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Integration and recovery processes contribute to the temporal selectivity of neurons in the midbrain of the northern leopard frog, *Rana pipiens*

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Abstract This study examined the mechanisms underlying amplitude modulation selectivity in the anuran auditory midbrain. Single units were recorded extracellularly in the torus semicircularis of the northern leopard frog, *Rana pipiens*. Two physiologically distinct classes of neurons were identified, based on their response latencies and their selectivities to pulse repetition rates. Cells in one group had short response latencies (median = 31 ms) and responded best to pulse repetition rates below 40 Hz. Tuning to low amplitude modulation rates was largely determined by recovery processes and phasic response properties. Cells in the second group had much longer latencies (median = 81 ms) and were generally selective for pulse repetition rates greater than 40–50 Hz. Sensitivity to higher amplitude modulation rates resulted from integration processes; these units only responded when a threshold number of pulses were presented at a minimum pulse density (amplitude modulation rate). At amplitude modulation rates above their best rate, their responses decreased, apparently due to inadequate recovery time between pulses.

Key words Temporal processing · Anuran auditory · Amplitude modulation · Sensory processing · Mate choice

Abbreviations *AM* amplitude modulation · *BEF* best excitatory frequency · *pps* pulses per second · *PRR* pulse repetition rate · *PSP* postsynaptic potential · *SAM* sinusoidal amplitude modulation · *VDC* variable duty cycle

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Introduction

The temporal structure of acoustic signals plays a crucial role in the communication of a variety of animals, including humans (Loftus-Hills and Littlejohn 1971; Emlen 1972; Simmons et al. 1979; Remez et al. 1981; Kay 1982; Weary et al. 1986; Langner 1992). It is important, therefore, to understand how temporal information is represented in the auditory system and how transformations in these representations are achieved. The anuran auditory system is well suited for investigating these questions. Acoustic signals are vital to the reproductive biology of anurans, allowing individual females to identify conspecific males (Wells 1977; Backwell and Jennions 1993) and to choose between them (Whitney and Krebs 1975; Ryan 1980; Sullivan 1983; Marquez 1995). Also, behavioral studies have shown that many species of anurans are able to discriminate between call types that differ only in temporal structure (Gerhardt 1988; Diekamp and Gerhardt 1995; Gerhardt and Schul 1999).

The main objective of this study was to further understand how amplitude-modulated (AM) signals are represented and processed in the torus semicircularis (homologue of the inferior colliculus; Wilczynski 1988) of anurans. There is a transformation in the manner in which AM is represented from the periphery to the torus semicircularis (Rose and Capranica 1983, 1985). Eighth-nerve fibers code the rate of sinusoidal amplitude modulation (SAM) in the periodicity of their discharges. With white noise as the carrier in the modulation, the mean spike rate of these fibers is independent of the modulation rate; in this stimulus regimen, long term spectral properties do not change with AM rate. At the level of the torus, however, the firing rate of most neurons is dependent on AM rate (Rose and Capranica 1983, 1985; Walkowiak 1988; Gooler and Feng 1992; Alder and Rose 1998). Band-pass selectivity for the rate of AM has also been observed for neurons in the midbrain of birds (Albert et al. 1989) and mammals

(Langner and Schreiner 1988; Rees and Palmer 1989). Remarkably, neurons that are most sharply tuned to AM poorly code the modulation rate in the periodicity of their discharges (quantified by calculating a synchronization coefficient; Rose 1995). Similarly, in mammals, there is a significant reduction of synchronization of spikes to the AM stimulus at the midbrain (Rees and Moller 1983; Langner and Schreiner 1988). The transformation from a periodicity code of AM rate in the peripheral nervous system to a temporal filter representation in the midbrain has been observed for frogs (Rose and Capranica 1985), birds (Langner 1981) and mammals (Langner and Schreiner 1988; Pinheiro et al. 1991). This transformation is of particular interest with regard to the abilities of anurans to discriminate between different patterns of amplitude modulation. The distribution of AM 'best rates' for toral neurons is species specific and nicely related to the range of pulse repetition rates (PRRs) observed in their calls (Rose and Capranica 1984; Rose et al. 1985), making band-pass neurons attractive as possible neural substrates for AM rate discriminations.

The mechanisms underlying temporal selectivities are poorly understood. One hypothesis concerning mechanism accounts for band-pass AM selectivity in terms of a neuron's underlying sensitivity to stimulus rise-time and duration. Individual 'pulses' (cycles of AM) decrease in rise-time and decrease in duration as the rate of SAM is increased. Band-pass selectivity could result if neurons, or cells upstream from them, were excited best for particular values of pulse rise-time or duration or both. Studies that have explored this hypothesis have been unable to account for the temporal selectivities of neurons in the torus, or upstream, solely in terms of their duration and rise-time sensitivities (Gooler and Feng 1992). The torus receives afferents from the dorsolateral nucleus (DLN, first-order auditory region) and from the superior olivary nucleus (SON, second-order region that receives auditory input from the contralateral DLN). Neurons in these two regions that respond phasically to tone bursts show preference for stimulus rise times < 25 ms (Condon et al. 1991; Hall and Feng 1991). In the SON, some phasic neurons are band-pass to SAM (Condon et al. 1991). These units, however, are also band-pass when square-wave AM is used and their rise-time sensitivity appears, therefore, to only account for the slight differences in the shapes of band-pass functions for these two stimulus sets at the lowest modulation rates (e.g., 5 Hz AM). In addition, most band-pass neurons were insensitive to the duration of tone bursts. Finally, many species of anurans produce call types that differ in PRR but not in the temporal structure of individual pulses, i.e., pulse duration and rise/fall characteristics are highly similar. For example, two of the call types produced by *Rana pipiens* consist of a series of pulses, each approximately 10–15 ms in duration. One is an advertisement call, pulsed at about 5–30 pulses per second (pps), and the other signals aggressive intent, and is pulsed at about 50–100 pps (chuckle call); the exact rate is dependent on temperature. In other words, to alter PRR these frogs change the duty cycle (ratio of pulse duration to inter-pulse interval) of their pulses. Because the pulses of these two call types are similar in spectral composition and shape, the calls appear to be differentiated solely on the basis of PRR. What mechanisms might account for discrimination of call types that differ only in PRR?

Earlier studies (Gooler and Feng 1992; Penna et al. 1997) provide evidence that recovery processes may contribute to the band-pass filtering properties of toral neurons. There are few cases, however, where this mechanism has been related quantitatively to SAM band-pass selectivity. Towards this objective, we have investigated further the role of recovery processes in the selectivity of toral neurons to AM tuning. Also, we have previously shown evidence for pulse integration processes in the midbrain of *R. pipiens* and the Pacific treefrog, *Hyla regilla*, which might underlie AM selectivity for higher rates in these animals (Alder and Rose 1998). This mechanism will be reexamined in more detail in *R. pipiens*, in order to shed more light on the issue of temporal pattern discrimination.

Materials and methods

Neurophysiology and anatomy

Surgery and animal preparation

Northern leopard frogs (*Rana pipiens pipiens*) were anesthetized by immersion in 3% urethane. Following the loss of all reflexes, the animals were wrapped in moist gauze to facilitate cutaneous respiration. After topical application of a local anesthetic (Lidocaine HCL), a small opening was made through the dorsal surface of the skull to expose the optic tectum. The hole was then sealed with a cap made from Gelfoam (Upjohn) and tissue adhesive (Vetbond, 3 M), after which the animals were allowed to recover overnight from the anesthesia. They were then immobilized by intramuscular injection of *d*-tubocurarine chloride (6 μmg g^{-1} body weight), wrapped in moist gauze and placed on a platform in a soundproof room (Industrial Acoustics). The frog's body temperature was maintained at 17–18 °C.

Stimulus generation

Acoustic stimulus sets were constructed using Tucker Davis Technologies (TDT) System II hardware and custom made software on a Pentium 90 computer. These sets consisted of: (1) sinusoidal amplitude modulation, (2) natural AM, (3) variable duty cycle (VDC), (4) rise/fall times, and (5) duration. These stimuli are described below, and are shown in Fig. 1 (except duration). Tone and noise carriers used in stimulus generation were created using a TDT AP2 card. The sampling rate for these carriers and all modulating waveforms was 25 kHz.

1. *SAM stimulus* was generated by multiplying a white-noise or pure-tone carrier with a sinusoidal modulating waveform, which contained a d.c. offset equal to 1/2 its peak-to-peak amplitude. Stimulus duration was held constant at either 300 ms or 400 ms. When AM rate was increased, stimulus pulse number increased, and pulse duration and rise time decreased, while maintaining constant energy to within 0.1 dB.

2. *Natural AM* stimulus was generated by digitally multiplying a pure-tone carrier with a modulating waveform that represented the

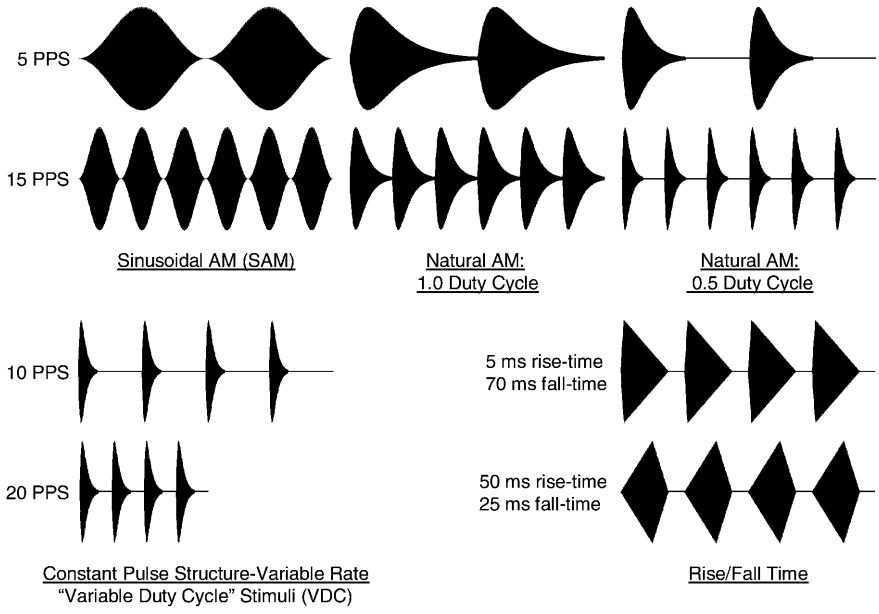

Fig. 1 Oscillograms showing examples of stimuli used

natural pulse envelope. Based upon analysis of field-recorded calls, a single pulse waveform was generated using the following equations:

$$V = k\left[e^{-t/\tau 1} - e^{-t/\tau 2}\right]$$
$$\tau 2 = (\tau 1)/2 \quad (1)$$

where $\tau 1$ and $\tau 2$ define the relative rising and falling phases of the waveform. This was then repeatedly copied to produce the modulating waveform. This stimulus was then presented at a constant duty cycle (ratio of pulse to interpulse interval) throughout the range of PRRs. Total stimulus energy (within 0.1 dB) was maintained with changes in rate. Neurons were typically tested using modulation duty cycles of 1.0, 0.5, and 0.25. Pulse duration and rise/fall times vary with rate, although the changes in rise/fall times are minimal compared to SAM due to the shape of the natural envelope. As PRRs decrease, pulse duration increases; as with SAM, the number of pulses changes as AM rates change in order to maintain constant stimulus duration.

3. *Variable duty cycle* stimulus was also generated using the natural pulse shape described above; however, in this case pulse duration and shape were held constant at natural dimensions while the PRR was varied. When AM rate was increased, pulses moved closer together, decreasing both duty cycle and total stimulus duration. Since pulse shape, duration and number did not vary with PRR, total stimulus energy remained constant.

4. *Rise/fall time stimuli* were generated by multiplying a triangular modulating waveform with a pure-tone carrier. The rise and fall times were inversely proportional to each other; as rise time increased, fall time decreased. This relationship was used in order to maintain constant pulse energy. Note that at one end of this continuum the rise/fall times approximate the proportions of the natural pulse; therefore, while duration remains constant, the pulse energy is distributed further away from the pulse onset as rise time is increased. One to ten pulses were generally used, presented at the rate to which the cell was most responsive. Various pulse durations were used in order to obtain a maximum response from the neuron and ensure that rise-times were long enough to observe a change in the firing pattern.

5. *Duration stimuli* consisted of a single pure tone burst with 1-ms rise and fall times gated on and off at the zero crossings. Durations ranging from 1 ms to 400 ms were generally used. Obviously total energy increases as duration increases. A fast rise time was used in keeping with the rapid onset of the natural pulse.

Stimulus presentation

Stimuli were amplified (Symetrix A-220) and presented free-field in an Industrial Acoustics audiometric room (2.2 m × 2.2 m × 2.0 m high) via a Bose speaker situated 0.5 m from the frog, contralateral to the recording site. Reflections in the booth were minimized by covering the walls and ceiling with acoustic foam (absorption coefficients from 250 Hz to 4000 Hz: 0.60–0.97). The corner directly opposite the speaker was covered with fiberglass insulation (3 1/2" Owens Corning R-11, absorption coefficients from 250 Hz to 4000 Hz: 0.85–0.98) covered with cotton cloth. Echoes were attenuated by at least 30 dB relative to the stimulus for frequencies greater than 500 Hz. Correspondingly, stimuli were presented at levels not exceeding 30 dB above each unit's threshold. A microphone (ACO Pacific, with Cetec Ivie IE-2P preamp) situated above the frog was used to measure stimulus levels via a sound level meter (Cetec Ivie IE-30A). Stimuli were presented once every 2.5 s, unless repetitions subsequent to the first consistently produced an attenuated response, in which case the rate was decreased. A Wavetek 19-function generator was used to time presentation rates, and sound level was varied using a TDT PA-4 programmable attenuator. Stimuli were presented at various RMS amplitudes across each neuron's dynamic range in order to test the intensity dependence of temporal tuning. Modulation rates presented to the animal varied from 5 Hz to 200 Hz. SAM was used as the search stimulus; carrier frequency was systematically varied from 300 Hz to 2200 Hz while modulation frequency was varied from 20 Hz to 100 Hz.

Neurophysiology

Microelectrodes were constructed from alumina-silicate glass (1.0 mm o.d., 0.75 mm i.d.) on a Brown-Flaming puller. Tips were approximately 2 μm in diameter, and were back filled with either 3% Biocytin (Sigma) or 3% Biotinylated Dextran (10,000 MW, lysine fixable Molecular Probes) in 2 mol l^{-1} NaCl. Shanks were then filled with 2 mol l^{-1} NaCl. (1–4 MΩ impedances). Electrodes were advanced remotely via a hydraulic microdrive (Narishige MO-11) through the torus semicircularis until a unit was isolated. Slight suction was then applied to improve the recording. Best excitatory frequency (BEF: frequency at which the unit has its lowest threshold) and threshold were then determined. Threshold is defined as the sound-pressure level necessary to evoke at least one

spike during 75% of the presentations of repetitive SAM bursts, at an AM rate that is determined audio-visually to produce the greatest response. Because many toral neurons respond poorly or not at all to pure tones, the unit's BEF was determined by varying the frequency of the carrier (fc) while amplitude modulating the carrier at the optimal rate (fm); the presence of side bands in the AM stimulus precludes absolute identification of the unit's BEF; conventional methods of constructing a frequency tuning curve cannot be used for these neurons. Also, because sidebands are present at fc – fm and fc + fm, changes in AM rate result in changes in the spectral structure of AM tone stimuli. Changes in activity stemming from such spectral differences can lead one to conclude erroneously that a neuron is selective for the temporal property, AM rate (Rose and Capranica 1985; Rose 1995). Thus, AM white noise was used routinely to determine whether a neuron was actually temporally selective. The limitation of white noise, however, is that energy is not present continually in any spectral band. This property leads to underestimation of the AM selectivity of the neuron at low AM rates. Therefore, once the temporal selectivity of a neuron had been established, amplitude modulated pure tone carriers were used in further tests.

Temporal tuning was then measured using the SAM stimulus; SAM was used in order to facilitate comparisons with previous anuran auditory work. A unit was considered temporally selective if the number of spikes at the least preferred rate was at most 50% of that recorded at the most preferred rate (Rose and Capranica 1983, 1985). The frog was then presented with a variety of stimulus sets (see above) at 12 dB above the neuron's threshold. Responses were amplified (Grass Instruments P15), displayed on an oscilloscope, broadcast over a loudspeaker monitor and stored on VHS tape, along with the stimulus on a separate channel, with a PCM-video recorder (Vetter 3000). Recording sites were marked by iontophoresing the dye from the electrode tip using DC current supplied by a 9-V battery.

Histological procedures

Following the recording session, the frogs were deeply anesthetized by immersion in 5% urethane and perfused through the heart with a physiological saline/heparin solution followed by a 1:1 mixture of 8% paraformaldehyde and 0.2 mol l^{-1} phosphate buffer (pH 7.4). The brains were then removed, fixed overnight in the paraformaldehyde solution, and sliced into 100-μm sections on a Vibratome. The sections were then processed using an avidin-biotin technique to form a dark reaction product: the sections were incubated overnight in a solution of 0.3% Triton X-100 in phosphate-buffered saline (PBS) plus the A and B reagents of the Vectastain Elite kit (Vector Labs). They were then given three 10-min washes in 0.01 mol l^{-1} PBS and processed using a Vector VIP kit. The slices were allowed to incubate until they began to turn a light gray. The reaction was then stopped by three 10-min washes in 0.01 mol l^{-1} PBS. Sections were then mounted dehydrated, cleared in xylene and cover-slipped. Locations of recording sites were then verified using an Olympus BH-2 microscope.

Data collection and analysis

Spikes were discriminated (SA Instrumentation model 13-T dual window discriminator) and acquired, along with the stimulus waveform, using a 12-bit analog-to-digital converter (Cambridge Electronic Design 1401+). Spikes were then counted over an analysis period from stimulus onset to offset plus 100 ms for each repetition. Responses of toral neurons to these stimulus sets generally return to baseline within 100 ms of stimulus offset. Spike counts were then averaged for each AM rate, duration or rise-time. Spike2 software (Cambridge Electronic Design) was used for spike counting and histogram generation. Response latency was also measured for each neuron; this was defined as the time from stimulus onset to the time of the initiation of the first spike. Pulse integration thresholds were determined from smoothed response histograms, where the bin number equaled the total number of pulses in the stimulus, i.e., bin width equaled the interpulse interval; threshold was defined as the first bin to have an average of 1.0 spike per stimulus repetition. Histograms were smoothed according to the function $Ri = [R(i-1) + Ri + R(i+1)]/3$. All data generated from Spike2 analysis were plotted using SigmaPlot (Jandel Scientific, ver 3.0). Raw data traces were generated using both Spike2 with the associated 1401+, and Signal software (Engineering Design, ver 2.29) with a 12-bit data acquisition board (Data Translation model DT21-EZ).

Results

Extracellular recordings were made from 106 neurons in the torus semicircularis of 19 northern leopard frogs, *R. pipiens pipiens*. For comparison with previous work, AM-tuning was initially determined using SAM stimuli. Responses to this stimulus fell into five categories: 56% band-pass, 21% high-pass, 11% all-pass, 6% low-pass and 6% band-suppression.

Roles of rise time and duration in AM rate selectivity

Band-pass selectivity to SAM could result if neurons, or cells upstream from them, were excited best for particular combinations of pulse rise-time and duration; individual pulses (cycles of AM) decrease in rise time and duration as the rate of SAM is increased. Previous work suggested that band-pass selectivity could not be entirely explained by underlying sensitivities to these two parameters (Gooler and Feng 1992). This hypothesis was investigated further by first recording responses of 67 toral neurons to tones of various durations (2–200 ms). Of these, 17 neurons did not respond to tone bursts and, therefore, could not be included in the duration analysis. When the response levels of the remaining 50 units were normalized for stimulus energy, i.e., divided by stimulus duration, all cells responded best to durations less than 50 ms (median 2 ms; range 2–50 ms). Eleven of these units were band-pass with respect to stimulus duration (median best duration 12.5 ms; range 4–50 ms).

Forty-three toral neurons were also tested with various stimulus rise/fall times. Sixteen of these units responded at similar levels for all stimulus rise-times that were employed (2–100 ms). The responses of 25 cells decreased as rise time was increased (median best rise-time 5 ms; range 1–50 ms); the 2 remaining units responded best for the longest rise times tested.

From these data, one would expect most units to respond best to AM rates above approximately 100 Hz; only at these high AM rates are pulses of the optimal duration and rise-time. Most AM-selective cells, however, preferred much lower AM rates (median 40 Hz; $n = 40$; range 10–160 Hz); units that were band-suppression to SAM were excluded from this analysis. Two examples are shown in Fig. 2. These units had similar best AM rates, despite differing markedly in their

sensitivities to stimulus duration and rise-time. Further, the cell that was tuned to 30 Hz AM (filled circles) responded poorly to AM rates greater than about 50 Hz, even though its responses to tone bursts were strongest for durations less than 6 ms and for fast rise times. The other cell, paradoxically, showed less decline in response for AM rates greater than its best rate despite preferring tone bursts of longer duration. If AM tuning is a direct result of duration and rise-time sensitivities, then units that show similar AM tuning should exhibit similar responses to duration and rise-time stimuli. This was not the case, however, and even the 11 cells that responded best for particular stimulus durations (i.e., were tuned) showed no correlation between duration tuning and AM tuning ($r^2 = 0.05$). If pulse duration selectivity was a primary factor in determining AM tuning in these cases, BMR should have been inversely proportional to duration tuning; i.e., units that were tuned to higher SAM rates should have preferred shorter durations.

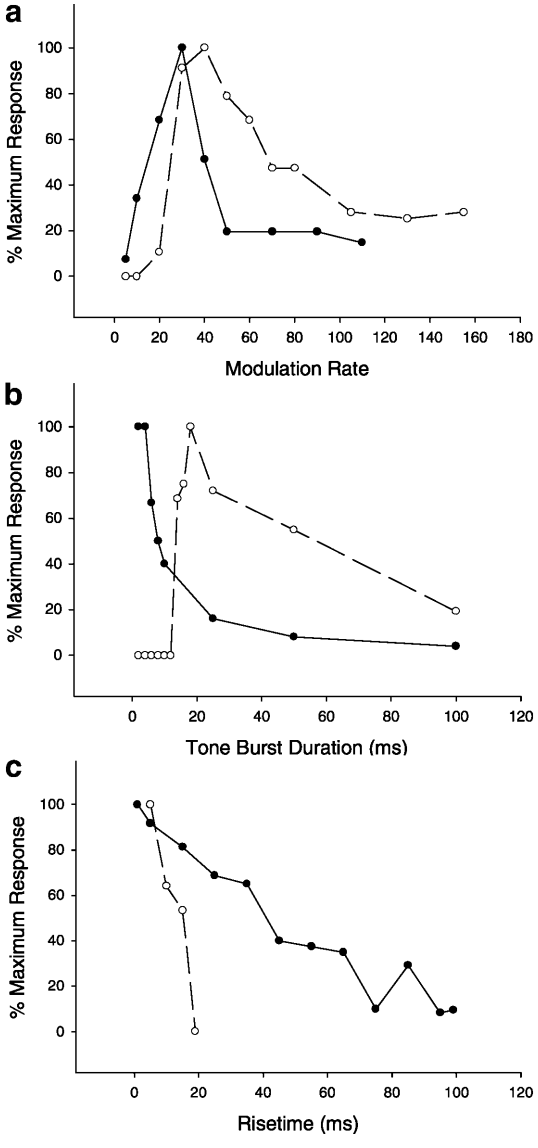

Fig. 2a–c Sensitivities of two units for pulse duration, rise time and amplitude modulation (AM) rate. **a** Tuning to sinusoidal amplitude modulation. Responses were normalized with respect to the maximum spike rate for each neuron. The units were tuned to 30 Hz (*filled circles*, 2.1-kHz carrier) and 40 Hz (*open circles*, 0.6-kHz carrier). Stimulus duration was 400 ms in both cases. **b** Sensitivity to pulse duration. Responses were first normalized with respect to stimulus energy by dividing the mean number of spikes per stimulus repetition by the tone burst duration. These results were then normalized with respect to maximum response in order to facilitate comparison. One unit (*filled circles*) responded maximally to 2-ms and 4-ms pulses. The second unit (*open circles*) did not respond to tone burst durations less than 14 ms, reaching its maximum at 18 ms. **c** Sensitivity to pulse rise-time. Responses were normalized with respect to the maximum spike rate for each neuron

In the final test of the duration/rise-time hypothesis, pulse structure and number were held constant and only the PRR was varied. This stimulus will be referred to as VDC. Figure 3 shows the close correspondence of VDC to a sample of the natural advertisement call of *R. pipiens*, along with a sample of SAM. Responses of units to VDC fell into five categories ($n = 90$): 59.0% low-pass; 23.3% band-pass; 4.4% high-pass; 3.3% all-pass; and 3.3% band-suppression. Six units (6.7%) showed no response to VDC stimuli. Thus, the response levels of all but three neurons were dependent on PRR, most being either low-pass or band-pass in response to VDC stimuli. Factors other than duration and rise-time selectivity, therefore, must underlie their temporal selectivity.

Roles of recovery and integration processes in AM rate selectivity

Units that were low-pass to VDC stimuli (Fig. 4a, light bars) generally had shorter response latencies (median 31 ms; $n = 52$; range 17–87 ms) than those that were band-pass to VDC stimuli (Fig. 4a, dark bars; median 81 ms; $n = 21$; range 34–174 ms; Mann-Whitney *U*-test, $P < 0.001$). Neurons that were low-pass to VDC stimuli

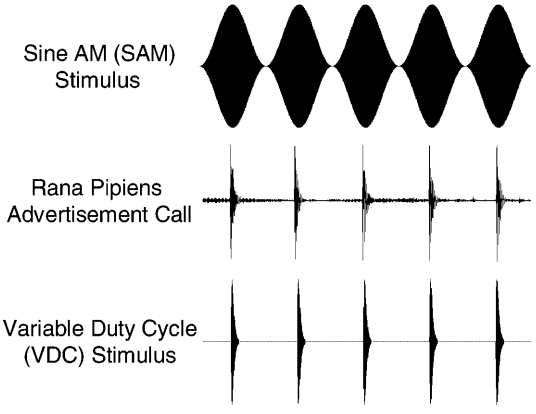

Fig. 3 Comparison of a five-pulse section of the natural *R. pipiens* advertisement call to five pulses of sinusoidal amplitude modulation and stimuli where pulse structure and number were held constant and only pulse repetition rate was varied (variable duty cycle, VDC, stimulus). Pulses from all three selections are shown at the same repetition rate, approximately 10 pps (pulses per second). Duty cycle for the bottom two traces is approximately 0.1

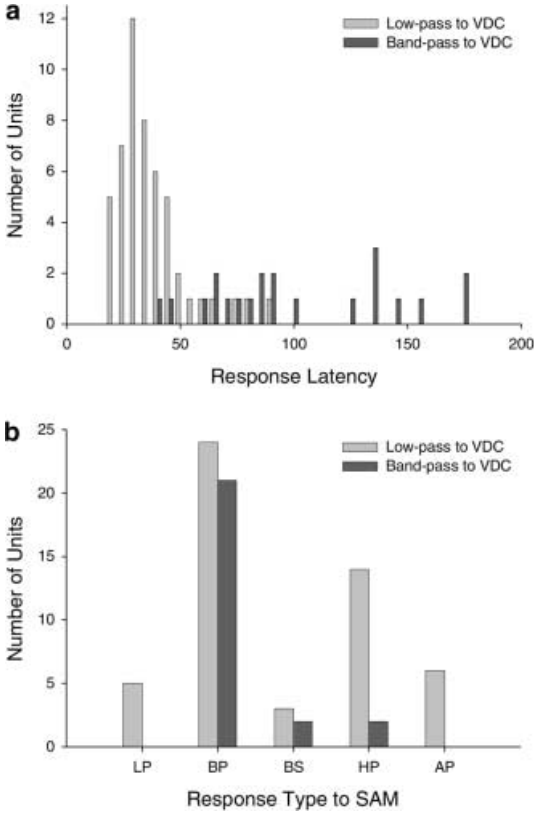

Fig. 4 a Response latency histogram showing comparison between midbrain units that were low-pass to variable duty cycle (VDC) stimuli (*light bars*) versus those that were band-pass to VDC stimuli (*dark bars*). **b** Distribution of response types to sinusoidal amplitude modulation for units that were low-pass (*light bars*) and band-pass (*dark bars*) to VDC stimuli

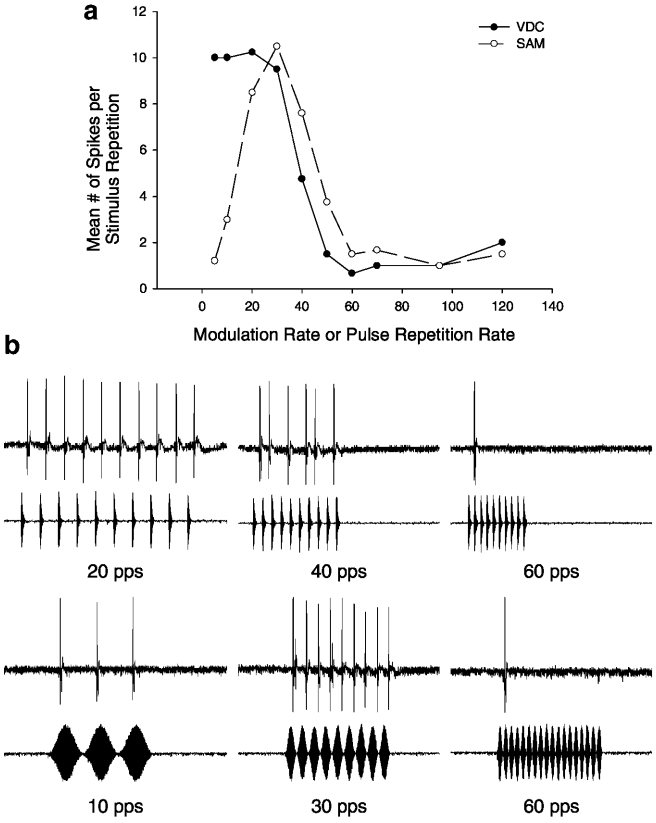

Fig. 5a, b Recovery properties of a typical midbrain neuron tuned to low AM rates. **a** Response level of a single unit versus the rate of amplitude modulation of a 2.1-kHz carrier signal. Carrier amplitude was modulated sinusoidally (*open circles*) or with a modulation envelope that closely approximated the relative rise and fall times of the natural call (*filled circles*, VDC). In the latter stimulus, pulse duration was held constant at 10 ms. Stimulus duration was 300 ms for the sinusoidal signal. Because the VDC stimulus maintained a constant number of pulses (ten), the stimulus duration varied with pulse repetition rate. **b** Recordings from this neuron in response to VDC stimuli (*top two traces*) and sinusoidal stimuli (*bottom two traces*). The top trace in each case shows the neuronal response, while the bottom shows the stimulus

showed a variety of selectivity profiles to SAM, although most were band-pass (Fig. 4b, light bars). Cells that were band-pass to VDC stimuli, however, were almost exclusively band-pass to SAM as well (Fig. 4b, dark bars). Results of further analyses of the mechanisms that underlie temporal selectivity in these two groups are presented below.

Neurons that were low-pass to VDC stimuli

Fifty-two units were low-pass to VDC stimuli. These units responded phasically with respect to individual pulses at low rates; the representative unit shown in Fig. 5 (filled circles, top raw traces) responded with 1 spike per stimulus pulse. These units also responded phasically to pure tones, regardless of the duration of the stimulus. At low PRRs or rates of AM, the activity of this cell, therefore, was directly related to the number of pulses in the stimulus. For AM rates greater than approximately 35 pps, the neuron failed to produce a spike for each stimulus pulse; at rates greater than about 55 pps, the unit responded with 1 spike per stimulus repetition. The only temporal parameter that changes with PRR for this stimulus is inter-pulse interval, suggesting that the response-limiting factor at high rates was the time between pulses. Of the units that were low-pass to VDC, 46.2% responded in a band-pass manner when presented with SAM. As with the VDC stimuli, the units responded phasically to each modulation cycle at low rates, and then responded only at the beginning of each stimulus repetition at higher rates (Fig. 5, open circles, bottom raw traces). Because stimulus duration was held constant, the number of modulation cycles in the stimulus decreased with the rate of AM. Phasic cells, therefore, produced fewer spikes (per stimulus repetition) for AM rates below their best rate. The decrease in response at high AM rates was similar to that seen with the VDC stimuli (right-most traces Fig. 5), suggesting that there was insufficient recovery time between pulses.

Twenty neurons that showed low-pass selectivity to VDC stimuli, were either high-pass or all-pass when presented with SAM. Their maximum level of response to SAM stimuli, however, was approximately equal to

their minimum level of response to the VDC stimuli (Fig. 6). This representative cell only followed, in its responses, pulses below 15 pps. The response to SAM remained at relatively low levels because, at rates below 15 Hz, there were few pulses in the stimulus. Classification of a unit of this type with regard to selectivity to SAM stimuli, therefore, depended heavily on the range of AM rates tested and the stimulus duration; if the total number of pulses had been increased by increasing the stimulus duration, and lower rates tested, the cell would likely have been classified as low- or band-pass. Of the 20 units that were either high-pass or all-pass to SAM, 15 showed these same characteristics; i.e., the level of the maximum response to SAM was similar to the level of the minimum response to VDC.

A third subset of the neurons that were low-pass to VDC consisted of five units that showed low-pass selectivity to SAM stimuli. These units all showed an increase in the number of spikes per pulse at low rates, dropping to ~1 spike per pulse at medium rates. This spike increase at low rates offset the fewer number of pulses as PRR was decreased, resulting in low-pass, rather than band-pass response characteristics (data not shown). Thus, units that were low-pass to VDC stimuli appear to be fundamentally either low- or band-pass to SAM, differing primarily in their recovery times and their phasic response properties.

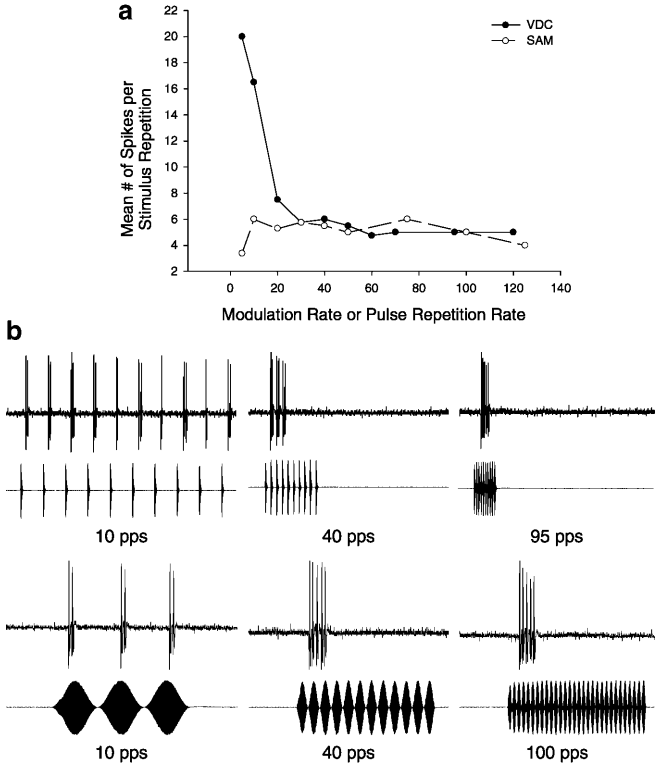

Fig. 6 An example of an auditory midbrain neuron that responded well to low pulse repetition rates, but poorly to all rates of sinusoidal AM. Description as in Fig. 5. The carrier signal was 1.1 kHz

In order to more closely examine the recovery mechanisms that underlie low-pass selectivity to VDC stimuli, 14 units were tested with VDC stimuli wherein pulse duration varied between stimulus sets. In 5 cases, increasing pulse duration caused an overall increase in response. These 5 units produced more spikes as tone burst duration was increased, in 2 cases up to 200 ms. Seven units showed no change in response as pulse duration was varied (Fig. 7a). This representative unit responded with ~1 spike per pulse to all pulse durations tested (5 ms, 10 ms, 15 ms, and 20 ms), up to 20 pps, above which it decreased sharply to ~1 spike per stimulus repetition. This would suggest, at least for the durations tested, that recovery processes in these cases were dependent on inter-pulse interval rather than the silent gap between pulses. Correspondingly, the tuning of this cell for the rate of AM was highly similar for duty

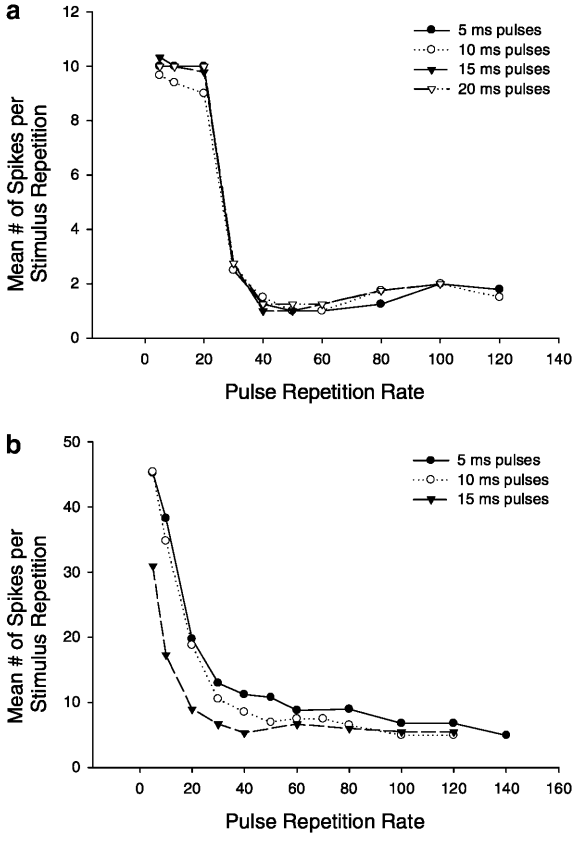

Fig. 7a, b Responses of midbrain neurons to VDC stimuli of different pulse durations. Pulse repetition rate was varied while holding pulse duration constant. Except for pulses of 5 ms, pulse duration exceeded the inter-pulse interval at high pulse repetition rate. In these cases, pulses were truncated at the ends to permit the start of the next pulse. **a** Response level of a single midbrain neuron versus the pulse repetition rate of the VDC stimulus for 5 ms (*filled circles*), 10 ms (*open circles*), 15 ms (*filled triangles*) and 20 ms (*open triangles*) pulses. The stimulus consisted of ten pulses, and had a carrier frequency of 1100 Hz. **b** Response of another midbrain neuron versus the pulse repetition rate of the VDC stimulus for 5 ms (*filled circles*), 10 ms (*open circles*) and 15 ms (*filled triangles*) pulses. The stimulus consisted of ten pulses, and had a carrier frequency of 2.2 kHz

cycles of 0.25, 0.5, and 1.0; in this stimulus regimen, lower duty cycle values provided longer gaps between the end of one pulse and the beginning of the subsequent pulse. Finally, 2 units showed a decreased response as pulse duration was increased (Fig. 7b). This representative unit's response decreased as the pulse duration was increased above 10 ms, indicating that recovery was a function of the silent gap between pulses. Both neurons of this type responded with 3–5 spikes per pulse at low rates, whereas units that showed no change in AM selectivity for different pulse durations produced 1 spike per pulse at low rates.

The relation between the 'recovery' characteristics of units and their tuning to SAM was quantified by determining the low-rate slope of the AM response curves for all units that were low-pass to VDC stimuli and band-pass to SAM. This slope was taken as the increase in AM rate required to drive the neuron from 10% to 90% of its maximum response; the 1.0 duty cycle stimulus was used in order to minimize possible rise-time effects. Because these units were phasic, responding with one spike per pulse at low rates, the slope decreased monotonically with increasing BMR (best modulation rate; Fig. 8). In other words, units that had shorter recovery requirements reached their response maximum at higher rates. Thus, neurons with higher best modulation rates showed broader AM tuning functions, and did not respond exclusively for these higher rates. As will be shown below, this differs markedly from results of the same analysis of neurons that are band-pass to VDC stimuli.

Neurons that were band-pass to VDC stimuli

Twenty-one units were band-pass to VDC and constant duty cycle stimuli. Two other cells appeared to belong to this response category; however, insufficient VDC data were acquired to make this determination. Neurons that were band-pass to VDC stimuli were tuned to significantly higher AM rates (median best rate 78 pps, range 15–110 pps) than cells that were low-pass (median best rate 5 pps, range 5–50 pps) to VDC stimuli (Mann-Whitney U-test, $P < 0.001$). Two representative cases are shown in Figs. 9 and 10. One neuron (Fig. 9) responded best when the rate of AM or PRR was approximately 60 Hz; no responses were recorded for rates of 20 Hz or less. The other cell was tuned to 100 pps (Fig. 10). In this case, spikes were not elicited until the PRR or AM rate exceeded about 60 Hz, and the response peaked near 100 Hz. The steepness of this aspect of the AM selectivity function is a hallmark of this category of neurons. The former unit responded in a tonic manner to AM stimuli (Fig. 9, raw traces), as did 80% of the units of this class; the other 20% responded with a burst of ~4–6 spikes (Fig. 10, raw traces). Interestingly, both units had response latencies of at least 70 ms (see traces below

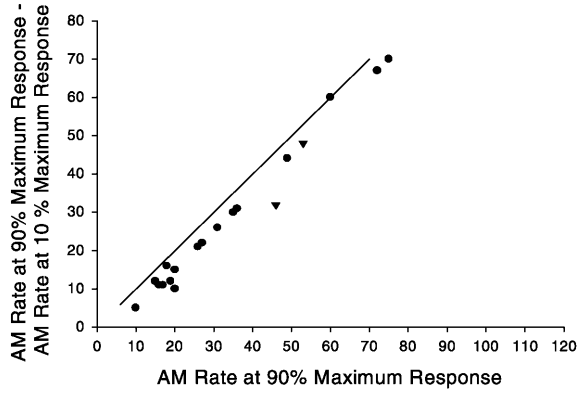

Fig. 8 Relation between the low rate slope of a unit's AM tuning curve and its best modulation rate. This slope was calculated as the change in AM rate required to drive a 'recovery' neuron from 10% to 90% of its maximum response versus the AM rate at 90% maximum response. All data were taken from each unit's responses to 1.0 duty cycle natural AM (*filled circles*) except for two cases where sinusoidal AM was used (*filled triangles*). The *solid line* represents a 1:1 slope from the origin

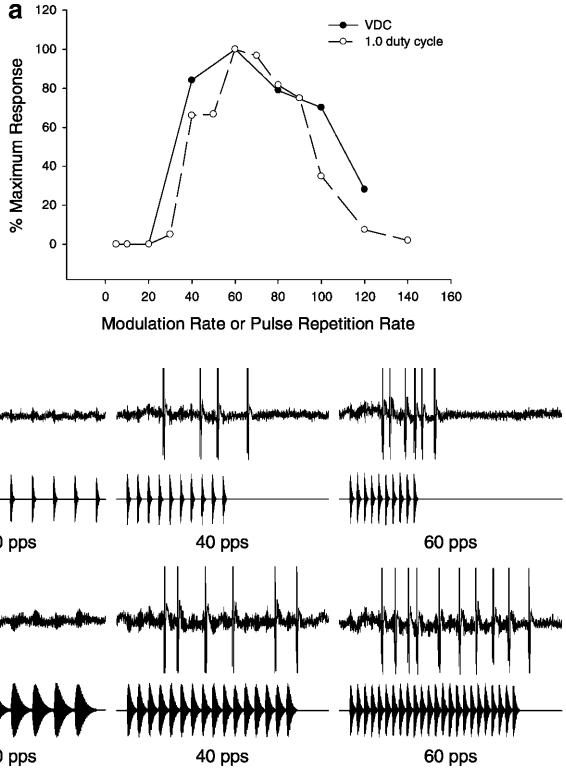

Fig. 9a, b Responses of a tonic 'pulse-integrating' neuron to VDC and natural AM (1.0 duty cycle) stimuli. **a** Normalized response level versus the rate of modulation of the amplitude of a 1.1-kHz carrier signal. Responses were normalized with respect to the maximum spike rate for each type of modulation. Signal amplitude was modulated so that the relative rise and fall characteristics of pulses resembled those found in natural calls. Pulse duration was either held constant at 10 ms (VDC stimulus, *filled circles*) or varied with pulse repetition rate to maintain a duty cycle of 1.0 (*open circles*). Stimulus duration was held constant at 400 ms for the latter stimulus. **b** Recordings from this neuron showing the response to VDC stimuli (*top two traces*) and 1.0 duty cycle natural AM stimuli (*bottom two traces*)

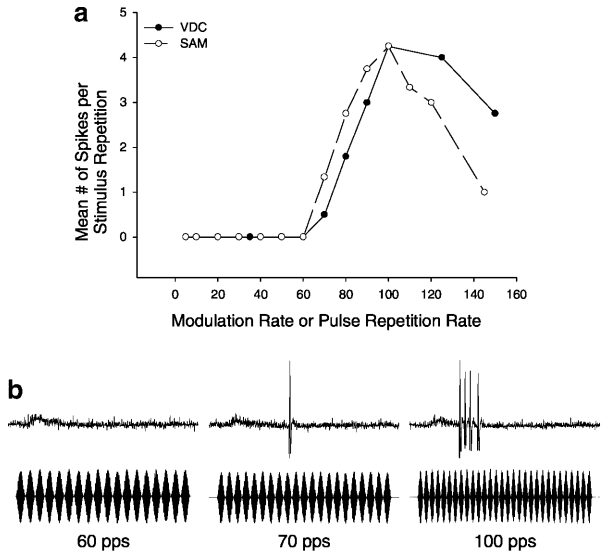

Fig. 10a, b As in Fig. 9, for a cell with a 'phasic' response. **a** Normalized response level versus the rate of modulation of the amplitude of a 1.1-kHz carrier signal. Responses were normalized with respect to the maximum spike rate for each type of modulation. Signal amplitude was modulated sinusoidally (*open circles*) or so that the relative rise and fall characteristics of pulses resembled those found in natural calls (*filled circles*). Pulse duration varied with AM rate for the sinusoidal stimulus, and was held constant at 10 ms (VDC stimulus) for the latter stimulus. Stimulus duration was held constant at 400 ms for the sinusoidal stimulus, and varied with pulse repetition rate for the VDC stimulus, which consisted of ten pulses. **b** Recordings from this neuron showing the response to sinusoidal amplitude modulation

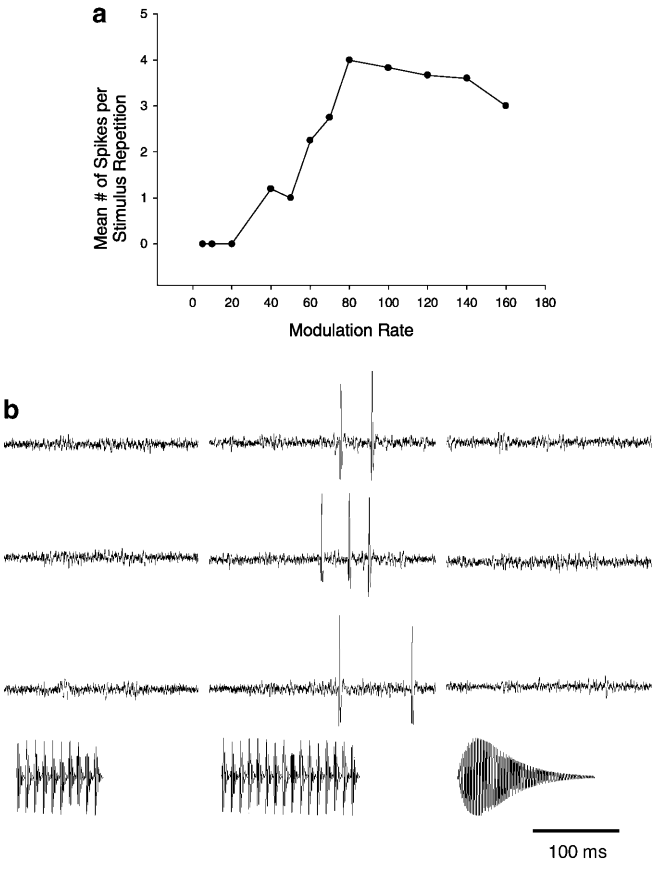

Fig. 11a, b Pulse-integrating properties of an auditory midbrain neuron. **a** Response level versus the rate of modulation of the amplitude of a 0.6-kHz carrier signal. Signal amplitude was modulated so that the relative rise and fall characteristics of pulses resembled those found in natural calls. Pulse duration was varied with pulse repetition rate to maintain a duty cycle of 1.0. Stimulus duration was held constant at 400 ms. **b** The left and center columns show recordings of responses from this neuron to three repetitions (*top three traces*) of 1.0 duty cycle natural AM stimuli (*bottom trace*) that consisted of 10 and 16 pulses, presented at 100 pps. This unit's pulse number threshold was 12. The *right column* shows recordings of responses to three repetitions of a single 160-ms pulse, with the same total energy as the 16 10-ms pulses in the center column

graph, Fig. 9). Tonic unit responses terminated shortly after the end of each stimulus, indicating that the long latency did not simply reflect the time required for transmission of information to this region of the brain.

The hypothesis that the long response latencies of these neurons were due to integration processes was examined by varying the number of pulses per stimulus presentation. The unit in Fig. 11 only responded when the number of pulses, presented at a rate of 100 pps, was 12 or more (raw traces). The center column of Fig. 11 shows the unit's consistent response to 16 pulses, each 10 ms in duration. A single, 160 ms pulse (right column) of the same symmetry and total energy as the sequence of 16 pulses failed to excite the neuron, indicating that the system was not simply integrating information about stimulus intensity. This result, coupled with the failure of the unit to respond to 10 pulses (left column), further demonstrates that the long latencies of these neurons are not due to conduction time from the periphery to the torus. These data are consistent with the finding that neurons of this type do not respond to tone bursts (Alder and Rose 1998). For the population, a median of 8 pulses (range 2–16) were required to elicit activity above spontaneous levels.

Pulse number threshold vs BMR for the entire population is shown in Fig. 12. This plot shows that most neurons of this type were tuned to PRRs above 40–50 pps, and that neurons tuned to higher rates generally required more pulses for eliciting activity ($r^2 = 0.341$, $P < 0.01$). These data provide evidence for an integration process underlying AM rate selectivity; but what is being integrated? The system may integrate stimulus intensity that is distributed in a specific temporal pattern, or it may integrate information relating to the number and temporal density of pulses. To examine these possibilities further, the variable pulse number experiments (described above) were repeated at 12 dB and 18 dB above each unit's threshold. Data from a representative case are shown in Fig. 13a. At both amplitudes the unit required a minimum of six pulses to respond, indicating that the integration process was independent of stimulus amplitude. Across the population, there was good correspondence between the pulse number thresholds at these two relative intensities (Fig. 13b).

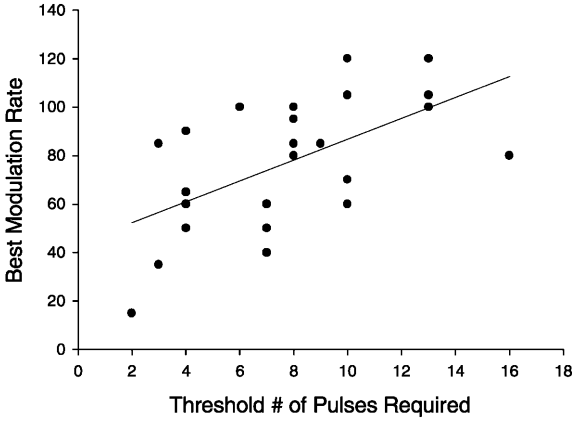

Fig. 12 The number of pulses required for a midbrain neuron to respond versus its best AM rate. Pulse number threshold was determined using 1.0 duty cycle natural AM stimuli. *Solid line* is a linear regression ($r^2 = 0.34$)

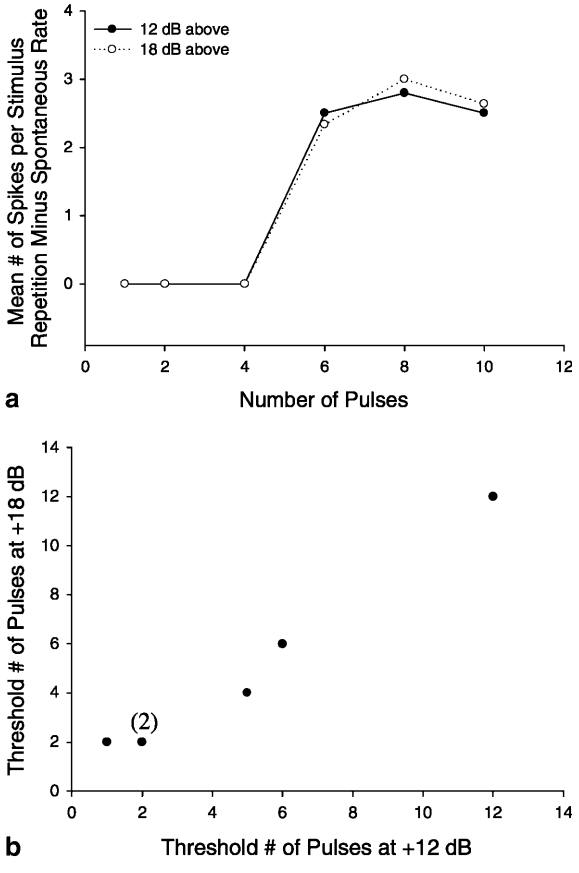

Fig. 13a, b Energy independence of the pulse-integration process. **a** The response levels of a single representative midbrain neuron for stimulus amplitudes 12 dB (*filled circles*) and 18 dB (*open circles*) above threshold, as a function of the number of pulses in the stimulus. Pulse repetition rate was 80 pps. The carrier frequency was 1.1 kHz. **b** Data from six midbrain neurons showing pulse number thresholds at signal amplitudes that were 12 dB and 18 dB above threshold

The low-rate slopes of the AM response curves were analyzed for all units that were band-pass or high-pass

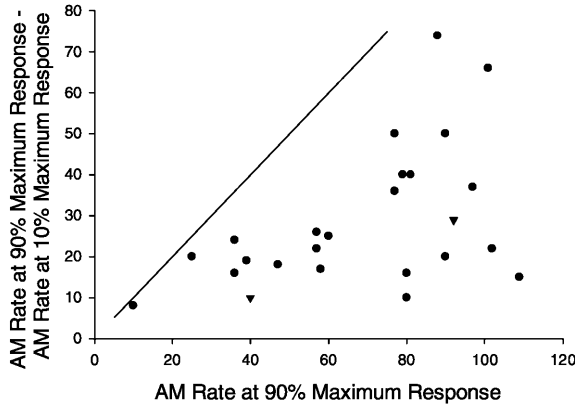

Fig. 14 The change in AM rate required to drive a 'pulse integration' neuron from 10% to 90% of its maximum response versus the AM rate at 90% maximum response. All data were taken from each unit's response to 1.0 duty cycle natural AM (*filled circles*) except for 2 cases where sinusoidal AM was used (*filled triangles*). The *solid line* represents a 1:1 slope from the origin

to both VDC stimuli and SAM. Figure 14 shows the increase in AM rate required to drive the neuron from 10% to 90% of its maximum response; the 1.0 duty cycle stimulus was used in order to minimize possible rise-time effects. Unlike units that were low-pass for VDC stimuli, 'pulse integration' neurons have very steep low-rate slopes on their AM tuning curves; the response level of 75% of these neurons increased from 10% to 90% of maximum over a change in PRR of less than 30 Hz. In many cases, the slopes of neurons tuned to high AM rates were as steep as those from neurons tuned to low rates. Although most pulse-integration neurons were tuned to high AM rates, it should be noted that a few were selective to low AM rates, indicating that this mechanism can also contribute to selectivity in this range.

Response latency versus best pulse repetition rate summary

Based on their responses to VDC stimuli, toral neurons could be divided into two discrete groups. One group consists of neurons that had short response latencies and were low-pass to VDC stimuli (Fig. 15a, open circles), the other had long response latencies and were tuned to relatively high PRRs (closed triangles). This plot reveals that these two groups of units form discrete clusters in this two-dimensional physiological space. This physiological separation is less clear when measurements of response latency and best PRR are determined from constant duty cycle stimulation (e.g., SAM and natural AM; Fig 15b). This overlap is due to the fact that many units that responded with short latencies and were low-pass to VDC stimuli showed tuning to mid and high modulation rates when SAM or natural AM were used as stimuli.

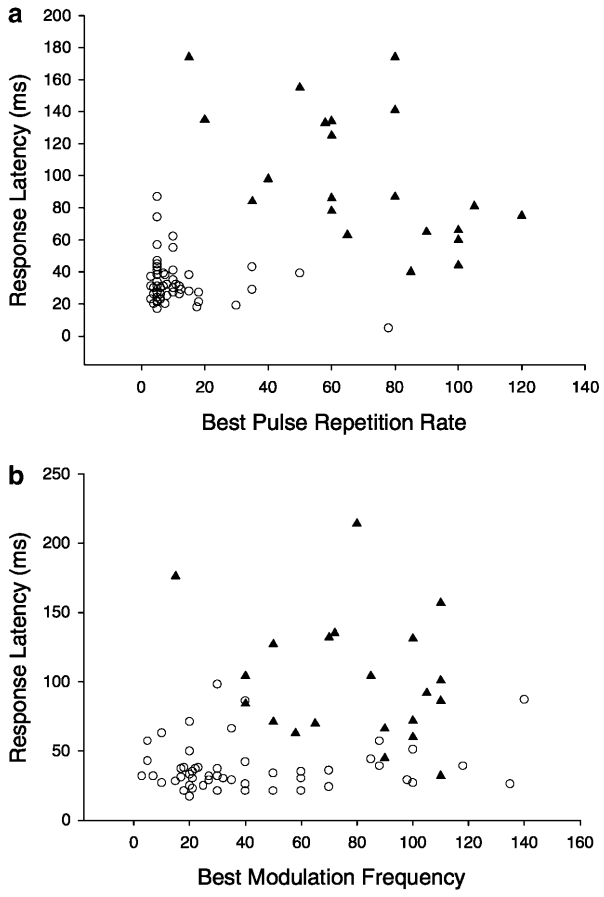

Fig. 15a, b Population plot of AM tuning as a function of response latency for neurons that were low-pass (*open circles*) and band-pass (*filled triangles*) to VDC stimuli. **a** Response latency and best pulse repetition rate values were determined from responses to VDC stimuli. Best pulse repetition rate for the low-pass units was defined as the AM rate prior to the point at which the response dropped below 90%. **b** Response latency and best modulation frequency values were determined from responses to constant duty cycle stimulation (e.g., sinusoidal AM and 1.0 duty cycle natural AM)

Discussion

We examined the mechanisms underlying the AM rate selectivity of neurons in the torus semicircularis of *R. pipiens*. The principal findings were that (1) the sensitivities of cells to stimulus rise-time and duration generally did not account for their tuning to SAM; most units preferred durations less than approximately 10 ms and fast rise-times, yet were tuned to SAM rates below about 50 Hz, (2) neurons that were tuned to these slow SAM rates showed low-pass selectivity when only the rate of repetition of pulses was varied; pulse duration, shape and number were held constant, and (3) other neurons were tuned for AM rates or PRRs above about 40–50 Hz and, remarkably, most failed to respond to rates below about 40 Hz. These units also failed to respond to pure tones. Holding the PRR near the optimal value, these cells only responded after a threshold number of pulses had been delivered.

Gooler and Feng (1992) found that the AM selectivities of toral neurons were generally not attributable to underlying sensitivities to stimulus rise time and duration. In this earlier study, relatively few neurons were recorded that were tuned to SAM. The present work strengthens this conclusion for AM-tuned cells. Also, Condon et al. (1991) recorded from phasic units in the SON that were band-pass to SAM and to square-wave AM. The responses of phasic cells to these two stimulus regimens differed only slightly at the very slow AM rates, indicating that rise-time sensitivity contributed little to their temporal selectivity.

Relative to previous investigations, AM band-pass units were recorded much more frequently in the present study (Rose and Capranica 1983, 1985; Walkowiak 1988; Gooler and Feng 1992). Several factors may have contributed to these differences. In previous studies, sharp pipettes were used, thereby increasing the probability of recording from toral afferents. Because we used patch-type electrodes and attempted to seal extracellularly onto neurons, it is likely that we recorded principally from neurons in the torus. Also, the search stimuli apparently differed among these studies. One group of neurons did not respond to tone bursts, and would not have been found if tones were used as a search stimulus. In the present study, the search stimulus consisted of a pure tone that was sinusoidally amplitude modulated. The frequencies of the carrier and the modulating signals were varied while advancing the electrode. This procedure attempted to minimize the probability of bypassing an AM-tuned neuron.

The relative abundance of band-pass neurons notwithstanding, as in previous studies, neurons were distributed among a variety of temporal selectivity classes on the basis of responses to SAM e.g., all-pass, low-pass, high-pass, but not for VDC stimuli. This diversity appears to be a consequence of the nature of the SAM stimulus and the fundamental temporal selectivities of these neurons. This issue will be addressed in detail below.

Mechanisms of AM selectivity

Selectivity to sinusoidal AM versus selectivity to VDC stimulation

In this study, AM rate or PRR were varied while holding energy per stimulus presentation constant. Under this condition, most toral units that were tuned to low rates of SAM showed low-pass selectivity when only PRR was varied (VDC stimuli), i.e., pulse number, shape and duration were held constant. These cells responded phasically to individual pulses. Their response level at low AM rates, therefore, was largely a function of the number of pulses (cycles of AM) in the stimulus. Because the number of pulses in the SAM stimulus decreased linearly with AM rate, the overall

level of response of these units decreased for rates of SAM less than their best rate.

The phasic response properties of toral neurons may be the result of processes upstream. The torus receives afferents from the DLN (first-order auditory region) and from the SON (second-order region that receives auditory input from the DLN; Wilczynski 1988). Many neurons in these two regions respond phasically to tone bursts and show preference for stimulus rise-times <25 ms (Hall and Feng 1988; Condon et al. 1991). These properties appear to account for the diminished responses of these cells to low rates of SAM. Hall (1994) showed that bicuculine injections, presumably reversing their phasic properties, in some cases transformed neurons from band-pass to low-pass, and high-pass to all-pass.

Other cells that were low-pass to VDC responded to SAM in a high-pass or all-pass fashion. Compared to their responses to VDC, however, these cells were only weakly excited by SAM stimuli; the level of maximum response to SAM was approximately equal to the level of the minimum response to VDC stimuli. If longer stimulus durations and lower AM rates had been employed, these units would most likely have been classified as band-pass or low-pass when presented with SAM stimuli. Also, some units that were low-pass to VDC were also low-pass to SAM stimuli. These units increased the number of spikes per pulse at low SAM rates, thus offsetting the decrease in pulse number.

Recovery mechanisms: selectivity to low AM rates

Neurons that are tuned to low SAM rates are low-pass to VDC stimuli and have short response latencies. For VDC stimuli, only the interval between pulses differs as the PRR rate is varied. The decline in response level of these cells as the PRR rate exceeded a critical value indicates that insufficient recovery time was available between pulses.

What is the nature of the mechanism underlying the selectivity to inter-pulse interval seen in these neurons? The decline in response above a minimum inter-pulse interval may be a result of the mechanism that generates the phasic nature of these cells. In one model, short latency excitation is followed by inhibition. The minimum recovery time would be related to the time required for the inhibition to decay; the faster the decay the shorter the required recovery time. In another, not mutually exclusive, formulation, the time-constant of recovery of afferents from synaptic depression (O'Donovan and Rinzel 1997; Fortune and Rose 2000) determines the maximum PRR that a neuron can 'follow' in its discharges. The experiments conducted in this study do not clarify the relative importance of these two processes in generating the recovery times of toral neurons. According to the synaptic depression model, longer pulse duration should prolong the recovery time. Consistent with this prediction, some units responded less vigorously to the VDC stimuli when pulse duration, and, therefore, duty cycle, was increased. The low-pass selectivity of most cells for VDC stimuli, however, was not substantially altered by changes in pulse duty cycle, i.e., different pulse durations. This result could, however, still be compatible with a role of synaptic depression, provided that it occurs after the phasic response pattern has been generated.

Pulse integration mechanisms: selectivity to high AM rates

Most neurons that responded best to SAM rates greater than approximately 40 Hz also showed band-pass selectivity to VDC stimuli. Most of these units did not respond to AM rates or PRRs below about 40 Hz, and had best rates of about 70–100 Hz. Each neuron responded only after a threshold number of pulses were delivered at its best rate, suggesting that an integration process contributed to its temporal selectivity. 'Pulse-number' thresholds are invariant over at least a 16-dB range in stimulus amplitude (Alder and Rose 1998). Thresholds do not, therefore, simply reflect the integration of stimulus intensity, even when the best PRR is used. These results raise the question, what is being integrated? Future studies should address whether these neurons respond best at particular PRRs because (1) an optimal temporal density of pulses exists, i.e., a particular number of pulses are presented within a specific time window, or (2) the interval between pulses is optimal. In the latter model, a neuron begins to respond when a threshold number of consecutive correct interpulse intervals have occurred.

Cellular processes that might underlie pulse integration include (1) facilitation (presynaptic), (2) postsynaptic temporal summation, and (3) postsynaptic accumulation of second messengers that amplify conductance changes. To investigate these issues more fully, intracellular recordings are needed. Facilitation would be supported if PSP amplitude increases when a sequence of correct interpulse intervals is presented. Calcium-dependent facilitation has been demonstrated at synapses between parallel fibers and Purkinje cells, where PSP amplitude reaches its maximum after approximately eight to ten pulses (direct stimulation of fibers; Kreitzer and Regehr 2000). Frequency-dependent facilitation is a short-term synaptic enhancement of neurotransmitter release that occurs during a train of presynaptic action potentials, and decays following the termination of the train (Delaney and Tank 1994; Zucker 1999). These increased levels of neurotransmitter release have been shown to be due to a build up of Ca^{2+} in the presynaptic nerve terminal (Atluri and Regehr 1996; Fischer et al. 1997; Dittman et al. 2000; Kreitzer and Regehr 2000).

Pulse integration neurons respond either tonically (Fig. 9) or phasically (Fig. 10) with respect to the pulse train once they reach threshold. The phasic response is

similar to that at the CA3 to CA1 Schaffer collateral synapse in the hippocampus, in which EPSC amplitudes facilitate and then decrease during the 50-Hz stimulus train. The combined actions of facilitation and synaptic depression appear to underlie the phasic responses of these hippocampal cells (Dittman et al. 2000).

Postsynaptic integration processes would be implicated if PSPs overlap and summate over time. In addition, the consequent depolarization might activate voltage-dependent (Fortune and Rose 1997) or second-messenger-related conductances that amplify PSP amplitude. Recent experiments on the sensory to motor neuron synapse in *Aplysia* show that postsynaptic processes can contribute to short-term facilitation (Bao et al. 1997, 1998). In these studies, short-term synaptic enhancement was abolished by postsynaptic hyperpolarization or BAPTA injection.

Evaluation of the rise-time and duration hypothesis

We have shown evidence for two classes of neurons in the anuran midbrain, selective to different ranges of PRR. Evidence that the mechanisms underlying the generation of these AM selectivities do not primarily result from sensitivities to pulse duration and rise/fall time include (1) neurons with similar AM tuning often have very different duration and rise/fall time response patterns; (2) relatively few neurons are tuned to duration, and these show no correlation between their best durations and their best SAM rates; (3) recovery processes underlie neural selectivity to low PRRs; and (4) pulse-integration mechanisms are responsible for the generation of AM selectivity to higher PRRs, and these neurons do not respond to tone bursts.

Nevertheless, data presented in Fig. 2 (open circles) gives some indication that it is possible for AM tuning to result from pulse duration and/or rise/fall time selective processes. The weak responses of this cell to low rates of SAM are consistent with its insensitivity to tone bursts of slow rise-time and long duration. Also, the weak responses to high rates of SAM are consistent with its insensitivity to tone bursts of short duration. Thus, it appears, in this case, that rise-time and duration selectivity contributes to the unit's tuning to SAM stimuli. The question, therefore, arises, why isn't this mechanism commonly used? The inadequacy of mechanisms that are based on rise-time and duration sensitivities can be understood by examining the temporal structure of anuran vocalizations. The calls of *R. Pipiens*, as well as many other anurans, consist of pulses with very steep rises and exponential falls. These pulses are similar to those found in calls from several species of *Bufo* (e.g., *B. regularis*, *B. gutturalis*, *B. brauni*, *B. pentoni B. gariepensis*, *B. bufo*), which are representative of the primitive condition (Martin 1972). Pulse shape in these cases is a result of passive opening and closing, due to tracheal airflow, of the arytenoid cartilages. For anurans that produce calls in this manner, pulse shape does not vary drastically between calls that differ significantly in PRR. Calls of different PRRs cannot be differentiated, therefore, on the basis of pulse shape. For example, the Pacific treefrog, *Hyla regilla*, produces two types of advertisement calls and an encounter (aggressive) call (Allen 1973). These call types consist of a series of pulses, each approximately 10–12 ms in duration, repeated at about 100 pps and 25 pps, respectively. Similarly to *R. pipiens*, these frogs change the duty cycle of their pulses to alter the PRR of their calls. Because the pulses of these two call types are nearly identical in spectral composition and shape, the calls must be differentiated solely on the basis of PRR. Behavioral studies have shown that two discrete sensory 'channels' exist for processing these two call types (Brenowitz and Rose 1994; Rose and Brenowitz 1997). Correspondingly, neurons have been found in this species that, similar to those found in *R. pipiens*, discriminate very selectively between high and low PRRs (Alder and Rose 1998). It is possible, therefore, that toral neurons cannot generate the very steep filtering characteristics required to perform this discrimination using solely pulse shape information. In other words, it may be difficult to generate pulse-duration selectivity that is sharp enough to allow the level of AM filtering that is required to differentiate between calls.

Certain species of *Bufo* (e.g., *B.coniferus*, *B. ibarrai*, *B. haematiticus*, *B. coccifer*, *B. viridis*) do amplitude modulate their calls in a more-or-less sinusoidal fashion. Martin (1972) has shown that this call type is a derived condition. Pulses in these calls are produced by the active contraction of the internal and external oblique muscles and they do not exhibit steep rise-times because the arytenoid cartilages are deactivated as a result of muscular contraction. While the duration of 'passive calls' is limited by lung volume, this active mechanism allows for longer duration calls, in some cases up to 30 s (Blair 1964; Capranica and Rose 1983). Toral neurons from two species possessing these calling characteristics, *B. a. americanus* and *B. woodhousii fowleri*, have been shown to have long response latencies (Rose and Capranica 1984). This would suggest that pulse integration mechanisms are responsible for rate discrimination, even in anurans that produce pulses that vary in shape with the AM rate of the call. Because the AM selectivity mechanisms were already in place in the primitive state, apparently there was no need to discriminate calls based on pulse structure. It should be noted that this situation, at least for rise time, might not apply to all anuran species. The gray treefrog (*Hyla versicolor*) produces and behaviorally prefers calls with slower rise-times (Diekamp and Gerhardt 1995; Gerhardt and Schul 1999). This anuran, interestingly, is a cryptic tetraploid species thought to have arisen from the diploid anuran, *H. chrysoscelis* (Wasserman 1970; Gerhardt 1994a). *H. versicolor* may have taken advantage of preexisting rise-time-sensitive mechanisms in order to discriminate between very similar calls (e.g., 25 pps versus 35 pps) in this sympatric situation.

Further evolutionary considerations

We have shown that pulse integration units require a threshold number of pulses in order to respond, and that a majority of these cells continue to respond, once this threshold is reached, tonically throughout the duration of the stimulus. Tonic neurons, therefore, should respond best for longer calls. This would be a more motivating stimulus to female anurans, an important factor to many *Bufo* species that must attract mates across long distances to temporary pools (Wells 1977). It has been shown in the gray treefrog (*H. versicolor*), that females behaviorally prefer long duration calls presented at slower calling rates to short duration calls presented at faster calling rates (Klump and Gerhardt 1987; Gerhardt 1994b). Preference for longer-duration calls would appear to be a case of 'sensory exploitation' (Ryan et al. 1990; Ryan and Rand 1993), where males exploit the existence of a pre-existing sensory mechanism (pulse integration) in order to enhance the attractiveness of their calls.

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