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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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 measured 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 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 side and measuring the rate of self-stimulation behavior as a function of the *C-T* interval, and then by comparing the results with those obtained by delivering both pulses to the same side of the brain, it should be possible to test the following prediction: Refractory period limitations present in the condition involving unilateral delivery of both pulses should not hold for the bilateral condition, with the consequence that temporal summation of self-stimulation behavior should now be observed even at the shortest *C-T* intervals.

Sprague-Dawley rats were bilaterally implanted in the medial forebrain bundle with indwelling monopolar electrodes insulated to within 0.1 mm of their tips (*8*); an uninsulated jeweler's screw placed rostrally in the skull served as the ground. They were screened for self-stimulation after 1 week of recovery. Wires running from the stimulator (*9*) were attached to an animal's electrodes and he was placed in the test apparatus, a 12 inch square (30 cm square) Plexiglas box with a lever. Each lever press during screening delivered a half-second train of *C* pulses, each pulse being 0.1 msec in duration with an intensity of 150 μa and separated from the onset of the preceding *C* pulse by a 5-msec interval, called the *C-C* interval. To be included in the study, an animal had to stimulate himself via each electrode at a rate of at least 30 presses per minute. This was to ensure the effectiveness of the stimulation as a positive reinforcer for both sides of the brain. The four qualified animals were then trained over ten 1-hour sessions to adapt them to some of the self-stimulation procedures which would prevail in the test itself. These included the priming procedure which preceded each trial, the method of initiating and terminating trials by inserting and withdrawing the lever, and certain changes in the duration of both the train of pulses and the *C-C* interval.

There were four experimental test conditions, presented in the following order: (i) unilateral stimulation, both *C* and *T* pulses delivered via the right electrode; (ii) bilateral stimulation, the *C* pulse delivered via the right electrode and the *T* pulse via the left electrode; (iii) bilateral stimulation, the *C* pulse delivered via the left electrode and the *T* pulse via the right electrode; and (iv) unilateral stimulation, both pulses delivered via the left electrode. Each condition was tested for four sessions administered over consecutive days. Each session consisted of 36 1-minute trials, each trial being separated by a 1-minute rest with the lever absent. During a session the train duration per press was always 2.0 seconds and the *C-C* interval was held constant at 120.0 msec. Five *C-T* intervals were tested, plus a single pulse condition with the intervening *T* pulse omitted. These intervals were varied from trial to trial in a 6 by 6 Latin square design. The single pulse condition was a control in that it provided a baseline from which the effectiveness of the addition of a *T* pulse at varying *C-T* intervals could be evaluated. The actual sizes of the *C-T* intervals tested were 0.1, 0.4, 1.2, 5.0, and 60.0 msec. These values were chosen because they have already been proven in unilateral studies of the self-stimulation system to yield data representative of refractory periods and temporal summation (*3*, *10*).

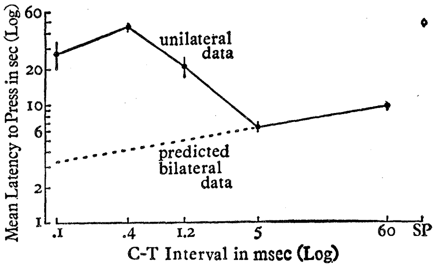

Fig. 1. A behavioral determination of the refractory period and homosynaptic temporal summation in the self-stimulation system of the hypothalamus where both the *C* and the *T* pulses are delivered unilaterally through the same electrode. The vertical lines about each point represent the standard deviations. The *SP* (single pulse) on the abscissa represents a *C-C* interval of 120 msec with the *T* pulses omitted. See the text for details.

Just before inserting the lever at the beginning of each test trial the animal was primed with a 10-second train of pulses of the same *C-T* interval at which he would be able to self-administer pulses during that trial. The latency to the first lever press and the number of trains of reinforcement obtained were recorded on each trial. The reinforcement score was subsequently converted into a measure of the mean interstimulation time between presses (*11*). The level of current to be employed for a given animal was defined before testing as that threshold intensity of current required to maintain a criterion rate of at least seven reinforcements per minute with trains of pulse pairs at a 60.0-msec *C-T* interval. This same current applied to all four experimental conditions described above.

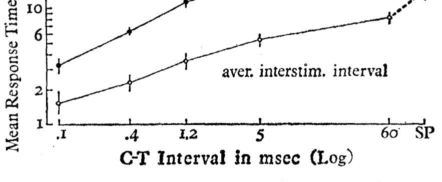

Fig. 2. A behavioral determination of heterosynaptic temporal summation in the self-stimulation system of the hypothalamus where the *C* and the *T* pulses are delivered to opposite sides of the hypothalamus. The vertical lines about each point represent the standard deviations. The *SP* on the abscissa represents a *C-C* interval of 120 msec with the *T* pulses omitted.

The results from this experiment are summarized in Figs. 1 and 2. Since earlier evidence in our laboratory has indicated that the curve exemplifying temporal summation of behavior has the form of a power function (*2*, *12*), we expected that the same kind of curve might obtain in the present experiment. Therefore, the data here are represented on a log × log scale because such a scale transforms power curves into straight lines, thereby offering a convenient visual check on our expectations.

The results of unilateral stimulation are portrayed in Fig. 1. The curve represents the averages of data from both of the unilateral conditions for all four animals. This curve has the following features: (i) latencies at 0.4-msec *C-T* interval and single pulse do not differ signficantly, indicating an absolute refractory period; (ii) latencies decrease from 0.4 through 5.0 msec (Duncan Multiple Range Test = DMRT, $P < .001$), suggesting that the axons are still in a relative refractory period at intervals less than 5.0 msec; (iii) the curve reaches a minimum at 5.0 msec and then rises through to 60.0 msec (DMRT, $P < .05$), demonstrating a decreasing temporal summation as the *C-T* interval is lengthened from 5.0 to 60.0 msec; (iv) the existence of latent addition (*13*) is shown by the faster latency at 0.1 msec than at either single pulse or 0.4 msec (DMRT, $P < .001$). The same pattern of results was found in all animals and essentially parallels the findings of Smith and Coons (*3*) and Kestenbaum *et al.* (*2*).

If the left-hand portion of this curve does indeed represent neuronal refractory periods and the right-hand portion synaptic summation, the bilateral stimulation should eliminate the high latencies in the left-hand portion and reveal, as explained earlier, a straight line falling all the way to the left and reflecting only synaptic summation: the shorter the *C-T* interval the shorter the latency. We generated the predicted bilateral effect by linearly extending the obtained 5.0- to 60.0-msec summation segment of the unilateral curve to the shorter intervals in Fig. 1. The results of bilateral stimulation, as averaged over the two bilateral conditions for all four animals, are presented in Fig. 2. The upper line represents the latency data and the lower line the mean interstimulation time. The nearly perfect parallelism of these two curves strongly suggests that the phenomenon we are observing is quite general, since it possesses the same function under two different measures. The difference in the heights of the two curves merely shows that it takes less time for a rat to press again, having just pressed, than it does to make the first press on a given trial. From a general comparison of these lines with the predicted line in Fig. 1, it is obvious that the obtained results comply remarkably well with the general expectation: over the entire range of *C-T* intervals the shorter the interval the shorter the latency. The log × log linear component of this trend was significant at the $P < .001$ level, indicating that the data indeed describe a power function. Furthermore, the differences between the *C-T* intervals all proved to be significant (DMRT, $P < .01$). Thus, as in the acute studies of heterosynaptic summation, the data confirm that the use of the *C-T* technique in a bilateral paradigm circumvents the refractory period limitations of the unilateral paradigm in uncovering and in investigating the entire range of temporal synaptic summation of self-stimulation. Moreover, the data support the notion that there is convergence of the self-stimulation system from the two sides of the brain, because temporal summation from bilateral stimulation is only possible if convergence occurs. In further support of this conclusion, additional data from our laboratory have demonstrated that temporal summation cannot be evidenced in a bilateral preparation where only one of the two electrodes elicits self-stimulation.

Although the obtained and predicted bilateral latency curves proved to be generally similar, a more detailed comparison indicated some differences: (i) By a regression analysis the slope of the 0.1- to 1.2-msec portion of the obtained curve was significantly steeper than the predicted curve, but the reasons for this are as yet unclear. (ii) Although the obtained latency curve was virtually identical in slope to the predicted latency curve, it lay at a higher level. This was because the predicted curve was generated from the unilateral curve which at intervals beyond the refractory period produced shorter latencies than did bilateral stimulation. These differences in unilateral and bilateral stimulation can probably be attributed to the fact that convergence and summation of impulses upon a common pool of postsynaptic neurons in the self-stimulation system is less perfect via fibers from separate sides of the brain (that is, via heterosynaptic convergence) than via repeated firing of the same fibers (that is, via homosynaptic convergence). However, it is of great interest in this connection that for latency data in the 5.0- to 60.0-msec range, the slope of the bilateral line and the slope of the unilateral line are virtually identical. The same temporal summative process is evidently involved in both instances (*14*). From this we infer that of the two possible mechanisms, presynaptic facilitation and postsynaptic summation, which can contribute to homosynaptic summation, only the latter is operative under the *C-C* condition of the present study, since otherwise the slope for the homosynaptic condition should be less steep than for the heterosynaptic condition in which only postsynaptic summation is possible.

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8. Electrode preparation and operative techniques have been detailed in E. E. Coons, thesis, Yale University (1964) (microfilm obtainable from University Microfilms Inc., Ann Arbor, Mich., order 64-13,166). The animal was anesthetized with Diabutal and its head was secured in a Kopf stereotaxic instrument adjusted so that the tooth bar was raised 3.1 mm above the intra-aural line. With the head thus positioned, the coordinates were 1.0 mm posterior to bregma, 1.5 mm lateral to the midline, and 8.5 mm ventral to the top of the skull.
9. Negatively going capacitance-coupled square wave pulses were produced by Digibit units [J. A. Deutsch, *J. Exp. Anal. Behav.* **9**, 399 (1966)] and administered through a 1.0 μf capacitor in series with a 68-kilohm resistor. A 50-kilohm variable resistor between the capacitor and the series resistor was connected to ground, as was the animal's indifferent electrode. Stimuli were monitored with a Tektronix oscilloscope.
10. J. A. Deutsch, *J. Comp. Physiol. Psychol.* **58**, 1 (1964); C. R. Gallistel, E. Rolls, D. Greene, *Science* **166**, 1028 (1969).
11. The reinforcement score was converted into a measure of the mean interstimulation time between presses by multiplying the 2-second duration of each train by the number of reinforcements to obtain the total stimulation on-time per trial. This value plus the latency measure were then subtracted from the trial's duration of 60 seconds to obtain the total stimulation off-time that accumulated in the trial after the animal had begun responding. This new value when divided by the number of reinforcements yielded a quotient representing the average time to obtain a new reinforcement train once the succeeding train had terminated.
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13. A nerve fiber can store and summate charge capacitatively on its membrane for as long as a 0.2-msec interval, termed the period of latent addition [K. Lucas, *J. Physiol.* **39**, 461 (1910)].
14. Temporal summation is generally mediated by the summation of excitatory postsynaptic potentials on the postsynaptic membrane in response to the total level of excitatory neurotransmitter active over the entire membrane at a given time. Heterosynaptically, this level is governed by the number of and temporal spacing between the separate presynaptic endings which fire during the period of the transmitter's lifetime. Homosynaptically, this level is governed by the number of and temporal spacing between the presynaptic endings which refire during this lifetime. Since increases in repetitive firing of the same presynaptic ending can lead to an increased presynaptic mobilization and release of transmitter and to an increased transmitter concentration in the synaptic cleft that cannot exist in the heterosynaptic situation, the summation of excitatory postsynaptic potentials homosynaptically could well exhibit a different temporal characteristic than heterosynaptically. However, the equivalence of the two slopes suggests that the processes underlying the temporal summation of self-stimulation in our bilateral and unilateral situation are essentially the same. A further discussion of the mechanism underlying temporal summation is given in J. C. Eccles, *The Physiology of Synapses* (Springer-Verlag, Berlin, 1966), and in H. Grundfest (see *5*).
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