

## RESEARCH ARTICLE

# The effects of call-like masking diminish after nightly exposure to conspecific choruses in green treefrogs (*Hyla cinerea*)

Megan D. Gall<sup>1,\*</sup> and Walter Wilczynski<sup>2,3</sup>**ABSTRACT**

One of the major difficulties encountered by animals that select mates using acoustic signals is discriminating individual calls from the background noise generated by other conspecifics. Reducing the effects of conspecific masking could improve discrimination of individual calls from background noise. We used auditory evoked potentials to investigate the effects of forward masking on the responses to artificial calls in male and female treefrogs (*Hyla cinerea*), as well as whether hearing advertisement calls over several nights, as happens in natural frog choruses, could modify the effects of masking. We found that response amplitude decreased with decreasing interstimulus interval when the masker was equal in amplitude to the stimulus. We also found evidence of a priming effect, whereby response amplitude at lower masker amplitudes was greater than when the target stimulus was not preceded by a masker. Finally, we found that the effect of masking was diminished by 10 nights of chorus exposure (i.e. responses were stronger to target stimuli), whereas there was no change in response in the control group. Our results show that hearing dynamic social stimuli, such as frog choruses, can alter the responses of the auditory periphery in a way that could enhance the detection of and response to conspecific acoustic communication signals.

**KEY WORDS:** Plasticity, Hearing, Audition, Lek, Social signals, Amphibian

**INTRODUCTION**

For organisms that communicate acoustically with one another in social groups, background noise generated by other signaling conspecifics can make detecting vocalizations and discriminating among them difficult (Gerhardt and Klump, 1988; Narins, 2013; Wollerman and Wiley, 2002). This phenomenon is generally referred to as the cocktail party problem and is shared by many taxa (Bee and Micheyl, 2008). For instance, many species of frogs form breeding leks (Hoglund and Alatalo, 1995) during the reproductive season. Males congregate near a suitable breeding location (often a small pond or other body of water) and advertise their presence and quality to females through vocalizations (Wells, 1977, 2007). Males will call for many hours at night and may overlap their calls with others at the lek, forming a frog chorus. This presents a difficult perceptual task for females that must detect and discriminate among males on the basis of their vocalization to select a mate, and for males trying to identify potential rivals encroaching on their calling site, as individual vocalizations are likely to be masked by the

chorus (reviewed in Bee, 2012, 2015). Chorus noise presents a particularly difficult discrimination task because chorus noise shares both temporal and spectral characteristics with the target stimulus. There are several strategies to combat the cocktail party problem that are common to many anurans (Bee, 2012, 2015), including spatial release from masking (Ratnam and Feng, 1998; Schwartz and Gerhardt, 1989) and dip listening, wherein individuals extract the maximal amount of information from target stimuli during periods of relatively low amplitude noise (Vélez and Bee, 2010, 2011, 2013; Vélez et al., 2012).

The way the auditory system processes stimuli mediates the species-specific ability to identify individual calls in background noise. In particular, the extent to which preceding stimuli influence the ability to detect a target stimulus (i.e. forward-masking) is likely to play a large role in the ability to detect calls in a chorus. Forward masking occurs when the auditory system experiences either persistent activity or adaptation in response to a stimulus, which decreases or eliminates the ability of the auditory system to respond to future stimuli (Wojtczak and Viemeister, 2005). Typically, masking increases with increasing amplitude of the masker and with decreasing intervals between the masker and the target stimulus. Any process that diminishes the effects of forward masking in the auditory system should enhance the ability of an individual to detect a target stimulus following noise generated by conspecifics. However, forward masking has been relatively understudied in anurans compared with other aspects of auditory processing.

Green treefrogs (*Hyla cinerea* Schneider 1799) are an excellent model system to investigate the cocktail party problem and forward masking, as they form leks and are thus faced with detecting and discriminating individual male calls within an acoustically complex chorus of multiple vocalizing males. Green treefrogs are also an excellent system in which to explore social modulation of auditory processing because they are prolonged breeders, with males congregating and calling in choruses at permanent bodies of water for many nights throughout the summer (Elliott et al., 2009; Garton and Brandon, 1975; Wells, 1977). Males are exposed to the calls of other males nightly, and females in vegetation surrounding the breeding ground may be exposed to the male chorus for days or weeks before breeding or between breeding attempts.

Previous work has shown that green treefrogs use several strategies in response to the cocktail party problem. For instance, females benefit from spatial release from masking; female phonotaxis to male calls improves when the call and background noise are spatially separated (Schwartz and Gerhardt, 1989). Interestingly, female green treefrogs may not benefit greatly from dip listening, as phonotaxis towards individual calls does not vary across noise with different levels of modulation (i.e. length of ‘dips’; Vélez et al., 2012), although male green treefrogs may employ dip listening when deciding to call in noisy conditions (Höbel, 2014). Additionally, context-specific changes in auditory processing may be used to enhance call detection in green treefrogs. Recent work

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suggests that acoustic experience can induce plasticity in the auditory periphery (Gall and Wilczynski, 2015). Hearing a dynamic assembly of conspecific advertisement calls over several nights, as would normally happen in a breeding chorus, increases the frequency sensitivity of the auditory periphery. Such a change makes the ear more sensitive to calls, hence increasing the ability to detect them in quiet conditions. However, this does not necessarily reflect how animals would process information in an acoustically complex chorus.

In a complex acoustic environment, the effects of masking (both simultaneous and forward) play a large role in determining whether animals can detect stimuli. Therefore, we used a repeated-measures design to investigate forward masking of synthetic calls in the auditory periphery of green treefrogs, as well as the modulation of forward masking by social stimulation. We predicted that increasing the masker amplitude and decreasing the time between the masker and the target stimulus would diminish the response to the target stimulus, as is common in forward masking. Furthermore, given our previous findings that exposure to social stimuli increases frequency sensitivity (Gall and Wilczynski, 2015), we tested whether there would be socially induced plasticity of forward masking such that the effects of forward masking would be diminished in animals exposed to nightly frog chorus.

## MATERIALS AND METHODS

### Housing and experimental design

Green treefrogs were acquired through a commercial vendor (Charles D. Sullivan) and housed at Georgia State University. Prior to the experiment treefrogs were housed in same sex groups of four in 40 l aquaria. During the experiment frogs were housed individually in custom-built acoustic isolation chambers. Both the aquaria and the chambers contained a shelter or rock, a large dish of fresh water, and artificial vegetation. The light cycle was set to 14 h light:10 h dark and the temperature mimicked summer breeding temperatures. Frogs were misted daily and fed with gut-loaded crickets twice weekly. All experiments were conducted in June and July of 2014 and approved by the Georgia State University Institutional Animal Care and Use Committee (IACUC protocol A12036).

We investigated whether sound exposure would alter the effects of forward masking in the peripheral auditory system using a within subjects design. Thus, we measured the effects of forward masking on auditory processing twice in each animal; once before and once after ten days of nightly sound exposure (Fig. 1A). Briefly, we measured auditory evoked potentials from each animal on day 0. Auditory evoked potentials are closely correlated with single unit and behavioral estimates of hearing in treefrogs (Buerkle et al., 2014; Schrode and Bee, 2015; Schrode et al., 2014). After auditory testing, animals were placed in custom-built sound isolation chambers where they received nightly sound exposure (stimuli described below) to either a simulated treefrog chorus (4 frogs: 2 males and 2 females) or random tones (5 frogs: 2 males and 3 females) for 10 consecutive days (days 1–11). The chorus and/or tone stimuli were presented at an amplitude of 80 dB (RMS) for 6 h during the dark phase of the light cycle. The animals remained in the isolation chambers in silence for an additional day to prevent possible habituation effects (day 12). Finally, we repeated our measurements of auditory evoked potentials on day 13.

### Sound exposure stimuli

The chorus was a 12 min compilation of natural treefrog calls combined into artificial ‘choruses’ of varying densities. We first

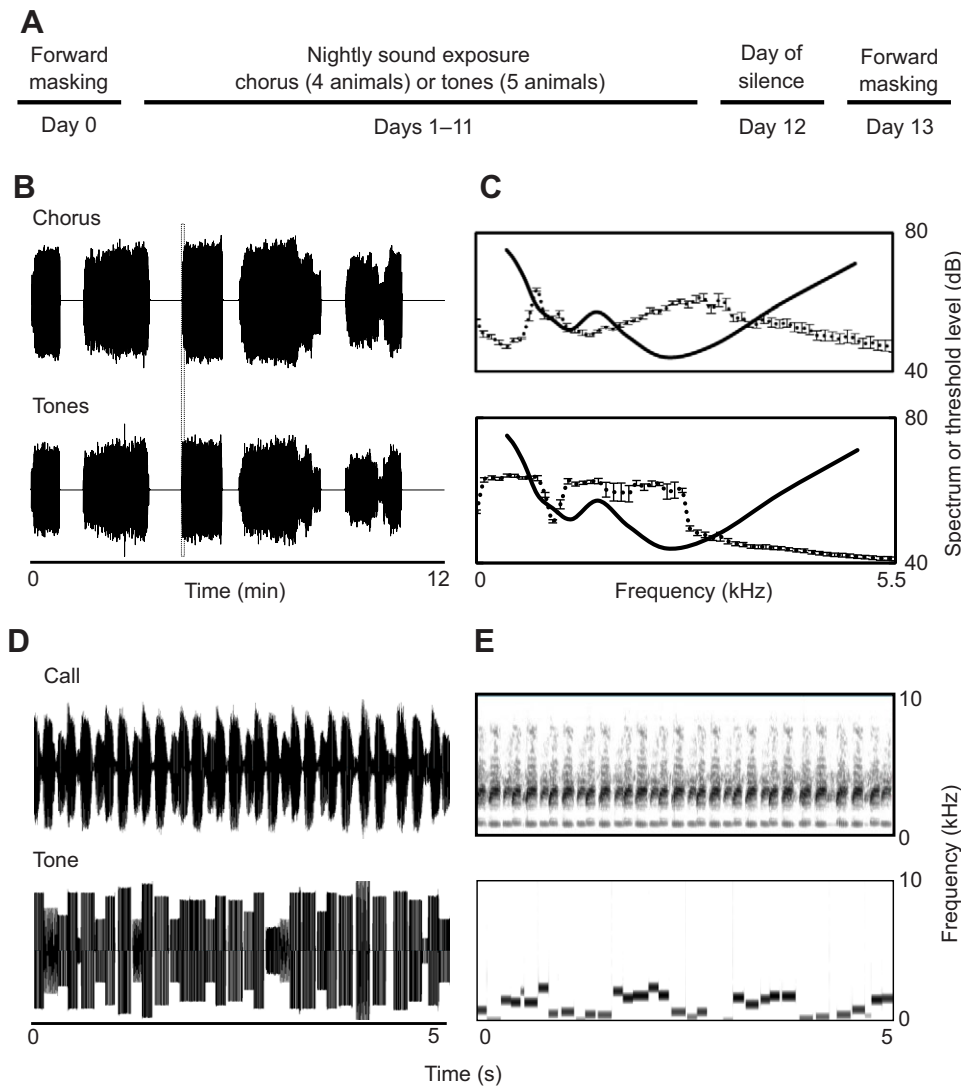
used individual calls of male frogs to create the chorus. Individual calls were approximately 100 ms in duration and the interstimulus interval (time between individual calls or tones) ranged from less than zero (overlapping calls) to 750 ms (Fig. 1B–E). As in many frogs (reviewed in Wells and Schwartz, 2007), closely spaced green treefrog males engaging in male-male vocal interactions alternate calls or overlap slightly as calling intensity increases (Jones et al., 2014); calls from more distant males sometimes may overlap in a random fashion. It was most common for calls in our stimulus chorus to overlap or have a separation of up to 50 ms. Longer interstimulus intervals were less common in our stimuli, although longer intervals could occur in low-density choruses. Additionally, in green treefrog choruses, individuals tend to begin and cease calling at similar times (Jones et al., 2014). This leads to bouts of calling interspersed with silent intervals. Our stimulus chorus featured five calling bouts separated by 25–75 s of silence.

We then generated tones that matched the amplitude and duration of each call element within the chorus exactly. Thus the timing and amplitude of this file matched exactly the chorus file. The tones all fell within the hearing range of the green treefrogs, although the frequency spectrum was slightly different to that of the chorus (Fig. 1C). Green treefrogs will respond strongly to artificial calls (those matching in frequency and duration) (Gerhardt, 1974, 1981); therefore, exact frequency matches do not allow us to adequately investigate the effect of chorus versus random sounds.

### Auditory evoked potentials

All of our evoked potential experiments were conducted in an IAC Acoustics audiology booth. Prior to testing, animals were given an intramuscular injection of sterile filtered (Millex Syringe Filter; EMD Millipore) tubocurarine hydrochloride pentahydrate (Sigma) dissolved in saline ( $6.4 \pm 1.3 \mu\text{g g}^{-1}$ , mean  $\pm$  s.d.). We applied a topical anesthetic (2.5% lidocaine) and inserted three subcutaneous needle electrodes when the animal was sufficiently immobilized (reduced muscle function, but ventilating normally). We placed one electrode at the apex of the head (positive), and one in each auditory meatus directly below the tympanum (inverting and ground). We then placed the subject in a small Petri dish that was lined with moistened paper towel and loosely draped the animal with an additional moistened towel to prevent dehydration and facilitate cutaneous respiration. The Petri dish was placed on a heating pad (Snuggle Safe) to maintain a constant temperature ( $26 \pm 1^\circ\text{C}$ ) between the heating pad and the ventral surface of the animal, which, in turn, was surrounded by pyramidal acoustic foam in the center of a Faraday cage.

We used a TDT System 3 (Tucker Davis Technologies, Alachua, FL, USA) to conduct our auditory evoked potential experiments. The system consists of a RZ6 processor connected via fiber optic cables to a computer fitted with a POE5 signal processing card. Stimuli generated on the computer (using SigGenRZ) were fed through the processor and then out to a Soundware XS satellite speaker (Boston Acoustics; frequency response: 0.15–20 kHz) that was positioned approximately 30 cm above the subject. Evoked responses were fed from the needle electrodes to a RA4LI head stage and RA4RA preamp, which fed into the RZ6 processor via a fiber optic cable. Responses were low-pass filtered at 3 kHz and digitized at a sampling rate of 24.4 kHz. To calibrate the system, we first generated a 1 V, 1 kHz tone in BioSig and measured the peak amplitude of the stimulus output of the speaker with a Larson Davis SoundTrack LxT sound level meter with a flat weighting. We then used the calibration feature in BioSig to flatten the frequency response of the speaker. Following calibration, we measured the



**Fig. 1. Conditioning sound exposure stimuli in green treefrogs.** (A) Timeline of the within-subjects experiment.

Waveforms (B) and corresponding long term average spectra (C) (means $\pm$ s.e.,  $N=6$ ; dotted lines) of the entire chorus and tones conditioning stimuli. Also shown in C are the AEP-derived auditory thresholds of green treefrogs (solid lines). The tones had greater spectral energy between 1 and 3 kHz than the green treefrog chorus. Waveforms (D) and spectrograms (E) of a 5 s section of the chorus and tone stimuli (dotted box in B) that illustrate the timing between individual calls. In general, individual calls were separated by 50 ms or less.

amplitude of tones in 1/3 octave bands with the sound level meter. All frequencies were within  $\pm 1.5$  dB of the calibrated amplitude.

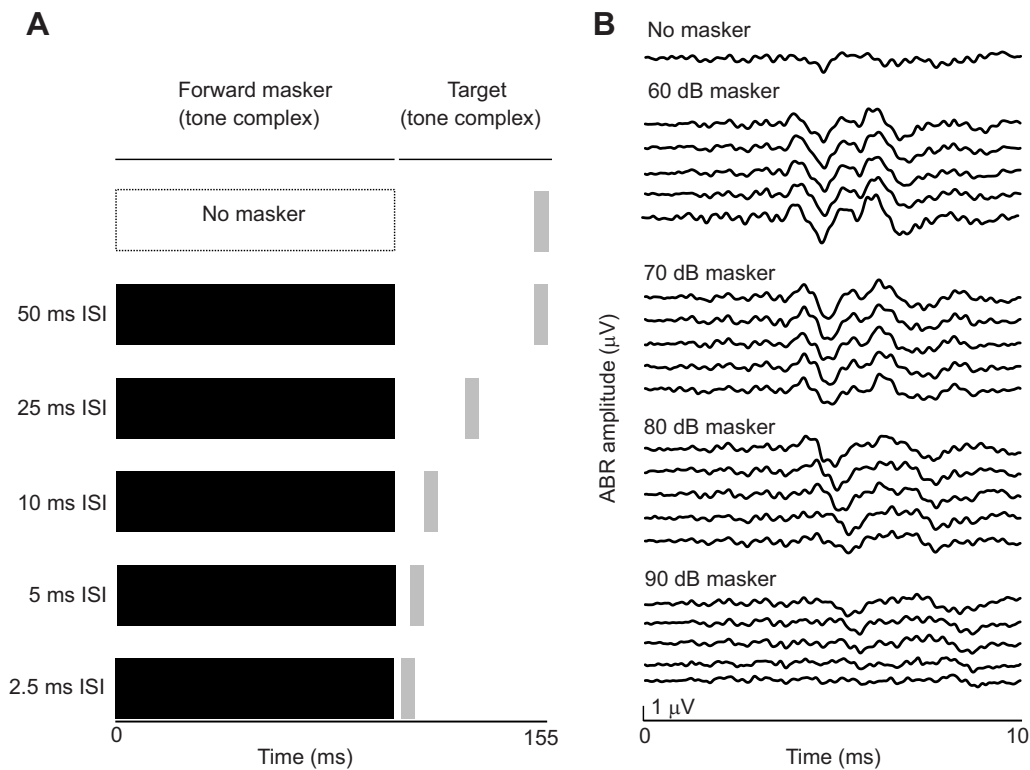
The forward masking stimuli were designed to mimic the masking that occurs in a natural chorus, where an individual is attending to the call of a nearby male, which can be masked by the preceding calls of near or distant males. The stimuli consisted of a test stimulus preceded by a masker (Fig. 2A). Both the test stimulus and maskers were structurally similar to green treefrog calls and consisted of two tones, with one randomly selected tone in a low frequency band (0.9–1.1 kHz) and one in a high frequency band (2.7–3.3 kHz) for each of the 400 presentations. The masker and target were not always the same frequency, as would be the case in a natural chorus. The test stimulus was 5 ms in length with a 1 ms onset/offset ramp and the masker was 100 ms in length with 10 ms onset/offset ramps. The offset of the masker was separated from the target stimulus by an interstimulus interval of 2.5, 5, 10, 25 or 50 ms. These intervals are within the range separating calls of males in naturally interacting green treefrogs (Jones et al., 2014). The target stimulus was always presented at 90 dB SPL, whereas the masker varied in amplitude from 60 to 90 dB in 10 dB steps. These amplitudes range from the average amplitude of a low-density chorus to the amplitude of an individual call at slightly less than 1 m

from the caller (Gerhardt, 1975). We also presented the target stimulus with no masker during each trial for each animal.

We analyzed the auditory evoked potentials offline in Praat 5.3.55 (available at <http://www.fon.hum.uva.nl/praat/>). Specifically, we analyzed the auditory brainstem response (ABR) of the animals to the onset of the test stimulus (Fig. 2B). The ABR is composed of several peaks which are generated primarily by the auditory nerve and the brainstem. The earliest peak is generally thought to be generated entirely by the auditory nerve. In mammals, this was determined by lesion studies (Buchwald and Huang, 1975); in birds and anurans, the latency of the first peak is similar to the latency of spiking of the auditory nerve in response to sound (Buerkle et al., 2014; Henry and Lucas, 2008). Thus, we chose to investigate the amplitude of the first peak of the ABR, as it represents a peripheral response to sound. To determine amplitude, we measured the voltage difference between the positive peak and the subsequent negative peak. We expected the amplitude of the response to decrease as the masker increased in amplitude and as the interstimulus interval decreased.

#### Statistical analysis

We used repeated-measures (subject=frog id) mixed models in SAS 9.3 to analyze our data. The dependent variable was the amplitude



**Fig. 2. Forward masking experiment.** (A) Experimental procedure. We generated 100 ms two-tone complexes that mimicked treefrog calls as a masker. A 5 ms target stimulus (two-tone complex) at interstimulus intervals (ISIs) of 2.5–50 ms followed the masker. Here, the tone and masker are the same amplitude (90 dB). The masker was also presented at 60, 70 and 80 dB. We recorded the summed response of the frog auditory nerve to the onset of the target stimulus. (B) Example traces of auditory brainstem responses (ABRs) from a single frog. Shown are responses to the target stimulus at four masking amplitudes (60, 70, 80 and 90 dB SPL). For each masking amplitude, responses to the probe tone are shown when the probe tone is separated from the masking stimulus by 50, 25, 10, 5 or 2.5 ms (traces arranged top to bottom).

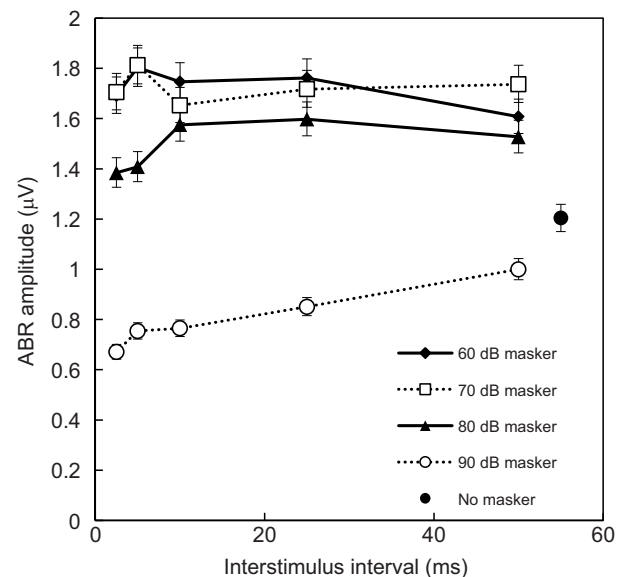
of the ABR. The independent variables were the within-subject factors of time (before and after exposure), masker amplitude (60–90 dB) and interstimulus interval (2.5–50 ms), the between-subject factor stimulus type (chorus or tones) and all interactions. We removed non-significant higher-order interactions from the model according to  $P$ -value and the resulting AIC value for the new model. ABR amplitude data were log transformed to achieve normality and homogeneity of variance. We therefore report back-transformed marginal means ( $\pm$ s.e.) throughout. Significant interactions were investigated using the SLICE or diffs option in the LSMEANS statement. *Post hoc t*-test  $P$ -values were adjusted using the Tukey method.

## RESULTS

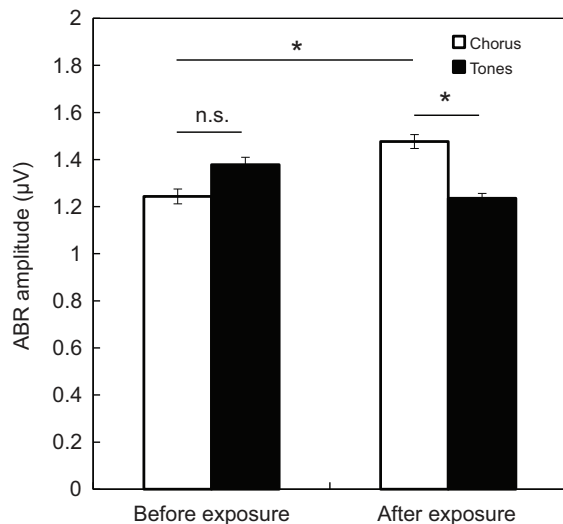
We found a significant main effect of masker amplitude ( $F_{4,64}=253.6$ ,  $P<0.001$ ), but no significant main effects of stimulus type ('chorus' versus 'tone':  $F_{1,15}=3.65$ ,  $P=0.075$ ), time (before versus after:  $F_{1,7}=4.23$ ,  $P=0.079$ ) or interstimulus interval ( $F_{4,64}=1.03$ ,  $P=0.39$ ). We also found significant interstimulus interval $\times$ masker amplitude ( $F_{13,191}=4.17$ ,  $P<0.001$ ), stimulus type $\times$ time ( $F_{1,7}=52.63$ ,  $P<0.001$ ) and time $\times$ masker amplitude ( $F_{4,32}=4.11$ ,  $P=0.009$ ) interactions.

The interstimulus interval $\times$ masker amplitude interaction was driven primarily by a difference between lower amplitude (60–80 dB) and high amplitude maskers (90 dB). At lower levels (60 dB masker:  $F_{4,96}=1.59$ ,  $P=0.18$ ; 70 dB masker:  $F_{4,96}=1.02$ ,  $P=0.40$ ), the effect of the masker on the amplitude of the ABR to the tone burst was similar across all interstimulus intervals (Fig. 3). In fact, low amplitude maskers actually elevated the amplitude of the ABR

relative to the ABR evoked by a target stimulus with no masker (all intervals:  $t_{96}>5.4$ ,  $P<0.001$ ), particularly at short interstimulus intervals ( $\sim 5$  ms), suggesting a priming effect of lower amplitude



**Fig. 3. Auditory brainstem response amplitudes as a function of both masker amplitude and interstimulus interval.** ABR amplitude (mean $\pm$ s.e.) to a 90 dB tone with no masking is shown offset from the other response. All data are averaged across all individuals ( $N=9$ ).



**Fig. 4. Auditory brainstem response amplitudes as a function of time and stimulus exposure.** ABR was measured before or after 10 nights of stimulus exposure (chorus,  $N=4$ ; random tones,  $N=5$ ). ABR amplitudes (means $\pm$ s.e.) are averaged across all masker amplitudes and interstimulus intervals. \* $P<0.05$ ; n.s., not significant.

stimuli. However, when the amplitude of the masker was either 80 dB ( $F_{4,96}=3.07$ ,  $P=0.02$ ) or 90 dB ( $F_{4,96}=12.04$ ,  $P<0.001$ ), ABR amplitude diminished as the interstimulus interval decreased (Fig. 3). When the masker amplitude was 80 dB, the ABR response was enhanced compared with the no-masking condition at longer intervals (intervals $\geq 10$  ms:  $t_{96}>4.67$ ,  $P<0.002$ ), but did not differ from the no-masker condition at short intervals (interval $\leq 5$  ms:  $t_{96}<2.73$ ,  $P>0.44$ ). When the masker amplitude was 90 dB, the ABR response was diminished compared with the no-masking condition at all interstimulus intervals ( $t_{96}<-5.3$ ,  $P<0.001$ ).

The time $\times$ masker amplitude interaction was driven by an increase in ABR amplitude after stimulus exposure (both chorus and tones) following low intensity maskers (60 dB:  $F_{1,32}=5.84$ ,  $P<0.025$ ). However, when masker amplitudes were greater, there were no changes in the ABRs of animals (again, with chorus and tones animals grouped together;  $F_{1,32}<2.93$ ,  $P<0.096$ ). This could be due to a stronger enhancement at low masking intensities in chorus animals following exposure, which would lead to a difference, even when ‘tones’ animals were included. Alternatively, in tones animals, there may be a small enhancement following stimulus exposure that is most evident when masker amplitudes are low.

The stimulus type $\times$ time interaction was driven by plasticity in the chorus group (Fig. 4). The tone and chorus exposure groups did not differ in the amplitude of the ABR prior to stimulus exposure ( $t_7=3.3$ ,  $P=0.05$ ). However, the two groups did differ after stimulus exposure ( $t_7=7.75$ ,  $P<0.001$ ). This is likely to be a result of an increase in ABR amplitude to the target stimulus increasing in the chorus animals ( $t_7=5.87$ ,  $P=0.002$ ), but not in the tones animals ( $t_7=3.18$ ,  $P=0.06$ ). This indicates that previous experience hearing chorus sounds, but not non-social sounds, facilitates release from masking in future masking conditions.

## DISCUSSION

In this study, we investigated forward masking from call-like noise in the periphery of the green treefrog, as well as whether experience with an acoustic social stimulus could modify the effect of masking.

We found that when the masker level was below the level of the target stimulus there was little difference in the effect of masker amplitude or interstimulus interval on the response to the target stimulus. Additionally, at low masking amplitudes there was an enhanced response to the target stimulus, with a particularly large enhancement at an interstimulus interval of 5 ms. This type of peripheral enhancement is also seen in mammals (Henry, 1991). When the masker and target stimulus were presented at the same amplitude (90 dB SPL), the response to the target stimulus decreased with decreasing interstimulus intervals, a pattern traditionally seen in forward masking (Wojtczak and Viemeister, 2005). Finally, we found that the overall effect of masking was decreased by 10 nights of chorus exposure, whereas there was no change in response in the control group. This lack of change could be due to our small sample size, although our previous work (Gall and Wilczynski, 2014, 2015) suggests that the tones may not be a salient enough stimulus to lead to the same large changes we see in animals that had exposure to the chorus. These results suggest that experience with vocal social signals would improve an individual’s ability to detect acoustic stimuli by reducing the masking effects of other acoustic signals.

Although we included both males and females in our study, we did not have sufficient statistical power to test for a sex difference in masking. Inspection of the data did not suggest the presence of a sex difference and our previous study (Gall and Wilczynski, 2015) did not find a sex difference in the effects of social experience on changes in peripheral auditory sensitivity in this species. However, that study did note a sex difference in baseline peripheral thresholds and several sex differences in peripheral auditory processing have been reported in various frog species (reviewed in Wilczynski and Burmeister, 2016), although no such data exist for auditory masking. A thorough investigation of potential sex differences in baseline masking parameters and in the potential effects of social experience on both simultaneous and forward masking is clearly warranted.

## Auditory masking and temporal resolution in treefrogs

The subject of auditory masking has been of interest to researchers studying anuran communication for some time because female anurans must be able to detect signals in noise with similar spectral and temporal features to the target signal. Simultaneous masking has been investigated physiologically in auditory nerve fibers (Ehret and Capranica, 1980; Lin and Feng, 2001; Ratnam and Feng, 1998) and midbrain cells (Goense and Feng, 2012), as well as behaviorally (Ehret and Gerhardt, 1980). In both low and high frequency auditory nerve fibers, tones begin to be masked at spectrum levels of 15–25 dB  $\text{Hz}^{-1}$ ; however, mid-frequency fibers do not begin to be masked until spectrum levels reach 35 dB  $\text{Hz}^{-1}$ . This translates to critical ratios between 10 and 20 dB for fibers with center frequencies below 0.8 Hz and critical ratios of 20–30 dB for fibers with center frequencies at or above 0.8 kHz (Ehret and Capranica, 1980). Ehret and Capranica (1980) found that the critical bandwidths of the auditory nerve fibers estimated from these critical ratios agreed well with the behavioral estimates. This suggests that peripheral physiological estimates of masking, such as those in this paper, correlate well with masking at the behavioral level of the animal. Furthermore, Ehret and Gerhardt (1980) found that artificial calls presented with low levels of low frequency noise were actually more attractive to females than calls presented alone, suggesting an enhancement effect of low noise levels. Interestingly, we also found that there appears to be enhancement (increased amplitude) of the ABR

when masker levels were low and particularly when they preceded the target stimulus by a short interval.

Forward masking has been relatively less well studied in comparison to simultaneous masking and has not previously been investigated in green treefrogs. However, it has been investigated in the auditory midbrain of gray treefrogs (*Hyla chrysoscelis*). In gray treefrogs, maskers that are lower in amplitude than the target tone did not elicit masking, rather they needed to be at or above the level of the target tone (Hillery and Fay, 1982). The masker and the target tone also needed to be separated by 20 ms or less for masking to be observed. This also appears to be the case in the auditory periphery of the green treefrogs we studied. However, at lower levels of masking, there did not appear to be the same enhancement of midbrain response that we found in the periphery of green treefrogs.

Auditory masking is a phenomenon that is related to auditory temporal resolution, as the two phenomena share several underlying mechanisms. The relationship between the temporal properties of calls and auditory temporal resolution has recently been investigated in green treefrogs and Cope's gray treefrogs using auditory evoked potentials (Schrode and Bee, 2015). Green treefrog calls have faster modulations than Cope's gray treefrog calls. Interestingly, the temporal resolution of green treefrogs was found to be greater than the temporal resolution of Cope's gray treefrog, with green treefrogs having a minimum resolvable interstimulus interval of 1.6 ms and gray treefrogs having a minimum resolvable interstimulus interval of 2.0 ms. Thus, we might predict that gray treefrogs might be more affected by forward masking than green treefrogs. This could explain, in part, why gray treefrogs appear to benefit more from long dips in chorus noise than do green treefrogs (Vélez and Bee, 2013). Future work should investigate this relationship between temporal resolution, forward masking and dip listening in these two species.

#### Forward masking and socially induced plasticity

Our findings that the effects of forward masking are diminished in animals that have had experience with an acoustic social stimulus adds to a growing body of work indicating that the auditory periphery is much more plastic than was originally thought. There is considerable work showing that there are seasonal and hormonally induced changes in peripheral auditory sensitivity in fish, frogs and songbirds (Coffin et al., 2012; Gall et al., 2013; Henry and Lucas, 2009; Miranda and Wilczynski, 2009; Rohmann et al., 2013; Sisneros et al., 2004; Vélez et al., 2015; Zhang et al., 2012). In these taxa, breeding attempts occur seasonally and attention to mating-related stimuli increases in importance during these times. For instance, in midshipman fish, house sparrows and frogs, frequency sensitivity is upregulated during the breeding season, or under conditions that mimic the breeding season such as administration of steroid hormones (Henry and Lucas, 2009; Miranda and Wilczynski, 2009; Sisneros et al., 2004). Although most work has focused on frequency sensitivity, frequency and temporal resolution have also been shown to change seasonally, with frequency resolution increasing during breeding periods at the expense of diminished temporal resolution.

Our recent work suggests that experience with social stimuli can also induce plasticity in both the auditory midbrain (Gall and Wilczynski, 2014) and in the auditory periphery by changing response strength to conspecific call frequencies (Gall and Wilczynski, 2015). The current results show that socially induced auditory plasticity extends to facilitating release from forward masking, which may be particularly important in real-world complex acoustic conditions. Socially induced plasticity, as with seasonal and hormonally induced plasticity, should facilitate

detection and/or recognition of the conspecific vocal signals that are crucial for guiding reproduction. It is not yet clear whether these effects are mediated by socially induced hormone changes (Burmeister and Wilczynski, 2000; Lynch et al., 2006) or occur independently. Nevertheless, our data add to the evidence that the social stimulation that occurs during a breeding season is an important factor in shaping the responses of the peripheral auditory system.

#### Effects of masking on communication

In the previous work described above, maskers were continuous noise with a flat amplitude envelope and were presented in the same spatial location as the target tone. However, in real world situations chorus noise consists of a complex assemblage of calls from multiple animals, which would be conducive to forward masking. Forward masking has been relatively less well studied than simultaneous masking, particularly in the context of the cocktail party problem. In our physiological experiments we mimicked the forward masking that can occur in even low density natural choruses, where male calls may be preceded by the calls of rival males. Our results show that forward masking of peripheral auditory responses have implications for acoustic communication in this species.

Peripheral auditory responses play an important role in determining the salience of conspecific signals as the auditory periphery acts as a series of filters that gate acoustic information reaching higher order auditory processing areas. Most previous work has described seasonal-, hormonal- or experience-based changes in peripheral sensitivity (reviewed in Wilczynski and Burmeister, 2016), which increase the ability of the ear to pass on acoustic information to the central nervous system. Animals with diminished forward masking may also be better at passing information to the central nervous system, helping them to detect conspecific vocalizations in temporally fluctuating noise. Forward masking can be an important factor call processing in species like green treefrogs in which males engage in antiphonal calling when interacting vocally within a breeding chorus (Jones et al., 2014; Wells, 2007; Wells and Schwartz, 2007). In this type of social system, small groups of spatially close males intersperse their calls to fit into the intercall intervals of others resulting in temporally close, but generally non-overlapping calls. Our data show that such call timing leads to forward masking when males are in close proximity, making trailing calls less salient than leading calls in antiphonal call bouts. In several species, females have been found to prefer (in terms of approaching and mating with) males producing the leading calls (Bosch and Marquez, 2002; Dyson and Passmore, 1988; Grafe, 1996) and this also appears to be true in green treefrogs (Höbel, 2010, 2011; Höbel and Gerhardt, 2007). Our results show that hearing calls would mitigate, but not eliminate, this effect. Furthermore, calling from more distance males (which will have an amplitude lower than the target stimulus) may elevate the attractiveness of target male calls.

#### Conclusions

We believe this is the first investigation of forward masking in green treefrogs, as well as the first report of plasticity in masking that is related to natural social stimuli. We found that masker amplitudes at or above the amplitude of the target stimulus were required for forward masking to occur. Interestingly, we also found an enhanced response when the forward masking stimulus was presented at amplitudes lower than that of the target stimulus. Our results show that hearing dynamic social stimuli, like frog choruses, can alter the

responses of the auditory periphery in a way that could enhance the detection of and response to conspecific acoustic communication signals, particularly in low density choruses with temporally fluctuating background noise.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

W.W. and M.D.G. jointly conceived the project. W.W. designed the social stimulus exposure paradigm and M.D.G. designed, executed and analyzed the data from the auditory evoked potential experiments. W.W. and M.D.G. jointly wrote the manuscript.

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#### References

- Bee, M. A. (2012). Sound source perception in Anuran Amphibians. *Curr. Opin. Neurobiol.* **22**, 301–310.
- Bee, M. A. (2015). Treefrogs as animal models for research on auditory scene analysis and the cocktail party problem. *Int. J. Psychophysiol.* **95**, 216–237.
- Bee, M. A. and Micheyl, C. (2008). The cocktail party problem: What is it? How can it be solved? And why should animal behaviorists study it? *J. Comp. Psychol.* **122**, 235–251.
- Bosch, J. and Marquez, R. (2002). Female preference function related to precedence effect in an amphibian anuran (*Alytes cisternasii*): tests with non-overlapping calls. *Behav. Ecol.* **13**, 149–153.
- Buchwald, J. S. and Huang, C. (1975). Far-field acoustic response: origins in the cat. *Science* **189**, 382–384.
- Buerkle, N. P., Schrode, K. M. and Bee, M. A. (2014). Assessing stimulus and subject influences on auditory evoked potentials and their relation to peripheral physiology in green treefrogs (*Hyla cinerea*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **178**, 68–81.
- Burmeister, S. and Wilczynski, W. (2000). Social signals influence hormones independently of calling behavior in the treefrog (*Hyla cinerea*). *Horm. Behav.* **38**, 201–209.
- Coffin, A. B., Mohr, R. A. and Sisneros, J. A. (2012). Sacculus-specific hair cell addition correlates with reproductive state-dependent changes in the auditory sacculus sensitivity of a vocal fish. *J. Neurosci.* **32**, 1366–1376.
- Dyson, M. L. and Passmore, N. I. (1988). Two-choice phonotaxis in *Hyperolius marmoratus* (Anura: Hyperoliidae): the effect of temporal variation in presented stimuli. *Anim. Behav.* **36**, 648–652.
- Ehret, G. and Capranica, R. R. (1980). Masking patterns and filter characteristics of auditory nerve fibers in the green treefrog (*Hyla cinerea*). *J. Comp. Physiol. A* **141**, 1–12.
- Ehret, G. and Gerhardt, H. C. (1980). Auditory masking and effects of noise on responses of the green treefrog (*Hyla cinerea*) to synthetic mating calls. *J. Comp. Physiol. A* **141**, 13–18.
- Elliott, L., Gerhardt, H. C. and Davidson, C. (2009). *The Frogs and Toads of North America: A Comprehensive Guide to Their Identification, Behavior, and Calls*. Boston, MA: Mariner Books.
- Gall, M. D. and Wilczynski, W. (2014). Prior experience with conspecific signals enhances auditory midbrain responsiveness to conspecific vocalizations. *J. Exp. Biol.* **217**, 1977–1982.
- Gall, M. D. and Wilczynski, W. (2015). Hearing conspecific vocal signals alters peripheral auditory sensitivity. *Proc. R. Soc. B Biol. Sci.* **282**, 20150749.
- Gall, M. D., Salameh, T. S. and Lucas, J. R. (2013). Songbird frequency selectivity and temporal resolution vary with sex and season. *Proc. R. Soc. B Biol. Sci.* **280**, 20122296.
- Garton, J. and Brandon, R. (1975). Reproductive ecology of the green treefrog, *Hyla cinerea*, in Southern Illinois (Anura: Hylidae). *Herpetologica* **31**, 150–161.
- Gerhardt, H. C. (1974). The significance of some spectral features in mating call recognition in the green treefrog (*Hyla cinerea*). *J. Exp. Biol.* **61**, 229–241.
- Gerhardt, H. C. (1975). Sound pressure levels and radiation patterns of the vocalizations of some North American frogs and toads. *J. Comp. Physiol. A* **102**, 1–12.
- Gerhardt, H. C. (1981). Mating call recognition in the barking treefrog (*Hyla gratiosa*): responses to synthetic calls and comparisons with the green treefrog (*Hyla cinerea*). *J. Comp. Physiol. A* **144**, 17–25.
- Gerhardt, H. C. and Klump, G. M. (1988). Masking of acoustic signals by the chorus background noise in the green tree frog: a limitation on mate choice. *Anim. Behav.* **36**, 1247–1249.
- Goense, J. B. M. and Feng, A. S. (2012). Effects of noise bandwidth and amplitude modulation on masking in frog auditory midbrain neurons. *PLoS ONE* **7**, e31589.
- Grafe, T. U. (1996). The function of call alternation in the African reed frog *Hyperolius marmoratus*: precise call timing prevents auditory masking. *Behav. Ecol. Sociobiol.* **38**, 149–158.
- Henry, K. R. (1991). Frequency-specific enhancement of the cochlear compound action potential: influence of the forward masker. *Hear. Res.* **56**, 197–202.
- Henry, K. S. and Lucas, J. R. (2008). Coevolution of auditory sensitivity and temporal resolution with acoustic signal space in three songbirds. *Anim. Behav.* **76**, 1659–1671.
- Henry, K. S. and Lucas, J. R. (2009). Vocally correlated seasonal auditory variation in the house sparrow (*Passer domesticus*). *J. Exp. Biol.* **212**, 3817–3822.
- Hillery, C. M. and Fay, R. R. (1982). Forward masking and suppression in the midbrain of the southern grey treefrog (*Hyla chrysoscelis*). *J. Comp. Physiol. A* **146**, 435–447.
- Höbel, G. (2010). Interaction between signal timing and signal feature preferences: causes and implications for sexual selection. *Anim. Behav.* **79**, 1257–1266.
- Höbel, G. (2011). Variation in signal timing behavior: implications for male attractiveness and sexual selection. *Behav. Ecol. Sociobiol.* **65**, 1283–1294.
- Höbel, G. (2014). Effect of temporal and spectral noise features on gap detection behavior by calling green treefrogs. *Behav. Processes* **108**, 43–49.
- Höbel, G. and Gerhardt, H. C. (2007). Sources of selection on signal timing in a tree frog. *Ethology* **113**, 973–982.
- Hoglund, J. and Alatalo, R. (1995). *Leks*. Princeton, NJ: Princeton University Press.
- Jones, D. L., Jones, R. L. and Ratnam, R. (2014). Calling dynamics and call synchronization in a local group of unison bout callers. *J. Comp. Physiol. A* **200**, 93–107.
- Lin, W.-Y. and Feng, A. S. (2001). Free-field unmasking response characteristics of frog auditory nerve fibers: comparison with the responses of midbrain auditory neurons. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **187**, 699–712.
- Lynch, K. S., Crews, D., Ryan, M. J. and Wilczynski, W. (2006). Hormonal state influences aspects of female mate choice in the tungara frog (*Physalaemus pustulosus*). *Horm. Behav.* **49**, 450–457.
- Miranda, J. A. and Wilczynski, W. (2009). Sex differences and androgen influences on midbrain auditory thresholds in the green treefrog, *Hyla cinerea*. *Hear. Res.* **252**, 79–88.
- Narins, P. (2013). Behavioral responses of anuran amphibians to biotic, synthetic and anthropogenic noise. *Proceedings of Meetings on Acoustics*, 010029–010029.
- Ratnam, R. and Feng, A. S. (1998). Detection of auditory signals by frog inferior colliculus neurons in the presence of spatially separated noise. *J. Neurophysiol.* **80**, 2848–2859.
- Rohmann, K. M., Fergus, D. J. and Bass, A. H. (2013). Plasticity in ion channel expression underlies variation in hearing during reproductive cycles. *Curr. Biol.* **23**, 678–683.
- Schrode, K. M. and Bee, M. A. (2015). Evolutionary adaptations for the temporal processing of natural sounds by the anuran peripheral auditory system. *J. Exp. Biol.* **218**, 837–848.
- Schrode, K. M., Buerkle, N. P., Brittan-Powell, E. F. and Bee, M. A. (2014). Auditory brainstem responses in Cope's gray treefrog (*Hyla chrysoscelis*): effects of frequency, level, sex and size. *J. Comp. Physiol. A* **200**, 221–238.
- Schwartz, J. J. and Gerhardt, H. C. (1989). Spatially mediated release from auditory masking in an anuran amphibian. *J. Comp. Physiol. A* **166**, 37–41.
- Sisneros, J. A., Forlano, P. M., Deitcher, D. L. and Bass, A. H. (2004). Steroid-dependent auditory plasticity leads to adaptive coupling of sender and receiver. *Science* **305**, 404–407.
- Vélez, A. and Bee, M. A. (2010). Signal recognition by frogs in the presence of temporally fluctuating chorus-shaped noise. *Behav. Ecol. Sociobiol.* **64**, 1695–1709.
- Vélez, A. and Bee, M. A. (2011). Dip listening and the cocktail party problem in grey treefrogs: signal recognition in temporally fluctuating noise. *Anim. Behav.* **82**, 1319–1327.
- Vélez, A. and Bee, M. A. (2013). Signal recognition by green treefrogs (*Hyla cinerea*) and Cope's gray treefrogs (*Hyla chrysoscelis*) in naturally fluctuating noise. *J. Comp. Psychol.* **127**, 166–178.
- Vélez, A., Höbel, G., Gordon, N. M. and Bee, M. A. (2012). Dip listening or modulation masking? Call recognition by green treefrogs (*Hyla cinerea*) in temporally fluctuating noise. *J. Comp. Physiol. A* **198**, 891–904.
- Vélez, A., Gall, M. D. and Lucas, J. R. (2015). Seasonal plasticity in auditory processing of the envelope and temporal fine structure of sounds in three songbirds. *Anim. Behav.* **103**, 53–63.
- Wells, K. D. (1977). The social behaviour of anuran amphibians. *Anim. Behav.* **25**, 666–693.
- Wells, K. D. (2007). *The Ecology and Behavior of Amphibians*. Chicago: The University of Chicago Press.
- Wells, K. D. and Schwartz, J. J. (2007). The behavioral ecology of anuran communication. In *Hearing and Sound Communication in Amphibians*, Vol. 28 (ed. P. M. Narins, A. S. Feng, R. R. Fay and A. N. Popper), pp. 44–86. New York: Springer-Verlag.

- Wilczynski, W. and Burmeister, S.** (2016). Effects of Steroid Hormones on hearing and communication in Frogs. In *Hearing and Hormones* (ed. A. H. Bass, J. A. Sisneros, R. R. Fay and A. N. Popper), pp. 53-75. New York: Springer-Verlag.
- Wojtczak, M. and Viemeister, N.** (2005). Mechanisms of forward masking. *J. Acoust. Soc. Am.* **115**, 2599.
- Wollerman, L. and Wiley, R. H.** (2002). Background noise from a natural chorus alters female discrimination of male calls in a neotropical frog. *Anim. Behav.* **63**, 15-22.
- Zhang, D., Cui, J. and Tang, Y.** (2012). Plasticity of peripheral auditory frequency sensitivity in Emei music frog. *PLoS ONE* **7**, e45792.