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Response of the Telencephalic Neurons of the Budgerigar *Melopsittacus undulatus* to Species-Specific Warble Song Elements

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ABSTRACT—The response of neurons of the budgerigar (*Melopsittacus undulatus*) was obtained in Field L, which is a laminated auditory structure of the caudal neostriatum in the telencephalon. Warble song of this species is comprised of a number of sounds (elements). The influence of sequence and silent intervals between elements on neuronal response was investigated. First, neurons in Field L were examined to know if neuronal response to isolated elements differed from that to the same elements in warble song, a sequence of elements. Sixty-two percent of the neurons exhibited increases of spike activity in response to elements in isolation compared with that to elements in warble song. These neurons thus exhibited temporally suppressed response. We then examined neuronal activity using the stimulation with paired elements, separated by various silent intervals (delta t msec). The spike activity in response to a specified element decreased as the interval was shortened. The responses of most neurons were strongly suppressed at the delta t of 80 msec, which is often seen in element intervals of warble song. In some neurons in Field L the response was suppressed, although they did not respond to the preceding sound. We hypothesize that temporally suppressed neurons may play a role in vocal discrimination.

INTRODUCTION

The budgerigar (*Melopsittacus undulatus*), a parakeet that lives in flocks, uses various vocal signals to coordinate social and reproductive behavior (Brockway, 1969; Wyndham, 1980). Warble song is vocalized in various contexts, including a precopulatory one (Brockway, 1964a; Trillmich, 1976; Wyndham, 1980). Both sexes vocalize warble song, although males warble more frequently than females (Brockway, 1969). Hearing of warble song promotes attainment of full gonadal activity in both sexes (Brockway, 1965, 1969), stimulates males' production of warble song (Brockway, 1968) and stimulates occupation of nest boxes in females (Brockway, 1969).

Acoustic analyses of warble song have also been conducted (Eda-Fujiwara and Okumura, 1992; Farabaugh *et al.*, 1992; Eda-Fujiwara *et al.*, 1995). Warble song is comprised of a sequence of elements or syllables with silent intervals between them (Fig. 2A). There is no fixed length of warble song, and a bout of warble song can last from a few seconds to several minutes. In warble song there are various types of acoustic structure of the elements, which are produced at characteristic shorter intervals. Acoustic analyses provide a basis for further research on the neural processing of warble song.

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In some songbirds, different types of syllables comprise a song, in which sequence of syllables is an information-bearing parameter (Konishi, 1985). Some species use duration of silent intervals between syllables as a parameter. Sequence and/or silent intervals between elements might be information-bearing parameters in warble song of the budgerigar. In Field L of the mynah bird (*Gracula religiosa*), temporal sequence of segmented sounds of human speech affects neuronal response (Uno *et al.*, 1991). Field L is a laminated auditory area in the caudal neostriatum of the telencephalon (Müller and Scheich, 1985; Brauth *et al.*, 1987). Temporal sequence and interval duration of elements may influence the response of auditory neurons of the budgerigar. In this paper, we obtained the response to warble song of single neurons in Field L. We compared the neurons' response to isolated elements with that to elements within warble song. We then analyzed neuronal response to a sequence of two elements while increasing the silent intervals between them.

MATERIALS AND METHODS

Preparation of birds and recording

We recorded the extracellular spike activities in Field L of the left caudal telencephalon in six adult male budgerigars.

The birds were anesthetized with 2 mg of urethane per g of body weight during surgery and during recording of neuronal activity. The brain was exposed through an opening (2 mm in diameter) made by removing a portion of the skull over the dorsal telencephalon. The bird was then placed in the stereotaxic instrument (Narishige). A metal

plate (50 × 26 mm) with a hole (14 mm in diameter) was cemented to the portion of the skull surrounding the dorsal trepanation. During recording sessions, the head was immobilized by this metal plate. A glass-coated elgiloy microelectrode (3-5 MΩ at 1 kHz) was advanced with a hydraulic microdrive (WR-87, Narishige).

Acoustic stimuli

We recorded the warble song of a breeding male budgerigar using a cassette recorder (TC-D5PRO, SONY) with a condenser microphone (ECM-150T, SONY). All the subjects in the present study had not heard this male's vocalization. We analyzed and edited the recorded warble song, using a computer-based system (The Sound Master, Canopus) and a 16-bit computer (PC286-US, EPSON) or the Sound Edit Pro Program (Macromind) with an Apple IIvx computer. For repeated presentation in electrophysiological experiments, we selected a segment about 2.8 sec in duration (the Segment) from a bout of warble song which lasted for a few minutes (Fig. 2A). Each element (1-11) of the Segment is numbered. We isolated five elements (Elements 2, 7, 8, 9 and 10) to present them in isolation.Acoustic stimuli were delivered binaurally via a closed acoustic system consisting of earphones (Denon) and metal housing. At each ear, the tapered end of the housing was firmly attached against the meatus. The system has flat frequency response over the range of 50-8000 Hz (±6 dB).

Experimental protocol

Experiment 1. During electrode advancement, the Segment was presented at peak values of 74 dB at 6-sec intervals. A neuron responding to the Segment was tested with the five types of elements presented in isolation. Element 2 in isolation was presented at the same frequency of occurrence and intensity as Element 2 in the Segment. After Elements 7, 8, 9 and 10 were presented in the same manner, the Segment was again presented.

Experiment 2. Element 2 was used as the search stimulus at 2-sec intervals at 70 dB. Then the sequence of Elements 1-2 was presented to each neuron. The interval of silence between the elements (delta t) was varied at 20, 40, 80, 160 and 320 msec. Element 2 was then presented again.

Histological verification of recording sites

For marking of the recording site, iron deposits were placed with electrical current. At the end of the experiment, the bird was injected with a lethal dose of sodium pentobarbital and perfused intracardially with 10% formalin. Serial frozen sections (30-μm thickness) were cut in the sagittal plane. The iron deposits were developed with the Prussian blue reaction, and the sections were counterstained with neutral red.

Field L is a trilaminar structure (Field L1, L2, L3). The central lamina L2 receives most of the afferents from the thalamic nucleus ovoidalis (Brauth *et al.*, 1987). In sections stained with cresyl violet and luxol fast blue, L2 is characterized by small neurons and many myelinated fibers (Fig. 1). Moreover, during recording sessions L2 neurons were easily identified audiovisually by powerful background activity. The data set in this study contains recordings from L1, L2 and L3.

Data analysis

The neural activity was digitized with 16-bit resolution at 20 kHz sampling rate and stored on a hard disk. Single-unit isolation was achieved with a window discriminator constructed on a personal computer (PC-286UX, Epson). Peristimulus time histograms (PSTH) were constructed from the responses of each unit. The number of repetitions was 10 in Experiment 1 and 20 in Experiment 2. Bin width was always 10 msec. Our objective was to compare a neuron's response to isolated elements with the same neuron's response to elements in sequence. Thus, in Experiment 1, the response of a neuron to each element was quantified as the spike count (SC) during a specified time, an element plus a subsequent silent interval, using PSTH. We calculated the ratio of SC (SC ratio) of an isolated element to that for the element involved in the initially presented Segment. In Experiment 2, spike was counted for the entire Element 2 (the entire

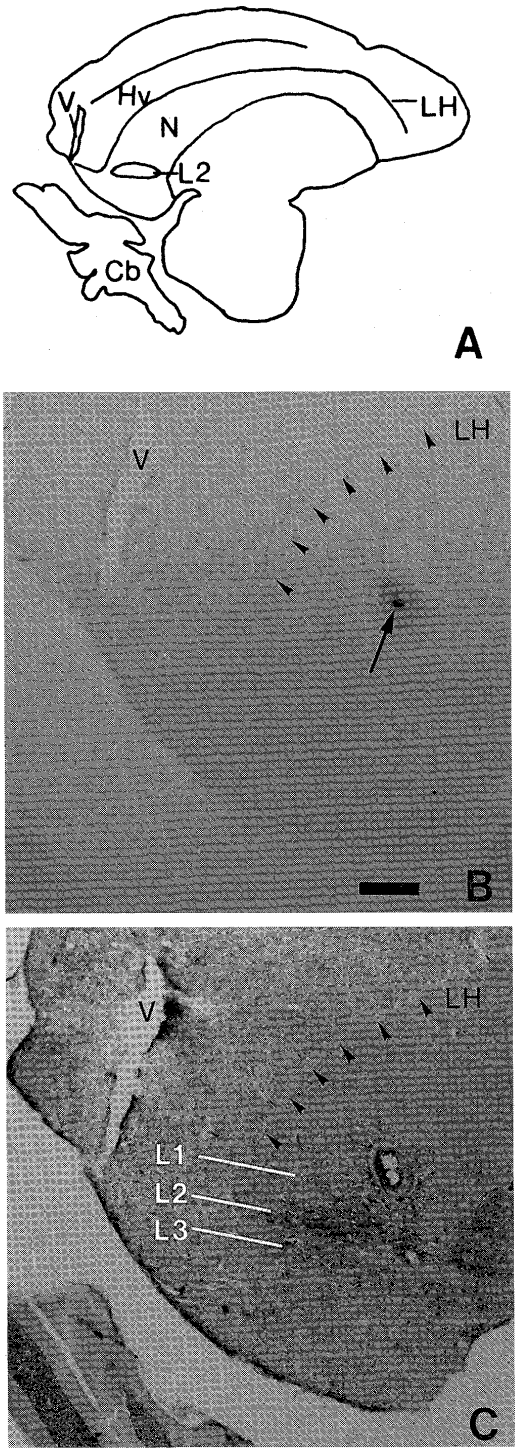


Fig. 1. (**A**) Schematic drawing of a sagittal brain section which illustrates position and orientation of Field L of the budgerigar. (**B**) A photomicrograph of a section stained with neutral red showing an iron deposit (arrow) in Field L at the level depicted in Fig. 1A. (**C**) A photomicrograph of an adjacent tissue section stained with cresyl violet and luxol fast blue. Many myelinated fibers of the Field L2 are intensely stained. Scale bar = 0.5 mm. Cb, cerebellum; Hv, hyperstriatum ventrale; L1, Field L1; L2, Field L2; L3, Field L3; LH, lamina hyperstriatica; N, neostriatum; V, ventricle.

SC) and for 50 msec after the Element 2 onset (the onset SC). We also calculated the SC ratios of Element 2 when paired with Element 1 to that for isolated Element 2 presented first.

RESULTS

Experiment 1: Responses to the five types of elements in isolation relative to responses to ones involved in the intact warble segment (the Segment)

We compared each unit's response to the five isolated elements with that to the elements involved in the Segment in 26 units isolated in Field L. Figure 2 illustrates response patterns of one of these neurons. The neuron 0606 responded with phasic on-response to Element 6 of the Segment, but not to the other elements (Fig. 2A). Element 2 within the Segment elicited no or only weak excitatory response, while the same element presented in isolation elicited strong response (Fig. 2B). When each of the four other elements (7, 8, 9 and 10) was presented in isolation, this neuron showed increased spike activity (Fig. 2C, D, E, F).

Of the total of 130 comparisons obtained from 26 neurons, 101 cases showed response to elements in isolation and/or to elements in the Segment. In these cases, we first examined the ratio of the spike count for the last Segment presentation to that for the initial one (as mentioned above, we presented the Segment twice) in order to ascertain the consistency of response to the same stimulation (Fig. 3A). The SC ratios were distributed around 1.0, and 79 of the comparisons (78%) ranged from 0.5 to 3.0.

SC ratios of element in isolation to that involved in the Segment are shown in Figure 3B. Some neurons (five neurons for Element 2, eight for Element 7, four for Element 8, five for Element 9 and three for Element 10) showed increased spike activity when elements were presented in isolation (SC ratios > 3, Fig. 3B). Some neurons exhibited temporally suppressed

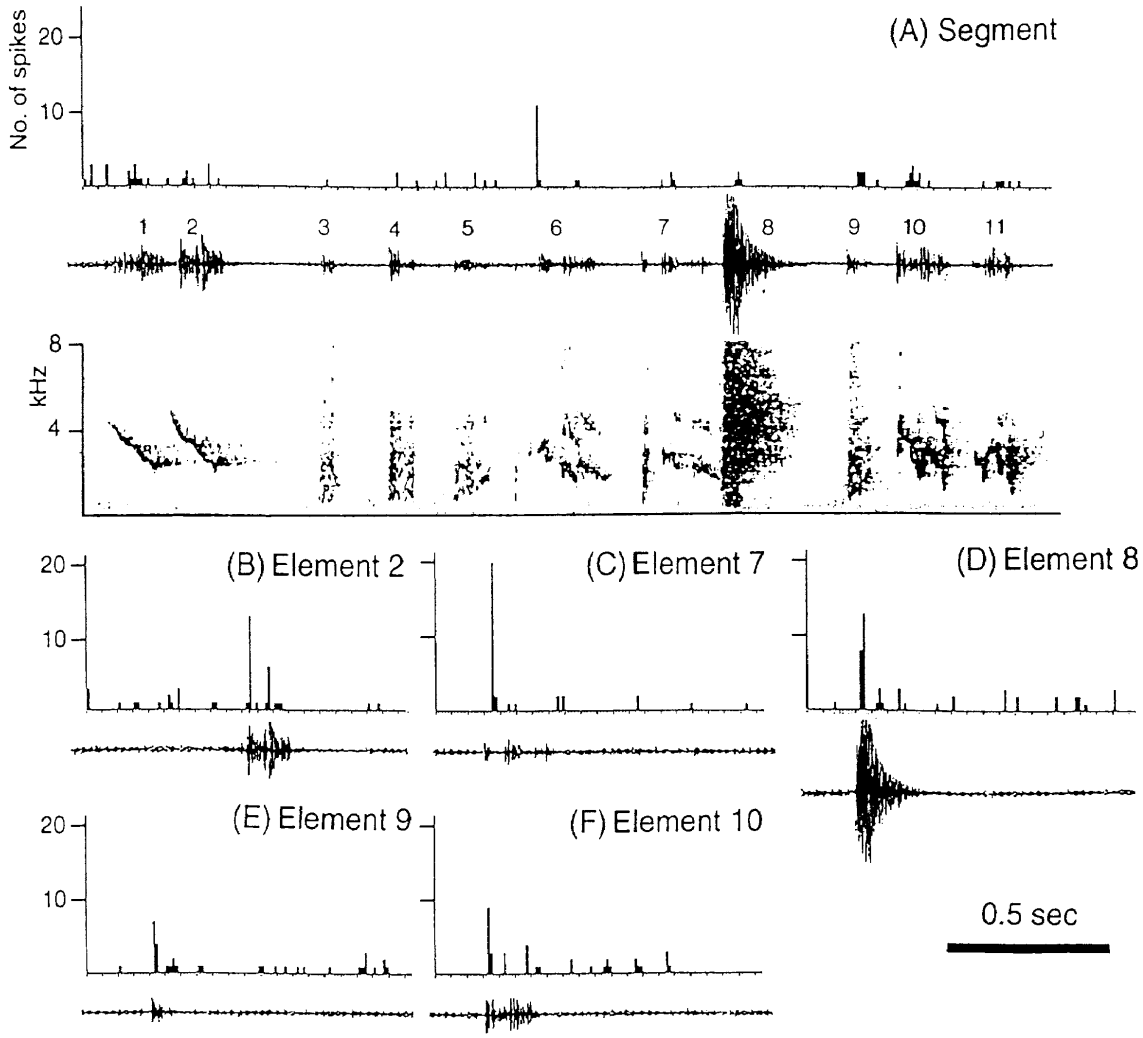
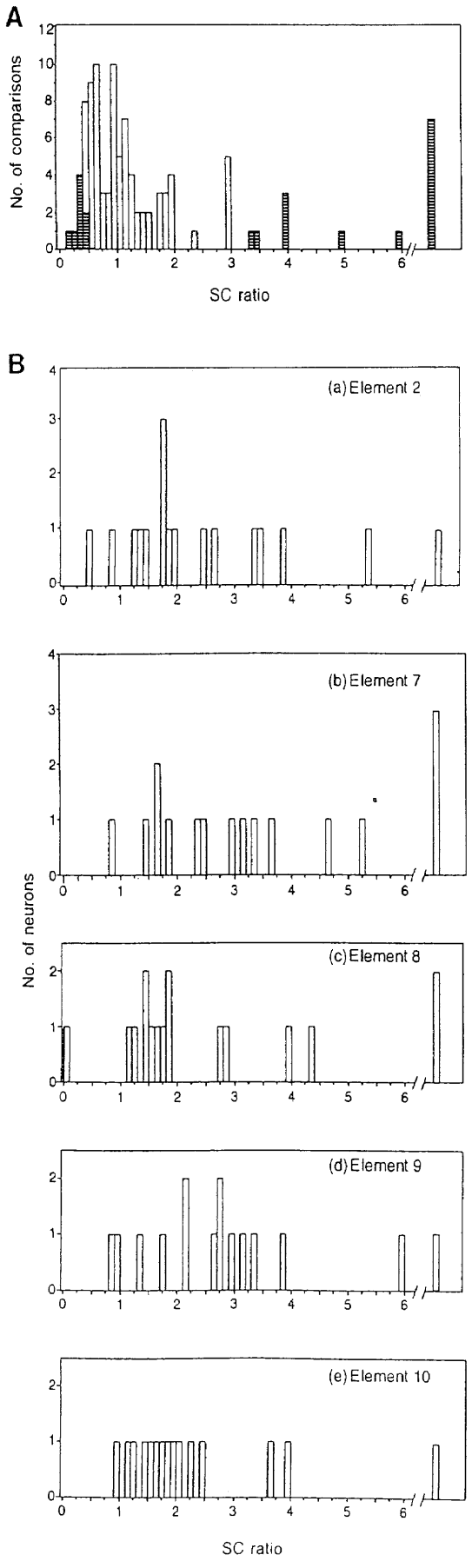


Fig. 2. The response of Field L neuron 0606. (**A**) The stimulus is the warble segment (the Segment). The PST histogram (top) is shown time aligned with the time waveform (middle) and the sonogram of Segment. The number of each element appears above the time waveform. Note that there is no or only weak response to most elements other than Element 6. (**B**) The stimulus is Element 2. Note the strong response to the element presented in isolation. For the other panels, the stimuli are Elements 7 (**C**), 8 (**D**), 9 (**E**) and 10 (**F**). Note the stronger response to Elements 7-10 than to the same elements in (**A**). All histograms show sequential 10-msec bins of the summed activity to 10 repetitions of the stimulus. The time bar represents 0.5 sec.



response to every element type (Element 2, 7, 8, 9 and 10), but others to only one element type. A total of 16 (62%) of the 26 neurons exhibited increased spike activity in response to elements in isolation compared with that to elements in the Segment. These 16 neurons were found in all three laminae of Field L. In one L3 neuron, on the other hand, the response to an element involved in the Segment was stronger than that to the isolated element (SC ratio < 0.5, Element 8 in Fig. 3B).

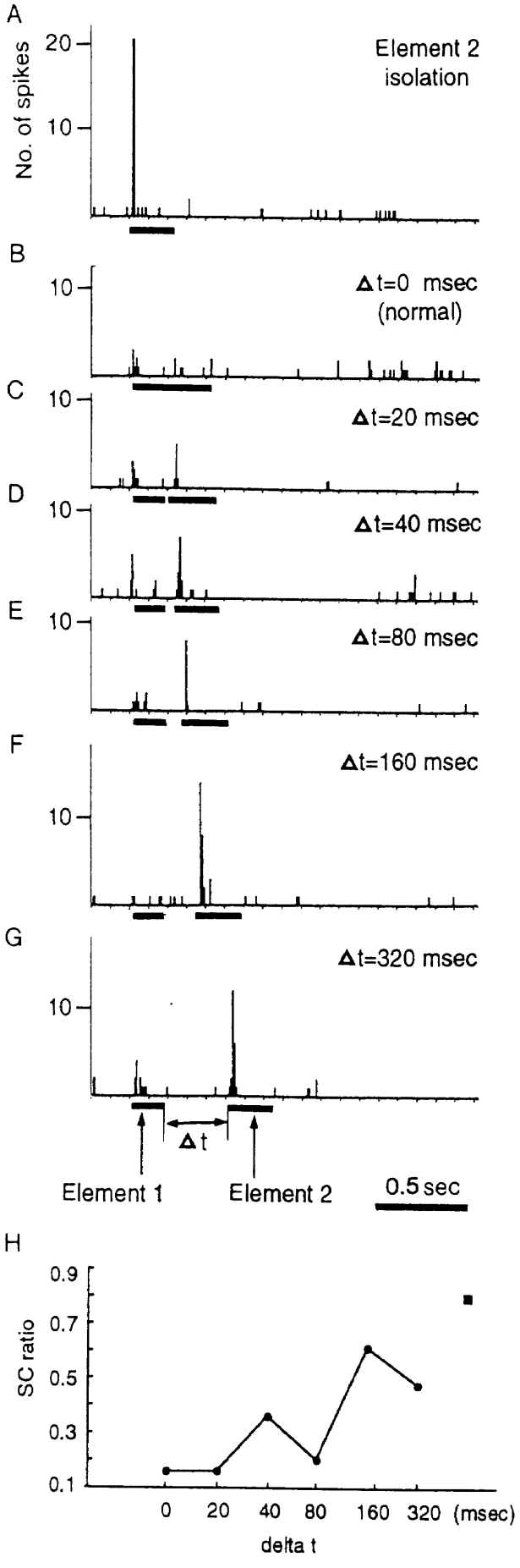
Experiment 2: Effects of preceding Element 1 on Element 2

We examined neurons' response to Element 2, when the interval of silence between the preceding Element 1 and Element 2 was systematically changed. Neuron 0702 exhibited a phasic on-response to Element 2 presented in isolation (Fig. 4A). This phasic response was suppressed when Element 1 preceded Element 2, with 0 msec of delta t (Fig. 4B). As the interval between the two elements was lengthened, the phasic on-excitation gradually recovered (Fig. 4C-G). The entire SC to Element 2 at 0 msec of delta t was decreased to 16% of that to isolated Element 2 and recovered to above 50% at 160 msec of delta t (Fig. 4H).

Five of the 14 neurons responding to Element 2 exhibited similar temporal suppression properties. In each of the five neurons the entire SC to Element 2 at 0 msec of delta t dropped to below 50% of that to isolated Element 2. The mean value of the entire SC ratios exceeded 50% at 80 msec of delta t (Fig. 5A). Since Element 1 did not influence the tonic part of the response to Element 2 but did affect the onset phasic part in some neurons, we analyzed the onset SC for the same 14 neurons. Onset SC to Element 2 at 0 msec of delta t was decreased to below 50% of that to isolated Element 2 in 13 neurons, including the five neurons mentioned above (Fig. 5B). On average, the suppressed response recovered to above 50% at 80 msec of delta t. Temporal suppression was especially evident in the range below 80 msec of delta t in the Field L neurons examined.

We examined relationship of Element-1-evoked responses to Element-2-evoked responses for the 14 neurons mentioned above. In some neurons response to Element 2 was suppressed after strong response to Element 1. On the other hand, five neurons were temporally suppressed without strong response to Element 1.

Fig. 3. (**A**) SC ratios of response to elements in the Segment presented again relative to the same elements in the initially presented Segment. Data for 101 comparisons (26 neurons) are shown. For most comparisons, SC was nearly the same in the two conditions. In the following analyses (**B**), we omitted 22 comparisons (solid bars) showing inconsistent response (SC ratios < 0.5 or > 3) or no response (SC of 0) for elements in the Segment. (**B**) SC ratios of response to elements presented in isolation in comparison to response to the same elements in the Segment. Each panel presents SC ratios from the neurons which showed nearly the same response to the identical element presented twice (open bars in **A**). (a) Element 2, (b) Element 7 (c) Element 8, (d) Element 9 and (e) Element 10.



DISCUSSION

Response to an element as affected by preceding elements

We found temporally suppressed neurons (TSN) with response to a warble element that was suppressed by a preceding element in Field L of the budgerigar. Overall structure of warble elements influenced the response of TSNs. Interval duration between elements affected the response of TSNs in Experiment 2.

Another type of effect of preceding elements or syllables on response to elements has been clarified in the vocal nucleus HVc in the dorsocaudal neostriatum of oscine songbirds (Margoliash *et al.*, 1994). Response of some HVc neurons to a syllable receives temporal facilitation from the preceding element. Uno *et al.* (1991) have demonstrated the presence of temporally facilitated neurons (TFN) in Field L1 and L3 of the mynah bird. Of 26 neurons in Experiment 1, only one L3 neuron exhibited stronger response to an element in warble song compared with that to the element in isolation. Response pattern of this L3 neuron of the budgerigar is similar to TFN of the mynah bird and might have been TFN.

A possible role of TSN in processing of vocalization

In Experiment 2, we examined the neuronal response to Element 2, when the silent interval between preceding Elements 1 and 2 was systematically increased. TSNs showed 50% recovery at 80 msec of delta t in entire SC and in onset SC. Although the functional significance of these neurons requires further clarification, a behavioral characteristic of the budgerigar is suggestive of a possibility. Budgerigars easily come to vocalize warble song after hearing others' songs (Brockway, 1968), but they respond with contact call after hearing contact call (Brockway, 1964b). Contact call is considered to aid in the coordination of synchronous group movement (Brockway, 1964b; Farabaugh *et al.*, 1994). One type of element resembling Element 10 is vocalized at longer intervals in contact call (Fig. 6). Eda-Fujiwara *et al.* (1995) measured the intervals between the onset of adjacent elements in warble song and contact call. The median interval was 260 msec in warble song and 1340 msec in contact call. A 260-msec interval between the onset of Element 1 and that of Element 2 corresponds to an 80-msec silent interval. The TSNs in Field L were suppressed in element intervals of warble song, but not in longer intervals as contact call. Thus the TSNs may play a role in vocal discrimination.

Fig. 4. The response of temporally suppressed neuron 0702. (**A**) Response of the neuron to Element 2 presented in isolation. (**B-G**) PST histograms in response to an Element 1-2 pair for different Δt values. Δt represents the silent interval between Elements 1 and 2 (shown in **G**). In **A** to **G**, the time bar represents 0.5 sec. (**H**) SC ratios obtained from the PSTH above are plotted as a function of Δt. ■ represents the SC ratio of response to the isolated Element 2 presented again to the response to the isolated Element 2 presented first.

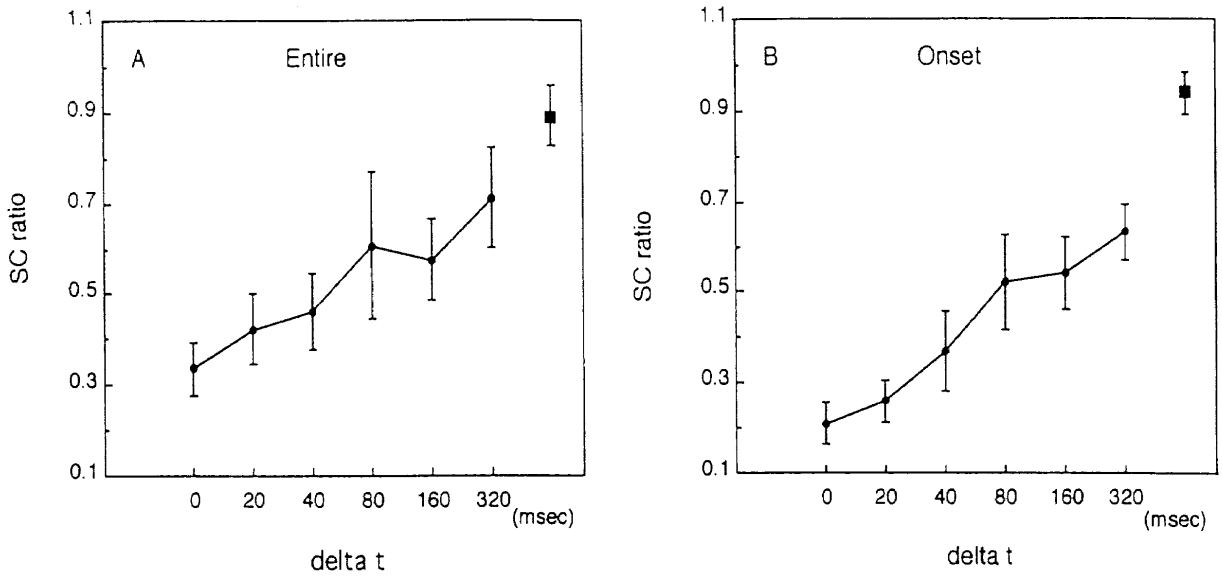


Fig. 5. Average SC ratio as a function of the length of a silent period (delta t) preceding Element 2. Spikes were counted for the entire duration of Element 2 (**A**) and for 50 msec after the Element 2 onset (**B**). ● represents mean for 5 units (**A**) and 13 units (**B**) whose SC ratios in 0 msec of Δt dropped below 0.5. Vertical bar represents standard error, and ■ represents SC ratio of response to the isolated Element 2 presented again relative to response to isolated Element 2 presented first.

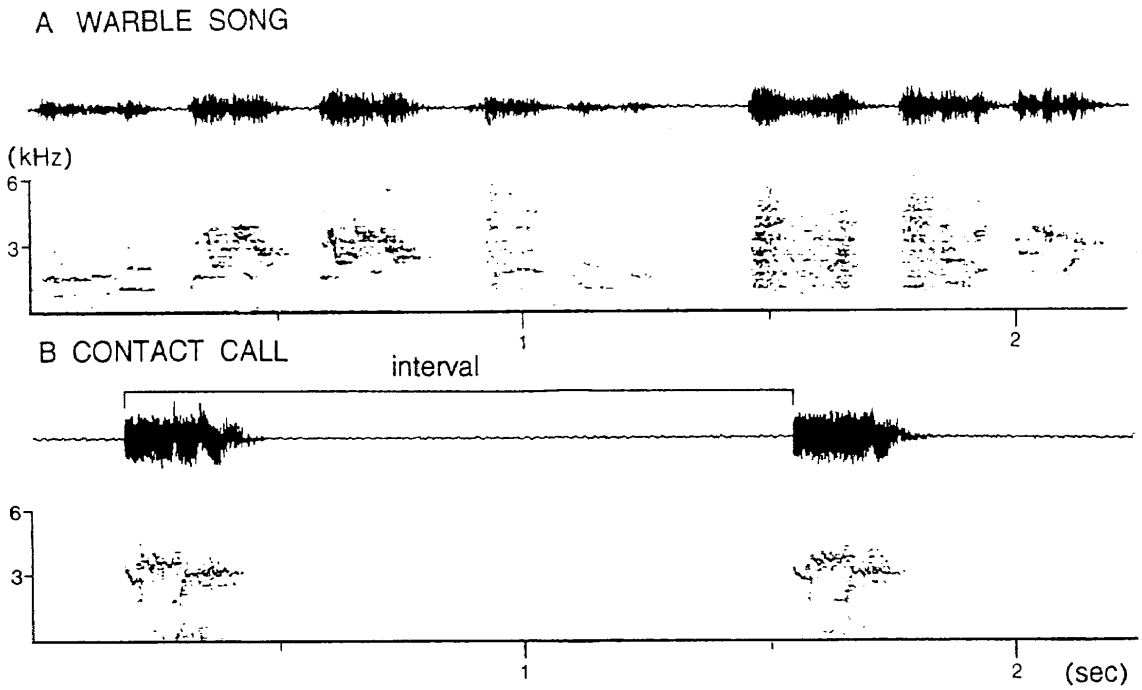


Fig. 6. Oscillograms (upper) and sonograms (lower) of warble song (**A**) and contact call (**B**).

Mechanism of the TSN responses

The phenomenon that a preceding sound (a masker) suppresses the response to a subsequent sound (a probe) is known as forward masking. Extensive studies of forward masking have been done on the auditory nerve in mammals (Smith, 1977; Harris and Dallos, 1979; Relkin and Turner, 1988). Forward masking of responses in auditory nerve fibers has been interpreted as a peripheral adaptation (Smith, 1977; Harris and Dallos, 1979). Auditory nerve fibers show a monotonic decrease in firing rate in response to the probe as the firing rate to the masker increases (Harris and Dallos, 1979). Studies in the cochlea nucleus complex (CNC) of the medulla oblongata showed that central auditory responses also exhibit forward masking (Watanabe and Simada, 1971; Boettcher *et al.*, 1990; Kaltenbach *et al.*, 1993; Shore, 1995). Relationship of probe-evoked responses to masker-evoked responses was examined in the CNC neurons of guinea pigs using paired tones separated by delta t of 100 msec (Shore, 1995). In contrast with auditory nerve fibers, some neurons show the decrement in the response to the probe which is

unrelated to the increment in response to the masker. This non-monotonic relationship is suggestive of the involvement of inhibitory process in addition to simple peripheral adaptation.

In Field L of the telencephalon we, for the first time, found neurons in which the response to the probe was suppressed without response to the masker. Since these neurons exist in central nervous system, the suppression without masker-evoked response could be attributed to the same mechanism as that in the mammalian CNC. The responsiveness of these neurons may involve inhibition in addition to adaptation.

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