

in evaporative cooling. His observations were made in June and July, when maximum air temperature in the shade reached 41°C. In our study at the same time of the year, the maximum outdoor air temperature was 31° to 32°C. Since our birds were very active during the midday hours, the activity reduction in the field may be a specific response to high temperatures, rather than an invariable feature of the activity pattern (that also prevails in cool weather). The tendency of the activity parameters to peak before sunset may be indicative of enhanced late afternoon activity in the field, possibly related to heavy predation on insects. The low level of early morning activity correlates with the known propensity of roadrunners to sunbathe at this time (14).

The inactivity of our birds during outdoor twilights and during indoor early dawn, late dusk, dim light, and darkness suggests that the visual system of the roadrunner is unsuited to dim-light vision. The fact that the birds almost always ran during the bright-light periods and always retired during the dim periods of a continuous cycle of 3-hour "days" suggests, at the least, that light conditions are highly restrictive for roadrunners and that sleeping birds are "aware" of marked changes in the ambient light level. Two findings suggest that roadrunner activity tends to increase with increasing light level: (i) greater activity on clear than on overcast days; and (ii) 56 percent greater activity of bird No. 1 at 3000 lux than at 200 lux in half-hour alternation tests.

Since roadrunners will accept an activity wheel as an outlet for locomotion, the way may be open for quantitative comparative studies of the influences of environmental factors on bird activity (15), at least for other running birds. The roadrunner may be an extreme diurnal case with pure-cone vision and little or no summation in the retina. Nocturnal birds and diurnal birds with some rods in their retinas may, like many mammals, show a greater range of responses to changes in ambient light intensities.

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8. The light intensities were measured at the bottom of the wheel, 125 cm from the incandescent lights and 185 cm from the nearest fluorescent lights. The 200-lux value (Spectra Candela X-100 lightmeter) is for a 100-watt incandescent bulb supplemented by 480 watts of ceiling fluorescent lamps. In some experiments one or two 150-watt General Electric cool-beam (PAR38/2FL) or regular (PAR/FL) flood lamps were used (about 1500 lux per lamp). The night light was a General Electric No. 44 incandescent bulb energized at 1.5 volts.
9. The sunrise and sunset times referred to are the arbitrarily defined (although exactly definable) and biologically unimportant almanac times (unobstructed horizons and zero elevation above the earth's surface). The use of astronomical sunrise and sunset as reference times would be more pertinent, since these are the times of beginning and ending of the sun's contribution to the light level, that is, the ending and beginning of the night. The astronomical times in Los Angeles during June and July occur roughly 43 to 49 minutes earlier for sunrise and 49 to 51 minutes later for sunset.
10. J. L. Kavanau, *Ecology* 44, 95 (1963).
11. Bird No. 2 exceeded 90 percent unidirectional-

ity on only 1 day; bird No. 1 exceeded 80 percent on only 4 days.

12. When a 150-watt cool beam was alternated with a regular flood lamp (1500 lux each), the magnitudes of the three measured activity parameters were slightly greater in the cool beam, possibly in part because the animal tended to spend more time perching on the axle in the sunning posture in the warmer light of the regular flood lamp.
13. Although the bird followed the 3-hour "day" closely with regard to times of activity and inactivity, its main activity was concentrated in a period of 10 to 14 hours that tended to begin later each day and was 9 hours out of phase with the previous rhythm after 84 hours of continuous cycles. This suggests that the animal began to shift to a 26- to 27-hour endogenous rhythm once the 24-hour exogenous light cycle ceased.
14. A. C. Bent, *Life Histories of North American Cuckoos, Goatsuckers, Hummingbirds, and Their Allies* (Dover, New York, 1964).
15. In the past, quantitative studies of bird activity in the laboratory have depended heavily on recording from spring-mounted perches and other substrata, which register all kinds of minor movements.
16. The percent active time for the last point for bird No. 2 outdoors is corrected to allow for the time when activity ceased, rather than being computed for the full hour.
17. Supported by NSF grant GB 7750 and by a NASA training grant to J.R.
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## Temporal Summation and Refractoriness in Hypothalamic Reward Neurons as Measured by Self-Stimulation Behavior

**Abstract.** *A neurophysiological technique of double-pulse stimulation has been applied to freely moving rats with chronic indwelling electrodes in the hypothalamic reward area. Self-stimulation thresholds, measured as a function of the interpulse interval, generated curves with time constants characteristic of refractory periods and temporal synaptic summation. The results indicate a way of studying central neuronal processes for which the overt behavior of the animal is the dependent variable.*

It is generally true of the overt behavior of an organism that the strength of a response varies as some function of the rate and pattern of the stimulus eliciting it. Similarly, it has been shown that a complex relationship, termed the neural poststimulation excitability cycle, exists between (i) the rate and pattern in which neurons and synapses are stimulated and (ii) their ability to transmit this information. Since overt behavior is mediated by way of neural pathways, it seems plausible that the response strength-stimulus rate functions for a given type of overt behavior and for the behavior of the neurons and synapses in these pathways might be the same in shape. Kestenbaum, Deutsch, and Coons (1) have, indeed, obtained evidence that such is the case at least in the pain system. The purpose of this study was to ascertain whether the isomorphism between strength-rate functions for overt and for neuronal behavior holds for a reward system as well.

The general plan involved implanting

electrodes in the medial forebrain bundle of the hypothalamus, a structure which rats find rewarding to stimulate (2). Through these electrodes various patterns and rates of brief electrical pulses could be administered as samples of what the rat could then administer to himself by pressing a lever. With any fixed number of reinforced presses per minute defined as the overt behavior criterion of reward, the intensity of current required to elicit this criterion at each given rate or pattern of stimulation became the response-strength measure for generating the strength-rate function holding for self-stimulation behavior. Since the expectations regarding the shape of such a function were that it should resemble the shape of the excitability cycle already established for individual neurons and synapses, certain predictions were possible against which to test the validity of these expectations. For example, the more rapidly a neuron is stimulated, the more likely it is that the postsynaptic neuron will fire, because the neurotransmitter

in the synapse summates faster than it can be destroyed; thus, the strength of the electrically elicited overt behavior should be greater as the interval between stimulus pulses is shortened. However, since neurons cannot fire to all the stimulus pulses if they follow each other too closely, because neurons require a time to recover after each firing (the refractory period), the strength of the electrically elicited overt behavior should not continue to increase indefinitely as the interval between stimulus pulses is shortened, but should even decrease at those intervals falling within the refractory period. The details of our test of these predictions follow.

The techniques employed in our experiment were based upon Sherringtonian procedures of neurophysiology, in which a pair of 0.1-msec negative-going electrical pulses (one being the conditioning or *C* pulse, whose onset is followed at a parametrically varied interval by another, the test or *T* pulse) has been used as a probe to explore the poststimulation excitability cycle of axons and synapses in the peripheral nervous system. In our experiment, however, in which the aim was to probe the central neural pathways mediating self-stimulation behavior, we adopted a modified *C-T* technique employed by Deutsch (3), who administered trains of such *C* and *T* pulses rather than only a single pair. This departure from the classical technique was necessary because administering a single pulse pair in the self-stimulation situation does not elicit overt behavior. However, as later demonstrated, the pattern of response as a function of the size of the *C* to *T* (*C-T*) interval was the same for overt behavior when a train of pulse pairs was used as the pattern that has been recorded in cells when a single pair of pulses was used.

The measure of the behavioral strength as a function of size of *C-T* interval was the threshold level of current intensity, in microamperes, just necessary to elicit a criterion rate of lever pressing. For each *C-T* interval tested this threshold was determined by the method of limits. Just before each trial the animal was given, with no lever present, a 20-second sample of pulse pairs at the current intensity at which he would later be able to stimulate himself once the trial began. The animal then had access to a lever for 1 minute during which each lever-press produced a 5-second train of pulse pairs. These trials were separated by 1-minute rest

intervals. The current level for any given trial and the sample beforehand was determined by whether or not criterion was met on the preceding trial; current level was increased 60  $\mu$ a following failure to meet criterion and decreased 60  $\mu$ a following success. Threshold was defined as the second time in this sequence of trials that the animal met the criterion. To ensure that the strength-rate function so obtained was not idiosyncratic to the criterion chosen, two criteria were employed: five presses per minute and seven presses per minute. Although it was expected at all *C-T* intervals that a higher threshold of current would be necessary to induce an animal to meet a seven-press than to meet a five-press criterion, the shape of the function in both cases was expected to be the same. The two criteria were not tested on the same session but were tested from day to day in an ABBA order to ensure that if there was a general tendency for threshold to change over time, this change would be distributed equally over both conditions.

Four male Sprague-Dawley albino rats met the screening requirements of the study. These rats, first, had been implanted with indwelling monopolar electrodes insulated to 0.5 mm of their tips and aimed at the medial forebrain bundle with the use of the Krieg rat brain atlas according to the following coordinates: AP, -1.0 mm; H, 8.5 mm; and L, 1.5 mm. An uninsulated jeweler's screw placed rostrally in the skull served as the ground. Then, 1 week after implantation these animals were screened for self-stimulation behavior. Screening consisted of placing the rat in the apparatus, a 12 inch square (30 cm square) Plexiglas box, and allowing him to press a retractable lever there which delivered a half-second train of pulses per press. During screening the pulses within a train were spaced 5 msec apart. To be included in the study the animals had to press the lever at a rate greater than 30 presses per minute for a current level under 80  $\mu$ a. This was to ensure the effectiveness of the stimulation as a positive reinforcer. Finally, the animals meeting this qualification were trained over 15 2-hour sessions to press the lever for trains in which the pulses were spaced progressively farther and farther apart in 20-msec steps until a 200-msec separation had been attained. At no point prior to testing was the animal ever exposed to the double-pulse stimulation to be used in the test

condition, although all other features of the training situation were the same as for the test situation.

Immediately after training, testing was begun. At this point the pulse-pair *C-T* procedure was instituted. The separation between the *C* pulse of one pair and the *C* pulse of the succeeding pair (the *C-C* interval) was kept constant at 400 msec. This separation between the first pulses of the pairs was long enough to allow us to look at the effect of *C-T* separation within a pulse pair with relatively little interaction between pulse pairs and yet was short enough to evoke and maintain reliable self-stimulation behavior from the animals. The *C-T* interval was varied from 0.1 msec to 200 msec. In addition, a single pulse control interval was employed in which the *T* pulses were omitted from the train, thus yielding a pulse every 400 msec.

The current threshold required to induce an animal to meet the five-press and the seven-press criteria of self-stimulation at the single-pulse interval and at each of the *C-T* intervals constituted the experimental data of this study. Eleven values of intervals were used; these are indicated along the abscissa of Fig. 1. These intervals were presented according to a randomly determined 11 by 11 Latin square. One arm of the Latin square was run each day.

The results of the experiment are plotted in Fig. 1, which portrays the thresholds of current necessary at the various *C-T* intervals to meet each of the two criteria of self-stimulation. The data are presented for individual animals. A two-way analysis of variance performed on the data for each animal showed that the threshold current required to meet criterion varied both as a function of *C-T* interval and as a function of number of presses to criterion, but that there was no interaction effect of these two factors. In other words, while the threshold curve lay at a higher current level for the seven-press than for the five-press criterion, the shape of this curve as a function of *C-T* interval did not differ for these two criteria. An analysis of variance for pooled data yielded the same results.

As analyzed for the Duncan New Multiple Range Test ( $P < .05$ ), each curve can be separated into three statistically distinct portions as follows: (i) Portion I, a period of high threshold ranging from *C-T* intervals of 0.1 to 0.6 msec during which performance is indistinguishable from that obtained

with single-pulse stimulation; (ii) Portion II, a period covering *C-T* intervals from 1.2 to 30.0 msec over which there is first a sharp drop and then a leveling off in the threshold curve; and (iii) Portion III, a period characterized by a gradual rise in threshold from *C-T* interval of 60 to 200 msec. Portion I and II contain behavioral evidence for the axonal phenomenon of absolute inexcitability of a fiber immediately after its firing. The duration of this interval varies inversely as the diameter of the fiber stimulated (4, 5). In the self-stimulation system of the

hypothalamus this refractory period has been found by Deutsch (3) and by Gallistel *et al.* (6) to be either about 0.6 msec or 1.0 msec, depending respectively upon whether reinforcement or drive subsystems are being examined. Within this period fibers having fired to the *C* pulse should not fire again to a *T* pulse no matter how high the current. Thus, thresholds at *C-T* intervals of 0.6 msec or less should not be discernibly different from the single-pulse thresholds. Our findings for Portion I are in agreement with this prediction, and thus support prior

work. The fact that fibers which recover from absolute refractoriness do not immediately return to normal excitability, but pass through a moderately long period of relative refractoriness in which only currents of greater than normal intensity will elicit a response, suggests that we should find a gradual rather than a sudden drop in the thresholds as the *C-T* interval is lengthened from 0.6 msec to some considerably greater value. In Portion II of Fig. 1 the negatively decelerating decline in threshold between 0.6 and 30.0 msec confirms this expectation.

The gradual rise in thresholds in Portion III is thought to reflect events taking place at the synapse rather than on the axon, since at these long intervals the *C-T* separation exceeds the refractory period, and the fibers should conduct both pulses to the synapse. Since this rise in current necessary to elicit criterion rates of self-stimulation mirrors so closely the shape of the temporal summation decrement observed at peripheral synapses at progressively longer *C-T* intervals, we interpret our results to be due to similar temporal summation factors acting at central neurons mediating reward (5, 7). These results are also concordant with one preliminary test of temporal summation in the self-stimulation system by Deutsch (8). One mechanism for such temporal summation is that the smaller the *C-T* interval the more the neural transmitter tends to be released into the synapse faster than it can be disposed of, with the result that the transmitter accumulates on the postsynaptic membrane and elevates the firing rate from the postsynaptic neuron. Another mechanism for temporal summation is a presynaptic change wherein closely spaced pulses may each cause the release of more packets of transmitter from the presynaptic terminal (9, 10). This mechanism has also been identified with a related phenomenon termed temporal facilitation (9) which may also be operating. By either mechanism the gradual loss of synaptic effectiveness of the stimulation as the separation between pulses is increased would produce a declining strength of behavioral response requiring an increasing amount of current to produce a criterion response. However, in this study a confounding variable of spacial summation may also be involved, since changes in current to meet criterion involve changes in current spread through tissue, so that at higher currents more fibers conduct-

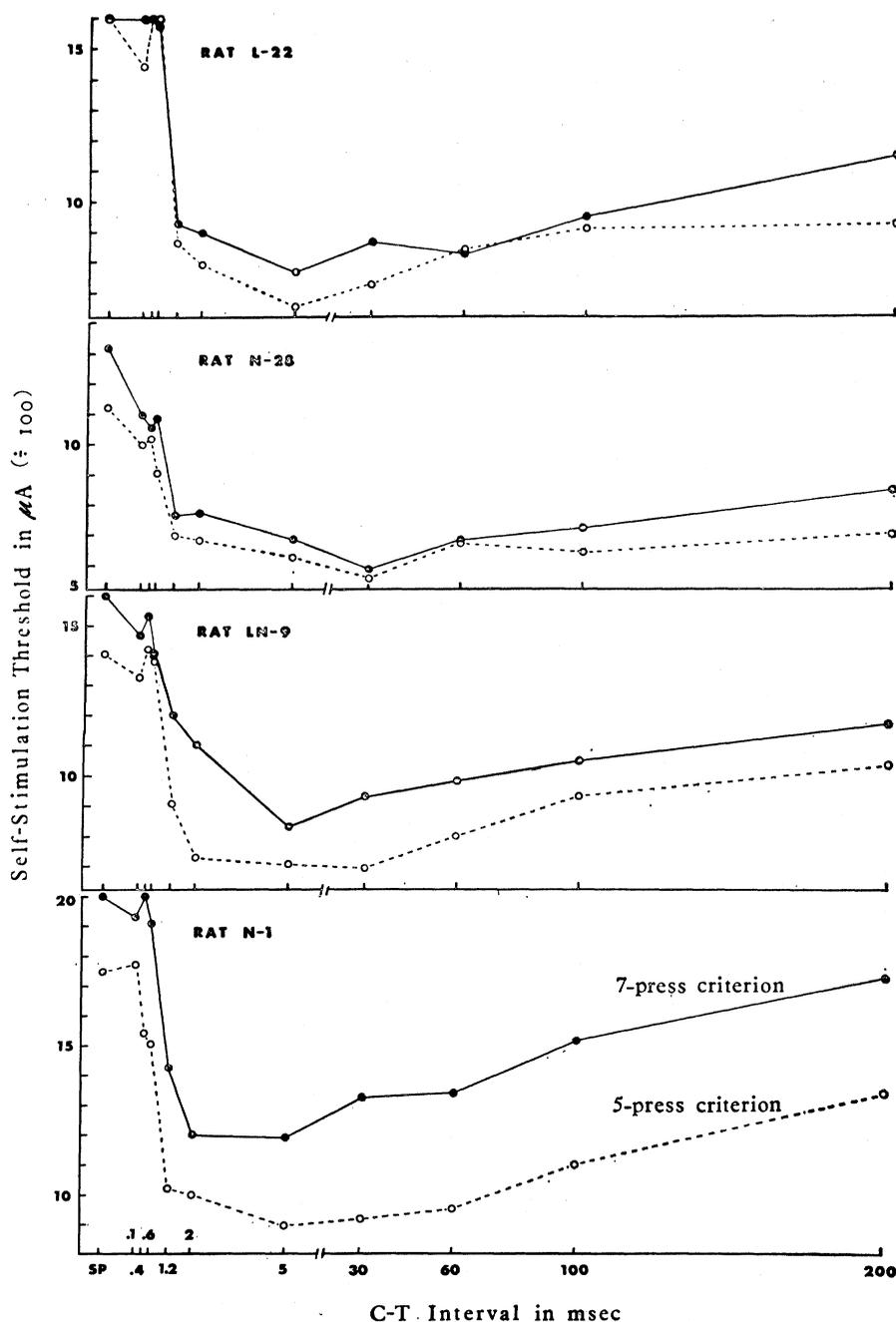


Fig. 1. Behavioral threshold determinations of the refractory period and temporal summation in the self-stimulation system of the hypothalamus. The *SP* (single pulse) on the abscissa represents a *C-C* interval of 400 msec with the *T* pulses omitted. See the text for details.

ing to common neuronal junctions may have been stimulated.

Thus, for the self-stimulation system as well as the pain system, there exists a remarkable isomorphism between the strength-rate functions for overt behavior and the strength-rate functions previously demonstrated to hold for axons and synapses. The major difference between these data and the peripheral neural excitability model is that the time constant for the summatory portion of the behavioral curve for temporal summation is longer than has been found electrophysiologically for peripheral systems (7, 11). This, however, is consistent with previous electrophysiological work in the central nervous system where processing seems also to take longer than in the periphery (9, 10, 12).

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the preparation used: both the *C* and *T* pulses were delivered via the same electrode, that is to say, the impulses arriving at a postsynaptic neuron were all generated on the same set of fibers. Since fibers once fired are absolutely refractory to being fired again for periods as long as 2.5 msec (4), a *T* pulse following a *C* pulse within such a period cannot be conducted to the synapse. Consequently, in these prior studies once the *C-T* interval was shortened to within the refractory period the rate of instrumental responding actually declined sharply rather than continuing to show the increase that is characteristic of temporal summation.

The purpose of the present study was to test a way to circumvent the limitation imposed by the presynaptic fiber's refractory period as it affects the postsynaptic neuron's ability to receive closely spaced impulses, and thereby to reveal the course of temporal summation at short as well as long intervals. The following facts suggested a way in which this could be accomplished: (i) Impulses may summate upon a postsynaptic neuron not only by repeated firing of the same presynaptic neuron (homosynaptic summation), but also by converging upon the postsynaptic neuron from separate presynaptic neurons firing within a brief period of each other (heterosynaptic summation) (5). (ii) Summation arising from heterosynaptic summation is not constrained by refractory period limitations inasmuch as the separate synapses which converge on the postsynaptic neuron are not activated by the same fibers (6). Hence, if we could deliver the *C* and the *T* pulses not to the same but to separate sets of fibers which converge onto common postsynaptic neurons, then responding even within the refractory period range should continue to increase as the *C-T* interval is decreased.

To meet the condition necessary to test this prediction we chose to study self-stimulation of the brain, because the structure to which rats will most readily administer pulses of current, the medial forebrain bundle, is represented bilaterally in the brain and is believed to converge from two widely separated sides of the hypothalamus to a common neuronal pool in the ventral tegmental area of the midbrain (7). This bilateral system therefore meets the criteria for studying heterosynaptic summation. By delivering the *C* pulse to the medial forebrain bundle on one side of the brain and the *T* pulse to the medial forebrain bundle on the other

## A Behavioral Measure of Homosynaptic and Heterosynaptic Temporal Summation in the Self-Stimulation System of Rats

**Abstract.** *Bilateral stimulation of the medial forebrain bundle with pulses of varying interpulse intervals elicited a pattern of self-stimulation behavior in the rat indicative of temporal synaptic summation: the shorter the interval the greater the response. In contrast, the effectiveness of unilateral stimulation at very short intervals was limited by neuronal refractory periods. The results support the notion that there is convergence of the medial forebrain bundle self-stimulation system from the two sides of the brain and suggest ways of studying the degree of convergence. They also suggest a technique for behaviorally comparing heterosynaptic and homosynaptic mechanisms of summation.*

From neurophysiological work it is known that the faster the excitatory impulses from presynaptic neurons impinge upon a postsynaptic neuron the higher the probability that this neuron will fire. The phenomenon is called temporal summation and is thought to be due to the release of neural transmitter at synapses more rapidly than it can be disposed of, such that there is a summation of excitatory postsynaptic potentials toward firing threshold. It has been shown that a pair of 0.1-msec electrical pulses (one, the conditioning or *C* pulse, being followed at a parametrically varied interval by another, the test or *T* pulse) can be used as a probe to explore the course of this temporal summation. Classically, the technique has been applied to acute preparations in which the response mea-

sured as a function of the interval size separating the onsets of the *C* pulse and the *T* pulse (the *C-T* interval) has mainly been the electrical behavior of neurons lying postsynaptic to the site of stimulation (1).

Recent studies have demonstrated that the same pattern indicative of neuronal temporal summation can also be evidenced in either instrumental escape (2) or self-stimulation responding (3) of freely moving rats when they are electrically stimulated in the appropriate central systems by a train of *C* and *T* pulses separated from each other by *C-T* intervals identical to those classically employed. However, in these experiments temporal summation could not be studied at *C-T* intervals less than 2.5 msec in duration because of refractory period limitations inherent in