Sensory processing and the evolution of female preference in Neoconocephalus

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By

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The undersigned, appointed by the dean of the Graduate School,

have examined the Thesis entitled

SENSORY PROCESSING AND THE EVOLUTION OF FEMALE PREFERENCE IN NEOCONOCEPHALUS

Presented by Garrett Andrew Hartman

A candidate for the degree of

Master of Arts

And hereby certify that, in their opinion, it is worthy of acceptance.

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Professor Johannes Schul

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Professor Robert Sites

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Professor David Schulz

ACKNOWLEDGMENTS

It was four years ago that I step foot in Columbia and the MU campus, and what an interesting trip it has been. From the many friends I have been fortunate enough to make, to the steadfast administrative prowess of the departmental staff (e.g., Nila), my time here has been incredibly fulfilling. There are times when I felt like I was completely in the dark, and had no idea where things were going, but I knew I was in a good academic group who would support me to the finish, as well surrounded by caring friends[1][2] and family who would be there for me to lean on, even when I didn't know I would need it.

My experiments were done in Lefevre Hall, where the 2nd floor neurobiology crew was extremely helpful, if only for the occasional lunch or chat by the water fountain. My working office was located on the 2nd floor of Tucker Hall, where there was an equally eclectic atmosphere[3]. It's nice to be able to walk down the hall and see a

[1] Before I entered graduate school, I took an undergraduate biology class at MU and my TA was Mari Morales. She eventually graduated with a PhD, but before that happened, I would stroll on up to the 4th floor of Tucker and borrow her time in between gels and imaging yeast cells. She was a down-to-earth graduate student whom I felt comfortable chatting with, and she always was ready to sit and drink some tea. I would ask her general questions about graduate school and science stuff, and she helped ease the weirdness of my new job. Through her, I met her husband Jon, and fellow friends Kathy and Bandhu. The informal get together were a welcome break from the deadlines and classes.

[2] When I first arrived in 2010, I met four postdocs, Patrick Guerra, Tim and Dani Ostrowski, and Janez Presern. These four, along with Lily Tesfai and fellow graduate student Tim Pale and Autoosa Salari (Doctor Salari, to me: when me and Tim Pale were attempting to change the oil in my car and I suffered a severe metal splinter under my fingernail, she proved her mettle with a pair of tweezers and a dissecting microscope during a 30 minute out-patient surgery) formed a welcoming group of science folks who cushioned my landing into graduate school. Tim and Dani were great hosts for everything from Halloween parties to birthday parties. And their new addition, Zoe, made everything even more special. Through Tim and Dani I met Manuel and Mariana and later little León, who were another source of friendship and helped to keep me sane.

[3] While I did make it occasionally to the 1st, 3rd, and 4th floor, it was the 2nd floor that housed most of the ecology/evolution labs. I was particularly lucky to have a neighboring office with Jeremy Gibson, who one day asked me to get some lunch, and we've been meeting up for lunch ever since. Me and Jeremy learned a lot about each other, in that we do not have a lot of things in common. He likes light beer, I like

friendly face every day. My office mates were an interesting bunch[4]. Between retelling my egg experiment 14 times, starting a twitter account, or just sharing professional and personal stories, there was never a dull moment. Never. A fly on the wall of that office might think it sometimes was a little too undull (if flies could think), but for me, I wouldn't trade these times for all the gold in the world.

If this was a ship, then Johannes was the captain. I feel grateful to have been under his wing learning science, neurophysiology, and evolution. I think back on all the one-on-one time he spent with me, and I wonder, who really came out with the better deal? I can only say that I probably got way more out of our conversations than he did.

Lastly but not least, I have to thank my family. While I did not come from an academic family background, their support was unwavering. My brother's curiosity about science stuff always kept me grounded. If I couldn't explain what I had learnt to him, what hope was there to explain it to anyone, much less myself? To say my parents were steadfast throughout my time here at MU would be an understatement. I can only liken them to a calm, peaceful lake, which has all the patience in the world. I suppose when I am in their position, I will try to match their capacity to care and love and support.

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dark. He is a realist, I am a romantic. All this makes me wonder, "why do I hang out with this guy so much?" My only answer is, that maybe we are similar in what really matters: in the heart. To put it another way, if I did a brain transplant with Jeremy, I would start thinking very very differently. But if we had heart transplants I don't think my attitude or my values of what is important in life would change very much.

[4] My labmates were, in no particular order, Katy Frederick, Megan Murphy, Gideon Ney, Nathan Harness, and Nathan Thompson. Katy was the wise old grad student, and if I had a nickel every time she offered to help me out on either my science or by giving some random organic food item, I would be independently wealthy. Megan and I started the program together, and we relied on each other (at least I her) to make sense of this new world we had both stepped into. Gideon and Nathan motorcyclized me, and now I have no excuse to not be on, much less own, some sort of 400 cc roadster. Nathan Thomson and Elise provided me with a place to go and enjoy spicy food and relax outside of work. These folks supplied equal doses of congeniality, guffaws, Godfather/Back to the Future quotes, "what muffin did you have today" questions, McGinty's stories, and lightheartedness. A good time was had by all.

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ABSTRACT

While the importance of the female preference for exaggerated male traits is largely understood, the evolutionary origins remain under debate. During the evolution of the female preference for leading signals via a sensory bias, an important factor is the processing of sensory information. In katydids, contralateral inhibition on two bilaterally paired ascending auditory neurons (AN1 & TN1) acts to enhance directionality of neural responses to a male call, but may also act to suppress responses on the side of following calls. Here, I investigate the sensory processing underlying the preference for leading calls (LP) in *Neoconocephalus* katydids and test for a sensory bias in a phylogenetic and behavioral context. When presented with three different chirp patterns, AN1 and TN1 responded directionally, but no leader bias was found in AN1. There was a strong leader bias in TN1, but only for the call model of *N. ensiger*, a species with LP. These results for AN1 and TN1 were similar in all species tested, both with and without LP. The strength of the leader bias in TN1 was reduced after removing contralateral auditory inputs by cutting the contralateral leg. The leader bias in *N. ensiger* correlates to LP, suggesting that this LP may be due to a sensory bias mechanism but only after TN1 was incorporated into call recognition. Alternatively, TN1 might not be important for LP if higher centers in the auditory system extract the location of the leading call from other auditory neurons, such as AN1.

INTRODUCTION

Females of many species and groups have preferences for exaggerated male traits (Andersson 1994), such as plumage colors (Saetre et al. 1994), tail length (Andersson 1982, Basolo 1990), call length (Helversen & Helversen 1994, Gerhard 1991), or call rate (Grafe 1997). While the importance of such preferences on the male signal is largely understood, the evolutionary origin of female preferences for exaggerated male traits remains under debate. Several explanations have been proposed for the evolution of a female preference: direct (non-genetic) or indirect (genetic) benefits (Andersson & Simmons 2006), Fisherian runaway selection due to genetic linkage (Kirkpatrick 1982), or a pre-existing sensory bias in the ancestral lineage (Endler & Basolo 1998, Ryan 1998). These explanations are not mutually exclusive. While the validity of these explanations has been amply demonstrated, it is not clear whether they are comprehensive, i.e. whether additional mechanisms of preference evolution exist. To gain a complete picture of the evolution of a novel preference it is important to consider all important aspects in a phylogenetically informed approach.

One important influence on the evolution of female preferences is the underlying sensory processing. For example, the sensory system may respond to certain signal parameter stronger than to others, resulting in a sensory bias for these signals. Such a bias can be the result of selection in a different context (e.g., predator detection) or evolve as a side effect of a different process. If some males produce a signal that as matches the bias, this signal would quickly spread in the population through female choice (Ryan 1998).

Here, I investigate the sensory processing underlying a novel female preference and test for the existence of a sensory bias, in a phylogenetic and behavioral context.

In acoustic communication systems, female preferences for temporal and spectral call characteristics are well documented (review: Gerhardt & Huber 2002). In some species females have preferences for the relative timing of the calls of neighboring males (Greenfield 1994, Greenfield 2005). In many acoustically advertising species males produce discontinuous calls composed of rhythmically repeated chirps and neighboring males often synchronize their chirps (Greenfield 1990). This synchrony is imperfect and the chirp of one male will precede the chirp of the neighboring male, resulting in a leader-follower relationship of the calls of the two males. In many species of anurans and insects, female have a preference for the call in the leading position (Grafe 1996, Klump & Gerhart 1992, Greenfield 1997); some species show preferences for following calls (Wells & Schwartz 1994, Grafe 1999).

When one signal precedes another, the following signal may be suppressed in the sensory system. An example of this is the precedence effect, found in humans and insects (Wallach 1949, Greenfield 1994, Wyttenbach & Hoy 1993). This suppression originates through forward masking at low levels in the auditory pathway (Sobel & Tank 1994, Bee & Micheyl 2008). Similarly, lateral inhibition in the context of directional processing may lead to the suppression of responses to the follower and may thus cause a preference of leading calls (Römer et al. 2002). This suppression would create a stronger response in the ascending pathway ipsilateral to the leading caller. The ascending activity then influences the neural networks which make the decision in which direction to turn.

In katydids of the genus *Neoconocephalus*, the ancestral call pattern is continuous (Snyder et al. 2009). Discontinuous calls have independently evolved twice. LP has appeared at least once within each of these clades (Greenfield & Roizen 1993, K. Frederick-Hudson unpublished). However, several species with discontinuous calls do not have a LP (*N. exciliscanorus*, *N. nebrascensis*, *N. triops*) and species with continuous calls also did not show a LP when tested with discontinuous call models (S. Bush unpublished, K. Frederick-Hudson unpublished). This pattern of presence and absence of LP in *Neoconocephalus* provides an opportunity to study LP evolution in a comparative manner.

In *Neoconocephalus*, sensory neurons in the ear excite ipsilateral auditory interneurons in the prothoracic ganglion. Three auditory interneurons (AN1, AN2, TN1) make ascending connections to the brain (Triblehorn & Schul 2009), where call recognition and localization decisions are probably made (Stumpner & Helversen 2001). TN1 has phasic response properties and responds only to the beginning of stimulus or to slow pulse rates; it functions likely as predator or change detector (e.g., bat cries, Faure & Hoy 2000, Kilmer et al. 2010, Schul & Sheridan 2006, Schul et al. 2012). Both AN1 and AN2 respond during the length of a stimulus and to fast pulse rates characteristic for *Neoconocephalus* calls (Triblehorn & Schul 2009). AN2 is tuned to frequencies above 15-20 kHz and thus responds only weakly to male calls (Triblehorn & Schul 2009). In contrast, AN1 is tuned to the spectrum of the male calls and faithfully transmits the temporal pattern of the male call to the brain. Auditory receptor cells also connect to the local auditory interneuron ON1, which inhibits contralateral auditory interneurons, including its mirror image. This activity enhances the difference between the activity of

left and right auditory interneurons and is an important part of the directional processing in the sensory system (Stumpner & Helversen 2001).

In a katydid with LP, *Mecapoda elongata*, the TN1 on the side of the leading call responded stronger than the TN1 on the side of the following call (Siegert et. al 2011). Consequently, when inputs to the contralateral ON1 were removed, this leader bias in TN1 was reduced. Here, contralateral inhibition is thought to suppress the TN1 on the side of the following call. The relative increase in TN1 activity on the side of the leader also correlates to the leader preference behavior in *M. elongata*. Thus LP behavior may be the result of the biased response in TN1 which is mediated by contralateral inhibition.

In this study I measured the ascending auditory response to leading and following calls. I test whether or not a neural bias exists for leading calls in AN1 or TN1 and if directional processing is necessary to produce a biased response. In addition, I examine if the neural responses were affected by the temporal pattern of a call. I demonstrate that under certain stimulus conditions a leader bias exists in the ascending auditory pathway for species of *Neoconocephalus* with and without LP, and that this leader bias relied in part on the contralateral inhibition. This bias could explain the LP in one species.

METHODS

Animals

I collected males and female of 5 species of *Neoconocephalus* [*N. bivocatus* (Walker, Whitesell and Alexander), *N. ensiger* (Harris), *N. exiliscanorus* (Davis), *N. nebrascensis* (Bruner), and *N. retusus* (Scudder)] near Columbia and Kirksville, Missouri, USA during July–October of 2011 and 2012. I identified individuals using morphological and bioacoustics traits (Froeschner 1954, Walker 2011). Animals were kept in 20-25° C with a light:dark cycle of 14 h:10 h and supplied ad libitum cut grass, cricket food (Flukers, USA), and apples. I used animals no earlier than 2 weeks after the final molt to ensure sexual maturity.

Neck connective recordings

Animals were anesthetized with CO2 and fixed ventral side up on a metal holder with the legs in a walking position using yellow sticky wax (Kerr Corporation, USA). I removed the maxillae and labrum and the cuticle above the connectives of the prothoracic ganglion. Exposed tissue was covered with saline (after Fielden 1960). I then cut connectives below the subesophegeal ganglion to reduce extraneous neural activity during recording. In some preparations, I also cut the connectives descending from the prothoracic ganglion. I placed one neck connective on a tungsten hook electrode (approximately 100 μm diameter), insulated the recording site with silicone gel (Baysilone, Bayer AG, Germany), and placed the preparation in an anechoic Faraday cage (1.2 m x 1.0 m x 1.5 m) at 20-25° C. In about 25% of the insects, I recorded from the right and left connective consecutively. In these cases, I treated each connective as an

independent data point. Recordings were amplified using a custom-made amplifier, band-pass filtered from 100-4000 Hz (model 3384, Krohn-Hite), digitized (14 bits/sample, 20 kHz sampling; Instrunet 100B, Omega Engineering, USA), and stored for analysis.

Stimulation

I played stimuli from loudspeakers (KSN1005B, CTS Corporation, USA) using a custom built DA converter and amplifier system (16 bits/sample, 250 kHz). Signal amplitudes were calibrated using a sound level meter (model 2231, Brüel & Kjaer, Denmark) and a 0.25 inch microphone (type 40BF, G.R.A.S., Denmark) in the place of the animal oriented along its caudal-rostral axis. All stimuli were presented at 80 dB peak SPL (re: 20 µPa).

I created 4 types of stimuli with chirp patterns based on natural male call patterns.

1) Chirps of 1000 ms durations were composed of a train of pulses, each 3 ms in duration (including 0.5 ms rise and fall times) separated by inter-pulse intervals of 2 ms. I repeated these chirps three times and separated them by 150 ms. These stimuli were used in behavioral experiments with *N. triops* and species with continuous calls (*N. bivocatus*, *N. retusus*).

2) These 1000 ms chirps were also presented with 800 ms inter chirp intervals, mimicking the calls of *N. nebrascensis*. 3) I generated 36 ms chirps from continuous noise bursts (i.e., they did not consist of individual pulses) with 16 ms rise and 6 ms fall time. These chirps were repeated 40 times with 44 ms inter-chirp intervals. These signals represented the temporal pattern of *N. ensiger* calls.

4) Similarly, I generated chirps of 76 ms duration (including 2 ms rise and fall time) form a noise burst which mimicked the call pattern of *N. exiliscanorus* and *N. spiza*. These were repeated 9 times with chirp intervals of 324 ms.

The envelopes of these four chirp types were filled with frequency filtered noise. I used a frequency range from 10-20 kHz for *N. retusus* and *N. ensiger*, and 9-15 kHz for *N. nebrascensis, N. bivocatus,* and *N. exiliscanorus*. These noise bands correlated to the frequency content of each species' call (Schul & Patterson 2003, K. Frederick-Hudson, unpublished).

All stimuli were presented 25 times with stimulus periods of 5 s (stimulus 1, 3, 4) or 6 s (stimulus 2). The last 1 s of each stimulus presentation, which contained silence, was used as 'no stimulus' for the purposes of data analysis.

Experiment 1: Fixed speaker stimulation

Here, two loudspeakers were located 53 cm and 55° fronto-lateral of the animal. I presented either one or two stimuli simultaneously. During simultaneous playback, I delayed the signal of one speaker by 0-300 ms to create leader-follower relationships between stimuli (Fig. 1). Three stimulus situations were presented in one series. First, the stimulus was presented from the speaker ipsilateral to the recording site (control). Next I presented the same stimulus from both speakers, with the stimulus on the contralateral side delayed. Last, the stimulus was again presented from both speakers, but this time delayed on the ipsilateral side. This sequence was then repeated 25 times.

During the series with 0 ms delays, I presented a stimulus from the ipsilateral side alone, then from the contralateral side alone, and finally with the stimulus from both

speakers simultaneously. I presented all delay values of the same chirp type (see above) consecutively, with the delay values in random order.

Experiment 2: Rotating speaker stimulation

Here, one speaker was placed frontal to the insect (0º) and the second speaker was mounted on an arm rotating around the insect, both located at 43 cm from the insect. I presented first a stimulus from the fixed speaker at 0° and then from the rotating speaker, and repeated this 25 times. I presented the series with the rotating speaker at +90º, +60º, +30º, +15º, 0º, -15º, -30º, -60º, -90º. I randomly varied the angle of the rotating speaker between the series.

TN1 analysis

Extracellular recordings contained all ascending activity including AN1, TN1 and AN2. The TN1 interneuron produces the large spikes in extracellular recordings of the neck connective (second trace, Fig. 2) (Schul, 1997, Faure & Hoy, 2000, Triblehorn & Schul 2009). To quantify TN1 activity I identified TN1 spikes with a voltage threshold criterion and recorded the time for each TN1 spike. These times were used for all further TN1 analysis. I measured TN1 activity by counting the spikes that occurred during all chirp periods of the stimuli with 1000 ms and 76 ms chirp duration. I excluded the first chirp period during the 36 ms chirp stimulus from analysis. In addition, animals with average TN1 spike rates less than 3 spikes/s during the 36 ms chirp control stimuli were excluded from TN1 analysis.

Analysis of AN1 activity

To quantify the activity of auditory neurons (mainly AN1) with smaller spike amplitudes, I first removed TN1 spikes from the recordings. I calculated an average spike based on all TN1 spikes in stimulus series (i.e., the 25 repeats of the ipsilateral-alone, ipsilateral-leading, ipsilateral-following stimuli described above in the fixed speaker stimulation section). This average spike was then subtracted at each TN1 spike occurrence. Averaging the spike over short periods of time (6-8 minutes) minimized the artifact in the resulting signal, as changes in recording quality were small, if at all detectable. I further minimized the artifact of TN1 removal by adjusting the size of the averaging window and the threshold criterion for TN1 detection.

After TN1 removal, the recording was rectified and averaged over the 25 repetitions of each stimulus situation. I then integrated the area under the averaged recording as a measure of response strength. The integration window started 12.5 ms after the onset of the stimulus to account for the response latency. I used 75, 250, and 1135 ms lengths of the integration window for 36, 76, and 1000 ms chirps, respectively. These lengths included the chirp duration and the longest delay used. I excluded the first chirp of each stimulus from the analyses to quantify the steady state responses.

I determined the level of background neural activity (no stimulus) using the last second of each stimulus presentation, which did not contain any sound. I applied the same procedure as above with a 1 s integration window. I subtracted the background activity level adjusted for the length of the integration window from the activity during the stimulus windows. The resulting value represents the activity caused by the stimulus of auditory neurons other than TN1. Both AN1 and AN2 likely contribute to these responses. However, AN2 responses are negligible since this neuron is tuned to

frequencies above the stimulus (Triblehorn & Schul 2009). Therefore I consider the results of this analysis as representative for AN1 responses and refer to accordingly in the text. I do, however, acknowledge that other auditory units may contribute to these responses.

Leader-follower analysis

I presented the response strength during stimulation with leader-follower duets relative to the response during ipsilateral only stimulation. To quantify a potential bias for either leading or following calls, I calculated the leading index (LI) after this formula:

$$LI = (A_{iL} - A_{cL})/(A_{iL} + A_{cL})$$

where A_{iL} is the relative response strength when the leader was ipsilateral to the recording site, and A_{cL} is the relative response strength when the leader was contralateral. Positive LI scores indicate a stronger response to the side of the leader, whereas negative scores indicate a stronger response to the side of the follower. I used the LI rather than a simple ratio of A_{iL}/A_{cL} because it results in symmetrical data for reciprocal ratios (e.g., 2/1 and 1/2 ratios result in LI of 0.33 and -0.33, respectively).

Directionality analysis

To determine the directionality of AN1 responses, I calculated the response strength for stimulation from the directional loudspeaker and the stationary loudspeaker separately. Then, for each direction, I divided the response strength to the rotating speaker by the response strength to the stationary speaker. This corrected for changing recording quality over the time of the experiment. The corrected response strengths to the

rotating speaker were then used to describe directionality. Directional responses are given relative to the responses at 0º.

RESULTS

Directionality of AN1 responses

AN1 responses to the three call models had a tonic time course and reliably encoded the time course of the stimulus (Fig. 3). AN1 responses followed the fast pulse rate of the 36 ms stimulus. At 76 ms chirp duration a transient response component occurred at the beginning of each chirp. This component is likely due to synchronized AN1 activity after the long inter chirp interval. AN1 responses did not decay during the long chirp duration (1000 ms chirp). The AN1 response properties to stimulation with one call model were similar for all species tested (data not shown).

During playback of contralateral stimuli, AN1 activity had the same time course as during ipsilateral stimulation (Fig. 3) but lower amplitude. For all three chirp durations, median amplitudes were between 50 and 85% relative to ipsilateral stimulation (Fig. 4). For the longest chirp duration (1000 ms), the difference between ipsilateral and contralateral stimulation tended to be smaller. Responses during contralateral stimulation were significantly smaller than during ipsilateral stimulation for all chirp durations, in all five species tested (Wilcoxon signed rank test, $p < 0.05$ in all cases). Directionality of AN1 responses was weakest in *N. retusus* (Fig. 4). When ipsilateral and contralateral stimuli were presented simultaneously (i.e., with 0 ms delay), AN1 responses were similar to ipsilateral only stimulation (Fig. 3) with amplitudes between 88 and 107% (Fig. 4).

To test the influence of contralateral inhibition on directional responses I quantified response strength before and after removing input from the contralateral ear by

severing the contralateral leg. After removal of the contralateral inhibition, responses during contralateral stimulation increased in almost all cases (Fig. 5). Significant increases occurred in *N. ensiger* and *N. bivocatus* (see Fig. 5 for statistics). Responses of *N. exiliscanorus* showed a strong effect of removing the inhibition, but the small sample size prevented statistical testing. Responses in *N. retusus* and *N. nebrascensis* (1000/150 ms chirp/gap duration) had little directionality to begin with and were only weakly affected by the loss of inhibition. These results indicate that AN1 receives significant contralateral inhibition, most likely mediated by the contralateral ON1.

AN1 responses to leader - follower stimulation

The time course of AN1 activity during leading and following stimuli varied with the duration of the delay (Fig. 6). As leading and following chirps overlapped, AN1 amplitudes were similar to ipsilateral only responses. Where chirps did not overlap, amplitudes roughly matched the respective ipsilateral only or contralateral only responses. Thus AN1 activity during playback of a leading and following call can be approximated as the temporal combination of how AN1 responds during playback of ipsilateral only, ipsilateral and contralateral (0 ms delay), or contralateral only stimulation.

I compared AN1 responses to leader-follower stimuli to ipsilateral only responses, across the different delays. The responses during ipsilateral leading and contralateral leading stimulation exhibited a similar trend: as the length of the delay increased, AN1 responses increased. This increase was correlated to the increase in total stimulation time during the leading and following chirp. For example, for 36 and 76 ms chirp durations,

responses at the 130 ms delay increased by 31 and 76%, respectively, in *N. ensiger* (Fig. 7, c and d). For the 1000 chirp duration, the same delay resulted in a much smaller increase (11%) (Fig. 7, e).

The response strength between the ipsilateral signal leading and contralateral signal leading was similar in individual preparations (Fig. 7, a and b) and at the population level (Fig. 7 c, d, e). The AN1 response properties for this paired stimulation were similar among the five species tested. Because the other species are well represented by the *N. ensiger* data shown, I do not present them here.

Median LI scores of AN1 activity were near 0 for all chirp types, delays, and species (Fig. 8). These LI scores indicate no difference in response strength whether the leading or following call was ipsilateral to TN1.

Directionality of TN1 responses

During ipsilateral only stimulation with the different call models, TN1 spiking occurred mostly during the onset of chirps (Fig. 9); only for the 1000 ms chirp duration a significant numbers of spikes occurred later in the course of the chirps. When the 36 ms chirps were presented from the contralateral side, TN1 spiking activity was much reduced (Fig. 9, middle) to 16 to 59% of the response strength during ipsilateral stimulation (Fig. 10, a). During stimulation with 76 and 1000 ms chirps, TN1 produced typically only one spike at the beginning of each chirp, while no responses occurred during the remainder of the chirps (Fig. 9, middle). This lead to decreased spiking relative to ipsilateral stimulation for the 1000 ms chirps with medians ranging from 49 to 73% of the responses to ipsilateral stimulation (Fig. 10, b). For 76 ms chirp duration the responses during

contralateral stimulation were less strongly reduced (medians of 81-91%, Fig. 10, c). Median responses during contralateral stimulation for all three chirp durations in all five species were significantly lower than during ipsilateral stimulation (Wilcoxon signed rank test, $p < 0.05$ in all cases). TN1 spiking activity during simultaneous presentation of ipsilateral and contralateral stimuli was similar to the response during ipsilateral only stimulation, for all three chirp durations (Fig. 9, right). During the presentation of these simultaneous stimuli, median TN1 responses were between 78-100% of ipsilateral only responses and were always larger than the contralateral only response (Fig. 10).

To test the importance of contralateral inhibition on TN1 directionality, I quantified responses before and after severing the contralateral leg. After removing the contralateral leg, directionality was strongly reduced and responses to contralateral stimulation were almost as strong as during ipsilateral stimulation (Fig. 11). Here, there was an increase in median relative responses during contralateral stimulation (Fig. 11, a and c). Because directionality was weak during stimulation with 76 ms chirps in intact animals, removal of the contralateral leg had only a minor effect (Fig. 11, b).

During the 36 ms control stimuli, TN1 spiking rates for *N. ensiger* showed variation among individual TN1 responses (Fig. 12, a). Intra-individual spike rates were fairly consistent among the four presentations of the control stimulus (approximately 30 minute time course) (Fig. 12, b). As the duration of the entire experiment increased (up to 120 minutes), there was a decrease in the average TN1 spiking rate for all four 36 ms control stimuli (Fig. 12, c). Using this measure, individuals clustered into either low (3-10 spikes/s) or high (>15 spikes/s) average spiking rates (Fig. 12, c).

TN1 responses to leader - follower stimulation

TN1 spiking occurred most frequently during the beginning of the leading chirp for both ipsilateral and contralateral leading stimuli (Fig. 13). The spiking activity of TN1 when the leading call was ipsilateral was similar to the ipsilateral only (control) response for all three chirp patterns (Fig. 13, first and second row). Median TN1 responses during ipsilateral leading calls were 91% of the ipsilateral only response when the 36 ms chirp pattern was delayed by 12 ms (Fig. 14, a). When the leading chirp was presented on the contralateral side with the same 12 ms delay TN1 spiking was reduced (Fig. 13, a) and the median response was 9% of the control response (Fig. 14, a).

I compared the responses for the 76 and 1000 ms chirp patterns presented with the leading chirp ipsilateral and preceding the following chirp by 80 ms (Fig. 13). Here, TN1 spiking was similar to the control responses (Fig. 13, b and c). Median responses for these two chirp patterns at 80 ms delay was 103 and 81%, respectively, compared to control (Fig. 14, b and c). When these same stimuli were presented with the leading call on the contralateral side, TN1 activity showed less spiking during the 1000 ms chirp pattern compared to the 76 ms chirp pattern (Fig. 13, b and c). Median TN1 responses during these stimuli were 54 and 77% of the respective control response (Fig. 14, b and c).

LI scores for the 36 ms chirp duration were similar in all species: LI scores for 12 and 24 ms delays were between 0.84 and 0.93 and for 36 ms delays the LI scores were near zero (Fig. 15, a). This represents a 12- to 28-fold increase in TN1 activity on the side of the leading call. LI scores during the 76 and 1000/150 ms chirp durations were closer to zero for all species and at every delay (Fig. 15, b and c). LI scores were positive, but

near zero, for the 76 and 1000 ms chirp durations in *N. ensiger* and *N. retusus* (Fig. 15, b and c). In *N. exiliscanorus* LI scores were also near zero and positive for the 1000 ms chirp duration and (Fig. 15, c). During the 1000 ms chirp with an 800 ms chirp interval, LI scores for *N. nebrascensis* were between -0.08 and -0.23 (Fig. 15, b).

To measure the effect of contralateral inhibition on LI scores, I compared the TN1 response before and after removing the contralateral leg in *N. ensiger* and *N. retusus*. Here, LI scores during 36 ms chirps during 12 and 24 ms delays decreased after contralateral leg removal (Fig. 16). LI scores for 12 and 24 ms delays, respectively, were reduced by 0.57 and 0.37 in *N. ensiger* and by 0.56 and 0.45 in *N. retusus*. At the 12 ms delay in *N. ensiger*, the reduction in the LI from 0.96 to 0.39 corresponds to a decrease in the difference between the leading and following side activity after removing contralateral inputs. In *N. retusus* there was also a decrease between leading and following side activity at the 12 ms delay (reduction in the LI from 0.86 to 0.27, Fig. 16).

To describe how the individual variation (Fig. 12) affected LI scores for 36 ms chirps in *N. ensiger* (Fig. 15, a), I compared relative TN1 responses between individuals with high and low average TN1 spiking rates (see above). Median TN1 responses during ipsilateral or contralateral leading stimuli were weaker in the low-spiking group across all delays (Fig. 17, a). However, the LI scores in both groups showed the same trend (Fig. 17, b). Median LI scores were the highest at 12 ms delays in the low and high spiking groups, 0.68 and 0.96, respectively (Fig. 17, b). For both groups LI scores were the lowest for 36 ms delays and were always near zero (Fig. 17, b).

DISCUSSION

Here I measured the responses in the ascending auditory pathway during the presentation of leading and following stimuli. I tested for differences in AN1 and TN1 responses depending on whether the neuron was ispilateral or contralateral to the leading stimulus. The responses of AN1 (Fig. 4) and TN1 (Fig. 10) had distinct directionally to the three chirp patterns tested. During stimulation with leading and following calls, AN1 did not have a leader bias for any of the chirp patterns (Fig. 8). TN1 also showed no leader bias during stimulation with the 76 and 1000 ms chirp pattern (Fig. 15). There was, however, a leader bias in TN1 responses during the short 36 ms chirp pattern (Fig. 15). These responses in AN1 and TN1 were the same in all of the species tested.

Responses during directional or leading and following stimuli

The directional response of AN1 and TN1 for the *Neoconocephalus* species used in this study (Fig. 4, 10) were similar to the directionality found in many katydid and cricket taxa (review: Stumpner & Helverson 2001). Thus *Neoconocephalus* probably shares the same mechanism of directional processing via ON1 contralateral inhibition (Schul 1997, Pollack 1998, Römer & Krusch 2000). This, in addition to the decreased directional responses after removing the contralateral leg (Fig. 5, 11), suggests that inhibition is acting on AN1 by the contralateral ON1.

While contralateral inhibition enhances interaural intensity differences during directional stimuli, there is no difference in total sound power between both ears during the leading and following stimulation. There is, however, a temporal difference between the onset of the leading and following stimulus (Fig. 1). This temporal offset may affect

how each ON1 inhibits the respective contralateral neurons. Inhibition from the ON1 ipsilateral to the leading call could potentially reduce activity of the contralateral ON1 and AN1 during the presentation of the following chirp, resulting in a leader bias (Römer et al. 2002). However, the inhibition from the ipsilateral ON1 must last until the end of the following stimulus. If inhibition does not persist long enough, then the contralateral ON1 and AN1 will not be suppressed during the following chirp. Without inhibition during the following chirp, the ON1 neurons produce equal amount of inhibition during leading and following stimulus, resulting in no large difference in activity between the AN1 on the side of the leader or follower (Fig. 6). Despite the presence of contralateral inhibition, then, the lack of a leader bias in AN1 (Fig. 8) may be due to the timing of inhibition on the contralateral AN1 and ON1.

When the leading 36 ms chirp preceded the following chirp by 12 or 24 ms, the response of the TN1 on the following side was suppressed (Fig. 15). Removing the contralateral inputs strongly reduced this leader bias (Fig. 16). Here, inhibition may play a large role in establishing the leader bias in TN1. Thus, in intact preparations, the ON1 on the side of the leading 36 ms chirp probably inhibits contralateral auditory neurons. This inhibition would result in reduced activity in the TN1 contralateral to the leading call, compared to the TN1 on the ipsilateral side.

While suppression of TN1 activity on the side of the following call was observed during the 36 ms chirp pattern, this suppression was not present during 76 or 1000 ms chirps (Fig. 15). Specifically, these responses were observed when the delays were between 12 and 24 ms. During the 12 and 24 ms delays for the 36 ms stimulus there was a strong leader bias in TN1, but when 76 and 1000 ms chirps were delayed by 15 ms

there was no leader bias (Fig. 13, 14). For the initial phase of each leader and follower stimuli at these short delays, there is no difference between the three chirp patterns. There is, however, a difference among the three chirp patterns in the inter chirp interval. This silent chirp interval was shortest during 36 ms chirps, at 44 ms, compared to the 324 and 150 ms inter chirp intervals during the 76 and 1000 ms chirps, respectively. Thus, the specific combination of chirp and interval durations determine whether or not a leader bias occurs in TN1 responses.

The silent interval between chirps may affect TN1 responses in a variety of ways, including changes in the response properties of sensory receptors or of TN1, or how the silent interval interacts with the timing of contralateral inhibition. However, shorter silent intervals tend to decrease receptor (J. Schul unpublished observations) and TN1 (Faure and Hoy 2000) responses. Decreased receptor responses may have a variety of effects, since different receptor populations connect to different areas in the prothoracic neuropile (Hennig & Stumpner 2004). Future studies should measure differences in the activity of receptor which innervate ON1 and TN1 during different silent chirp intervals. Decreased response in TN1, on the other hand, would probably still result in a leader bias, as seen in the individuals with low TN1 spiking rates in this study (Fig. 17, a).

If the silent interval is longer than the duration of effective contralateral inhibition, TN1 activity may not be suppressed. During the preceding leading chirp, inhibition from the ON1 ipsilateral to the leading chirp would suppress contralateral TN1 activity, as seen during the 36 ms chirp pattern (Fig. 13). If the chirp interval was longer than the duration of this inhibition, such as during the 76 and 1000 ms chirp pattern, there would be no suppression of activity in the contralateral TN1. Here, the timing of

contralateral inhibition acts to suppress the TN1 on the side of the follower at short chirp intervals but not at longer chirp intervals.

Evolution of leader preference in Neoconocephalus ensiger

The preference for leading calls (LP) in *N. ensiger* (K. Frederick-Hudson unpublished observations) corresponds to the bias for leading calls in TN1 activity during the *N. ensiger* call model. The LP in *N. ensiger* may be due to a sensory bias in TN1 (sensu Römer et al. 2002) which results only after the evolution of the *N. ensiger*-like chirp pattern and the integration of TN1 activity into call recognition. TN1 directionally encodes predator signals of less than 20 Hz (e.g., bat cries; Faure and Hoy 2000, Kilmer et al. 2010, Schul and Sheridan 2006, Schul et al. 2012). Before the evolution of discontinuous calls, the TN1 response to male calls would not be important for call recognition, since the ancestral state in *Neoconocephalus* is continuous calling (K. Frederick-Hudson, unpublished). As discontinuous calling evolved, only the short chirp intervals, such as with *N. ensiger* calls, would probably result in a leader bias in TN1. If TN1 had already been integrated into call recognition after the evolution of *N. ensiger*-like chirps, then this leader bias could produce LP in *N. ensiger*.

Localization of a male call compares the activity level and the call pattern in the left and right ascending inputs, most likely in the brain (Stumpner & Helversen 2001). If TN1 is important for recognizing chirp rate, suppression of TN1 could affect the perception of interaural chirp patterns. A leader bias in the activity of TN1 would effectively not encode the chirp pattern on the side of the follower. Thus during call localization this combination of factors would create a "deaf side" in the auditory

landscape. This would result in a phonotactic response towards the side of the leading call and explain LP in *N. ensiger*.

Mated females have reduced phonotactic responses for male calls in *Neoconocephalus* (K. Frederick-Hudson, unpublished), similar to crickets (Loher et al. 1993). Therefore the variation in TN1 responses among individuals (Fig. 12, 17) may be due to the reproductive state of females. Insects may reallocate resources away from reproductive functions (Zera & Harshman 2001) after mating. The reduced neural response in TN1 may be the result of a shift away from internal resources, which aid in finding mates, such as neural sensitivity to male calls. Reducing responses to male calls may sharpen the detection of predator signals such as bat cries. While in female *N. ensiger* the individual TN1 responses during control stimuli were consistent (Fig. 12, b), the variability among individuals may be the product of female receptivity.

Evolution of leader preference due to sensory bias or benefits?

Whether LP in *N. ensiger* evolved via a sensory bias or from benefits would depend on whether the female preference or the male trait evolved first (Endler & Basolo 1998). As described above, LP in *N. ensiger* may have evolved as a result of a sensory bias in TN1, mediated by directional processing and integrated into call recognition. Only after the evolution of discontinuous chirps with short silent intervals would the timing of contralateral inhibition result in a leader bias in TN1. In this case female preference evolved before the male trait.

It is not known, however, whether TN1 is important for call recognition in *N. ensiger*. An alternative explanation for LP evolution is that the leader bias is a product of

auditory processing higher in the nervous system (e.g., the brain). Here, the location of the leader as well as the chirp rate could be extracted from AN1 activity and then used for phonotactic decisions. In this scenario LP is not a product of the ascending sensory system but a mechanism of brain networks. In either case, if these processes are ancestral to the evolution of the male call trait, then LP most likely evolved due to a sensory or cognitive bias.

LP in *N. ensiger* may also have evolved due to fitness benefits from choosing leading male callers. The leading position is the outcome of competitive interactions (Greenfield and Roizen 1993) and thus likely an honest signal that may indicate superior genes or some direct benefit. After the evolution of discontinuous chirps, the chirp pattern could be extracted from AN1 rather than TN1 activity. Leader and follower information could also be extracted from the activity of right and left AN1 by cognitive filters, thus allowing females to perceive the location of the leading caller. Females who could detect and choose leading callers might realize higher fitness from mating with leading callers. In this scenario, LP evolved after the appearance of this male trait.

Comparative LP evolution in Neoconocephalus

In order to discern among the evolutionary pressures leading to LP in *N. ensiger*, investigations into the function of TN1 for female preference will be important. Experiments following the approaches of Schildberger & Hörner (1998) and Atkins et al. (1992) might reveal whether TN1 plays a role during phonotaxis. In addition, comparing the mechanism of female preference in *N. spiza*, a species which evolved LP independently of *N. ensiger* (Greenfield & Roizen 1993, K. Frederick-Hudson

unpublished observations), would shed light on the evolution of call synchrony and LP in *Neoconocephalus*.

Currently no data on AN1 and TN1 responses for *N. spiza* are available. However, given that response properties of the ascending pathway did not differ among the five species tested here, it seems that the, LP of *N. spiza* is not due to a leader bias of AN1 or TN1: the call models representing *N. spiza* calls (76 ms chirp duration) did not elicit any leader bias in any of the 5 species tested. Thus it appears likely the LP in *N. spiza* is generated in brain circuits rather than the ascending sensory pathway.

The mechanisms for LP in *N. ensiger* and *N. spiza* are probably not the same since there are independent origins for their LP evolution and only in *N. ensiger* does the male call pattern match with the leader bias in TN1. While LP in *N. ensiger* and *N. spiza* are likely not the result of a pre-existing sensory bias, it is possible that TN1 may be important for LP. In this case LP in *N. ensiger* would only be expressed after the evolution of a male call pattern which resulted in a leader bias in TN1. Therefore it will be important to investigate whether TN1 activity affects locomotion during phonotaxis (crickets: Schildberger & Hörner 1988, Atkins et al. 1992), and if so, whether this trait is associated with LP in *Neoconocephalus*. In addition, future studies in species with LP should investigate what genetic benefits, if any, exist for females that mate with males who produce leading calls. These two lines of study will clarify whether the sensory bias or an indirect benefit mechanism was most important in the evolution of LP, although both of these evolutionary processes, among others, could operate mutually to produce LP.

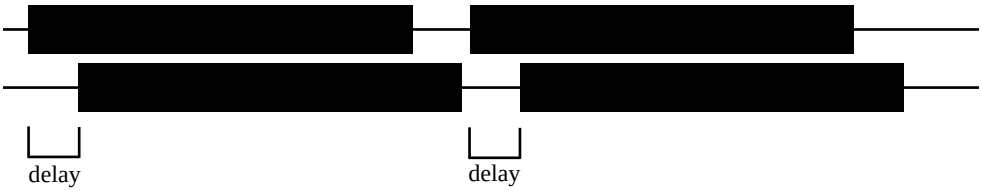

Figure 1. An example of leader-follow stimulus. Brackets indicate a 130 ms duration of delay for the following chirp. Shown is 1000 ms chirp with 150 ms inter chirp interval.

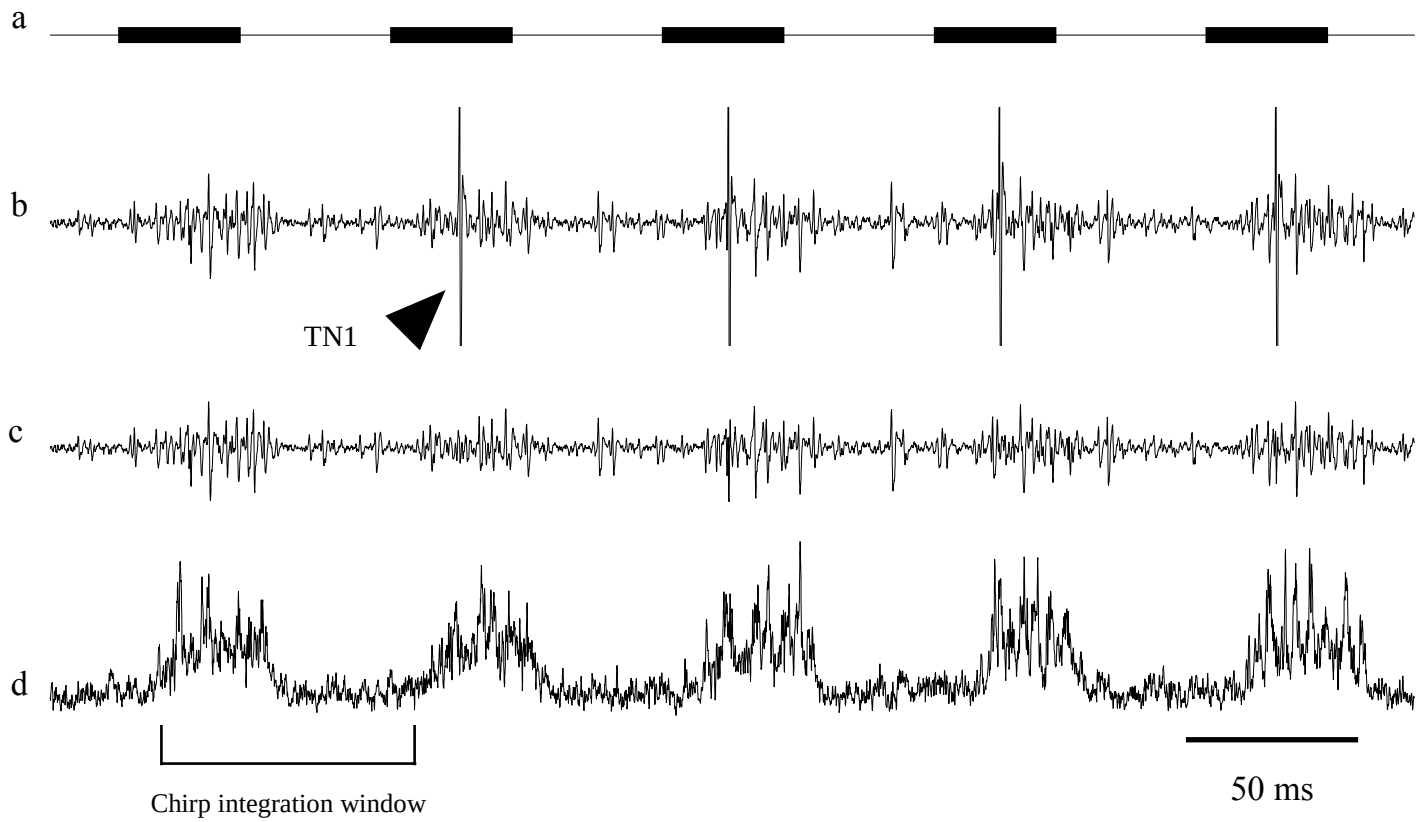

Figure 2. Recording and analysis of extracellular activity from a neck connective. *a*) The stimulus of the 36 ms chirp with 44 ms inter chirp intervals. *b*) The original recording shows large TN1 spikes (truncated) at the onset of stimulus. *c*) Recording after

subtraction of an average TN1 spike. *d*) Average of 25 rectified sweeps. Stimulus was presented from the speaker ipsilateral to the recording side. Recordings are from an individual *N. ensiger*.

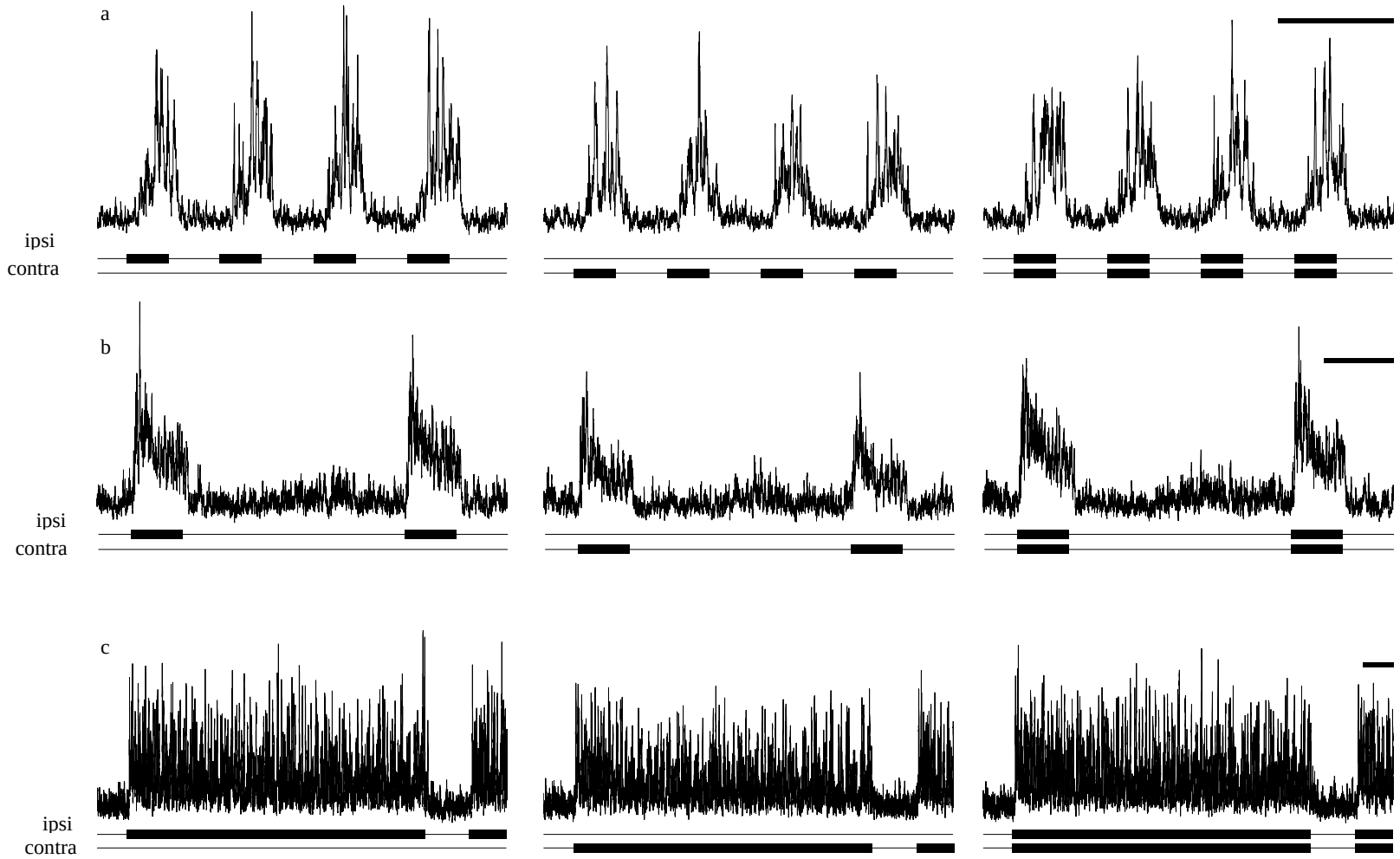

Figure 3. Averaged rectified AN1 activity during directional stimuli. Rows *a*, *b*, and *c* show responses during

the 36, 76, and 1000 ms chirp patterns, respectively. Individual *N. ensiger*, *N. exiliscanorus*, and *N. retusus* (rows *a*, *b*, and *c*, respectively) responses were averaged from 25 repetitions after removal of TN1 spiking activity. Scale bars are 100 ms.

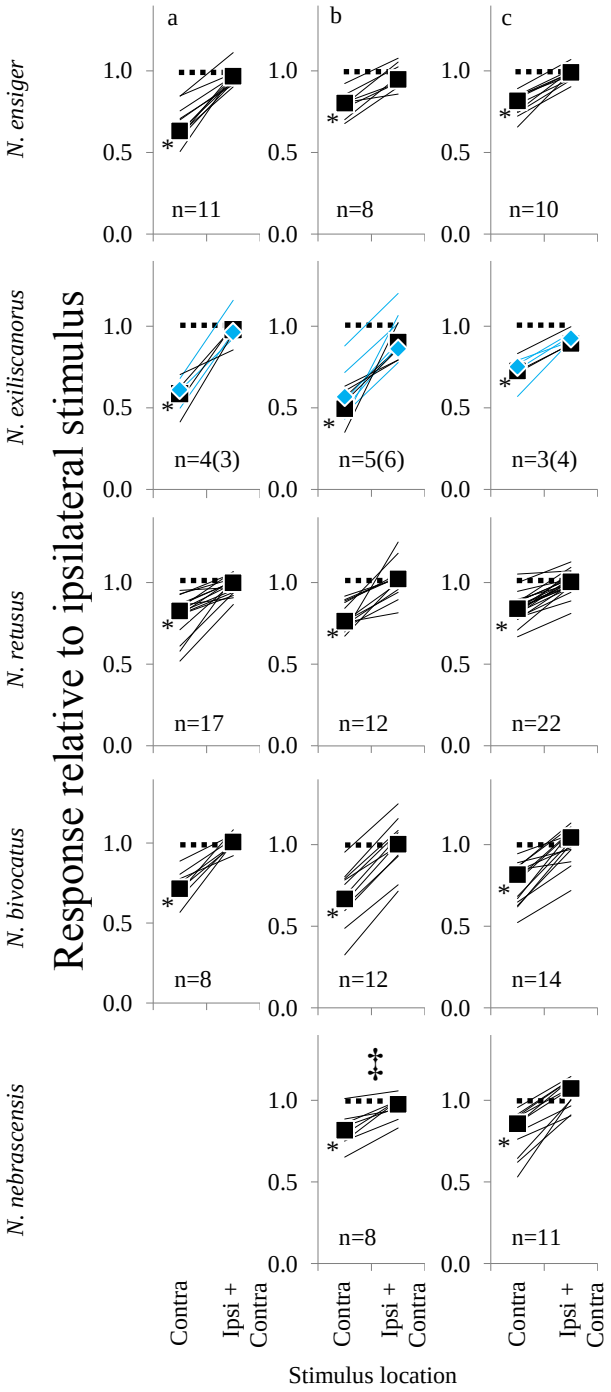

Figure 4. Relative AN1 responses to directional stimulus for 5 species of *Neoconocephalus*. A contralateral stimulus or an ipsilateral and contralateral stimulus with no delay were presented. Responses to 36, 76, and 1000/150 ms chirp patterns are

in columns *a*, *b*, and *c*, respectively, except for ‡ which indicates responses to the 1000/800 ms chirp pattern. *Lines* and *symbols* indicate individual and median responses; *black* and *blue* indicate female and male responses. *Dashed lines* indicate response relative to ipsilateral only stimulus. Sample sizes in each plot is n=female(male). Significant differences between ipsilateral only stimulus (combined male and female) are indicated by * ($p<.05$) (Wilcoxon signed rank test).

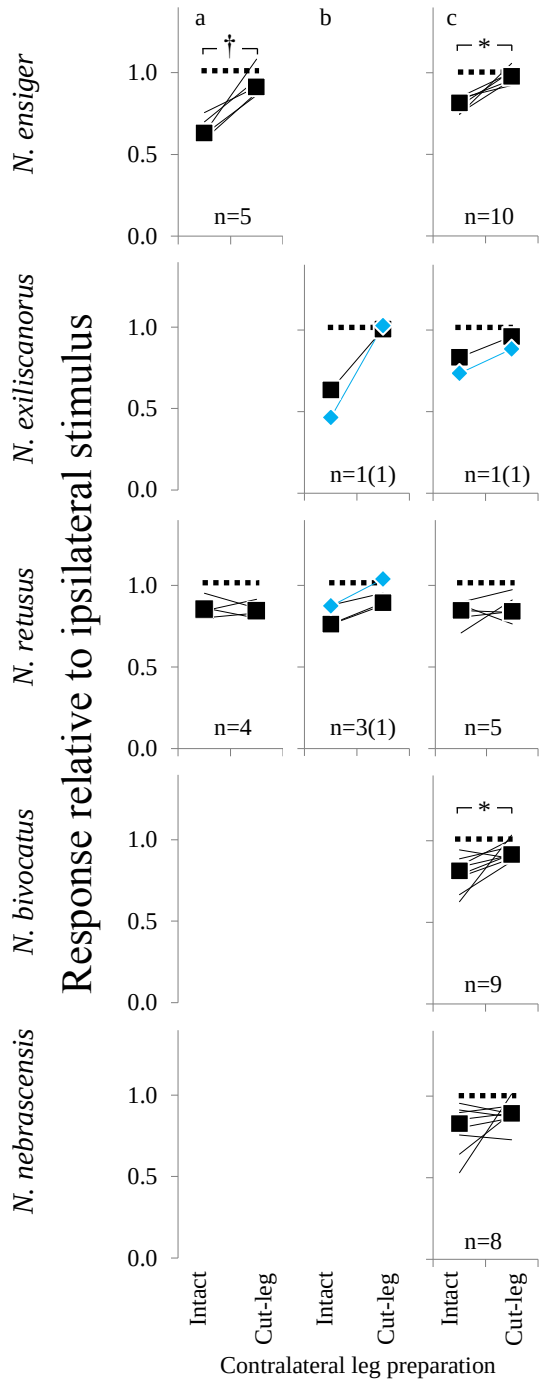

Figure 5. Relative AN1 response to directional stimuli before and after removing the contralateral leg in 5 species of *Neoconocephalus*. Contralateral stimulus or simultaneous ipsilateral and contralateral stimulus with no delay were presented. Rows

a, *b*, and *c* show responses during the 36, 76, and 1000/150 ms chirp pattern, respectively. Dashed lines indicate response relative to the control stimulus. In each plot sample size is given as n=female(male). Significant differences are indicated by † ($p<.10$) and * ($p<.05$) (Wilcoxon signed rank test).

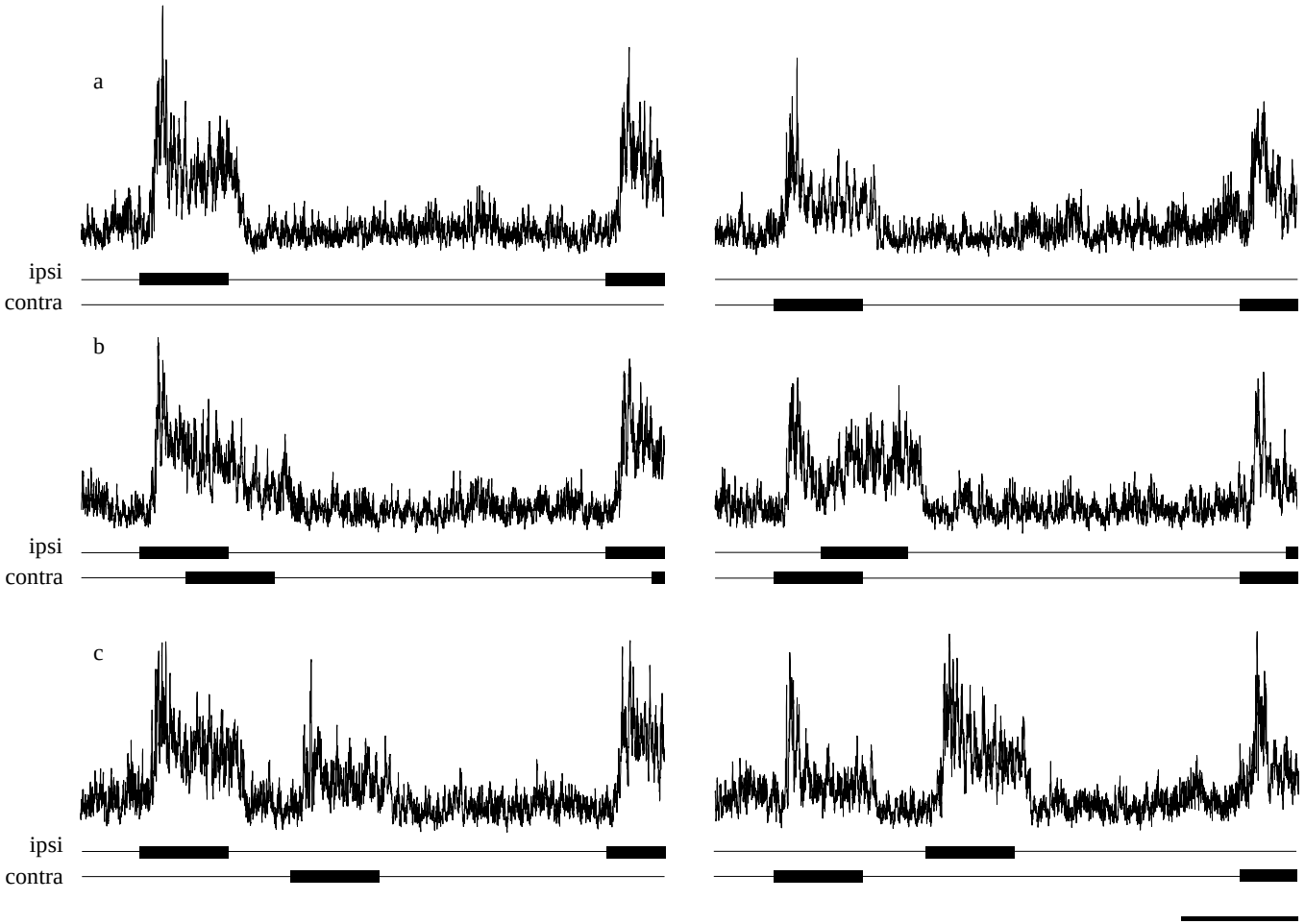

Figure 6. Average rectified extracellular response to leader-follower stimuli. Row *a* shows the response to a single chirp

stimulus. Rows *b* and *c* show the response to leader-follower stimulus incorporating a 40 and 130 ms delay of the following chirp, respectively. *Left side* stimulus presented the leading stimulus ipsilateral to the recording side and for the *right side* the leading stimulus was contralateral. Shown are individual *Neoconocephalus ensiger* responses to 76 ms chirp pattern. Scale bar is 100 ms.

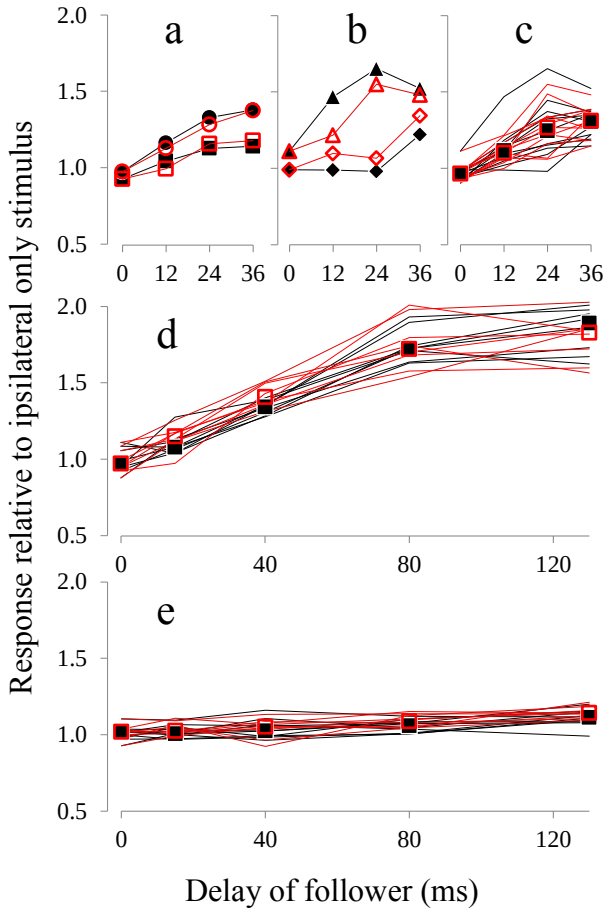

Figure 7. Relative AN1 responses in *Neoconocephalus ensiger* to stimuli with the leading chirp either ipsilateral or contralateral. Responses to ipsilateral leading stimuli are in *black*, contralateral leading stimuli are in *red*. Plots *a* and *b* show paired responses for each individual with the 36 ms chirp pattern. Paired responses for an individual are represented by the same symbol. Plots *c*, *d*, and *e* show median (*symbols*) and individual (*lines*) responses during the 36 (n=11), 76 (n=8), and 1000/150 ms (n=10) chirp patterns, respectively.

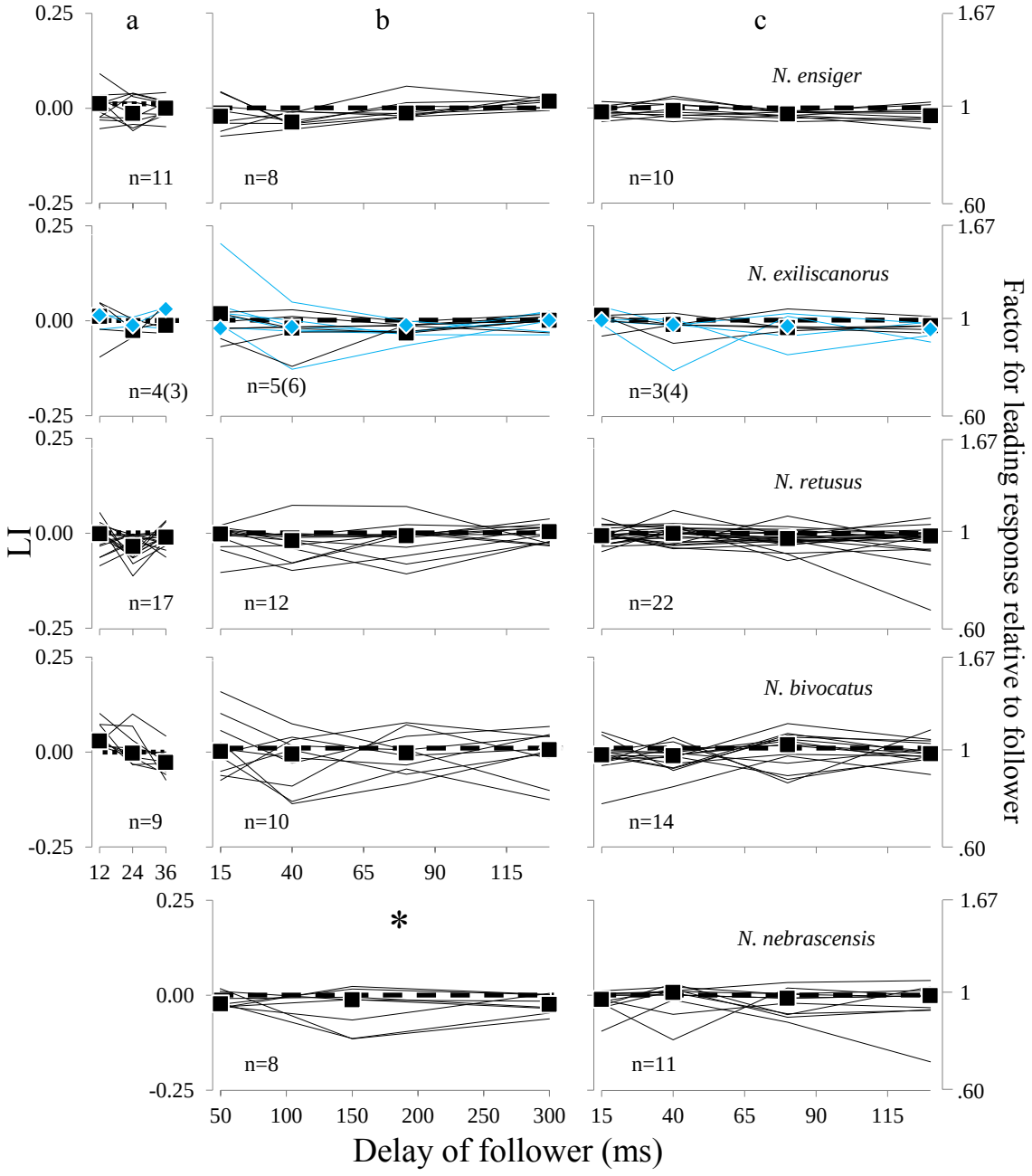

Figure 8. LI scores for AN1 during leader-follower stimuli for 5 species of *Neoconocephalus*. Columns *a*, *b*, and *c* show responses to the 36, 76, and 1000/150 ms chirp patterns, except for *, which is the 1000/800 ms chirp pattern. Each row represents a different species. Sample size given in each chart is n= female(male).

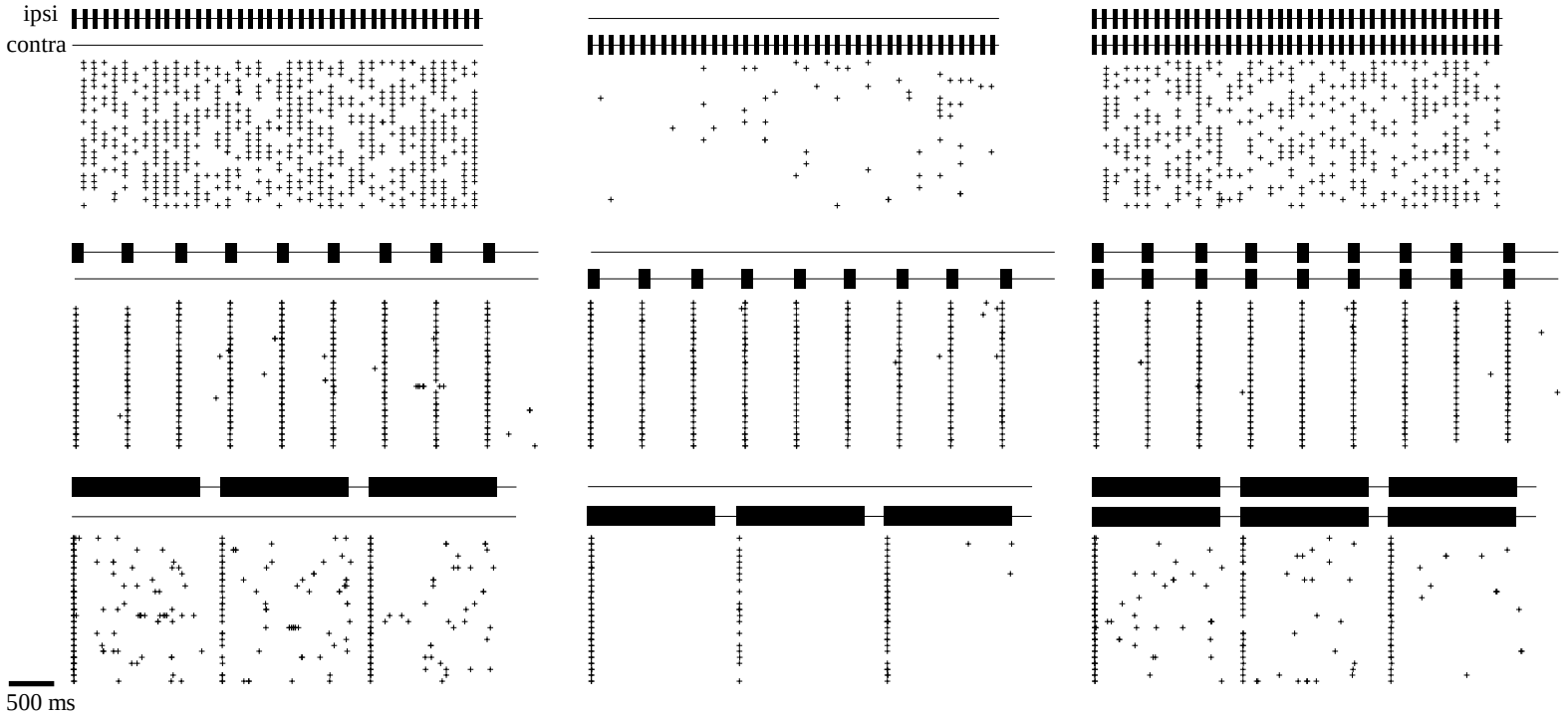

Figure 9. Representative TN1 activity in *Neoconocephalus ensiger* during different chirp types and directional stimuli. *Left column* is the speaker ipsilateral to the recording site, *middle* is contralateral speaker only, *right* is ipsilateral and contralateral. *Top*, *middle*, and *bottom rows* are responses to 36, 76, and 1000 ms chirps, respectively. Each plot shows the responses for 25 repetitions, beginning with the first repetition at the top of each plot. Response to the first 36 ms chirp is excluded.

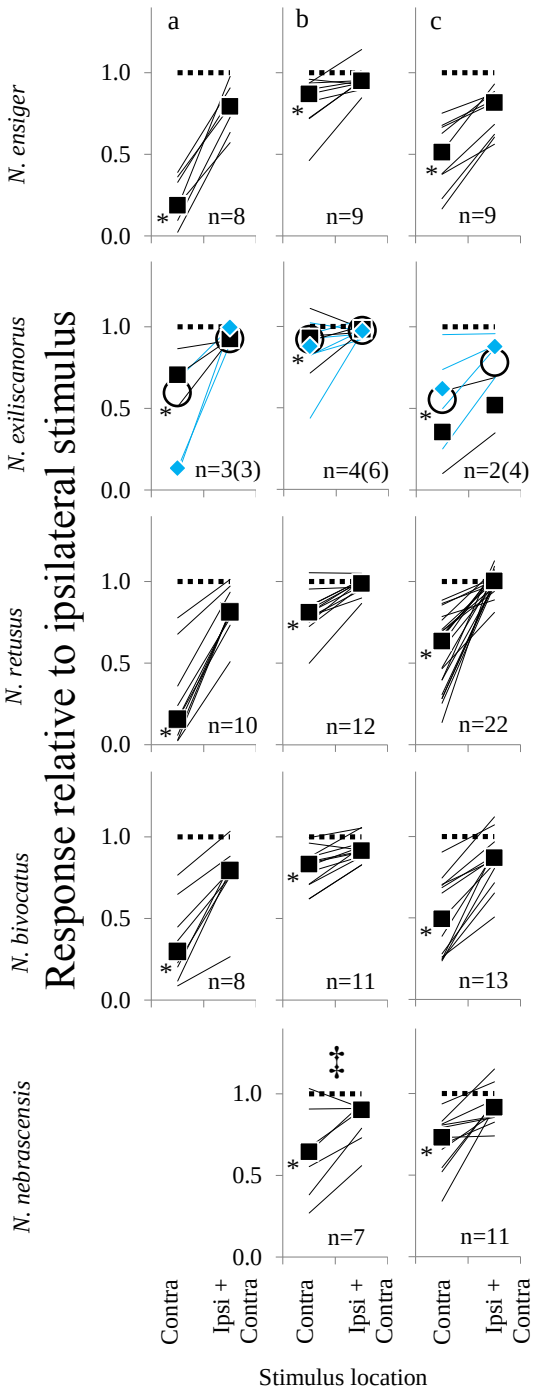

Figure 10. Relative TN1 responses to directional stimulus for 5 species of *Neoconocephalus*. Contralateral stimulus or simultaneous ipsilateral and contralateral stimulus with no delay were presented. Responses to chirp patterns of 36, 76, 1000/150

ms are represented by columns *a*, *b*, and *c*, respectively, except for ‡ which are responses to the 1000/800 ms chirp pattern. *Lines* and *symbols* indicate individual and median responses. *Closed black* and *blue* symbols indicate female and male responses; *open circles* indicate combined male and female median response. *Dashed lines* indicate response relative to the ipsilateral only stimulus. Sample size in each plot is n=female(male). Significant differences from the ipsilateral only response (combined male and female) are indicated by * ($p<.05$, Wilcoxon signed rank test).

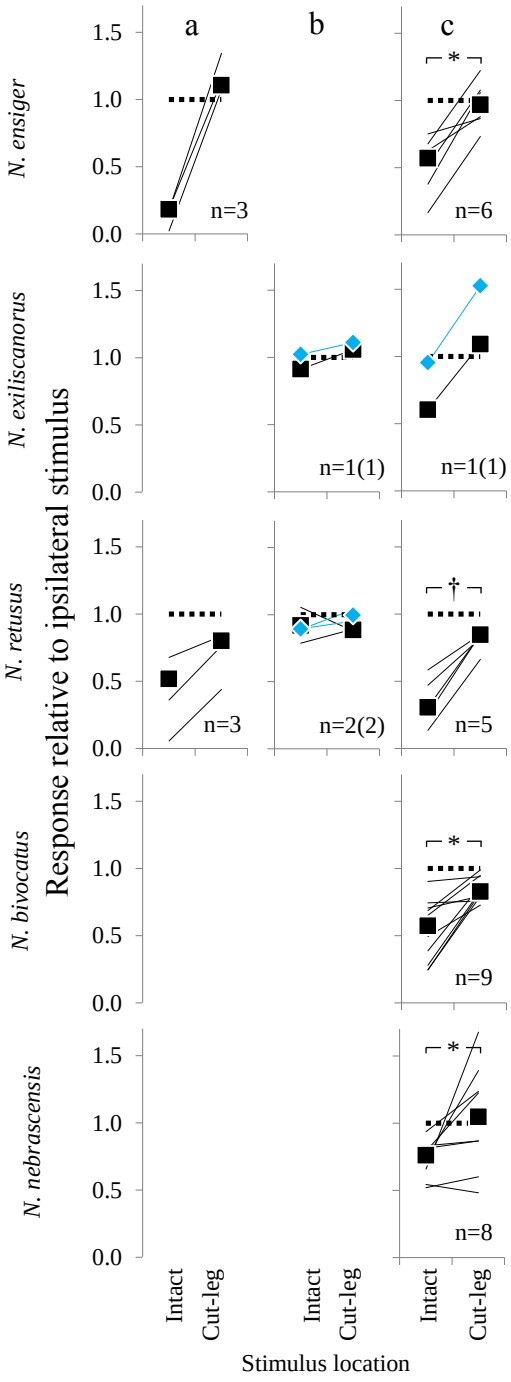

Figure 11. Relative TN1 responses to contralateral only stimulus for 5 species of *Neoconocephalus* before and after contralateral leg removal. Columns *a*, *b*, and *c* show responses to 36, 76, and 1000 ms/150 chirp patterns, respectively. *Dashed lines* indicate

response to the ipsilateral only stimulus. In each plot sample size is n= female(male). Significant differences (Wilcoxon signed rank test) between cut and uncut responses are indicated by * ($p<.10$) and † ($p<.05$).

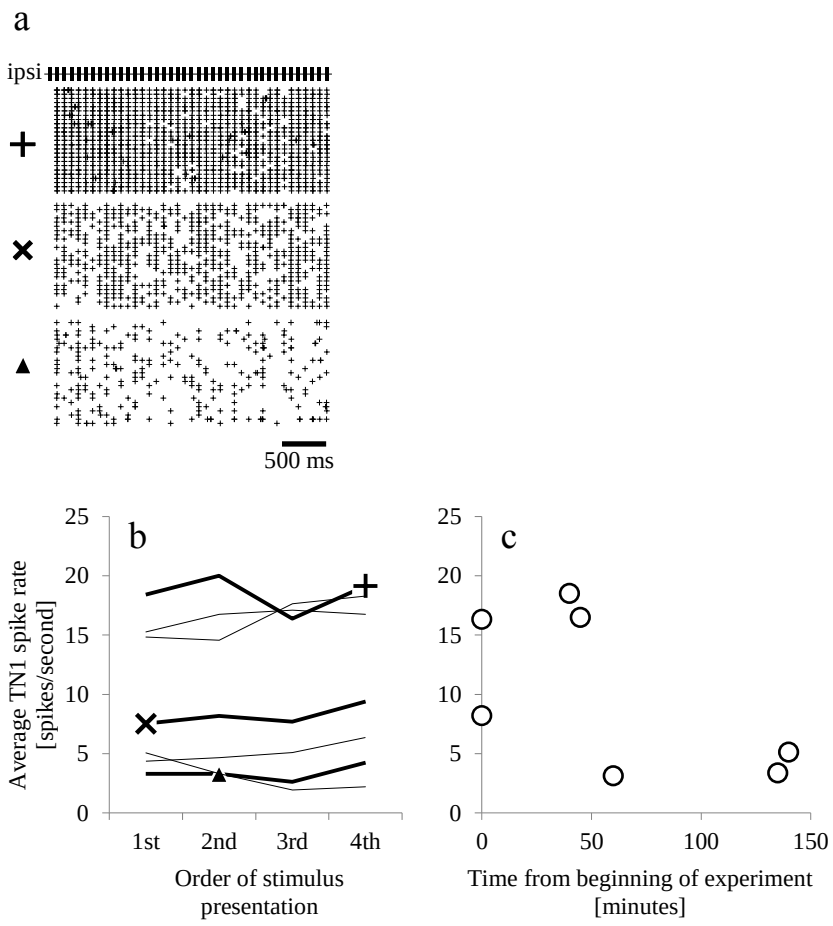

Figure 12. TN1 spiking rates for *Neoconocephalus ensiger* individuals during a presentation of 36 ms chirps. *a*) Raster plots of TN1 spikes in three different individuals for 25 repetitions of the leader-follower control stimulus. *Top trace* is stimulus of chirps presented from the ipsilateral side only; *symbols* to the left of each plot represent each individual in *b*. Responses during the first chirp period have been removed. *b*) *Lines* show average spike rates for individuals during the control stimului (in order of presentation). *Symbols* represent average spike rate from the respective individual in *A*; *thick lines* indicate the series for each symbol. Each series lasted approximately 7 minutes. *c)* Average spike rates of all control responses for an individual, throughout the time course of an experiment. Sample size is n=7.

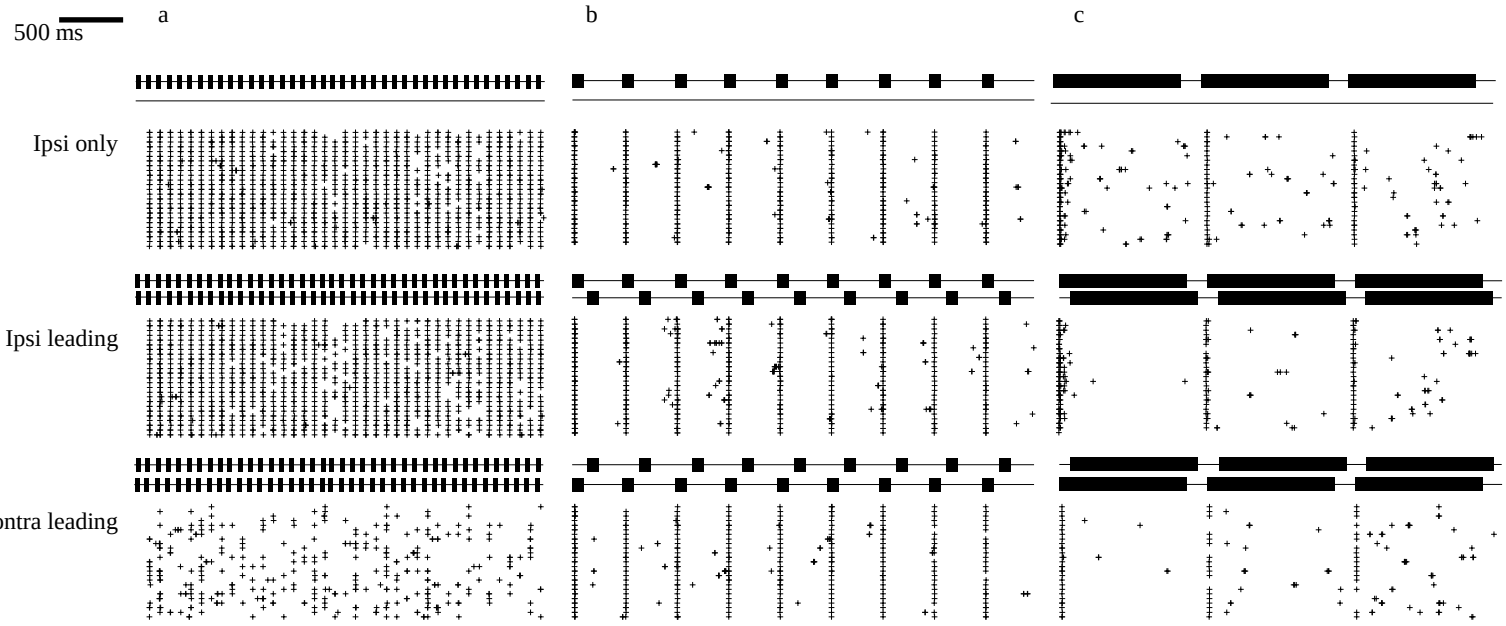

Figure 13. Representative TN1 spiking activity in *Neoconocephalus ensiger* during three different stimuli with and without leading and following chirps. Column *a*, *b*, and *c* show the activity during the 36, 76, and 1000 ms chirp patterns. Above each plot are the chirp stimuli from the ipsilateral (top trace) and contralateral (bottom trace) speakers. The *first row* shows the activity during the control. The *second* and *third row* show the activity when the leading call is ipsilateral and contralatereal, respectively. Following calls are delay by 12 ms (column *a*) or 80 ms (*b* and *c*).

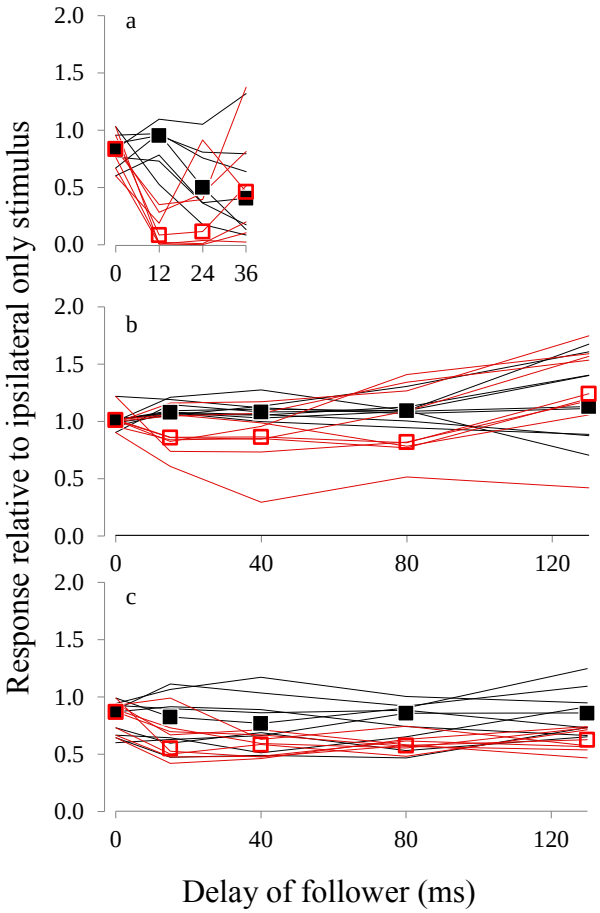

Figure 14. Representative relative TN1 responses to stimuli with the leading chirp either ipsilateral or contralateral in *Neoconocephalus ensiger*. Response to ipsilateral leading stimuli is in *black*, contralateral leading stimuli is in *red*. Plots *a*, *b*, and *c* show median (*symbols*) and individual (*lines*) responses during 36 (n=7), 76 (n=9), and 1000 ms (n=9) chirp patterns, respectively.

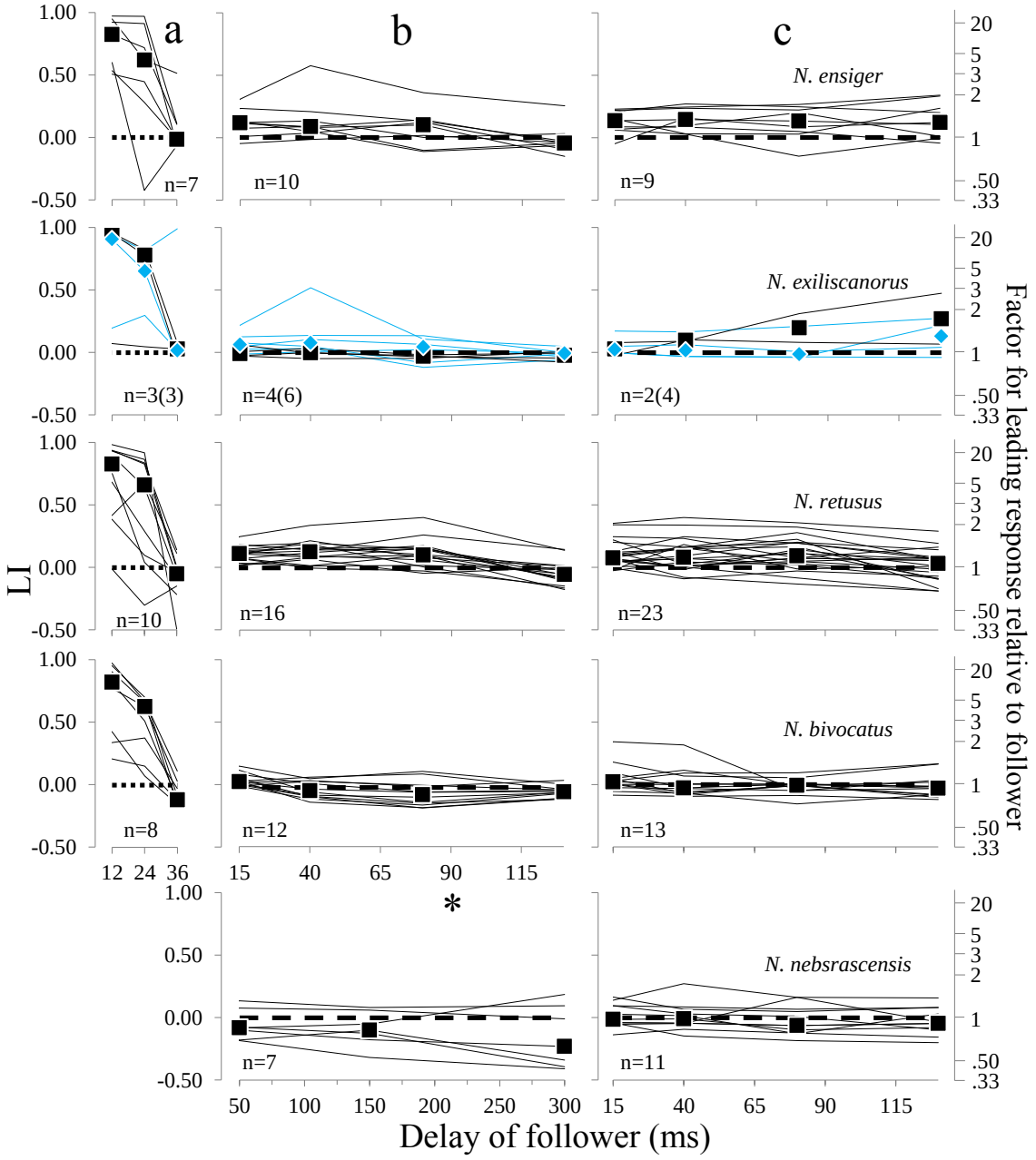

Figure 15. LI scores for TN1 activity during leader-follower stimuli for 5 species of *Neoconocephalus*. Positive LI scores indicate a stronger response to ipsilateral leading chirps while negatives scores result from stronger responses to following chirps. Responses during the 36, 76, and 1000/150 ms chirp patterns are represented by columns *a*, *b*, and *c*, respectively, except for * which is the 1000/800 ms chirp pattern. *Dashed*

lines indicate response to the control stimulus. Sample size in each plot is n=female(male).

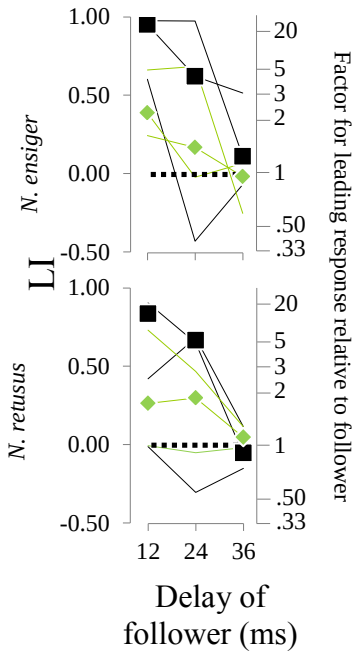

Figure 16. LI scores for TN1 during leader-follower stimuli of 36 ms chirp pattern before and after removal of contralateral leg in 2 species of *Neoconocephalus*. *Lines* and *symbols* indicate individual and median intact responses; *black* and *green* show intact and cut-leg responses. *Dashed lines* indicate response to control stimulus. Sample size is n=3 females for each plot.

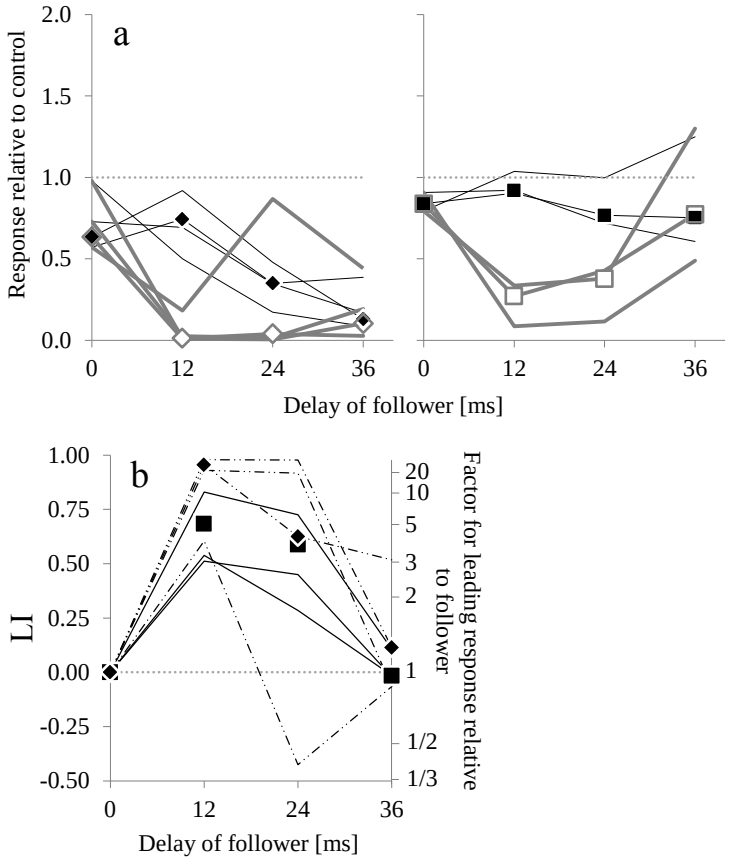

Figure 17. Comparison of responses to 36 ms leader-follower control stimuli among *Neoconocephalus ensiger* individuals. *a*) Relative TN1 response to leader-follower duets. *Left* and *right* plots shows the respective responses from individuals with low average TN1 spike rates (3-10 spikes/s; *diamond symbol*, n=4) or high spike rates (>15 spikes/s; *square symbol*, n=3) during control stimuli. *Lines* and *markers* indicate individual and median responses, respectively. *Filled symbols* and *thin black lines* indicate the median and individual responses, respectively, when leading calls are ipsilateral to the recording site; *open symbols* and *thick grey lines* are contralateral leading responses. *Dotted grey line* indicates equal response to control stimulus. *b*) LI scores for TN1 leader-follower duets. *Dashed* and *solid lines* indicate low and high spike

rates, respectively. The *dotted grey line* indicates equal response to leading and following calls.

APPENDIX

AN1 responses to changes in call angle

AN1 responses were directional for 36 and 1000 ms chirp patterns in *N. ensiger*. Median responses were near 1.25 and -0.75 when the stimulus was furthest (-90° call angle) and closest (+90°) to the recording side (Fig. A1). After removing the contralateral leg and repeating the experiments, directionality was reduced. The median responses before and after removal of contralateral inputs were very similar, resulting in relative responses near 1 for all call angles (Fig. A2).

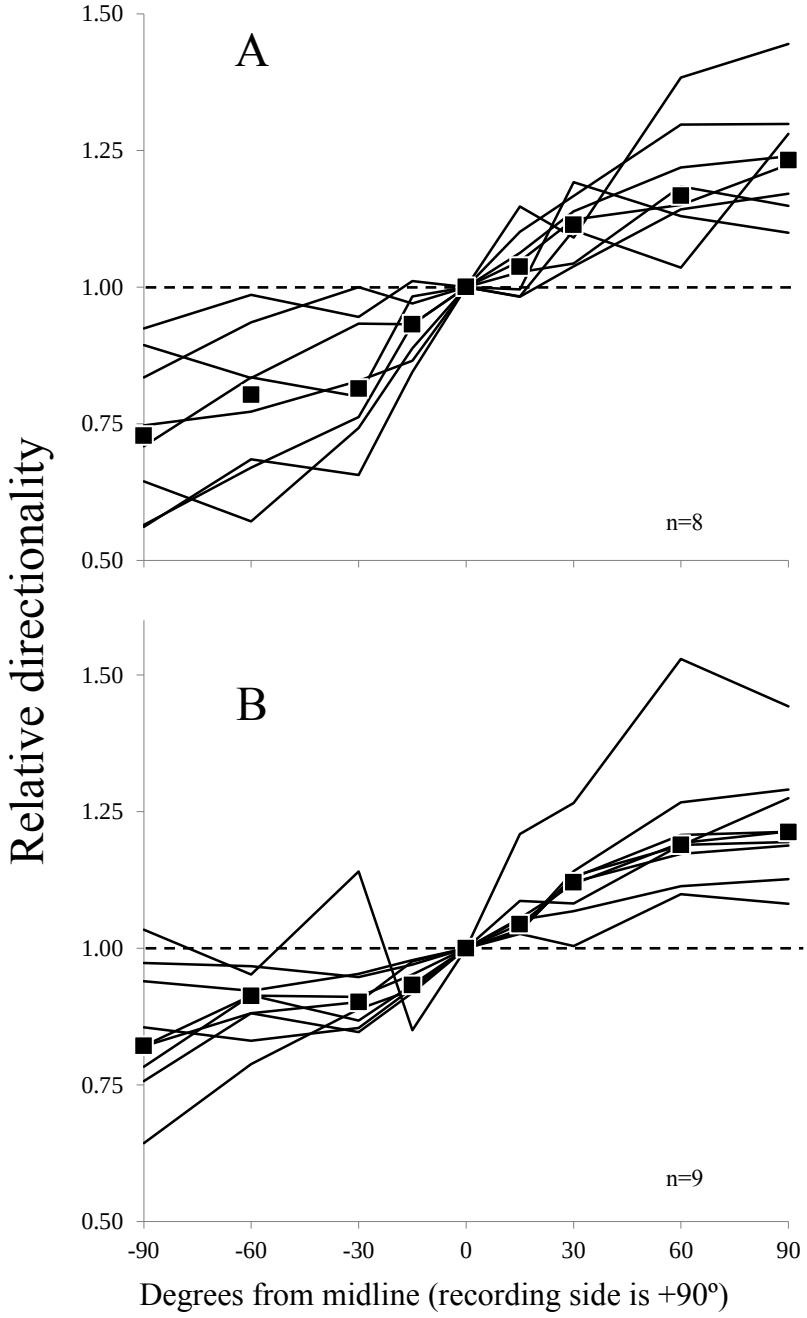

Figure A1. Responses of AN1 during changes in call angle for *Neoconocephalus ensiger*. Plots *A* and *B* represent stimuli with chirp lengths of 36 ms and 1000 ms, respectively. Markers represent medians and lines represent individual responses.

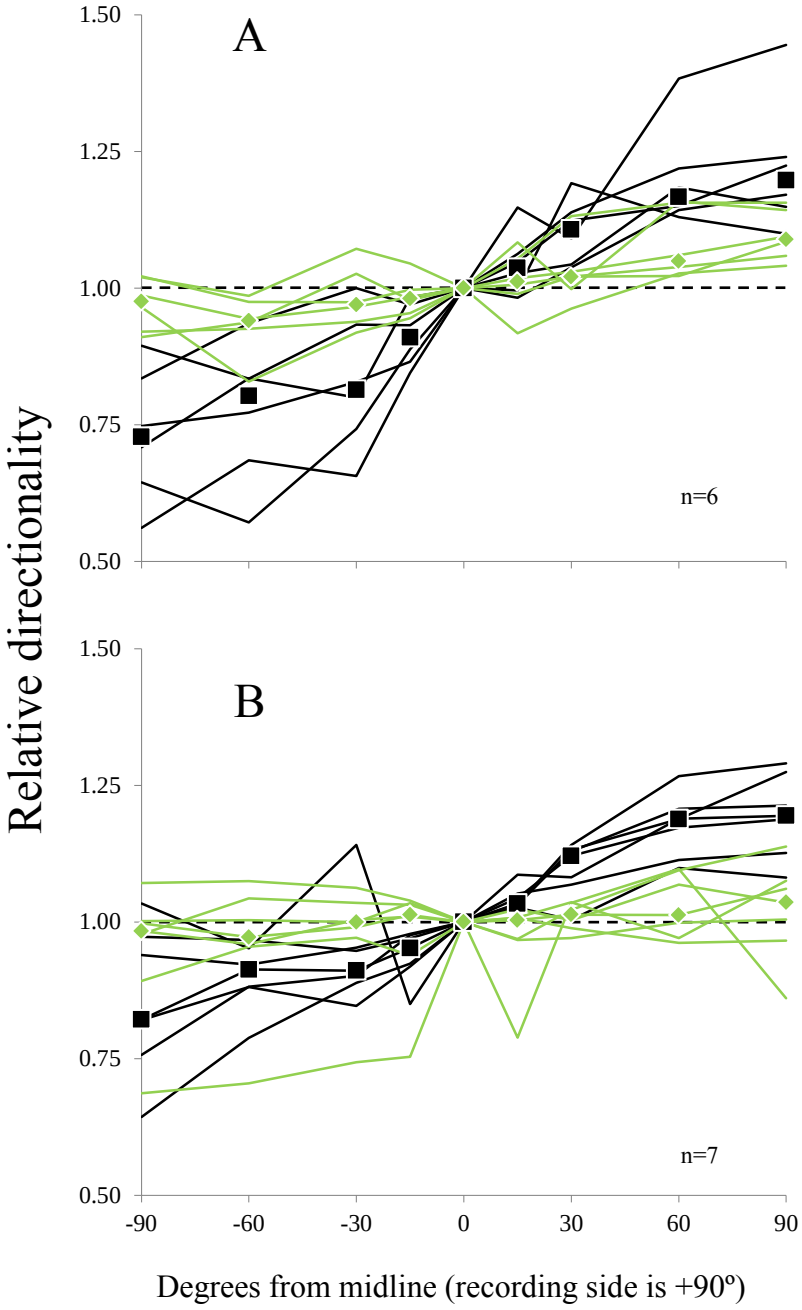

Figure A2. Responses of AN1 during changes in call angle *Neoconocephalus ensiger* before and after contralateral leg removal. Plots *A* and *B* represent stimuli with chirp lengths of 36 and 1000 ms, respectively. *Symbols* represent *medians* and lines represent individual responses. *Green* indicates cut-leg responses, *black* indicates intact responses.

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VITA

Rhett Hartman was born in Ft. Worth, TX, in 1975 and went to high school in the Kansas City area vis-à-vis primary schools in Turnersville, NJ and later Edmond, OK. Afterwards he graduated from Kansas State University in Manhattan, Kansas, with a BA in Interdisciplinary Social Science. During that time he studied abroad for 7 months in Giessen, Germany, although all he can remember is the ceramic course he took and the many weeks when the university went on strike.

After graduation, he worked part time while attending the University of Missouri-Kansas City (UMKC), taking computer programming and multimedia coursework. This eventually led him into freelance film production in the Kansas City area as a grip. Rhett took a break from all this to save money for school and enlisted in the Marines, stationed in Camp Pendleton, CA. He was deployed two times, once on the USS Peleliu (LHA 5) and later on the USS Bonhomme Richard (LHD 6) with stops in the Middle East. During these deployments he was part of the response to the tsunami in Banda Aceh, Indonesia. At the end of his 4 year enlistment, he studied for a personal training certificate (NASM). He returned to Missouri, eventually relocating for a year to Brooklyn, New York, for film production work. This route did not prove prosperous, though, and he instead focused on the work that paid the bills, working in the health care field. From personal training he moved to physical therapy and volunteering in the emergency department of New York Methodist Hospital.

These experiences inspired him to going back to school to study biology. For this he returned to UMKC in 2008 and, while working as a lab technician in the molecular

biology lab of Julia Chekanova for a year, he sat in or took basic science coursework in physics, chemistry, and biology. In addition, he learned to love non-calculus math from the open math help sessions from the UMKC math department. Even though he was considering a medical research career, these science classes persuaded Rhett to investigate basic science research at MU.

He moved to Columbia and enrolled in and sat in upper level biology and math classes. He met with various faculty members and talked about a master's project in the lab of Johannes Schul. In the meantime he assisted in MU undergraduate research projects with Tina Bloom in the School of Nursing School and with Robert Walker in the Department of Anthropology. He began the master's program in the Fall of 2010.