Absolute Refractory Period of Neurons Involved in MFB Self-stimulation

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The refractory period of the directly stimulated neurons whose excitation elicits behavior can be measured by variation of the intra-pair interval of trains of stimulating pulse pairs. It is shown that at high frequencies of stimulation (e.g. 200 pulses/sec) the current threshold of the neurons directly excited in self-stimulation may be approached. Thus to measure the absolute refractory period of neurons, the electrical stimulation must be at least twice the threshold value. In Experiment 2, using twice threshold currents, the absolute refractory periods of the neurons involved in the continuation of medial forebrain bundle (MFB) self-stimulation were found to be in the range 0.8-1.1 msec in six rats. The measure may represent a priming effect of the stimulation. This value of the absolute refractory period of neurons excited in lateral and posterior hypothalamic self-stimulation corresponds to the value for the neurons through which the brainstem arousal system is excited (see preceding report), and provides further evidence that this brainstem neural system is involved in MFB self-stimulation.

Deutsch ([1], Experiment 1) measured the refractory period of the neurons which probably mediate the priming effect. He measured the number of bar presses made in the second minute of a test period for trains of stimulating pulse pairs with different intra-pair intervals (the rationale of the refractory period determination is the same as that used in the indirect determinations in the preceding report [5]). The priming effect was measured essentially by whether any animal continued or ceased lever pressing for the stimulation during a relatively long period of two min. If the animal would only continue bar pressing in the subsequent short test period if the stimulation obtained were rewarding, then the number of bar presses made for weak brain stimulation reward of pulse pairs with different intra-pair intervals was measured in a subsequent one minute test period. Deutsch argued that in the presence of pro-active after-effects of the previous intense 60 Hz stimulation, an animal would only continue bar pressing in the subsequent short test period if the stimulation obtained were rewarding.
It is not clear how accurately the absolute refractory period of the neurons mediating the priming effect has been measured behaviorally. In one example ([1], Experiment 1), the lowest voltage was used, consistent with good rates of responding when the frequency of the stimulating pulses was high. As is shown here in Experiment 1, the voltage may have been close to the threshold of the excited neurons, and a relative rather than an absolute refractory period may have been measured. Therefore, the refractory period experiment described here used current at twice the threshold for self-stimulation. This ensures that at least some of the directly excited neurons will produce an absolute refractory period. In this way an estimate of the absolute refractory period of the neurons which probably mediate a priming effect in hypothalamic self-stimulation is obtained (Experiment 2). (A behavioral experiment reported earlier [3] was performed at more than twice the voltage threshold so that the absolute refractory period was probably approached, but only one rat was used.)

METHOD

Albino male rats were implanted with two monopolar electrodes aimed at medial forebrain bundle self-stimulation sites as described for the electrophysiological experiments [5]. Examples of the sites which were at the levels of the lateral and posterior hypothalamus are shown in Fig. 1. Constant current 0.1 msec pulses were delivered to the electrodes as in the acute experiments. The current and the voltage of the stimulation pulses were measured at all times with a Tektronix 502A oscilloscope. Animals were tested in an aluminum cage 10×8×10 in. which was housed in a large soundproof box. The rats were able to press a bar which delivered a 0.3 sec train of pulse pairs to the electrodes through flexible wires. The frequency of pulse pairs in the train, and the time interval between the members of each pulse pair (intra-pair interval) could be independently altered. In initial testing for self-stimulation with a train of single pulses at a frequency of 200 pulses per sec the electrode (lateral or posterior hypothalamic) which produced the most consistent responding was chosen for the experiments.

EXPERIMENT 1

The purpose of the experiment was to determine the relation between the frequency and the current of the stimulating pulses in a train of fixed duration which would just maintain self-stimulation. This clarifies the measurement of the absolute refractory period made in Experiment 2.

Procedure

In two rats the stimulating current of 0.1 msec pulses which would maintain self-stimulation for a train of fixed duration was determined for different pulse frequencies. An ascending and descending method of limits was used.

Results

Figure 2 shows the results for two rats with a train duration of 0.3 sec per bar press, and for one of the two rats with a train duration of 1.0 sec. It is clear that there is little change in the current required to elicit self-stimulation at frequencies above 200 pulses/sec. At these high frequencies there is a current threshold for self-stimulation below which neurons important in self-stimulation are not excited. Thus if a determination of the refractory period of the neurons which mediate the pro-active effect in self-stimulation is performed at a frequency of 200 pulses/sec [1], the current may be near the threshold of the neurons, and a relative refractory period may be measured.

EXPERIMENT 2

The purpose of the experiment was to determine the absolute refractory period of the neurons mediating the pro-active effect in self-stimulation, by performing the determination...
at twice the current threshold for self-stimulation. It has been shown [5] that under the monopolar stimulating conditions used, the absolute refractory period of directly excited neurons is reached at a twice-threshold current. The experiment also extends an earlier study which used mainly tegmental self-stimulation [1], by using self-stimulation sites near the lateral and posterior hypothalamus.

Procedure

The lowest current for consistent self-stimulation at a frequency of 200 pulses per sec was determined for each rat. Further reduction in the current, even when the pulse frequency or the train duration were increased, brought cessation of bar pressing. Thus this current level was assumed to be at or above the threshold of neurons important in self-stimulation.

A determination of the refractory period of neurons concerned with the maintenance of self-stimulation was then made at twice the current threshold for each rat by the following method. The twice threshold 0.1 msec pulses were delivered in a 0.3 sec train for every bar press. The repetition frequency of the pulses was reduced until a frequency was found where bar pressing just ceased. At double this critical frequency the rats pressed consistently for the intra-cranial stimulation. Then pulse pairs were delivered at the critical frequency, with different intra-pairs interval, in order to determine the intra-pair intervals for which the animal would maintain responding. The different intra-pair intervals were introduced as follows. For each trial the animal was given free or priming stimulation with an intra-pair interval of 2 msec until responding started, after which self-stimulation was allowed to continue for 30 sec with this same intra-pair interval. Then the intra-pair interval was changed to a value between 0.4 and 2 msec chosen in random order, and the number of bar presses in the next 90 sec was counted. Then the current was switched off, and the subject was left in the cage for a period of 90 sec, after which another trial started with priming at the 2 msec intra-pair interval. For comparison with the number of presses made in the 90 sec testing period at the different intra-pairs intervals, trials with the second pulse of a pair omitted (1P) and trials at twice the critical frequency (2F) were included in the random order of trials. In some rats extra trials were then run in random order to define the results more precisely.

Results

The threshold currents for the different rats were between 200 and 500 μA, and the critical frequencies at twice threshold current were between 15 and 25 pulses per sec (4–7 pulses per train). The results for each of 6 rats are presented in Fig. 3, where the numbers of presses in the 90 sec test periods are plotted as a function of the intra-pair interval. There is a large increase in the number of presses in the period for each animal for intra-pair intervals of and greater than 0.9–1.0 msec, when compared with responding at lower intra-pair intervals or with 1P. The small numbers of presses at 1P and intra-pair intervals below 0.9 msec reflect cessation of responding during the test period—the large number of presses at intra-pair intervals greater than about 1.0 msec and at 2F indicate that the animals are continuing to respond during the 90 sec test period. Thus, increasing the intra-pair interval of the pulse pairs in the stimulating train beyond about 0.9–1.0 msec produces self-stimulation more like that for the critical frequency (1F). That this occurs at twice the threshold current for self-stimulation is evidence that the absolute refractory period of the neurons which maintain bar pressing for hypothalamic stimulation is near 0.9–1.0 msec.

As the refractory period is exceeded for animals 32, 41, 14 and 26 the number of bar presses in the test period becomes immediately like the 2F results. In animals 12 and 35 the number of presses gradually approaches the 2F results as the intra-pair interval is increased beyond the refractory period. This gradual increase in the effectiveness of the stimulation found as the intra-pair interval was raised above the refractory period was not found when the stimulating current was raised further. Therefore the gradual slope found in these two animals is probably due to the relatively refractory state of the stimulated neurons distant from the electrode. The stimulation of these peripheral neurons is discussed below.

DISCUSSION

The values of refractory period found were in the range 0.8–1.1 msec: 0.8–0.9 for one rat, 0.9–1.0 for three rats, and 1–1.0–1.1 msec for two rats. They correspond to the refractory periods of the brainstem neurons which are directly excited through MFB self-stimulation electrodes (see preceding paper, [5]). The majority of these units have absolute refractory periods in the range 0.78–1.0 msec. They also correspond to the indirect refractory period determinations in the preceding paper: that is, to the refractory periods of the neurons through which the indirectly driven arousal units are activated. These indirect determinations were in the range 0.8–1.1 msec. This provides evidence that the brainstem neural system traced in the preceding paper mediates the self-stimulation effect measured here. Thus one effect of increasing the stimulation of the brainstem neural system is to increase the rate of self-stimulation.

The behavioral refractory period determination described here is appropriate for comparison with the electrophysiological results presented in the previous paper for three reasons. First, the behavioral and electrophysiological results were both obtained with self-stimulation of the MFB region at the level of the lateral and posterior hypothalamus. An earlier study used mainly tegmental self-stimulation [1]. Second, by using a twice–threshold stimulating current, the absolute refractory period of the directly stimulated neurons is probably approached, and this can be compared with the electrophysiological results. Because the current was near threshold in an earlier study (11, Experiment 1), a relative refractory period may have been measured. In that case, the absolute refractory period would have been shorter and would not have had the same value as that of the system traced electrophysiologically [5]. Third, the present study used a group of rats rather than the single animal used previously [3].

The rate of self-stimulation was the dependent variable in the refractory period experiment. Halving the frequency of stimulation of the directly excited neurons with refractory periods of 0.8–1.1 msec in the experiment resulted in cessation of self-stimulation within about 30 sec, rather than continuation of bar pressing for at least 90 sec. This frequency change occurred on the trials in which after 30 sec the pulse-pair intra-pair intervals of 2 msec were reduced to values of less than 0.8–1.1 msec (or to 1P). The measure, of the extent to which an animal would continue to press for relatively long periods (90 sec) for low level brain stimulation is
FIG. 3. Number of bar presses in a 90 sec test period for a 0.3 sec train of twice threshold pulse pairs as a function of the intra-pair interval of the pulse pairs. Results for six rats.
similar to that used by Deutsch to measure a drive-like or priming effect in self-stimulation ([1], Experiment 1). The measure also gave a value for the refractory period which is similar to that for the priming effect in a runway situation in one rat [3]. Therefore the aspect of self-stimulation measured in the experiments described here may be a priming effect. This may be distinguished from a reward effect in self-stimulation (see Introduction).

It is of interest that Deutsch’s determinations of refractory period [1], performed with electrode placements in the ventral tegmental area (except for one rat with a lateral hypothalamic placement), gave a refractory period for the ‘drive’ class of neurons (i.e. the priming or pro-active effect class, and the class measured here by the continuation of responding) very similar to that obtained in this study using lateral and posterior hypothalamic placements. This indicates that in two different areas of the brain the neurons producing a similar behavioral effect in self-stimulation have similar refractory periods. Alternatively, the same neurons may be stimulated at different points in the two experiments.

The question of how closely these techniques can measure the absolute refractory periods of neurons must remain open. For as current is increased the stimulation frequency at which self-stimulation no longer occurs decreases (Fig. 2). This implies that self-stimulation can be obtained with a lower stimulation frequency, if more neurons are excited. Thus, when an animal just ceases to respond as frequency is decreased at twice threshold current, there may be neurons at the periphery of the field of stimulation which are stimulated at less than twice threshold current. If both neurons close to the electrode and distant neurons must fire twice for every pulse pair for the behavioral effect under examination, then the determination could yield a longer refractory period than the absolute value, and the peripheral neurons would move the determinations toward a relative refractory period. Four points favor the present technique. First, the twice threshold current must mean that some of the neurons are excited twice at their absolute refractory period, and this may lead to a behavioral effect. Second, the stimulating frequency used is lower, and neurons are better able to follow the pulses at their absolute refractory period if the frequency is low (personal observation). Third, further increase in the stimulating current does not lead to a reduction of the behaviorally refractory period to below 0.8 msec. Thus, although at three times threshold current the behaviorally measured refractory period of rat 12 had decreased from 1.0–1.1 msec (see Fig. 3.) to 0.8–0.9 msec, a further increase of stimulating current to five times threshold gave a behaviorally measured refractory period of 0.75–0.85 msec—very little lower than at three times threshold. Fourth, the behavioral method used to measure the refractory period of directly stimulated neurons is analogous to the electrophysiological method using the response of indirectly driven neurons [5], and the results from the two methods are therefore comparable.

A further method of determining the voltage threshold of all neurons functionally relevant to a behavioral effect is to increase stimulating current until a second pulse at a delay of 0.2 msec from the first does not result in an increased behavioral effect. If ‘latent addition’ is absent, this ensures that there are not distant neurons which mediate the effect and which are below threshold for the first pulse (C. R. Gallistel, personal communication). Thus, raising the current to twice the level at which latent addition disappears would ensure that all relevant neurons were stimulated with twice-threshold current.

REFERENCES