A novel coding mechanism for social vocalizations in the lateral amygdala

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Gadziola MA, Grimsley JM, Shanbhag SJ, Wenstrup JJ. A novel coding mechanism for social vocalizations in the lateral amygdala. J Neurophysiol 107: 1047–1057, 2012. First published November 16, 2011; doi:10.1152/jn.00422.2011.—The amygdala plays a central role in evaluating the significance of acoustic signals and coordinating the appropriate behavioral responses. To understand how amygdalar responses modulate auditory processing and drive emotional expression, we assessed how neurons respond to and encode information that is carried within complex acoustic stimuli. We characterized responses of single neurons in the lateral nucleus of the amygdala to social vocalizations and synthetic acoustic stimuli in awake big brown bats. Neurons typically responded to most of the social vocalizations presented (mean = nine of 11 vocalizations) but differentially modulated both firing rate and response duration. Surprisingly, response duration provided substantially more information about vocalizations than spike rate. In most neurons, variation in response duration depended, in part, on persistent excitatory discharge that extended beyond stimulus duration. Information in persistent firing duration was significantly greater than in spike rate, and the majority of neurons displayed more information in persistent firing, which was more likely to be observed in response to aggressive vocalizations (64%) than appeasement vocalizations (25%), suggesting that persistent firing may relate to the behavioral context of vocalizations. These findings suggest that the amygdala uses a novel coding strategy for discriminating among vocalizations and underscores the importance of persistent firing in the general functioning of the amygdala.

persistent firing; response duration; communication calls; bat; Eptesicus fuscus

IN HUMANS AND OTHER VERTEBRATES, acoustic signals play a primary role in social interactions. Social vocalizations carry significant information about the sender and behavioral context, including sender identity, affective state, location, and body size (Bastian and Schmidt 2008; Fichtel et al. 2001; Hauser et al. 1998; Morton 1977; Rendall et al. 2005; Seyfarth and Cheney 2003). Natural selection will favor animals who vocalize to affect the behavior of listeners, as well as listeners who acquire information from acoustic signals to make a behavioral or physiological response (Endler 1993; Seyfarth and Cheney 2003). A critical function of the nervous system is the evaluation of vocal and other social signals and coordination of appropriate behavioral responses (Maren 2007). Whereas the social signals are analyzed by discrete sensory systems, other centers evaluate their significance and coordinate a behavioral response. In particular, the amygdala plays a central role in establishing associations between sensory cues and biologically important events and orchestrating emotional responses. Although studies have examined how the amygdala responds to and learns the significance of previously neutral, simple acoustic stimuli (LeDoux 1993; Paré and Collins 2000; Quirk et al. 1995), little is understood about how the amygdala contributes to acoustic communication and the analysis of complex social vocalizations.

Auditory information from the medial geniculate body and auditory association cortex enters the amygdala via the lateral nucleus (LeDoux et al. 1991; Mascagni et al. 1993). In turn, the amygdala is situated to modulate auditory responses through direct projections to auditory cortex (Amaral and Price 1984), nucleus basalis (Sah et al. 2003), and in bats, the inferior colliculus (Marsh et al. 2002). The amygdala also coordinates emotional expression to sound, with the central nucleus sending widespread projections to hypothalamic, midbrain, and medullary nuclei (LeDoux et al. 1988; Veening et al. 1984). To understand how amygdalar responses modulate auditory processing and drive emotional expression, it is important to assess how amygdalar neurons respond to and encode information that is carried within complex acoustic stimuli.

This study characterized responses of lateral amygdalar neurons to species-specific vocalizations and simple acoustic stimuli in awake big brown bats. We show that neurons in the lateral nucleus typically respond to most social vocalizations presented but differ in spike rate and response duration. Of these response measures, response duration and the persistent firing that contributes to the response duration provide codes for discriminating among social vocalizations. This suggests a novel coding strategy, in which only select vocal signals evoke an extended discharge, and the duration of the extended discharge differs for the different stimuli. This transformation may be critical for general amygdalar functioning.

METHODS

Auditory responses of single neurons were recorded from the lateral nucleus of the amygdala in awake, restrained big brown bats (Eptesicus fuscus). Four adult bats (three females, one male), providing electrophysiological data, were captured from private residences in northeast Ohio. The procedures were approved by the Institutional Animal Care and Use Committee at Northeast Ohio Medical University.

Acoustic Recordings

For use as stimuli in neurophysiology experiments, we recorded and analyzed social vocalizations and associated behaviors of big brown bats in defined social interactions. Vocalizations were previously recorded from 10 adult bats (six females, four males) and eight pups (four females, four males) at McMaster University (Ontario, Canada). To optimize the quality of acoustic recordings, groups of three to four animals were housed in a recording cage (14 × 13 × 11
plots. Once auditory activity was identified audiovisually using search bandpass filtered (600 – 6,000 Hz), and sampled at 40 kHz with a hydraulic micropositioner. Extracellular potentials were amplified, roll-off of eral to the amygdala under study. The system response had a gradual were antialias filtered, attenuated, amplified, and then sent to a signals. Acoustic stimuli were downloaded to a digital signal processor (Gadziola et al. 2011). Duration of social vocalizations ranged from 7 ms duration) and previously recorded social vocalizations were pre-

Surgical Procedures

Before surgery, animals received an intraperitoneal injection of Torbugesic (5 mg/kg ip, Fort Dodge Animal Health, Overland Park, KS) and then were anesthetized to effect with Isoflurane (2–4%, Abbott Laboratories, Abbots Park, IL). Hair overlying the dorsal surface of the skull was removed using depilatory lotion. A midline incision was made, and the underlying muscle reflected laterally to expose the skull. A metal pin was cemented onto the skull to secure the head to a stereotaxic apparatus. A sharpened tungsten ground electrode was inserted through a small hole in the skull and cemented in place. Based on stereotaxic coordinates, a craniotomy (<1 mm diameter) was made to access the amygdala. The underlying dura was removed and the hole covered with sterile bone wax. Immediately following surgery, local anesthetic (Lidocaine) and antibiotic cream were applied to the surgical areas, and the animal returned to the holding cage for a 2-day recovery period before physiological experiments.

Acoustic Stimulation and Physiological Recording

General methods used for acoustic stimulation and physiological recording were described previously (Gans et al. 2009). Physiological experiments were conducted in a single-walled acoustic chamber. On experiment days, animals were placed in a custom-built stereotaxic device used to guide electrode penetrations and aid histological reconstruction of recording sites. Animals were secured with a non-adhesive wrap and placed inside a small tube, where they rested comfortably while head-fixed within the stereotaxic apparatus. Bats were not sedated during experiments. If an animal showed signs of discomfort or distress, the experiment was terminated for the day. Recording sessions did not exceed 6 h and were limited to one/day. Physiological recordings were obtained from both sides of the amygdala. On average, eight recording sessions were made from each animal (range: four to 12 sessions) on different days. Synthetic acoustic stimuli (noise and tone bursts, 0.5 ms rise-full fall time; 10–64 ms duration) and previously recorded social vocalizations were presented at one/s. Social vocalizations consisted of 11 species-specific syllables or a combination of syllables that were previously related to aggressive (eight calls) or appeasing (three calls) behavioral contexts (Gadziola et al. 2011). Duration of social vocalizations ranged from 7 to 126 ms. Peak amplitude was normalized across all social vocalizations. Acoustic stimuli were downloaded to a digital signal processor and converted to analog signals at 400 kHz and 16-bit depth. Signals were antialias filtered, attenuated, amplified, and then sent to a loudspeaker (Infinity EMIT-B, Harman International, Stamford, CT), placed 10 cm from the animal and 25° into the sound field centrad-
cerebral to the amygdala under study. The system response had a gradual roll-off of ~3 dB/10 kHz. Harmonic distortion components were not detectable at ~55 dB below the signal level. Single-neuron activity was recorded using micropipette electrodes filled with 1 M NaCl (tip diameters of 2–5 μm) and advanced by a hydraulic micropositioner. Extracellular potentials were amplified, bandpass filtered (600–6,000 Hz), and sampled at 40 kHz with a 16-bit resolution. Custom software recorded spike occurrence and displayed real-time peristimulus time histograms (PSTHs) and raster plots. Once auditory activity was identified audiovisually using search stimuli (noise bursts and social vocalizations), single-neuron activity was defined by spikes of constant waveform and amplitude. Peak voltage of spikes exceeded background noise by a factor of five. Initial data collection involved a standard series of rate/level functions in response to social vocalizations. Simple stimuli were used to assess onset latency and responsiveness to pure tones or broadband noise. Stimuli were typically presented in blocks of 20 or 32 trials.

Neurons characterized here responded robustly to sounds presented over successive trials; we did not attempt to characterize neuronal responses that habituated rapidly. Across a recording session, there was no significant difference in the average firing rates to the first and last sounds presented, suggesting that habituation throughout the experiment was not a factor.

Verification of Recording Location

Stereotaxic coordinates developed in our laboratory for this species guided electrode penetrations through the amygdala. Responses were recorded from an amygdala in several successive penetrations, the last of which was marked by a deposit of a neural tracer (Fig. 1A) (Fluoro-Gold, Fluorochrome, Denver, CO, or Fluororuby, Molecular Probes, Eugene, OR). Thus nearly all electrode penetrations were placed close to electrode tracks marked by deposits, which were made using a constant current source. The electrode was kept in position for an additional 10 min and then removed from the brain. Details of tracer-specific techniques have been described previously (Marsh et al. 2002; Yavuzoglu et al. 2010). Tracer deposit sites were photog-
hed with a SPOT RT3 camera and SPOT Advanced Plus imaging software (version 4.7) mounted on a Zeiss Axio Imager M2 fluores-
cence microscope. Adobe Photoshop CS3 was used to invert brightness levels and to adjust brightness and contrast globally.

Data Analysis

 Spike times were exported into MATLAB (MathWorks, Natick, MA) for further analysis. Since we observed that background discharge varied with stimulus history, we did not evaluate spike discharge based on an initial nonstimulus control period. Instead, we calculated background discharge for each block of trials featuring the identical stimulus type and level during a period of 50 or 100 ms prior to stimulus onset. The average background activity of recorded neurons measured at the lowest sound intensity presented was 4.8 ± 5.3 spikes/s, with the majority of neurons having background firing rates between 1.3 and 6.1 spikes/s. The criterion for a significant stimulus-evoked response was an average discharge exceeding 2 SD above mean background rate. For neurons with little or no background discharge, a response was required to exceed a discharge rate of 10 spikes/s. We obtained several additional measures of responsiveness to stimuli.

PSTH-based measures of response timing. We used a 20-ms sliding window, shifted in 5-ms increments, to measure response timing from the PSTH. Plots display the mean firing rate along with the 95% confidence interval (mean ± 2 SE) at each 5 ms time bin (Fig. 1, B and C). Response onset was calculated as the first 5 ms bin after stimulus onset, during which the neuron’s response was above the mean 95% confidence interval of the background discharge. Response duration was calculated as the duration in which the response re-

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Trial-by-trial variation in response duration. To assess the variability of response duration on a trial-by-trial basis, we computed single-trial, spike-density functions by convolving spike times with a Gaussian kernel having a 20-ms bandwidth. The mean firing probability prior to sound onset was calculated across trials featuring the identical stimulus type. Response onset was measured as the time at which the neuron’s firing probability exceeded 1 SD above the background. Response duration for single trials was calculated as the duration in which the response remained continuously above criterion. If multiple response peaks were present, response duration was only measured from the peak containing the bin with the maximum firing probability. The mean response onset and duration for a given stimulus could then be calculated by averaging across trials. For stimulus durations less than the Gaussian bandwidth, the difference was subtracted from the measured mean response duration to remove any bias toward persistent firing.

Spike rate. Because responses often extended beyond the end of the stimulus, we calculated the average discharge rate over a standard 200-ms window from stimulus onset. The 200-ms window well exceeded the longest stimulus duration (126 ms).

Modulation of response measures. We calculated the percent change in measures of rate or duration of firing across stimuli. For each neuron, stimuli were rank ordered from high to low based on each response measure, and the percent change between ranked vocalization pairs was calculated. These values were then averaged across the population. A high-percentage change value represents greater modulation of the response. Stimulus ranking could be different for different response measures from a single neuron or across neurons.

Mutual information. We used an information-theoretic approach to quantify the association between stimuli and neural responses. For each neuron, we computed the mutual information (MI) transmitted about the stimulus set (i.e., 11 social vocalizations) for three different neural response measures: spike rate, response duration, and persistent firing duration. The code to perform MI analyses was written in MATLAB using functions from the Information Breakdown Toolbox (Magri et al. 2009). In this context, MI reflects the ability of each of the response measures to discriminate among the different stimuli. When MI is zero, the responses are statistically independent, and the neuronal response measure does not encode information regarding the stimuli. If MI is equal to the entropy of the stimulus set (i.e., 3.5 bits for our 11 stimuli), it is possible to perfectly distinguish stimuli by the response on a single-trial basis. A second MI analysis using the same data set collapsed the vocal stimuli into one of two behavioral contexts (aggressive or appeasing) to assess whether these response measures could discriminate between vocalizations of different contexts. Because the grouping changes the entropy of the stimulus set, a MI value equal to one for these analyses would indicate that stimuli could be perfectly distinguished as being either aggressive or appeasing.

Due to limitations in the number of trials/stimulus, we corrected for the bias associated with small samples of the response probability distribution by using the method of Panzeri and Treves (1996), as implemented by the Information Breakdown Toolbox. To obtain a measure of 95% confidence intervals for the MI values for each neuron and response measure, a bootstrap method was implemented. Input data matrices were generated by sampling, at random and with replacement, from the original data matrix. The size of the bootstrap values was determined by examining the MI values across a range of bootstrap sizes. The mean and variance of the MI values stabilized at a size of ~175 resampled points, and a value of 200 was used for all neurons. The MI was then calculated, with the bias correction applied, from the bootstrapped data set, and the process was repeated 500 times to obtain a suitably normal distribution of MI values. The mean and 95% confidence intervals were then obtained for each response measure for each neuron from this distribution.

The contribution of stimulus-related information from combinations of the different response features is not addressed in the basic MI analysis. If two response features, R1 and R2, are completely independent, then the total information they convey together is simply the sum of information transmitted by each feature separately. That is, I(S; R1, R2) = I(S; R1) + I(S; R2). However, if the two response features are correlated, they are mutually redundant, and the information transmitted by the two features together will be less than the sum of the information transmitted by each feature alone. The measure of

\[
I(S; R_1, R_2) = \frac{1}{2} \log \sigma_2^2 + \frac{1}{2} \log \sigma_1^2 - \frac{1}{2} \log \sigma_0^2,
\]

where \(\sigma_0^2\) is the variance of the mutual information transmitted by the two features together, \(\sigma_1^2\) is the variance of the mutual information transmitted by R1, and \(\sigma_2^2\) is the variance of the mutual information transmitted by R2. Thus, the mutual information transmitted by two features is equal to the sum of their individual mutual informations minus twice their joint mutual information.

Fig. 1. Auditory-evoked responses obtained at a histologically identified recording site. A photomicrograph illustrating a Fluororuby deposit at 1 of the recording sites in the lateral nucleus of the amygdala; note that brightness is inverted in this monochrome image. Arrow indicates site of deposit. See list of anatomical abbreviations at end of figure legend. For the identified recording site in A, mean firing rate plots illustrate auditory-evoked responses with tonic excitatory discharge (B) and persistent firing (C) in response to different stimuli. Solid black lines represent the mean firing rate after applying a 20-ms sliding window to measure the onset and duration of significant excitatory discharge. Light gray outlines indicate the 95% confidence interval of the mean firing rate. Blue rectangles indicate response duration computed from this analysis (see METHODS). Vertical dashed lines mark the end of the period of response that would correspond to stimulus duration. Response durations that extend beyond these vertical dashed lines indicate persistent firing. Black rectangles above mean firing rate indicate timing and duration of the stimulus. Mean firing rates binned at 5 ms. Anatomical abbreviations: Bmg, magnocellular subdivision of the basal nucleus of the amygdala; Bpc, parvicellular subdivision of the basal nucleus of the amygdala; CE, central nucleus of the amygdala; CPU, caudate-putamen; ec, external capsule; int, internal capsule; L, lateral nucleus of the amygdala; Pir, piriform cortex; rf, rhinal fissure; Tem, temporal cortex. Bottom left orientation: D, dorsal; L, lateral.

\begin{align*}
&I(S; R_1, R_2) = \frac{1}{2} \log \sigma_2^2 + \frac{1}{2} \log \sigma_1^2 - \frac{1}{2} \log \sigma_0^2,
&\sigma_0^2 = \sigma_1^2 + \sigma_2^2 - 2 \sigma_1 \sigma_2 \rho,
&I(S; R_1) + I(S; R_2),
\end{align*}

where \(\rho\) is the correlation coefficient between R1 and R2.
Neurons in the lateral amygdala responded to a broad range of sounds, including social vocalizations and synthetic acoustic stimuli. From a total of 54 neurons, 21 neurons were tested with synthetic signals as well as social vocalizations. In these neurons, minimum response latency to tonal or noise bursts averaged 24 ± 13 ms (range: 12–48 ms). All tested stimuli were similarly effective at evoking a response in these neurons (Fig. 2A). We then determined the number of social vocalizations that evoked a response within each neuron. On average, nine of 11 tested social vocalizations elicited a response across the population of 54 neurons (Fig. 2B). However, within neurons, we observed clear differences in both firing rate and temporal pattern of discharge in response to different social vocalizations.

Encoding Features of Amygdalar Neurons

The neurons in Figs. 3 and 4 illustrate these differences well, responding to each of the 11 social vocalizations tested. Six responses are shown for each neuron, arranged vertically, according to increasing stimulus duration. For the neuron in Fig. 3, spike discharge within a 200-ms window varied from 4.5 to 34.8 spikes/s (Fig. 3B), and response duration varied from 20 to 250 ms (Fig. 3C). The neuron in Fig. 4 shows smaller variations in spike rate (6.3–17.3 spikes/s) and response duration (20–70 ms). In each of these neurons, there was no clear relationship between stimulus duration and either the spike rate or response duration measures.

To assess whether these variations in spike rate or response duration permit discrimination among social vocalizations by single neurons, we computed MI held in spike rate and response duration for each neuron’s responses to the suite of vocal signals (Fig. 5, A and B). To perform this analysis, we first obtained trial-by-trial estimates of response duration using spike-density functions (Figs. 3D and 4D); these estimates are typically smaller than those obtained using the plots of mean firing rate (Figs. 3C and 4C). We found that information held in response duration (mean ± SD = 1.95 ± 0.38 bits) was substantially and significantly greater than information in spike rate (0.38 ± 0.17 bits; independent samples t-test [t(106)] = −28.01; P < 0.001). In fact, all 54 neurons carried more information about stimulus identity in response duration than in spike rate (Fig. 5D).

The plots in Figs. 3 and 4 suggest that there is little relation between stimulus duration and either spike rate or response duration. We analyzed this further by computing for each neuron the correlation between the duration of the 11 vocal stimuli and either the evoked spike rate or response duration. Each response measure was very poorly correlated with stimulus duration (Fig. 6). Across neurons, Pearson correlations between stimulus duration and response duration averaged 0.02 ± 0.36 (mean ± SD), with only six of 54 neurons showing a significant correlation (Fig. 6A). On average, stimulus duration explains <1% of the variance in response duration across the sample. There is also a poor correlation between stimulus duration and spike rate (−0.02 ± 0.34), with only four of 54 neurons showing a significant correlation (Fig. 6B).

Since response duration holds significant discriminatory information about stimulus identity and since this information

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**RESULTS**

In response duration (mean ± SD = 1.95 ± 0.38 bits) was substantially and significantly greater than in spike rate (0.38 ± 0.17 bits; independent samples t-test [t(106)] = −28.01; P < 0.001). In fact, all 54 neurons carried more information about stimulus identity in response duration than in spike rate (Fig. 5D). The plots in Figs. 3 and 4 suggest that there is little relation between stimulus duration and either spike rate or response duration. We analyzed this further by computing for each neuron the correlation between the duration of the 11 vocal stimuli and either the evoked spike rate or response duration. Each response measure was very poorly correlated with stimulus duration (Fig. 6). Across neurons, Pearson correlations between stimulus duration and response duration averaged 0.02 ± 0.36 (mean ± SD), with only six of 54 neurons showing a significant correlation (Fig. 6A). On average, stimulus duration explains <1% of the variance in response duration across the sample. There is also a poor correlation between stimulus duration and spike rate (−0.02 ± 0.34), with only four of 54 neurons showing a significant correlation (Fig. 6B).

Since response duration holds significant discriminatory information about stimulus identity and since this information...
is unrelated to stimulus duration, neural processing appears to result in adjustments to response duration, which could be shorter or longer than stimulus duration. For the neuron in Fig. 3, differences in firing beyond stimulus duration (i.e., persistent firing duration) were particularly noteworthy, ranging from no persistent firing (e.g., isolation double-note syllable) to a persistent duration of 200 ms in response to the 5 broadband downward frequency modulation (5bDFM)-sinusoidal frequency modulation (sinFM) vocalization (Fig. 3C). Across the sample of 54 neurons, 49 displayed persistent firing to at least one stimulus, and 58% of all responses demonstrate some degree of persistent firing. Average duration of persistent firing

Fig. 3. This neuron responded to all tested social vocalizations but showed clear differences in magnitude and duration of response. For 6 social vocalizations (A), arranged vertically according to increasing stimulus duration, 3 response measures are illustrated. B: raster plots showing timing of spike discharge. Time zero occurs at stimulus onset. Black rectangles above rasters indicate timing and duration of stimuli, and adjacent numbers indicate average firing rates over a 200-ms window from stimulus onset. Note that responses did not habituate across blocks of 32 stimulus presentations. C: mean firing rate (black lines) and 95% confidence intervals (light gray outlines). Blue rectangles behind mean firing rate indicate response duration calculated from this measure. Mean firing rate binned at 5 ms. Inset (bottom panel): overlaid spike waveforms demonstrate well-isolated unit activity. D: trial-by-trial variation in response duration (gray histograms) using spike-density calculation. Average response duration across trials plotted as open bars with 95% confidence interval. Stimulus duration plotted as black bars. Note that the discharge pattern in response to 5bDFM-sinFM (bottom panel) switches to persistent firing that lasts at least 1 s. This neuron returned to a locked discharge pattern in response to subsequent stimuli (e.g., rBNB). All stimuli presented at 20 dB below maximum speaker output.
was 28 ± 25 ms (range: 5–200 ms). Like other response measures, persistent firing duration was not related to stimulus duration (Fig. 6C).

We next examined MI in persistent firing duration (Fig. 5C). There was significantly less information in persistent firing duration [0.60 ± 0.45 bits; t(106) = 16.82; P < 0.001] than in overall response duration but significantly more information than in spike rate [t(106) = −3.39; P = 0.001]. The former result is not surprising, since neurons appear to modulate response duration to be either shorter or longer than stimulus duration. What is noteworthy is that persistent firing was more informative than spike rate. Across the sample, the majority of neurons (33 of 54) showed greater information in persistent firing than in spike rate (Fig. 5E).

We further tested whether a combination of multiple response features carried more information about the stimulus (Table 1). The information transmitted by combinations of two or three features was always greater than the information carried by any one of the individual features alone. In almost all cases, the normalized synergy values were close to zero, suggesting that
each of the response features carried a significant amount of independent information about the stimulus. Unexpectedly, we found that spike rate did not add significantly to the information already provided by response duration, whereas both spike rate and persistent duration contributed a similar degree toward their total joint information (Table 1). Not surprisingly, response duration and persistent duration showed a higher degree of mutual redundancy, with response duration responsible for a substantial proportion of the total joint information. Thus response duration transmitted substantially more stimulus-related information than any other response feature.

Although MI analyses quantify the robustness of discriminatory information provided by stimulus-evoked differences in neural responses, they do not reveal how much a response measure is modulated by different stimuli. To compare the magnitude of changes in spike rate and persistent firing duration across different stimuli, we first rank-ordered a neuron’s responses to different vocal stimuli using either the spike rate or persistent duration measures. We then computed the percent change in the response measure between each rank-ordered pair: the first and second responses, the second and third responses, and so forth. Across all neurons, there was signifi-
Table 1. Comparison of information content carried in the response features of lateral amygdalar neurons

<table>
<thead>
<tr>
<th>Information Content</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI (S; spike rate)</td>
<td>0.38 ± 0.17</td>
</tr>
<tr>
<td>MI (S; response dur.)</td>
<td>1.95 ± 0.37</td>
</tr>
<tr>
<td>MI (S; persistent dur.)</td>
<td>0.60 ± 0.45</td>
</tr>
<tr>
<td>MI [S; (spike rate, response dur.)]</td>
<td>2.15 ± 0.40</td>
</tr>
<tr>
<td>MI [S; (spike rate, persistent dur.)]</td>
<td>0.93 ± 0.52</td>
</tr>
<tr>
<td>MI [S; (response dur., persistent dur.)]</td>
<td>2.22 ± 0.49</td>
</tr>
<tr>
<td>MI [S; (spike rate, response dur., persistent dur.)]</td>
<td>2.37 ± 0.50</td>
</tr>
<tr>
<td>Norm Synergy (spike rate; response dur.)</td>
<td>−0.08 ± 0.05</td>
</tr>
<tr>
<td>Norm Synergy (response dur.; spike rate)</td>
<td>0.82 ± 0.07</td>
</tr>
<tr>
<td>Norm Synergy (S; response dur., spike rate)</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Norm Synergy (spike rate; persistent dur.)</td>
<td>−0.03 ± 0.06</td>
</tr>
<tr>
<td>Norm Contrib (S; persistent dur., spike rate)</td>
<td>0.53 ± 0.17</td>
</tr>
<tr>
<td>Norm Contrib (S; spike rate, persistent dur.)</td>
<td>0.44 ± 0.19</td>
</tr>
<tr>
<td>Norm Contrib (S; persistent dur., response dur.)</td>
<td>−0.14 ± 0.09</td>
</tr>
<tr>
<td>Norm Contrib (S; response dur., persistent dur.)</td>
<td>0.11 ± 0.08</td>
</tr>
<tr>
<td>Norm Contrib (S; response dur., persistent dur.)</td>
<td>0.75 ± 0.15</td>
</tr>
</tbody>
</table>

Values are mean ± SD across all neurons for the mutual information (MI), normalized synergy (Norm Synergy), and normalized contributions (Norm Contrib) between the stimulus (S) and the different response features. dur., duration.

significantly greater modulation in persistent firing between ranked pairs than in spike rate (Fig. 7).

Contextual Features of Amygdalar Responses

The presence and duration of persistent firing may relate to behavioral or acoustic contexts of vocalizations. Persistent firing was present in 64% of responses to aggressive vocalizations but only 25% of responses to appeasement vocalizations. Furthermore, when stimuli were ordered from longest to shortest duration of persistent firing, two of three appeasement vocalizations were never ranked within the top 50% for any neuron. These results were reinforced by an analysis of MI. When the vocal signals were collapsed into their behavioral context as either aggressive or appeasing, we found that more information was held in response duration than in firing rate [0.29 ± 0.09 vs. 0.05 ± 0.03, respectively; \( t(106) = -18.46; P < 0.001 \)]. Persistent firing was also found to carry more contextual information than firing rate [0.10 ± 0.08; \( t(106) = -4.76; P < 0.001 \)]. This suggests that response duration may provide some discrimination between vocalizations of different contexts.

Single-neuronal responses may be particularly susceptible to the behavioral context of vocal signals. In some cases, we observed a dramatic difference in response when emotion-related acoustic cues were altered. For example, the sinFM syllable illustrated in Fig. 8A was observed during higher levels of aggression (Gadziola et al. 2011), and syllable duration varied according to the number of cycles of frequency modulation (range: 1–5 cycles; 9–73 ms duration). When this syllable was extended by 8 ms to include a second cycle of frequency modulation (Fig. 8B), the neuron’s response was better locked to the two-cycle stimulus and displayed an increase in response magnitude due to a substantial increase in the duration of persistent firing (from 41 to 168 ms). Although the increased stimulus duration and energy in the signal in Fig. 8B may be expected to evoke a stronger spike rate during stimulus duration, the increase in duration of persistent firing was unexpected based on signal acoustics alone. A more extreme case of persistent firing is shown in response to the 5bDFM–sinFM vocalization, a vocal sequence associated with aggression (Fig. 3). Averaged across stimulus trials, the duration of persistent firing for this neuron was measured as 200 ms. On examination of the raster plot, it is clear that after several presentations, the discharge pattern switched from locked with persistent firing to a firing pattern that persisted for at least 1 s, continuing into the presentation of the subsequent stimulus. These results suggest that some feature of the behavioral context or stimulus history associated with this signal may modulate the duration of persistent firing.

DISCUSSION

This study characterized responses to a set of social vocalizations and simple acoustic stimuli by lateral amygdalar neu-
rons in awake, restrained big brown bats. Amygdalar neurons in this species respond to many of the social vocalizations presented but differentially modulate both firing rate and duration of response. Firing rate has been shown to code differences among social vocalizations in higher-order auditory centers (Lu et al. 2001; Romanski et al. 2005). In the basolateral amygdala, we find that both firing rate and response duration provide codes for discriminating among social vocalizations. These codes appear to be mostly independent, and their combination is highly informative about vocal stimuli. However, a major finding was that response duration holds substantially more information. We show further that part of the modulation in response duration is due to persistent firing (i.e., lasting beyond stimulus duration). The present study proposes a novel strategy for coding social vocalizations in the amygdala and highlights the importance of persistent firing in the general functioning of the amygdala.

Response Duration

Measuring response duration can be challenging in neurons with variable spike discharge. The present study assessed response duration using two different measures. The first measure used a sliding 20-ms average of the PSTH to analyze response duration across a block of trials, a method commonly used for assessing response duration (Bendor and Wang 2008; Naumann and Kanwal 2011). Although derived, this type of measure uses confidence intervals to take into account the trial-by-trial variance, and it provides a more faithful representation of the original raster data. With the use of this response measure, we showed that response duration is unrelated to stimulus duration and can extend well beyond it. A second measure of response duration was used for the MI analyses; we measured the trial-by-trial variation in response duration by computing spike-density functions for each stimulus presentation, a method similar to that used by Parush et al. (2008). Although the trial-by-trial measure reduced the range of persistent firing durations observed, the MI analysis showed that information held in response duration was greater than in spike rate.

Stimulus information can be encoded by a variety of spike train features, including spike timing. In auditory cortex, features related to spike timing (e.g., first spike latency, mean response duration) have been found to transmit more stimulus-related information than spike count (Furukawa and Middlebrooks 2002; Nelken et al. 2005). In their study of basal ganglia neurons, Parush and colleagues (2008) found that response duration carried much of the information available in responses and was more informative than spike count in neurons with negative responses. Auditory cortical neurons are capable of firing in a sustained manner when driven by preferred stimuli but show more transient responses to nonpreferred stimuli (Wang et al. 2005). These sustained discharges also show greater stimulus specificity than onset discharges. In the present study of amygdalar responses to social vocalizations, we found that single amygdalar neurons can modulate response duration to be transient, sustained, or persistent. With the use of information theoretic methods, we show that response duration is substantially more informative than firing rate in the lateral amygdala.

Persistent Firing

From work in passively listening animals, it is clear that persistent excitatory discharge to auditory stimuli occurs in both primary and other auditory cortical fields (Bendor and Wang 2008; Campbell et al. 2010; Moshitch et al. 2006; Peña et al. 1999) and prefrontal cortex (Romanski et al. 2005). Some studies have found that a significant amount of stimulus information is coded within persistent firing in the auditory cortex when responses are pooled across stimuli or neurons (Campbell et al. 2010; Moshitch et al. 2006). Persistent auditory responses also occur elsewhere: recent work in the big brown bat found auditory-evoked, persistent activity in a small proportion of medial geniculate neurons and a much greater proportion of pontine gray neurons (Miller and Covey 2011). In studies of basolateral amygdalar responses, persistent firing is observed in auditory and other sensory stimuli (Bordi and LeDoux 1992; Bordi et al. 1993; Maeda et al. 1992; Naumann and Kanwal 2011) but is generally not characterized. Our results show that persistent firing in the lateral amygdala has the potential to carry discriminatory information for coding social vocalizations at the single-neuron level and that duration of persistent firing carried more information for discriminating among social vocalizations than firing rate. Even more important, in our view, is the finding that persistent firing in response to select acoustic stimuli occurs in nearly all lateral amygdala neurons tested. We believe that such firing is likely to be more dramatic when more realistic vocal sequences are used as stimuli (e.g., Fig. 3, bottom) and is a critical feature of general amygdalar functioning.

What is the basis for the persistent firing in lateral amygdalar neurons? Egorov and colleagues (2006) have shown, in vitro, that principal neurons generate persistent spike discharge that requires cholinergic inputs, acting through muscarinic receptors, which likely originate from nucleus basalis. The duration of the persistent firing, however, depends on the strength of depolarization. Emotional arousal can increase activity in nucleus basalis (Paré 2003), suggesting that persistent excitatory discharge depends on both sensory input and on the animal’s internal state. Bordi and LeDoux (1992) and Bordi et al. (1993) characterized auditory responses in the basolateral amygdala in response to simple acoustic stimuli, comparing both anesthetized and awake recording conditions. Inspection of their results reveals that persistent firing responses are present in the basolateral amygdala and may be more common during awake conditions, supporting the idea that the behavioral state of the animal may be a critical factor driving persistent firing. In awake, mustached bats (Pteronotus parnellii), basolateral amygdalar responses appear more selective for particular social vocalizations than in big brown bats (Naumann and Kanwal 2011; Peterson and Wenstrup 2008), with persistent firing commonly observed in response to a highly aggressive vocalization (Peterson and Wenstrup 2008). Although the significance of persistent firing during passive auditory processing remains unclear, our finding that social vocalizations related to aggression are more likely to evoke persistent firing and our observations of persistent firing in Figs. 3 and 8 further support the idea that persistent firing may be state and stimulus dependent. Specifically, the duration of persistent firing in response to social vocalizations may depend in part on the particular vocalizations but also on the level of emotional arousal evoked.
by vocalizations. Future studies are necessary to better understand how the behavioral state and the acoustic context influence the degree of persistent firing.

Persistent firing functions in several neural systems to sustain representations of a sensory stimulus for working memory (Frank and Brown 2003; Major and Tank 2004). It likely plays a crucial role in amygdala-mediated memory operations (Egorov et al. 2006) but may serve a more general function related to control of emotional expression and modulation of sensory processing. Through connections with intercalated cell masses, neurons in the lateral nucleus cause disinhibition of neurons in the central nucleus (Paré et al. 2004), the main amygdalar output region driving behavioral responses. Persistent firing could play an important role in transforming the rapid timescale of auditory stimuli to a timescale more appropriate for driving emotional expression to sounds. In the amygdala, persistent firing could also influence the activation and plasticity of auditory cortical responses to sounds, due both to direct projections from the basolateral amygdala to the auditory cortex and indirect projections through the cholinergeic basal forebrain (Amaral and Price 1984; Suga and Ma 2003; Weinberger 2004). Overall, persistent firing provides a mechanism by which particular vocal signals effectively drive behavioral responses and activate experience-dependent plasticity in the sensory cortex.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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