSA or RS in auditory systems of vertebrates, making the katydid TN-1 an excellent candidate for studying the cellular mechanisms underlying these processes.

Here, we study the neuronal processing leading to the detection of the deviant tones by the TN-1 interneuron in *Neoconocephalus*. First, we quantify the importance of carrier frequency and amplitude of standard and deviant pulses. Second, we examine the input pattern provided by auditory receptors to TN-1. Third, we test the importance of inhibitory

inputs for the detection of the deviant pulses. Based on our results, we conclude that the response properties of the receptor neurons cannot account for change detection by TN-1. Instead, we propose that dendritic mechanisms intrinsic to TN-1 serve as the primary mechanism.

Methods

Animals

We collected female *Neoconocephalus retusus* (Scudder 1862) as adults in Boone County, Missouri (USA) and identified them after Froeschner (1954). The insects were kept at 17–25 °C and a light:dark cycle of 14:10 h. Experiments were conducted within 2 weeks after collection.

Neurophysiology

The animals were anesthetized with CO₂ and mounted ventral side up on a freestanding metal holder with a wax/resin mixture. The front legs were fixed on a wire holder perpendicular to the body axis, middle and hind legs were fixed along the body wall. We exposed either the cervical connectives (for TN-1 recordings) or the prothoracic ganglion (for receptor cell recordings) by removing a small piece of cuticle. The exposed area was covered with saline (modified after Fielden 1960). The ganglion was stabilised for receptor cell recordings by a NiCr-spoon, which also served as an indifferent electrode. We inserted a silver wire in the abdomen as indifferent electrode during TN-1 recordings.

Experiments took place in an anechoic Faraday cage (1.2 m × 1.2 m × 0.7 m) at 22–24 °C. Due to its large axonal diameter, spikes of the TN-1 auditory interneuron (for the morphology of TN-1 see Stumpner 1999; Tribblehorn and Schul 2009) can be reliably detected in the summed activity of the cervical connective (McKay 1969; Schul 1997; Faure and Hoy 2000a). We recorded TN-1 spikes with a tungsten hook electrode (70- to 100-μm diameter). The connectives were severed caudal of the subesophageal ganglion. The recording site was covered with silicone-grease (Baysilone, Bayer) to prevent desiccation. The recorded signals were amplified using a custom-made amplifier and band-pass filtered (100–4,000 Hz, Krohn-Hite 3342).

Intracellular recordings of individual receptor fibers were made at the entrance of the leg nerve into the prothoracic ganglion using thick-walled borosilicate glass microelectrodes filled with 3-mol l⁻¹ KCl (resistance 20–40 MΩ). The recorded signals were amplified (Warner Instr. Corp. IE-210), low-pass filtered at 4 kHz (Krohn-Hite 3384).

Both types of recordings were digitized (10 kHz sampling rate, 14-bit resolution, InstruNet 100B, Omega Engineering, Inc., New York) and evaluated with custom-designed computer programs. The timing of spikes relative to the stimulus onset was marked with 0.1 ms temporal resolution. We describe the subsequent analysis of these data below.

Stimulation

Sound stimuli were delivered via one loudspeaker (Technics 10TH400C) located 50 cm from the preparation, perpendicular to the body axis, and ipsilateral to the recording site. The stimuli were generated using a 16-bit DA-converter system (National Instruments PCI-6251) at a sampling rate of 250 kHz using custom-designed software developed in LabView (National Instruments). Stimulus amplitudes were manipulated by a computer-controlled attenuator in steps of 3 or 6 dB.

The amplitudes of the stimulus signals were calibrated at the position of the insect using a B&K 2231 sound level meter (Brüel and Kjær, Nærum, Denmark) and a 1/4 in. free field microphone (G.R.A.S. 40BF). Sound measurements were obtained on the preparation site with no animal present. Signal amplitudes are given in dB peak SPL (re 20 μ Pa), which is, for sine waves, 3 dB above the respective root-mean-square value. At the recording site, slight echoic influences were unavoidable, but these influences did not alter the intensity or the envelope of the signals by more than ± 1 dB.

TN-1 threshold measurements

We measured TN-1 thresholds for sinusoids in the range of 6.3–60 kHz. Stimuli had a trapezoid-shaped envelope with a rise and fall time of 0.5 ms and a 2.5 ms plateau time. Stimuli were presented at a repetition rate of 2 Hz. Each frequency was played back at 18 different amplitude attenuations in 3-dB steps. Each frequency/amplitude combination was presented three times. Absolute stimulus amplitudes for each frequency were set so that the lowest amplitude tested was well-below threshold. We counted TN-1 spikes occurring within 35 ms after stimulus onset as responses. We defined threshold as the lowest stimulus amplitude that elicited spiking responses in two of the three presentations.

Quantifying detection of deviant pulses

We modeled the standard stimulus after the conspecific male call, and the deviant pulses after bat signals. Male *N. retusus* produce calls with very high pulse rates (140 pulses/s at 25 °C; Greenfield 1990), with spectral energy concentrated in a narrowband low-frequency component (Q_{3dB} between 5 and 6), centered around 15 kHz (Schul and Patterson 2003). The standard signal was a train of pure tone pulses (duration 3.5 ms, including 0.5 ms rise and fall time) repeated after silent intervals of 3.5 ms, equivalent to a pulse rate of approximately 140 Hz. The carrier frequency of the standard pulses was typically 15 kHz, but was varied in some experiments.

Echolocation cries of most aerially hawking bats (e.g., *Eptesicus fuscus*) are short frequency-modulated sweeps with most energy between 30 and 50 kHz (Fenton et al. 1998). During the search and approach phase, echolocation cries are repeated at low rates of 5–10 pulses/s (Fenton 1982). Katydid respond to bat cries at relatively low stimulus amplitudes, and bat-avoidance behaviors occur before the bat is likely to detect the insect's echo (Schulze and Schul 2001; Kilmer et al. 2010). We modeled the deviant tones after such echolocation cries as short pure tone pulses (duration 3.5 ms, including 0.5 ms rise and fall time), presented at a period of 140 ms (approximately 7-Hz pulse rate). The carrier

frequency of the deviant pulses was in most experiments 40 kHz, but was varied in some experiments.

To test the detection of the deviant pulses among standard pulses, we used the following stimulus situation (Fig. 1). The standard pulses were presented for a total of 8 s: the first 3 s alone (i.e., without deviant pulses inserted) and the following 5 s with deviant pulses inserted into the call model, for a total of 35 deviant pulses (i.e., 1 deviant pulse for every 20 standard pulses). The deviant pulses were inserted in the silent intervals between two standard pulses. Both standard and deviant pulses were constant in carrier frequency and amplitude during the 8 s presentation. We kept 112 s of silence between different stimuli to avoid long-term changes of TN-1 responses.

We quantitatively analyzed spiking responses to the latter 5 s of each stimulus (the part with deviant pulses inserted, Fig. 1). Spikes occurring within 35 ms of the onset of deviant pulses (i.e., within the first third of the deviant pulse period) were classified as responses to the deviant, while spikes occurring outside of this time window were classified as responses to the standard (Fig. 2). We present the fraction of the 35 presentations of deviant pulse that evoked spiking responses for each stimulus. Throughout our experiments, the mean response probability during the standard time windows was always <0.1 (mean, $n = 10$ in all cases; see Schul and Sheridan 2006 for the magnitude of the responses to standards), we therefore do not report these numbers in the “Results” section (see Fig. 2 for a typical TN-1 response). We chose response probability rather than spike/pulse as a measure, because we wanted to test the reliability of the encoding of the deviant pulses. Furthermore, TN-1 responses to the deviant were typically 1 action potential (AP) per pulse, the first few deviants, however, often resulted in burst of 4–8 spikes (see Fig. 2) and would unduly influence a mean spike count. The response probability, thus, is the more adequate measure for change detection in this system.

Experiment 1: importance of contralateral inhibition

TN-1 receives its main excitatory input from the auditory receptor neurons in the ear ipsilateral to its axon (Römer et al. 1988). In addition, it receives prominent inhibition from the axon-contralateral receptor cells, most likely mediated by the omega-neuron (Schul 1997; Römer et al. 2002). To test whether this contralateral inhibition contributes to the selective responses to deviant pulses, we compared TN-1 responses to one stimulus situation (standard pulses 15 kHz, deviant pulse 40 kHz) before and after severing the contralateral leg nerve, which contains the axons of the contralateral receptor cells.

Experiment 2: importance of the amplitude of the standard pulses

Here, we determined the detection thresholds of 40 kHz deviant pulses at four amplitude levels of 15 kHz standard pulses (50, 60, 70, and 80 dB SPL). At each amplitude level of the standard pulses, we presented a series of 8-s stimuli with amplitudes of the deviant pulses increasing from 30 to 72 dB SPL in 6 dB steps (8 stimuli/series). Before and after of each these series, we presented additional series with stimuli consisting only of the deviant pulses (i.e., without standard pulses), which were used as reference to determine the detection

threshold shift caused by the standard pulses. The sequence of the four series with standard pulses was varied among the ten animals tested.

For each experimental series, we constructed the amplitude response function of the deviant pulses. Detection thresholds were determined as the amplitude with 50 % response probability to the deviant pulses using linear interpolation. Detection thresholds are presented as threshold shifts relative to the threshold in the 'deviant only' series tested before and after. The detection threshold for the 'deviant only' situation was calculated from the fourth presentation of this series relative to the third and fifth presentation of the 'deviant only' series.

Experiment 3: frequency dependence of SSA

In this experiment, we varied the carrier frequency of the standard and deviant pulses independently of each other. Standard pulses ranged between 8.9 and 50.4 kHz and deviant pulses between 7.5 and 60 kHz. Carrier frequencies were spaced at 1/4 octave distance, i.e., the steps were equidistant on a logarithmic frequency axis. The amplitude of both standard and deviant pulses was kept at 70 dB SPL.

Receptor cell recordings

We characterized the frequency tuning of individual receptor neurons using iso-amplitude stimulation from 4 to 80 kHz (4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80 kHz) presented at amplitudes of 60 and 80 dB SPL. The stimuli, pure tone pulses with 12-ms duration (including 1 ms rise and fall time), were presented three times each. Spikes occurring within 35 ms of stimulus onset were counted as responses. The resulting iso-amplitude curves had a continuous frequency range in which the response strength was higher than the background activity; this range was wider for 80 dB SPL (Höbel and Schul 2007). We determined the best frequency (BF) of each receptor cell as the geometric center of this range on a logarithmic frequency axis. The BF was determined from the 60 dB SPL iso-amplitude function if it showed a distinct range with strong responses, otherwise the 80 dB SPL function was used. With few exceptions, the BF was also the frequency that elicited strongest responses.

We tested the representation of deviant pulses in receptor cell responses with the same 8-s stimuli as described above. We used three stimulus situations: (1) standard pulses with 15 kHz and deviant pulses with 40 kHz carrier frequency (both at 70 dB SPL); (2) standard pulses 40 kHz, deviant pulses with 15 kHz carrier frequency (both at 70 dB SPL); (3) standard and deviant pulses both at 15 kHz, with deviants having +15 dB higher amplitude (standard at 70 dB SPL). The temporal parameters were identical to the TN-1 experiments described above. We generated peristimulus time histograms (PSTH) (bin width 2 ms) for the responses of individual receptor cells and of several cells added together. To quantify the population response strength to the deviant pulses, we counted the number of APs occurring during the 7-ms time window that contained the deviant pulse and one standard pulse (N_{D+S}) and during the 19 other 7-ms windows (N_{S-only}), which contained only one standard pulse. These time windows were set after correcting for the latency of the receptor cells. We

calculated the ratio $N_{D+S}/N_{S\text{-only}}$ for each of the 19 $N_{S\text{-only}}$ time windows and present mean $\pm$ SD. This analysis was performed for the complete data set as well as for the group of six receptors presented in Fig. 6 (see below).

We recorded from a total of 22 receptor neurons in five individuals (4–7 receptors/individual). We present a subset of six receptors with BFs between 6 and 60 kHz, which is representative for the receptor cell population in *Neoconocephalus* katydids (Höbel and Schul 2007; Schul and Patterson 2003). The summed responses for this subset were similar to the summed response of all cells recorded.

Results

Influence of contralateral inhibition

In intact preparations, TN-1 responded selectively to the deviant pulses, while the standard pulses did not evoke ongoing responses (Fig. 2a). Responses to the standard pulses were limited to the beginning of the 8-s stimulus and subsided within <500 ms (see also Schul and Sheridan 2006). Cutting the contralateral leg nerve (and thus removing the contralateral inhibitory input on TN-1) did not change the TN-1 response pattern, and the response probability to the deviant pulses was slightly higher than that in the intact preparation (cut: 0.99 ± 0.01 , intact 0.92 ± 0.09 , mean $\pm$ SD, $n = 6$ each; Fig. 2c). The average responses to the standard pulses were <0.1 in either situation.

Amplitude dependence

Here, we tested how the amplitude of the standard pulses (15 kHz carrier frequency) influenced the sensitivity for 40 kHz deviant pulses. When we presented the deviant pulses alone, the intensity response function reached 50 % response probability at approximately 40 dB SPL (median, $n = 10$; open squares, Fig. 3a). Simultaneously presented standard pulses at 50 dB SPL did not change the sensitivity for the deviants noticeably. With increasing amplitudes of the standard pulses, the amplitude response functions to the deviants shifted toward higher amplitudes (Fig. 3a). Standard pulses at 60, 70, and 80 dB SPL shifted the median of the 50 % detection threshold of the deviant by 1.7, 5.8, and 13.9 dB relative to the ‘deviant only’ situation (Fig. 3b). These shifts were significant at 70 and 80 dB SPL (one-sided paired t test, $p < 0.001$ in both cases; $\alpha = 0.0125$ after Bonferroni correction for $n = 4$ tests); at 60 dB SPL the threshold difference was marginally significant ($p = 0.0128$, $\alpha = 0.0125$ after Bonferroni correction for $n = 4$ tests).

Frequency dependence

For this set of experiments, we first determined TN-1 thresholds for tone pulses presented at a rate of 2/s. TN-1 was broadly tuned from 6.3 to 60 kHz, with highest sensitivity occurring between 20 and 30 kHz ($n = 11$; data not shown). Between 7.5 and 60 kHz, TN-1 thresholds were almost always below 60 dB SPL in the 11 individuals tested. The only exceptions were two individuals with thresholds at 60 kHz of 67 dB SPL and one individual with threshold of 61 dB SPL at 7.5 kHz.

Next, we varied the carrier frequency of standard and deviant pulses independently between 7.5 and 60 kHz. All pulses were presented at 70 dB SPL, i.e., at least 10 dB above threshold (see above). No responses to deviant pulses occurred when the carrier frequencies were identical (black diagonal band from bottom left to top right in Fig. 4). Response probabilities increased with increasing difference in frequency between the standard and deviant pulses. This was true when the deviant pulses had lower and higher carrier frequencies than the standard pulses. When the carrier frequency of the standard was at or below 15 kHz, only deviant pulses with a higher carrier frequency elicited responses. For standard carrier frequencies of 30 kHz and higher, only deviant pulses with lower frequencies elicited responses (Fig. 4).

Receptor cell responses

To characterize the afferent input that TN-1 received, we determined the response pattern of auditory receptor neurons during stimulation with standard and deviant pulses. We collected data from 22 receptor cells in five animals. Six of these receptor cells had best frequencies (BF) below 10 kHz, seven between 10 and 20 kHz, five between 20 and 40 kHz and four above 40 kHz; this distribution is representative for the BF-distribution of receptor cells in the ear (Höbel and Schul 2007).

During stimulation with standard pulses at 15 kHz and deviant pulses at 40 kHz, receptor cells with BFs between approximately 10 and 25 kHz copied the temporal pattern of the standard, while receptors with higher BFs responded strongly to the deviant pulses (Fig. 5a, left column). In the inverse frequency situation (standard 40 kHz, deviant 15 kHz), receptor cells with BFs of 25 kHz or higher encoded the pulse rate of the standard, while receptors with lower BFs (10–25 kHz) responded strongly to the deviant pulses (Fig. 5a, middle column). In both situations, the TN-1 response probability to the deviant was high (mean > 0.8, $n = 10$, for both situations, Fig. 5c, left and middle column).

We also tested the situation where both standard and deviant pulses had 15 kHz carrier frequency, but the deviant pulses were presented at a 15 dB higher amplitude. TN-1 response probability to the deviant pulses in this stimulus situation was low (0.27 ± 0.27 mean $\pm$ SD, $n = 9$). Receptor cells with BFs between 10 and 25 kHz responded strongly to both standard and deviant pulses, while receptor cells with adjacent BFs (7–10 kHz and 25–40 kHz) responded mostly to the deviant pulses (Fig. 5a, right column).

The representation of the deviant pulses in the summed activity of the receptor cell population did not differ significantly among the three stimulus situations. The deviant pulse is well-detectable within the combined PSTH (Fig. 5b). During the 7-ms period that contained the deviant pulse (and one standard pulse) the number of elicited action potentials (APs) was 1.9- to 2.9-fold the APs elicited during an average 7-ms interval containing only one standard pulse (Fig. 6). The relative response to the deviant was largest in the third stimulus situation (15/15 + 15 stimulus combination; Fig. 6), both for the selected group of receptors shown in Fig. 5, and for all receptors recorded in this experiment ($n = 22$). Note that this was the stimulus combination that evoked weakest TN-1 responses to the deviant pulses. This means that TN-1 responses to the deviant pulse did not depend on the response

strength of the population of all receptor cells. Rather, the selective pattern of receptor cell activation appears important: strong TN-1 responses to the deviant occurred when standard and deviant pulses stimulated separate groups of receptor cells (Fig. 5 left, middle), while TN-1 responses were weak when standard and deviant pulses were transmitted by the same group of receptors cells.

Discussion

The auditory interneuron TN-1 responded selectively to deviant pulses with a slow repetition rate that were simultaneously presented with a fast pulse rate standard stimulus, if the deviants differed sufficiently in carrier frequency from the standard. The amplitude of the standard stimulus significantly affected the sensitivity to the deviant. TN-1 responses to the deviant were not determined by the strength of the response of the total receptor cell population. Responses occurred when the deviant pulse was transmitted by a different receptor cell population than the population activated by the standard pulses.

The response properties of TN-1 were characterized by rapid adaptation to the fast pulse rate of the standard pulses. Spiking responses occurred only at the onset of the standard pulse train and usually ceased within 500 ms (Schul and Sheridan 2006). Despite the strong suppression of responses to standard pulses, TN-1 remained highly responsive to deviant pulses. Deviant pulses of up to 25 dB lower than the standard pulse amplitude were detected (Fig. 3a) and standard pulses at lower amplitudes (50–60 dB SPL) had little, if any, impact on the deviant threshold (Fig. 3b). This sensitivity allowed TN-1 to reliably detect changes in the auditory scene.

Reduced neural activity in response to repeated stimulation while remaining sensitive to rare signals occurs in a wide range of organisms across various levels in the nervous system. It is commonly referred to a stimulus-specific adaptation (SSA, Nelken and Ulanovsky 2008). We use the term SSA without implying a specific underlying neural mechanism.

Several parallels occur between SSA in TN-1 and that in the auditory system of mammals. At earlier, subcortical stations of the mammalian auditory pathway (inferior colliculus), strongest SSA was found in neurons with broad spectral sensitivity (Malmierca et al. 2009). TN-1 is also broadly tuned and responded almost exclusively to the deviant stimulus. Responses to the deviant pulses were strongly phasic both in mammalian neurons (Antunes et al. 2010) and in TN-1. Finally, strong SSA occurred when the deviant had lower and higher carrier frequency than the standard (Malmierca et al. 2009; Antunes et al. 2010). These parallels make TN-1 an important system to study the cellular basis of SSA, since TN-1 is a primary interneuron, receiving direct excitation from auditory receptor neurons (Römer et al. 1988).

The ‘memory’ about preceding stimuli is likely localized in the dendrites

The selective responses to deviant pulses require some form of ‘memory’ about preceding stimulation. Such memory traces could be within the afferent responses or occur within the

neurons exhibiting SSA themselves. Alternatively, additional ‘memory neurons’ could mediate the SSA (Jääskeläinen et al. 2007).

TN-1 receives direct excitatory input from the auditory receptor neurons, as evidenced by the short and highly consistent latencies of TN-1 (Römer et al. 1988; Schul 1997). The receptor neurons could contribute to the SSA of TN-1 either through reduced spiking activity or reduced synaptic transmission to TN-1 in response to the standard stimulus. However, spiking responses of receptor cells did not decrease and responses to the standard pulses persisted throughout the 8 s of stimulation (Fig. 5). Several other auditory interneurons (e.g., ON, AN1) have tonic responses to stimulation with fast pulse rates (Tribblehorn and Schul 2009), indicating that synapses of receptor neurons are capable of transmitting the fast pulse rate of the standards.

It is possible that the synaptic transmission properties of the receptor cells’ are influenced by the post-synaptic neuron; one receptor cell could form synapses with one interneuron that reliably transmit the repeated signal for extended periods, while synapses with another interneuron show simultaneously synaptic depression (Killian and Murphey 1998). However, such a situation seems unlikely in TN-1. During the stimulus situation used in Fig. 3 (standard 15 kHz, deviant 40 kHz), different receptor cell groups transmit each carrier frequency (Fig. 5). At higher amplitudes, some receptor cells with intermediate BFs will be stimulated by both standard and deviant pulses (due to the V-shaped threshold curves of the receptor cells). However, near threshold, the deviant pulses will be transmitted by receptor cells not responding to the standard pulses (Fig. 5; note that the deviant was here at 70 dB SPL, 15–30 dB above threshold Fig. 3a). If the transmission of the standard pulses onto TN-1 ceased due to synaptic depression, increasing the amplitude of the standard pulse should not affect thresholds to deviant pulses. Thus, the significant shift of thresholds to deviant pulses at higher standard pulse amplitudes (Fig. 3b) supports the hypothesis that auditory receptor neurons are unlikely candidates for generating the SSA of TN-1.

Besides the excitatory input from ipsilateral receptor neurons, TN-1 receives prominent inhibition from contralateral receptor cells via the omega neuron (ON, Römer et al. 1988; Molina and Stumpner 2005) as well as inhibition from low-frequency (LF) receptor cells, likely mediated by dorsal unpaired medial (DUM) neurons (Stumpner 2002). Severing the contralateral afferents (Fig. 2b) did not affect SSA of TN-1. Thus, contralateral inhibition played little to no role in the generation of SSA. The inhibition from LF receptor cells also appears unlikely to contribute to SSA, since SSA occurs over a wide range of frequencies and when the deviant pulse carrier frequency is higher or lower than the carrier frequency of the standard pulses (Fig. 4).

Based on the above reasoning, we conclude that neither the properties of the receptor cells nor the known inhibitory inputs of TN-1 underlie SSA. We propose here that the ‘memory traces’ are intrinsic to TN-1 and that the processes underlying SSA of TN-1 occur within the dendrite of TN-1, activated by the pattern of excitatory input from the receptor cells. The response pattern of the receptor cell population (Figs. 5, 6) indicated that TN-1 responses did not depend on the absolute amount of receptor cell activity. TN-1 responded to the

deviant pulses only if they were transmitted by a different group of receptor cells than the standard pulses. The spatial arrangement of receptor cell projections onto the TN-1 dendrite (Römer et al. 1988) might play a role in the generation of SSA in the TN-1 dendrite.

Biological function of SSA in TN-1

Due to its large size and strong adaptation, the Tettigoniid TN-1 is generally considered to function in bat detection (Faure and Hoy 2000a, b). In *Neoconocephalus*, the strong adaptation is the main argument against a role of TN-1 in the processing of the continuous communication signals (Tribblehorn and Schul 2009). A role in reproductive communication appears possible only in systems with transient signals of low repetition rates (e.g., Schul 1997; Siegert et al. 2011). The correlation between bat avoidance behavior and TN-1 responses is weak, however, suggesting the involvement of other sensory neurons and the integration of these inputs at higher levels for decision-making (Schulze and Schul 2001; Kilmer et al. 2010). While the response properties of TN-1 efficiently segregate ultrasonic bat signals from a background of male *Neoconocephalus* calls (15 kHz carrier frequency) (Schul and Sheridan 2006), our results here show that the SSA of TN-1 is not limited to the detection of ultrasonic deviants in low-frequency backgrounds. Transient (i.e., deviant) sounds are detected as long as they differ sufficiently in spectrum from the background (i.e., standards). Thus, TN-1 might simply acts as a detector of change in the auditory scene; evaluation of the information present in the complete auditory pathway would then determine the appropriate behavioral responses. TN-1 should, thus, be considered a ‘novelty neuron’, which potentially could contribute to several behavioral contexts, rather than being a dedicated bat detector.

Such a general change detector would be evolutionarily advantageous. First, the mechanism would be tolerant to evolutionary change of the spectrum of the conspecific communication signals which are inherently a dominant part of the auditory scene, as this would not impact change detection. Second, if a novel signal with fitness relevance (e.g., a novel predator signal) occurs in the acoustic scene over evolutionary times, it would immediately be detected by TN-1, thus providing the opportunity to evolve appropriate response mechanisms.

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Fig. 1. Schematic view of the standard/deviant stimulus at second (*top*) and millisecond (*bottom*) time scales. The standard stimulus consisted of pulses (3.5-ms duration) repeated after a silent interval of 3.5 ms. After 3 s, a deviant pulse (3.5-ms duration) was inserted after every twentieth standard pulse into the silent interval. Note that standard and deviant pulses were played through the same loudspeaker

Fig. 2.

a, b Scatter plot (*top*) and peristimulus time histogram (*bottom*) of TN-1 responses during the 8 s of the standard/deviant stimulus. The *top traces* indicate the stimulus with 15 kHz standard pulses at 140 pulses/s (*open pulses*) and 40 kHz deviant pulses at 7 pulses/s (*filled pulse*). For generation of the plots, the 8 s stimulus was divided into 140 ms sweeps, each including 20 standard pulses. The first 21 sweeps contained only the standard pulses; sweeps 22–56 also included one deviant pulse. The *horizontal dotted line* in the raster plot indicates the beginning of the deviant presentation. The *dashed lines* indicate the 35-ms window used for calculating responses to the deviant pulse. **a** Before (= intact preparation) and **b** after removing contralateral inhibition by cutting the contralateral leg nerve. AP occurring within 35 ms after the onset of the deviant pulse was counted as response to the bat pulse; AP occurring outside of this time window (indicated by *vertical dotted lines*) as responses to the male (15 kHz) pulses. **c** Probability of the deviant pulse to elicit at least one AP during the 35 presentations in the before (*int*) and after (*cut*) cutting the contralateral leg nerve ($n = 6$, mean $\pm$ SD)

Fig. 3.

The importance of the amplitude of the standard pulses on the sensitivity of TN-1 for deviant pulses. Standard 15 kHz, deviant 40 kHz carrier frequency. **a** Response probability to the deviant pulse as a function of deviant pulse amplitude. Standard pulses were presented simultaneously with the deviant pulses at amplitudes of 50, 60, 70, or 80 dB; *open symbols* indicate stimulation with deviant pulses alone. **b** Thresholds relative to the stimulation with deviant pulses alone. Data is given as median and 10th and 90th percentile ($n = 10$).

Asterisks denote significantly higher thresholds than to the deviant only stimulus (paired t test, $p < 0.001$)

Fig. 4.

The importance of the standard and deviant carrier frequency on the sensitivity of TN-1 for deviant pulses. (TN-1 response probability to deviant pulses for carrier frequencies of standard and deviants between 7.5 and 60 kHz, varied in 1/4 octave steps. Mean response probability ($n = 10$) is color coded with *lighter colors* indicating stronger responses; hatched areas were not tested

Fig. 5.

Response of receptor cells to standard/deviant stimuli with three different frequency/amplitude combinations: *left column* standard 15 kHz, deviant 40 kHz, both 70 dB SPL; *middle column* standard 40 kHz, deviant 15 kHz, both 70 dB SPL; *right column* both 15 kHz, standard 70 dB SPL, deviant 85 dB SPL. *Top trace* shows the timing of standard pulses (open) and deviant pulses (black). **a** Peristimulus time histograms (PSTH) of the responses of six receptor cells representative for the receptor cell population; the characteristic

frequency (cF) of each cell is given. **b** PSTH of the summed responses of the six receptors shown in **a**. **c** PSTH of representative TN-1 responses

Fig. 6.

Response magnitude of the receptor cell population to the deviant pulses. The number of spikes occurring during the 7-ms period containing the deviant pulse and one standard pulse are given relative to the average spike number occurring in the 7-ms periods containing only one standard pulse. *Gray columns* give the values for the six receptor cells shown in Fig. 5, *open columns* indicate the results for all 22 receptor cells