In preparations which are not stained with silver, these bodies appear as empty spots within the nucleus; most of the incorporation of H^3-cytidine occurs in these bodies (Fig. 1, *E*, *F*). The data from silver-grain counts over larger pronucleolar bodies and over the chromatin of both daughter telophase nuclei of root-tip cells of *Nigella* show that the rate of incorporation of H^3-cytidine into the nucleolar bodies far exceeds that of the chromatin fraction (Table 1). Similar results are obtained in root-tip cells of *Allium*. The fact that these bodies incorporate H^3-cytidine, as do interphase nucleoli, suggests that they are pronucleolar bodies (*6*).

In root tips of both *Nigella* and *Allium* as long as nucleoli are still present in the prophase cells, the rate of nucleolar RNA synthesis remains normal; only the condensing chromosomes during prophase show reduced synthesis. In mitotic cells lacking distinct nucleoli there is practically no RNA synthesis. This synthesis resumes in late telophase or early interphase cells when pronucleolar bodies are formed. The rate of synthesis is much higher in the pronucleolar bodies than in the chromatin fraction, but not twice as great as in the interphase nucleoli (*7*). There is also a high rate of RNA accumulation in the early interphase nucleoli of root-tip cells of *Vicia faba* (*5*).

Thus, nucleoli are very active primary centers of RNA synthesis (*8*, *9*). The nucleolus does not merely function as a center for the accumulation of chromosomal RNA (*10*). The peripheral nucleoli of newt oocytes incorporate precursors of RNA even though such nucleoli are not attached to chromosomes at the time (*11*). Furthermore, RNA synthesis in the chromatin fraction can be blocked selectively without greatly affecting the synthesis in the nucleolus (*12*) which implies also that different RNA fractions are involved (*13*).

NIRMAL K. DAS
Department of Zoology, University of California, Berkeley

References and Notes

1. J. H. Taylor, *Ann. N.Y. Acad. Sci.* **90**, 409 (1960).
2. D. M. Prescott and M. A. Bender, *Exptl. Cell Res.* **26**, 260 (1962).
3. R. Baserga, *J. Cell Biol.* **12**, 633 (1962).
4. C. Konrad, thesis, Univ. of California, Berkeley (1963).
5. J. Woodard, E. Rasch, H. Swift, *J. Biophys. Biochem. Cytol.* **9**, 445 (1961).
6. N. K. Das, *Exptl. Cell Res.* **26**, 428 (1962).
7. D. Mazia, in *The Cell*, J. Brachet and A. E. Mirsky, Eds. (Academic Press, New York, 1961), vol. 3, p. 373.
8. J. Brachet, *Biochemical Cytology* (Academic Press, New York, 1957), p. 133.
9. J. L. Sirlin, in *Progress in Biophysics and Biophysical Chemistry* (Pergamon, New York, 1962), vol. 12, p. 38.
10. L. E. Feinendegen, V. P. Bond, W. W. Shreeve, R. B. Painter, *Exptl. Cell Res.* **19**, 443 (1960). J. H. Rho and J. Bonner, *Proc. Natl. Acad. Sci. U.S.* **47**, 1611 (1961).
11. H. G. Callan and J. G. Gall, unpublished.
12. R. P. Perry, *Second Annual Meeting of the American Society for Cell Biology, San Francisco, 1962*, abstract, p. 143.
13. Aided by National Science Foundation grant G-19538 and by U.S. Public Health Service grant RG-6025. Some of the present results were demonstrated at the second annual meeting of the American Society for Cell Biology, San Francisco, 1962 (see abstr. vol., p. 40). I thank Prof. Max Alfert for his advice and criticism, and Dr. E. G. Pollock of San Francisco Valley State College, Northridge, for providing *Nigella* seeds.

20 March 1963

Heart Rate Changes after Reinforcing Brain Stimulation in Rats

Abstract. *Cardiac responses to hypothalamic and septal stimulation are polyphasic, the first component being accelerative. As interstimulus intervals are decreased, only accelerative components appear, but prestimulus heart rates ("background levels") are decreased upon septal and are either increased or unchanged upon hypothalamic stimulation. The suggestion that parasympathetic responses accompany reinforcing brain stimulation was not supported.*

Recently, Malmo (*1*) described cardiac decelerations upon septal stimulation, and suggested that this "parasympathetic or quieting effect" had "reinforcing properties." This provocative suggestion led us to three successively more detailed experiments comparing cardiac responses to reinforcing septal and posterior hypothalamic stimulation. We expected cardiac acceleration upon posterior hypothalamic stimulation, because this area is generally considered to mediate sympathetic-like responses. Because this area is also one of the most reinforcing brain sites (*2*), the appearance of stimulus-produced acceleration would invalidate the notion that cardiac deceleration per se is linked to the reinforcement process involved in the brain self-stimulation experiment.

Bipolar, stainless-steel electrodes were implanted in rats in posterior hypothalamic and septal regions, with a technique previously described (*3*). Upon histological examination, "septal" placements were found to be in either the medial septal nucleus or the parolfactoria area, and "hypothalamic" placements were widely distributed in the lateral hypothalamus between dorsal medialis and the mammillary body.

The stimulus consisted of a half-second train of biphasic, rectangular pulses at 100 pairs per second. Pulse duration and interpulse interval were 0.2 msec.

In experiment 1, four rats were trained to bar-press for intracranial stimulation (ICS). For each rat at each of the neural sites, a stimulus intensity was chosen which produced consistently high rates of bar pressing. The animals were then habituated to and stimulated in an Econo-Cage restraining device. On each of 6 days, two blocks of stimuli were administered in counterbalanced order, first to one brain area, and then to the other. The average interstimulus interval was 20 seconds. Each stimulus-block was continued until 20 recordings free of movement artifact were obtained. Arterial pulsations from the tail were taken with an "Infraton" (Beckman-Spinco). The durations of the two pulse cycles immediately prior to, during, and directly after ICS were converted to beats per minute.

During hypothalamic stimulation, heart rate increased above prestimulus levels, average values (based on 120 stimuli per animal) for the individual animals being 9.6, 11.9, 3.3, and 1.5. These increases were significant in three of the four animals, individual p values being less than 10^{-6}, 10^{-8}, and .02 (*4*). In contrast to Malmo's findings, we found that heart rate also increased during septal stimulation: in the four animals, the average increases were 8.5 ($p < 10^{-5}$), 13.6 ($p < 10^{-8}$), 17.5 ($p < 10^{-8}$), and 4.9 ($p < 10^{-8}$). After cessation of septal ICS, moreover, heart

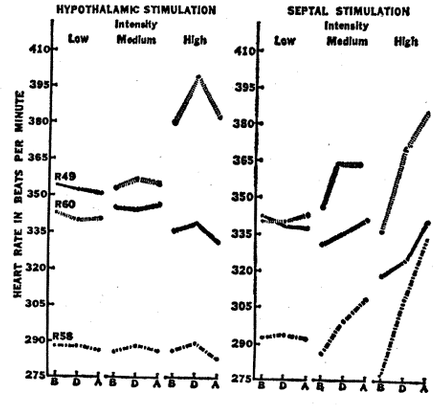

Fig. 1. Mean heart rate immediately before (*B*), during (*D*), and after (*A*) brain stimulation in experiment 2. Each point based upon 100 observations.

Table 1. Average bar presses per minute in experiment 2.

Animal	Hypothalamic ICS			Septal ICS		
	Low (30 to 75 μa)	Medium (100 to 175 μa)	High (200 to 300 μa)	Low (125 to 250 μa)	Medium (300 to 400 μa)	High (450 to 650 μa)
R 49	7.2	42.7	69.2	6.2	18.7	23.5
R 58	15.8	77.0	87.8	12.1	33.5	41.3
R 60	11.6	95.7	136.8	6.0	17.6	44.6

rate continued to increase. Average increases above rates during stimulation were 5.6 ($p < .01$), 21.7 ($p < 10^{-8}$), 17.5 ($p < 10^{-8}$), and 7.2 ($p < 10^{-8}$). The cardiac acceleration produced by hypothalamic ICS, however, did not persist after cessation of stimulus. In two animals heart rate decreased below rates during stimulation (averages of 14.8, $p < 10^{-8}$; and 8.0, $p < 10^{-7}$), and in two animals the rate increased (2.1 and 0.7 beats per minute) nonsignificantly. In this first experiment, then, we did not replicate the finding of cardiac deceleration upon septal stimulation. Instead, both septal and hypothalamic stimuli produced acceleration, and, indeed, accelerations were greater in magnitude and duration for septal stimulation.

In experiment 2, we investigated the role of stimulus intensity. By the procedure described above, cardiac changes were determined for three intensities at each neural site, for three of the four rats. The lowest intensity was at the reinforcement threshold and did not maintain consistent bar pressing; the intermediate intensity produced moderate bar-pressing rates; and the highest intensity was that used in the first experiment. Twenty-five responses at each stimulus intensity were obtained on a given day for one or the other of the sites. The intensities and sites were presented in counterbalanced order over 8 days. Bar-pressing rates were obtained on alternate days and are presented in Table 1. It can be seen in Fig. 1 that with increasing intensities of septal ICS the degree of acceleration increased both during and after the stimulus. However, with increasing hypothalamic ICS, the degree of acceleration increased only during stimulation.

Thus the findings of experiment 1 are replicated: both hypothalamic and septal stimulation produce acceleration; the acceleration is greater for septal stimulation, and persists beyond the period of stimulation.

Complete statistical analyses of all the interrelationships in Fig. 1 would be excessively space-consuming. It is important to stress, however, that there is no doubt that heart rate increased upon septal stimulation. For each of the rats, at both medium and high stimulus intensities, individually determined p values were all less than 10^{-8}, for both sets of comparisons (during and after stimulation).

Through personal communication with Malmo, it was learned that he continues to get deceleration with septal stimulation. His heart-rate measures, however, begin ½ second after ICS termination, thus omitting the very period in which we obtain acceleration. This procedural discrepancy suggested a study of the full temporal course of the cardiac response.

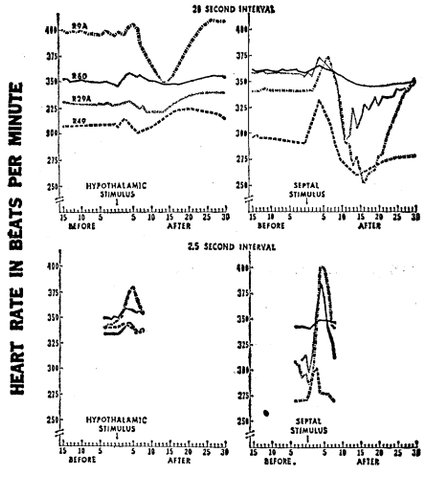

Fig. 2. Temporal course of cardiac response averaged for 100 ICS. Occasional movement artifact reduced the number of stimulations included in the averages. The brief decelerations immediately before septal ICS at 2.5 per second in R29A and R9A were due to "missed beats," possibly attributable to sino-auricular block produced by the preceding ICS. "Missed beats" were reported also by Malmo (*1*). Control recordings analyzed in identical fashion as experimental data (using mock stimulus-onset identification) demonstrated that no systematic cardiac changes occurred in the absence of stimulation.

In experiment 3, therefore, a beat-by-beat analysis was made, with two new subjects and two animals of the previous experiment. The effect of stimulation rate was also studied by presenting ICS in blocks of 25 stimuli, with interstimulus intervals within a block of 1, 2.5, 5.0, 10.0, or 20.0 seconds.

On a given day only one neural site was stimulated, with an intensity selected to yield near-maximal bar-pressing rates. Successive blocks of stimuli were presented either in ascending order from short to long intervals, or in descending order. The neural site was randomly selected to provide 4 days at each site; within a neural site, order of presentation of interstimulus intervals was alternated from day to day. Average bar-pressing rates per minute, determined after each experimental session, ranged from 32.4 to 95.2 for septal, and from 88.7 to 148.7 for hypothalamic ICS.

Electrocardiograms and cardiotachometric tracings were obtained from stainless-steel skin sutures. Simultaneously, between successive R-waves of the electrocardiogram intervals were recorded in milliseconds on punched tape with the Fels SETAR (*5*). An IBM 1620 computer translated each interval on the tape into beats per minute and separately averaged heart rate for the first cardiac cycle preceding and subsequent to ICS onset, the second cycle, and so on, for a maximum of 15 cycles preceding and 30 cycles subsequent to ICS.

Figure 2 shows the averaged cardiac response curves to the 20- and 2.5-second stimulation rates (*6*). The full cardiac response at the 20-second interval is polyphasic. For the period under analysis, hypothalamic ICS produced triphasic curves (acceleration-deceleration-acceleration), whereas septal ICS resulted in biphasic ones (acceleration-deceleration). However, as the interstimulus intervals were decreased toward rates achieved during self-stimulation (2.5-second interval) only an accelerative phasic response was evident (but, see discussion of "tonic effect," below).

In addition to these phasic responses, a tonic effect was noted as inter-stimulus intervals were decreased: the average prestimulus heart rate decreased upon repetitive septal stimulation but tended to increase upon hypothalamic stimulation. A comparable effect of intensity was observed in experiment 2.

High-intensity septal ICS depressed the prestimulus heart level (see Fig. 1). With septal stimulation, then, either high intensity or fast repetition rate lowers prestimulus heart rate ("background levels"). The effect of repetition rate was verified by the finding that for all intervals shorter than 20 seconds, each of the four rats showed progressively decreasing prestimulus heart rate levels as rate of stimulation increased.

This third study seems to begin to resolve the contradiction between the results of our first two experiments and Malmo's results. One source of the difficulty is temporal: the early effect of septal ICS is accelerative, but the late effect is pronouncedly decelerative. However, at rates of septal stimulation that would be achieved during bar-pressing, we observed only an accelerative phasic component which was superimposed upon a diminished overall heart rate level (tonic effect). With hypothalamic stimulation, moreover, which is generally regarded as more reinforcing, the accelerative component was superimposed on a heart level that either did not change or increased. These facts make it impossible for us to accept a linkage between the reinforcing properties of ICS and a "parasympathetic or quieting effect." Indeed, the polyphasic nature of the responses, both to septal and hypothalamic stimuli, makes it difficult to characterize the autonomic participation in the reinforcement process as exclusively "parasympathetic" or "sympathetic" (*7*).

WILLIAM J. MEYERS
ELLIOT S. VALENSTEIN
JOHN I. LACEY

Department of Psychophysiology-Neurophysiology, Fels Research Institute, Yellow Springs, Ohio

References and Notes

1. R. B. Malmo, *Science* **133**, 1128 (1961).
2. J. Olds, R. P. Travis, R. C. Schwing, *J. Comp. Physiol. Psychol.* **53**, 23 (1960).
3. E. S. Valenstein, W. Hodos, L. Stein, *Am. J. Psychol.* **74**, 125 (1961).
4. Wilcoxon's paired replicates test was used; see F. Wilcoxon, *Some Rapid Approximate Statistical Procedures* (American Cyanamid Co., Stamford, Conn., 1949).
5. N. T. Welford, *IRE (Inst. Radio Engrs.) Trans. Bio-Med. Electron.* **9**, 185 (1962).
6. Because two animals had seizures with septal ICS at the 1-per-second repetition-rate, the complete results available with stimulation at 2.5-per-second are presented. The data for the 1-per-second rate, however, completely verified the trend seen in Fig. 2.
7. Supported by grants M-623 (J.I.L.) and M-4529 (E.S.V.) from the National Institute of Mental Health. W.J.M. is a U.S. Public Health Service postdoctoral fellow (MF-8687).

25 March 1963

Heart Rate: Differential Effects of Hypothalamic and Septal Self-Stimulation

Abstract. *Heart rate in rats was recorded during self-stimulation with electrodes permanently implanted in both the hypothalamus and the septal region. Acceleration was observed during stimulation of the hypothalamus, and deceleration during stimulation of the septal region. In both areas self-stimulation reduced variability in heart rate.*

Malmo (*1*) suggests a relationship between the reinforcing properties of self-stimulation of the septal region and a parasympathetic "quieting" effect indicated by cardiac deceleration after stimulation. Our study compares the cardiac changes during self-stimulation of the septal region with such changes during self-stimulation at other "rewarding" brain sites (for example, hypothalamus) in the same animal.

Two enameled, stainless steel, bipolar electrodes were stereotaxically implanted (one in the septal region and one in the hypothalamus) in ten male albino rats approximately 120 days old, according to an established procedure (*2*). Four days after the operation all animals were trained to press a lever for brain stimulation. The experimental space consisted of a box, 12 by 12 by 14 inches, equipped with a microswitch actuated by a lever. A white light was positioned directly above the manipulandum. Brain stimulation resulted only when the white light was on. The electrical stimulus consisted of a bidirectional rectangular waveform (0.2 msec pulse duration) at a frequency of 100 cy/sec. The pulse train produced by a press of the lever lasted for 0.5 second. Current levels were determined individually for each animal within the following limits: 0.7 to 1.0 ma in the septal region, 0.2 to 0.5 ma in the hypothalamus.

Five rats were trained daily for 1 hour with septal self-stimulation, and this was followed later by daily training with hypothalamic self-stimulation. In the remaining five rats the procedure was reversed. Each session consisted of 5-minute periods of self-stimulation (light "on") alternating with 5-minute periods of "time-out" (light "off") when pressing the lever produced no self-stimulation. Lever responses were recorded separately for each on and off interval. Heart rate was recorded continuously with tantalum disk electrodes (1.5 cm in diameter, 1.0 mm thick) taped to the animals' side over the thoracic region. This method yielded polygraph tracings free from artifacts from which heart rate was determined by a count of the *R* waves in the electrocardiogram during each 20-second interval throughout both self-stimulation and timeout periods. Ectopic beats, randomly distributed throughout the stimulation and time-out periods, were not included in the heart-rate analysis.

Histological verification of placement of the electrodes was obtained after the experiments.

The results obtained with both hypothalamic and septal self-stimulation are summarized in Table 1. The data represent mean heart rate and lever-pressing values determined during one 50- to 70-minute session for each placement according to the alternating 5-minute on, 5-minute off procedure. Eight of the ten rats maintained stable lever-pressing rates for hypothalamic self-stimulation and nine of the ten animals responded consistently for septal self-stimulation. A comparison of the 5-minute alternating control and

Table 1. Mean heart rates and lever-pressing rates for each animal during experimental sessions involving self-stimulation in the hypothalamus and in the septal region.

Animal No.	Heart rate (beats per minute)		Lever-pressing rate (responses per minute)	
	Control	Self-stimulation	Control	Self-stimulation
	Hypothalamus			
1	359.8	428.2	0.6	43.8
2	409.5	424.6	1.6	47.9
3	322.6	352.2	1.5	13.0
4	349.6	469.8	1.6	28.0
5	457.9	464.3	2.0	39.4
6	477.7	486.1	2.1	40.7
7	438.5	413.6	2.3	24.3
9	490.0	464.0	2.1	21.6
	Septal region			
1	429.1	401.9	0.8	33.0
2	407.3	347.8	0.7	32.7
3	379.1	356.2	0.1	38.0
4	383.4	343.9	0.5	16.4
5	404.9	384.5	2.5	7.8
6	446.9	445.9	1.5	33.8
7	391.7	399.3	1.1	25.1
8	382.3	362.6	0.1	31.8
10	401.2	383.2	0.2	33.5