Echo-Detecting Characteristics of Neurons in Inferior Colliculus of Unanesthetized Bats

Abstract. *Neurons in the inferior colliculus of echolocating bats responded well to two stimuli presented in close temporal sequence. Favorable recovery of responsiveness was seen with stimuli having durations, intensities, and interpulse intervals similar to the natural biosonar signals reaching the ears during the various phases of echolocation. Some units responded to a subthreshold simulated echo but only when preceded by a loud initial pulse. These units appear to be specialized for echo-detection.*

Many bats of the suborder Microchiroptera orient in the environment, capture prey, and avoid obstacles by emitting loud frequency-modulated (FM) pulses and listening to the returning echoes, a form of biological sonar called echolocation (*1, 2*). First detecting objects at about 0.7 to 3.0 m, these animals must perceive faint echoes within milliseconds after the emission of a loud pulse and continue to derive echo information at progressively shorter time intervals as the target is approached (*1–3*).

The bat's auditory system must be capable of extracting requisite information from a series of pulse-echo combinations in which the interval between the emission of a pulse and the return of the echo to the ear is continuously decreasing. Neurophysiological studies indicate that many neurons in the nucleus of the lateral lemniscus respond well to the second of two tone pips presented in close temporal sequence (*4, 5*). Some units are remarkable in that mechanisms interposed somewhere between the auditory nerve and lateral lemniscus can actually amplify the response to a simulated echo, provided the sound returns within a specified time slot.

In contrast to neurons in the lateral lemniscus, units in the inferior colliculus generally responded poorly to the second of a pair of equally intense tone bursts when both signals were 70 to 80 db. In systematic studies, the vast majority of collicular units exhibited little or no recovery for periods of up to 20 msec unless the simulated echo was 10 to 20 db more intense than the pulse (*5, 6*), conditions surely rare in nature. Indeed, no collicular unit has been reported to have recovered well, even at long interpulse intervals (IPI's), when the first signal or simulated orientation cry was 70 to 80 db and the second signal or simulated echo was 40 to 50 db, signal intensities comparable to those received by bats during much of the echolocation cycle.

The neurophysiological studies described above are at once intriguing and puzzling. While exquisite specialization is found in the lateral lemniscus, an exceptionally large percentage of neurons in the inferior colliculus seem to be poorly suited for processing biosonar information, yet the central nucleus of the inferior colliculus is essential for echolocation (*7*). The question, therefore, is how the neurophysiological facts can be reconciled with the known behavioral capabilities of the animal and the requirement of intact colliculi.

Two methodological features of the previous experiments are important in this regard. (i) They were conducted on anesthetized preparations. Recent studies in the medial geniculate and inferior colliculus (*8*) of awake cats indicate that a barbiturate can enhance inhibitory activity, which suggests that recovery may have been adversely affected by drug action. (ii) In all previous recovery studies stimuli were of very short duration, typical of pulse durations emitted during the terminal phase of flight. During echolocation not only is the temporal relation of the pulses and echoes changing but the durations and intensities of the biosonar signals are also in dynamic state of change (*9*). Perhaps, as Friend *et al.* (*6*) have suggested, the colliculus is required for the search and approach phases of echolocation when pulse durations are relatively long, and only a small population of cells is responsive to the short pulses emitted during the terminal phase, stimuli to which neurons of the lateral lemniscus respond favorably.

We report here recovery data recorded from single neurons in the inferior colliculus of fully awake bats with stimuli designed to simulate the biosonar signals received during the various phases of echolocation. Seven Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, were prepared under Metofane anesthesia (Pitman-Moore, Inc., Washington Crossing, New Jersey). The skull was exposed and acrylic applied to the cranium as described by Henson and Pollak (*10*). Wisps of cotton soaked in procaine were placed on all open wounds. An indifferent electrode was placed in the skull overlying the cerebellum and cemented in place, and a small hole drilled over the inferior colliculus (*11*). The bat was placed in a Plexiglas chamber and the head was braced adequately by three metal rods cemented to the skull which allowed for a clear sound field to the ears. The bottom of the Plexiglas chamber had Nichrome wire loops placed in machined grooves which served as heating elements maintained at 34°C. Rectal temperature was monitored with a microthermocouple connected to a digital thermometer and was always 36° to 38°C. The bats tolerate these procedures well and recover within a few minutes after removal of the anesthetic (*12*).

Experiments were conducted in a small room with walls and ceiling covered with fiberglass to reduce echoes. Stimuli were delivered through a condenser loudspeaker (*13*) positioned about 36 cm from the bat's ear and oriented 45 degrees from the midline on the side contralateral to the exposed colliculus. Sound pressure levels were measured with a ¼-inch B & K condenser microphone placed where the bat's head had previously been, a technique similar to that of others (*4–7*). All sound intensities are expressed in decibels above 0.0002 dyne/cm^2 root-mean-square.

Two tones or FM bursts were generated by oscillators, pulse generators, and electronic switches. The timing of both tones was controlled by separate timers, and counters permitting the generation of a predetermined number of events and variation of the IPI, defined as the time separation between the beginning of the first tone (T_1) and the beginning of the second (T_2). All tone or FM bursts had 0.5-msec rise and fall times, and the frequencies of both oscillators were continuously monitored with two frequency counters.

Glass micropipettes filled with 1*M* NaCl with impedances of 5 to 10 megohms were visually placed on the colliculus. Action potentials from individual neurons were amplified with a high-input impedance amplifier and then sent to an oscilloscope, loudspeaker, and discriminator. The square pulses generated by the discriminator were fed to the *Z* axis of a second oscilloscope for brightening.

Extensive recovery behavior was examined in 30 neurons, each studied for at least 1 hour but more often for 2 to 4 hours. All units exhibited phasic "on" firing patterns. With most units the following stimulus parameters were varied: (i) the intensity ratio of the simulated pulse-echo pair from just below threshold to about 40 to 60 db above threshold, (ii) the stimulus duration from about 7 to 1.7 msec (*14*), and (iii) the values of all the above parameters for a number of selected IPI's (*15*). These experiments yielded 200 to 1000 frames for each unit examined, a frame being the photo-

graphed dot display generated by 16 presentations of a unique combination of stimulus parameters.

In general, four types of recovery patterns were observed. (i) In the poor recovery pattern the unit was not responsive to the second stimulus except at long IPI's. This pattern was usually seen when stimulus durations were 1 to 2 msec. (ii) In the independent recovery pattern the presentation of an initial signal had little or no effect upon the firing pattern evoked by a second signal when the initial signal was as high as 70 to 80 db, the intensity of the second signal was 2 to 7 db above threshold and greater, and the silent interval between the end of the first signal and beginning of the second was either long or as short as 0.5 to 0.0 msec. This pattern was observed in 20 of the 30 neurons sampled and was by far the most common form of recovery (Fig. 1, a and c). (iii) The selective recovery pattern was typified by poor recovery except at one particular combination of stimulus parameters at which recovery was dramatically improved (Fig. 1b). As was the case for the independent recovery pattern, recovery only occurred when the second stimulus was above threshold intensity, and was always observed when the first stimulus was 20 to 30 db more intense than the second. (iv) In the pulse-enhanced recovery pattern, a much louder initial stimulus facilitated firing in response to a second subthreshold stimulus (Fig. 2).

A given neuron did not necessarily fall into just one of the above types; rather, a unit would commonly exhibit different types of recovery depending upon stimulus conditions. For example, unit T-12-5-1C (Fig. 1) exhibited poor recovery with 1.7-msec stimuli, independent recovery with 6.9-msec pulses (Fig. 1a), and selective recovery with 3.4-msec pulses (Fig. 1b). This shows that a neuron may well be suited for processing pulse-echo information only during certain phases of echolocation and poorly adapted for others.

Of particular interest were the units exhibiting the pulse-enhanced type of recovery. These neurons responded favorably when both stimuli were above threshold. This pattern, however, was distinguished from independent recovery by firings in response to a subthreshold

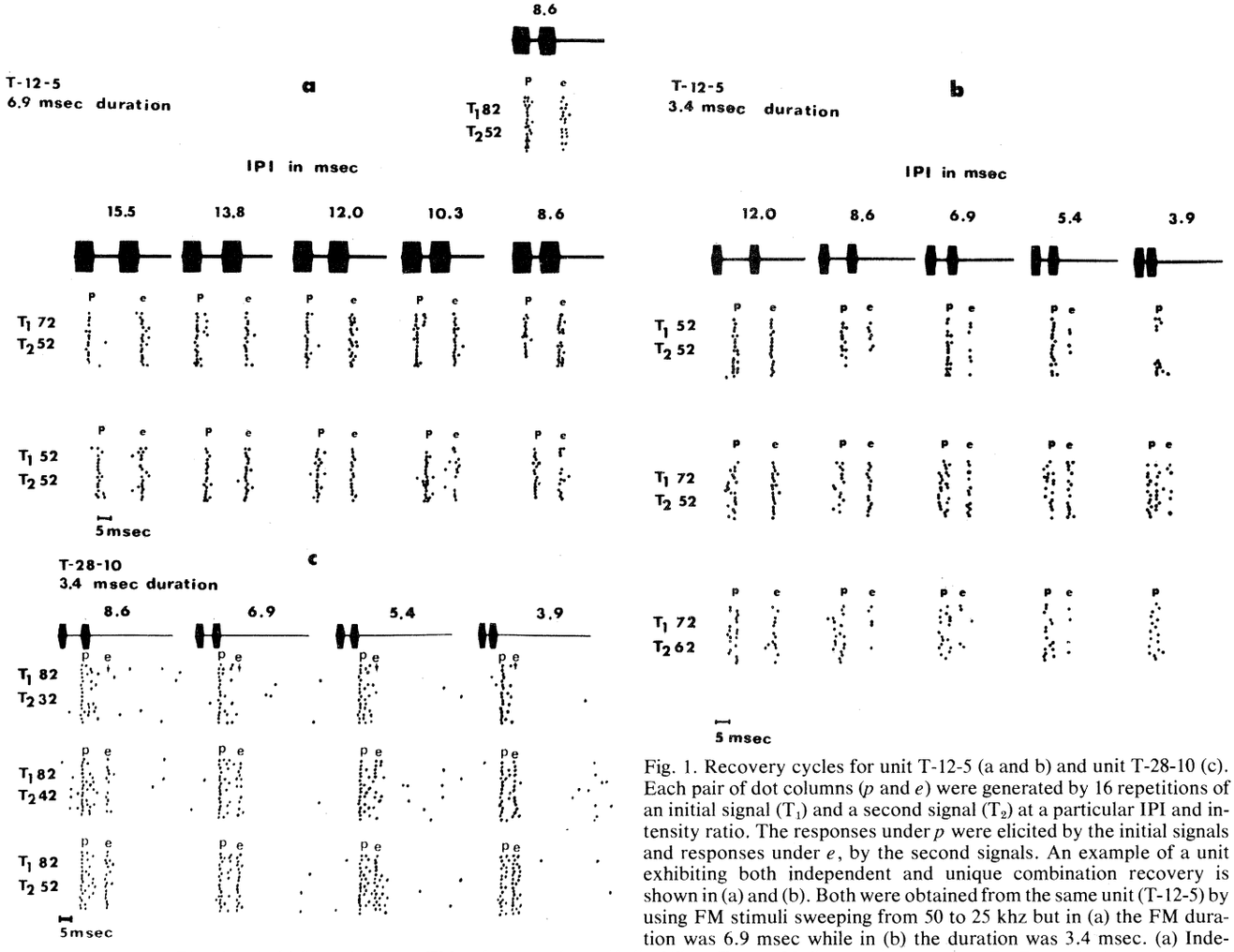

Fig. 1. Recovery cycles for unit T-12-5 (a and b) and unit T-28-10 (c). Each pair of dot columns (*p* and *e*) were generated by 16 repetitions of an initial signal (T_1) and a second signal (T_2) at a particular IPI and intensity ratio. The responses under *p* were elicited by the initial signals and responses under *e*, by the second signals. An example of a unit exhibiting both independent and unique combination recovery is shown in (a) and (b). Both were obtained from the same unit (T-12-5) by using FM stimuli sweeping from 50 to 25 khz but in (a) the FM duration was 6.9 msec while in (b) the duration was 3.4 msec. (a) Independent recovery. The threshold for a 6.9-msec FM pulse was about 42 db. When T_1 was 20 db more intense than T_2 (T_1 = 72 db; T_2 = 52 db), the neuron responded consistently to T_2 at all IPI's. A similar result was obtained when T_1 was 82 db and T_2 was 52 db (top right). When both stimuli were about 10 db above threshold (T_1, T_2 = 52 db), the unit was equally responsive to both signals. (b) Selective recovery. Recovery was consistently poor for IPI's of 8.6 msec or less except when T_1 was 72 db and T_2 was 52 db. Under this latter condition, recovery was excellent for IPI's as low as 5.4 msec and the unit even responded to T_2 at 3.9 msec but not quite as well. Reducing the intensity of T_1 by 20 db (T_1, T_2 = 52 db) did not improve recovery at the shorter IPI's; on the contrary, recovery deteriorated. Furthermore, increasing the intensity of T_2 by 10 db (T_1 = 72 db; T_2 = 62 db) also resulted in poor recovery. (c) Independent recovery pattern for unit T-28-10. All signals were 3.4-msec downward-sweeping FM pulses (50 to 25 khz). Threshold for a singly presented 3.4-msec FM pulse was 36 to 37 db. In top recovery cycle (T_1 = 82 db; T_2 = 32 db) the second signal was 4 to 5 db below threshold and the unit fired only in response to the initial signal. Arrows under *e* indicate where responses to second signals should have occurred. In middle recovery cycle the initial signal was 82 db and the second signal was 42 db (5 to 6 db above threshold). Under these conditions the unit consistently fired in response to T_2 even at the 3.9-msec IPI. A similar recovery pattern is seen when the second signal was 52 db (lowest recovery cycle).

second stimulus contingent upon the presentation of a much louder first stimulus.

Unit T-24-7-IC illustrates some of these features. The threshold of this neuron for response to a 26-khz tone burst was about 15 db (Fig. 2C). At low stimulus intensities it responded with a phasic "on" response but at high intensities it responded with a vigorous "on" response and a more variable "off" response (Fig. 2A). In Fig. $2A_1$ three control runs are shown in which 80-db tone pulses were presented alone, that is, without a second tone. When the same 80-db initial tone was followed shortly by a second tone, the neuron fired in response to both the first and second tones (Fig. 2, A_1 and A_2). The second tones or simulated echoes had intensities of 15 to 5 db, which are subthreshold and, in fact, close to the absolute threshold of hearing.

That the firing in response to a subthreshold second stimulus was contingent upon a loud initial stimulus is shown in Fig. 2B. When the initial tone was 70 db, the neuron failed to respond consistently to a second subthreshold stimulus, although occasional firings were seen.

Three neurons in our sample exhibited pulse-enhanced recovery and are noteworthy for several reasons.

1) Each responded to subthreshold simulated echoes having intensities of 5 to 20 db, but these responses were only observed if the 26-khz initial tone bursts were 70 to 80 db. This provides evidence that the bat's auditory system can respond to echoes as faint as 5 db shortly after the emission of a loud emitted pulse. Griffin (*1*) calculated that under natural conditions bats should be able to first detect insects in the open air with echoes of about 17 db, and our data show that the bat's nervous system does indeed possess the required sensitivity. Moreover, previous studies in which cochlear microphonic responses elicited by the bat's own emitted pulses were monitored showed 65 to 85 db to be the range of intensities that actually stimulate the bat's ear upon emission of loud pulses (*15*). The correlation between the pulse intensity reaching the ear, the echo intensity expected at the point of initial detection, and the behavior of pulse-enhanced responders is striking.

2) The fact that these neurons exhibited pulse-enhanced recovery when stimulated with 26-khz tone bursts has biological relevance. Many bats, including the species used in this study, tack a constant frequency component onto the FM sweep; this is especially prominent during the search phase of flight (*1, 16, 17*). Moreover, it was suggested on theoretical grounds that the constant frequency component serves to increase detection range (*17*). This is precisely the function suggested for the pulse-enhanced responders, and 26 khz is the frequency of the constant frequency component used by *Tadarida* (*16*). We argue, therefore, that these units seem to be specialized for echo detection.

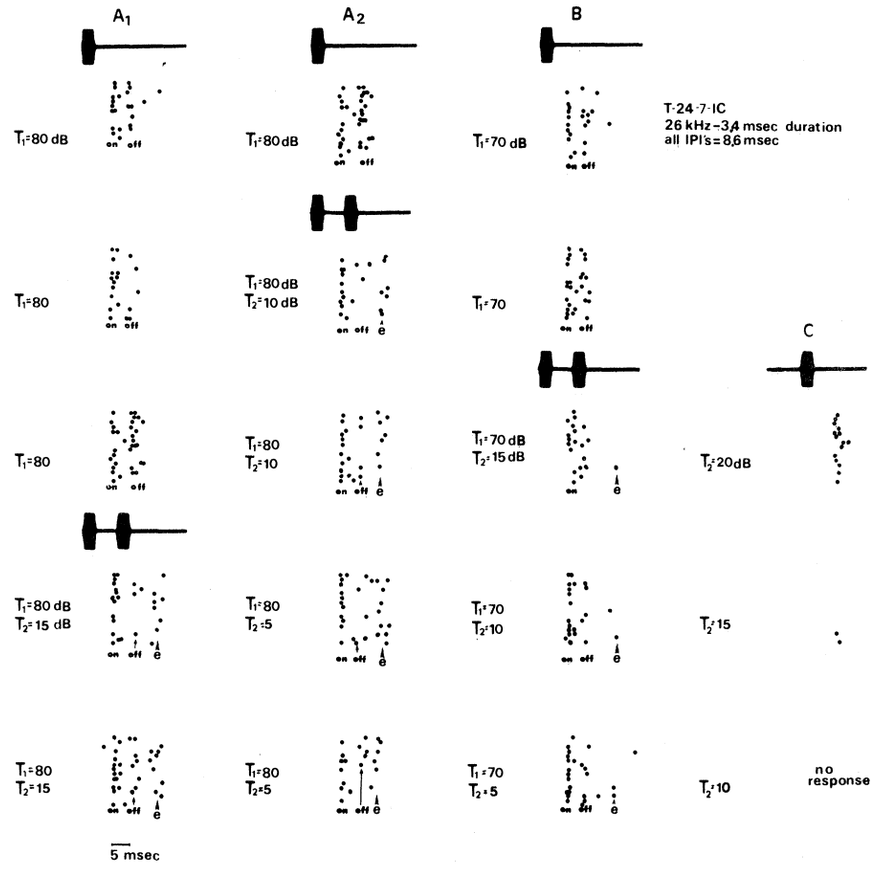

Fig. 2. Pulse-enhanced recovery, unit T-24-7-IC. All tone bursts are 26 khz and 3.4 msec in duration. (A_1) At top are responses to tone bursts at 80 db presented alone. Firings to the onset (on) and offset (off) of the tone burst are indicated. At bottom are responses to initial tone bursts (T_1) at 80 db followed 8.6 msec later by tone bursts at 15 db (T_2). Firings in response to the second tone (*e*) are indicated. (A_2) At top is the response to an 80-db tone burst presented alone. At bottom are responses to 80-db tone bursts followed 8.6 msec later by tone bursts at 10 and 5 db. Each condition is presented twice. There are consistent firings in response to second tones although these tones elicited few or no firings when presented alone. (B) At top are responses to 70-db tone bursts presented alone. Below are firing patterns when 70-db tones are followed 8.6 msec later by second tone bursts at 15, 10, and 5 db. There is no consistent firing in response to the second tone under these conditions. (C) Responses elicited by second tone at 10, 15, and 20 db when presented alone. The threshold of the neuron was considered to be about 15 db.

These data support the idea of a primary role for the colliculus during the search and goal-directed portions of flight. With few exceptions, every unit examined in the colliculus was well suited for responding to pulse-echo combinations at intervals within the range of echo detection suggested by behavioral studies and at pulse-echo intensities expected to reach the ear during echolocation.

Our results differ significantly from others for reasons that are at least partially understood. The use of an anesthetic was probably an important factor. There is strong evidence that recovery in the somatosensory, visual, and auditory systems (*18*) is substantially lengthened under barbiturate anesthesia. Also, in previous studies the recovery ability of collicular neurons may have been overlooked due to the employment of one, typically short stimulus duration.

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9. Bats change pulse designs throughout the various phases of echolocation (*1–3*). For bats whose orientation cries are composed primarily or exclusively of an FM component, the following changes have been observed in both laboratory and field studies. During the search phase, as the bat cruises with no apparent target holding its attention, pulses are relatively long (3 to 12 msec) and are emitted at a rate of about 10 per second. In the approach phase of goal-directed flight, the location, distance, size, and other target attributes are presumably evaluated. During this period the tempo of pulse emission increases and pulse durations are shortened, which precludes any overlap of the outgoing pulse and returning echo. In the terminal phase, the repetition rate can be as high as 200 pulses per second with pulse durations continuing to shorten as the bat holds its course for final interception. During this last 100 msec or so of goal-directed flight, pulse durations can be as short as 0.5 msec.
10. O. W. Henson, Jr., and G. Pollak, *Physiol. Behav.* **8**, 1185 (1972).
11. The inferior colliculus is greatly hypertrophied in *Tadarida* and can be seen through the thin skull as two white masses protruding between the cerebral cortex and the cerebellum.
12. In the Plexiglas restraining chamber, signs of an awake condition include (i) frequent spontaneous movements of the shoulders, wings, feet, and mouth, which differ from extreme struggling indicative of pain; in such cases, refreshing the cotton wisps with additional procaine results in a cessation of struggling movements; (ii) reflex withdrawal upon gently poking the wing or shoulder; and (iii) overt, coordinated drinking when water is applied to the lips with a dropper.
13. Designed as described by W. C. Kuhl, G. R. Schodder, F. K. Schroder, *Acoustica* **4**, 519 (1954).
14. In five units only one or two durations were tested. In three other units 0.86-msec pulses were used in addition to the longer durations.
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16. All stimuli were designed to mimic the natural biosonar cries of the bat. *Tadarida* emit pulses with durations ranging from about 9 to 0.5 msec. The first harmonic of the FM cries typically sweep from about 50 to 24 khz over the duration of the pulse. Constant frequency components of 26 khz having durations of several milliseconds are often present during the search phase of echolocation. The biosonar signals of *Tadarida* (family Molossidae) are similar to those of the big brown bat, *Epesicus fuscus* (family Vespertilionidae) (J. A. Simmons, personal communication).
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Pontine Reticular Formation Neurons: Relationship of Discharge to Motor Activity

Abstract. *The discharge correlates of pontine reticular formation units were investigated in unrestrained cats. In agreement with previous investigations using immobilized preparations, we found that these cells had high rates of activity in rapid eye movement sleep, and responded in waking to somatic, auditory, and vestibular stimuli at short latencies, many having polysensory responses and exhibiting rapid "habituation." However, despite the sensory responses of these cells, most unit activity could not be explained by the presence of sensory stimuli. Intense firing occurred in association with specific movements. Units deprived of their adequate somatic, vestibular, and auditory stimuli showed undiminished discharge rates during motor activity. Discrete sensory stimuli evoked sustained unit firing only when they also evoked a motor response. We conclude that activity in pontine reticular formation neurons is more closely related to motor output than to sensory input.*

Studies of pontine reticular formation (PRF) units, usually performed in anesthetized, decerebrated, or immobilized preparations, have emphasized the sensory responses of these cells (*1, 2*). It has been repeatedly demonstrated, in experiments using both natural and electrical stimulation of sensory systems, that many of these neurons are polysensory and show response attenuation with stimulus repetition (*2*). The present investigation studied the discharge correlates of medial PRF units in unrestrained, behaving cats. We find that most unit discharge does not result from sensory stimuli impinging upon the animal, but rather relates closely to the cat's motor activities.

Units were studied with previously described recording and sensory stimulation techniques (*3*). All cells considered in this study were localized to the pontine gigantocellular tegmental field ("FTG") (*4*), one of the most commonly explored areas in unit studies of the PRF (*1–3, 5*). A total of 70 units were recorded in nine cats.

In the waking cat, most PRF units were found to have a low level of background activity intermixed with phasic bursts of discharge that occurred in conjunction with spontaneous movements (Fig. 1A).

Table 1. Unit activity rates (discharges per second) before, during, and after a 3-minute period of stimulus reduction. The units' discharge rates were not reduced by the procedure. The standard deviation is that of the arithmetic mean.

Parameter	Before	During	After
Geometric mean	1.00	8.32	8.33
Arithmetic mean	4.94	11.94	12.59
Standard deviation	7.82	8.65	8.57

A subset of these units ($N = 21$) belongs to the previously described neuronal group which shows no background activity in waking or sleep (*3*). The remaining cells were active in sleep, particularly during phasic discharge bursts occurring in rapid eye movement (REM) sleep.

Unlike studies in acute preparations, which have reported a high proportion of unresponsive units (*1, 2*), we found that all PRF cells showed phasic discharge bursts during active waking. Sensory responses were examined in detail. Seventy-one percent of the PRF cells responded to somatic stimuli, 57 percent to vestibular stimuli, 40 percent to auditory stimuli, none responded to stroboscopic visual stimuli, and 57 percent were polysensory (*6*).

Discrete 0.5-msec shock stimuli were applied to the receptive fields of units responding to natural somatic stimuli. No response occurred in 4 of the 12 cells tested, the remaining units responding at a latency between 14 and 40 msec, in agreement with previous studies (*1, 2*). However, only one or two unit firings could be elicited by this stimulus, even at an intensity which produced a clear muscle twitch. Similarly, discrete auditory stimuli evoked responses between 15 and 33 msec, but never elicited more than two spike discharges. When the intensity of the somatic shock was raised to a level at which a behavioral response was evoked, a more sustained discharge occurred, but this was time-locked to the motor activity, rather than to the stimulus (Fig. 1B).

While natural stimuli could repeatedly elicit unit activity, the duration and intensity of unit discharge was not closely related to the eliciting stimulus. For example, stimulation applied by placing a cotton swab in the concha of the ear produced intense discharge in three PRF cells. However, the discharge normally continued with undiminished rate for 1 to 10 seconds after the swab was removed from the ear, ending abruptly with a change in ear position. Rhythmic movement of the stimulus did not produce rhythmic discharge in the unit. Furthermore, in most PRF units we observed discharges at rates equal to or exceeding those induced by our most effective stimuli during small, spontaneous movements. These observations suggested the hypothesis that unit discharge was related to motor activities. Therefore, we observed 35 units for periods of at least 2 hours, during active waking. We were able to identify consistent, individually distinctive motor correlates of discharge in 32 of the cells. These included head and neck ($N = 21$), ear ($N = 3$), forepaw ($N = 1$), scapula ($N = 2$), tongue (N