Brain Research, 109 (1976) 497–514

RESPONSES OF PRIMATE LOCUS COERULEUS AND SUBCOERULEUS NEURONS TO STIMULATION AT REINFORCING BRAIN SITES AND TO NATURAL REINFORCERS

DWIGHT C. GERMAN* AND EBERHARD E. FETZ

Regional Primate Research Center and Department of Physiology and Biophysics, University of Washington, Seattle, Wash. 98195 (U.S.A.)

(Accepted November 12th, 1975)

SUMMARY

In alert rhesus monkeys the activity of 64 neurons was recorded in the locus coeruleus and subcoeruleus region, collectively referred to as coeruleus (C) neurons. C neurons were identified physiologically by antidromic activation from electrodes in the medial forebrain bundle (MFB) and medial septal nucleus which sustained intracranial self-stimulation (ICSS) behavior, and/or anatomically by their proximity to microlesions at unit recording sites. The following results were obtained: (1) the current intensity that supported the highest rate of MFB and septal ICSS was similar to the current intensity for evoking antidromic responses in C neurons; (2) stimulation in the vicinity of the dopaminergic neurons of nucleus A10 did not activate C neurons; (3) C neurons were antidromically activated by ipsilateral and contralateral MFB shocks; (4) the C axons had slow estimated conduction velocities (1–5 m/sec), and a mean neural refractory period of 0.8 msec; (5) the behaviorally determined refractory period for MFB ICSS was also approximately 0.8 msec; and (6) mean firing rates while the monkey sat quietly were 15 $\pm$ 2 Hz (S.D.) for subcoeruleus cells and 5 $\pm$ 3 Hz for locus coeruleus cells, and activity of most cells changed negligibly during operant responding for apple-sauce reinforcement. These results suggest that the reinforcing effects of ICSS may be mediated by activation of coeruleus cells, but that these cells do not appear to be strongly involved in operant responding for natural reinforcers.

INTRODUCTION

The primate locus coeruleus (LC) and its rostroventral extension, nucleus sub-

* Present address: Department of Physiology, University of Texas Health Science Center, Dallas, Texas 75235, U.S.A. Reprint requests should be sent to this address.

coeruleus (SC), consist of catecholamine-containing neurons located in the dorsolateral pontine tegmentum[14,22]. Most LC cells are densely clustered medial and, to a lesser extent, lateral to the mesencephalic tract of the trigeminal nerve at the level of the decussation of the trochlear nerve. SC cells are more diffusely distributed rostroventral and somewhat lateral to LC and the mesencephalic tract of the trigeminal nerve. LC axons project to limbic, cerebral and cerebellar cortices, and to various brain stem and diencephalic nuclei in the rat[26,33,41,44], fetal human[29] and rhesus monkey[15]. SC axons project mainly to periventricular and preoptic areas of the hypothalamus[27,32]. Most of these neurons contain norepinephrine[9,24,25] and, functionally, the noradrenergic neurons in this region have been implicated in sleep[23], autonomic[9,10], and reinforcement[6,13,42] mechanisms.

The hypothesis that these noradrenergic neurons constitute one of the neural systems underlying intracranial self-stimulation (ICSS) — *i.e.*, operant responding reinforced by electrical stimulation of specific brain loci — is based primarily upon neuroanatomical mapping of reinforcing sites and neuropharmacological experiments[6,13,42]. High ICSS rates are sustained by stimulation of LC and SC[6,35,36], and of areas through which the LC and SC axons traverse, such as the lateral hypothalamic medial forebrain bundle (MFB) and the septal nucleus. Furthermore, ICSS rates are increased by drugs that facilitate noradrenergic transmission in the brain, and decreased by drugs that impair noradrenergic transmission (*cf.* German and Bowden[13]).

The reinforcing properties of both brain stimulation (at certain loci) and food have been suggested to depend upon the integrity of LC neurons. Septal ICSS[2,40] and MFB ICSS[31] are abolished by chronic lesions between the ICSS electrode and LC. Bilateral LC lesions can reduce the ability of rats to learn a runway maze for food reinforcement[1]. If LC cells mediate natural reinforcement, their activity might be affected by food reinforcement.

In the present experiment we investigated the responses of LC and SC cells recorded extracellularly in two alert monkeys. Units were tested for antidromic responses to stimulation at rostral ICSS loci, both ipsi- and contralaterally. The threshold and refractory period of these axons were directly measured and compared with the behaviorally determined measure of ICSS threshold and refractory period. The spontaneous activity of these cells was documented during inactivity and during operant responding for food reinforcement.

METHODS AND PROCEDURE

Initial training

Two 4-kg male rhesus monkeys (*Macaca mulatta*) were operantly conditioned with apple-sauce reinforcement. The reinforced response for monkey 'A' was a phasic elbow extension or flexion followed by a 1-sec hold, with each response being reinforced. The reinforced response for monkey 'B' was every tenth key press. The monkeys were seated in a primate chair in a sound attenuating booth. Two white lights in front of the monkey were turned on only when the operant response was to be reinforced, and a tone accompanied the delivery of each apple-sauce reinforcement.

For monkey 'B' a green light came on with the eighth key press. The monkeys typically responded for over 2 h.

Surgery

After stable operant responding was achieved, an electrode platform, recording chamber, and head-restraint fittings were secured to the skull with Vitalium screws and dental acrylic. A prefabricated dental acrylic platform[37] was stereotaxically placed above the skull and 22-gauge stainless steel guide tubes were inserted into the platform and tapped down to the skull surface at points indicated in Fig. 1 for monkey 'A'. A 10-mm diameter recording chamber (Trent Wells) was stereotaxically placed with its center on the midline at P-3.0, such that microelectrode tracks could be made in the vertical plane. This placement allowed exploration of the right and left LC over its entire anterior–posterior extent, and exploration of a major portion of SC.

Stimulation electrode implantation

Stimulating electrodes were made of 0.005-in. platinum wire (with 0.5 mm uninsulated tip) inserted into 28–gauge stainless steel tubing and protruding 1 mm beyone the end of the tubing. A few days after surgery, the monkey was anesthetized with ketamine and holes were drilled through the skull beneath certain guide tubes. After recovery from anesthesia, the stimulating electrodes were lowered into the brain through the guide tubes and ICSS behavior was tested. (The indifferent electrode was in the parietal skull.) The monkey could press a telegraph key to deliver a 300-msec train of 100-Hz, 0.1-msec biphasic shocks at 0.5 mA (Nuclear Chicago constant current stimulator) which is equivalent to 50 μA RMS current. The electrode was implanted at the depth which sustained ICSS. In monkey 'A', all of the electrodes on the right side were implanted before the right coeruleus region was initially investigated with microelectrodes. The left electrode was implanted before the subsequent exploration of the left coeruleus region. In monkey 'B', all stimulation electrodes were implanted prior to recording from LC.

ICSS testing

Each stimulating electrode was tested for the rate of key pressing at various current intensities. Each press incremented a counter and delivered electrical stimulation to the brain (same stimulus parameters as above). One electrode was tested per day, and always with increasing current intensities. At the beginning of each 1-min trial the animal was 'primed' with two 300-msec trains of brain stimulation at the current intensity that he could subsequently self-administer. A white noise generator was turned on at the beginning of a trial and turned off at the end of the trial. One minute elapsed between trials. The free operant rate of key pressing was defined as the number of presses per minute when no current was available with white noise on. The monkey's behavior in the sound attenuating booth was observed via closed circuit television throughout the entire experiment.

In monkey 'A', a behavioral refractory period was documented for the right

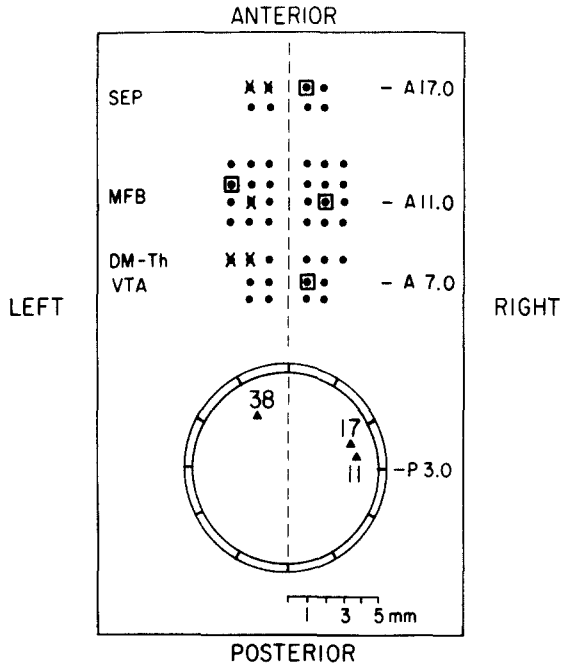

Fig. 1. Top view of electrode platform and recording chamber fixed to monkey 'A's skull. Dots represent guide tubes, symmetrically placed about the midline (dashed line). Squares represent electrodes that sustained ICSS, and Xs represent electrodes that did not. Recording chamber was centered on the midline at P-3.0. Triangles indicate coordinates of identified microlesions. The left LC unit, recorded in track 38, was antidromically activated only by shocks through the contralateral R-MFB electrode (1.6 msec latency 0.4 mA threshold). The right SC unit, recorded in track 17, was antidromically activated only by shocks through the R-SEP (4.1 msec latency) and R-MFB (3.2 msec latency) electrodes. The right SC unit, recorded in track 11, was antidromically activated only by shocks through the R-MFB electrode (3.0 msec latency).

MFB (R-MFB) ICSS electrode. Each key press delivered a 500-msec train of paired cathodal 0.1-msec shocks at 0.6 mA. The first shock of each pair was called the conditioning (C) pulse and the second, the test (T) pulse. The C–C interval was held constant at 20 msec, and the rate of key pressing per minute was recorded as a function of changes in the C–T interval. The behavioral refractory period was operationally defined as the longest C–T interval producing an ICSS rate not significantly different from that sustained by a train of conditioning pulses alone. Preceding each 1-min trial, the animal was primed twice with 500-msec trains of brain stimulation at the same parameters used in the subsequent trial. The longest C–T interval was always tested first, followed by progressively shorter C–T intervals, and the rate of responding for C–C sitimulation was tested next. To control for possible fatigue effects, the ICSS rate was tested for trains with 5-msec C–T intervals on the last trial each day, and high rates were always obtained.

Electrophysiology

Single units were recorded with 0.005-in. tungsten microelectrodes, insulated

with epoxy resin except for conical tips, which were 14–18 μm long and 14–18 μm across the base. With the monkey tranquilized by a light dose of ketamine (10 mg), a 60-mm-long 21-gauge sharp-tipped cannula was inserted into the brain. When the monkey was actively performing the operant response (usually within 20 min of receiving the ketamine), microelectrodes were lowered through the cannula into the brain with a remotely controlled hydraulic microdrive (Trent Wells). The microelectrode entered the brain at the level of the superior colliculus and recorded units to 10 mm below this point. In monkey 'A', 46 microelectrode tracks were made over a 6-month period; in monkey 'B', 40 microelectrode tracks were made over a 2-month period.

When the microelectrode was below the level of the trochlear nerve, isolated units were tested for response latency and threshold, absolute refractory period and antidromic activation from rostral stimulating electrodes (in monkey 'A'). The activity of these units in both monkeys was also observed during the performance of the operant task, and when the apple-sauce or apple slices were presented unconditionally. A Honeywell FM tape recorder recorded the unit action potentials, a voltage pulse triggered from the unit, arm position or key presses, apple-sauce feeder pulses, stimulus pulses, delayed trigger pulses occurring 1 sec after each elbow response, and voice. A Lab 8E computer (Digital Equipment Corp.) compiled off-line interspike interval and post-stimulus time histograms.

Histology

For histological location of the macro- and microelectrode tracks, the monkeys were anesthetized with Nembutal and perfused with 10% neutral buffered formalin. After removal of the skull and dura mater, with the head stereotaxically positioned, coronal knife cuts were made at A-22.0, A-4.0, and P-11.0. After the brain tissue was 'sunk' in formol-sucrose (30%), 40-μm thick frozen sections were cut to locate the stimulating electrode tips, microelectrode tracks, and microlesions (produced by passing 35 μA anodal current through the microelectrode for 20 sec) at or below unit recording sites. In monkey 'A', 3 microlesions were made at predetermined distances below unit recording sites; in monkey 'B', 3 microlesions were made directly at unit recording sites.

RESULTS

ICSS

Fig. 1 illustrates the platform attached to the skull of monkey 'A', and shows the anterior–posterior coordinates of the implanted electrodes. Four of the 9 stimulating electrodes supported ICSS. These electrodes were in the R-MFB, right ventromedial septal nucleus (R-SEP), the left MFB (L-MFB), and in the right ventral tegmental area (R-VTA). Electrodes in the dorsomedial nucleus of the thalamus and in the lateral ventricle adjacent to the septal nucleus did not support ICSS. One electrode in the L-MFB proved defective. The locations of electrode tips that sup-

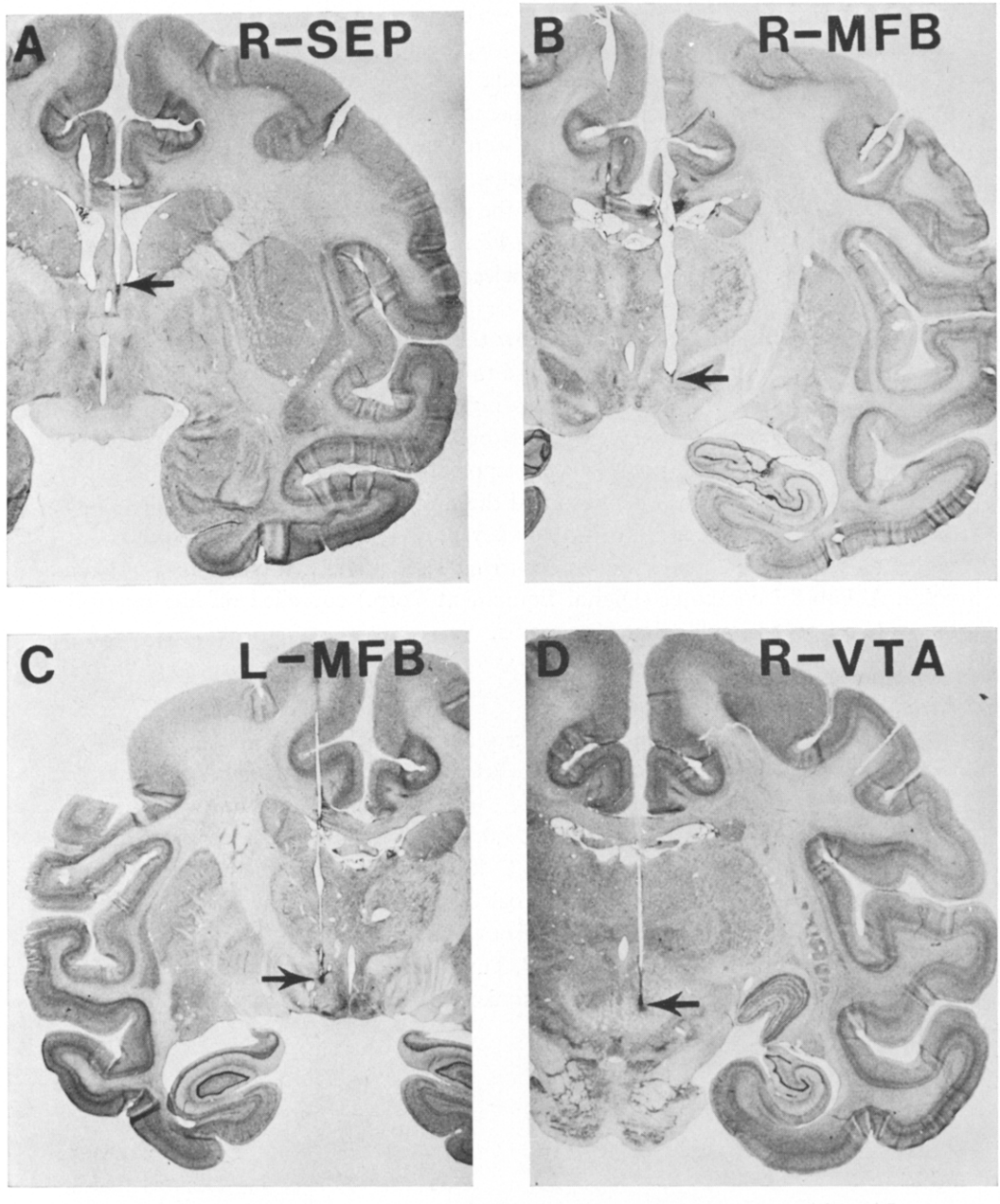

Fig. 2. Histological identification of ICSS electrode tracks and tips (arrows) in monkey 'A'. A: right medial septal electrode (R-SEP). B: right medial forebrain bundle electrode (R-MFB). C: left medial forebrain bundle electrode (L-MFB). D: right ventral tegmental area electrode (R-VTA).

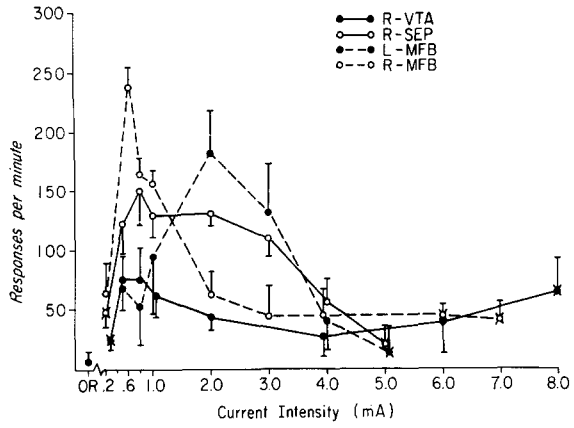

Fig. 3. Mean number of ICSS key press responses per minute (± S.D.) as a function of current intensity for different stimulation electrodes in monkey 'A'. Mean operant rate, with no brain stimulation, is indicated by OR. Means were compiled for five 1-min trials; those marked by X are means of 3 trials.

ported ICSS are illustrated in coronal brain sections in Fig. 2. In monkey 'B', reliable low threshold ICSS was not obtained because the electrodes were off target.

Fig. 3 plots the ICSS rate at each electrode as a function of current intensity. Two features of these data are noteworthy. (1) The current level that sustained the highest ICSS rate varied with electrode placement. The highest ICSS rate occurred at 0.6 mA for R-MFB, in contrast to 2.0 mA for L-MFB. This difference may reflect the asymmetrical placement of the two MFB electrodes with respect to the axons involved in ICSS. High rates of ICSS were sustained by R-SEP stimulation over a wide current range. The lowest ICSS rates were sustained by R-VTA stimulation. (2) At higher current intensities the ICSS rate decreased for all electrodes except the R-VTA (for this electrode there was an increase in ICSS rate from 4.0 to 8.0 mA; $t = 2.26$, $P < 0.05$, single tail test). These data suggest that higher current intensities may recruit fiber systems which decrease ICSS rates, and for R-VTA stimulation different fiber systems may be involved for ICSS observed at high and low current intensities.

Motor responses were elicited by ICSS trains at some electrode sites. Each R-VTA stimulation train evoked a right pupillary constriction at 0.5 mA and downward movement of the right eyebrow. At 2.0 mA, the head tilted backward and to the right. During R-MFB ICSS, lip smacking occurred occasionally at 0.6 mA, and reliably at 1.0 mA. Less stereotyped lip smacking behavior was elicited during L-MFB ICSS at a current of 3.0 mA. No elicited behaviors accompanied R-SEP ICSS, and no behavioral signs of seizure activity were seen at any of the current intensities tested.

The behavioral refractory period for R-MFB ICSS was measured with trains of pulse pairs as illustrated in Fig. 4A. Figure 4B shows the rate of ICSS as a function of changes in the C–T interval, and suggests that the neurons mediating ICSS have a refractory period of approximately 0.8 msec. When only conditioning shocks were available, at 50 Hz, the mean ICSS rate was 83 presses/min. When the pulse frequency

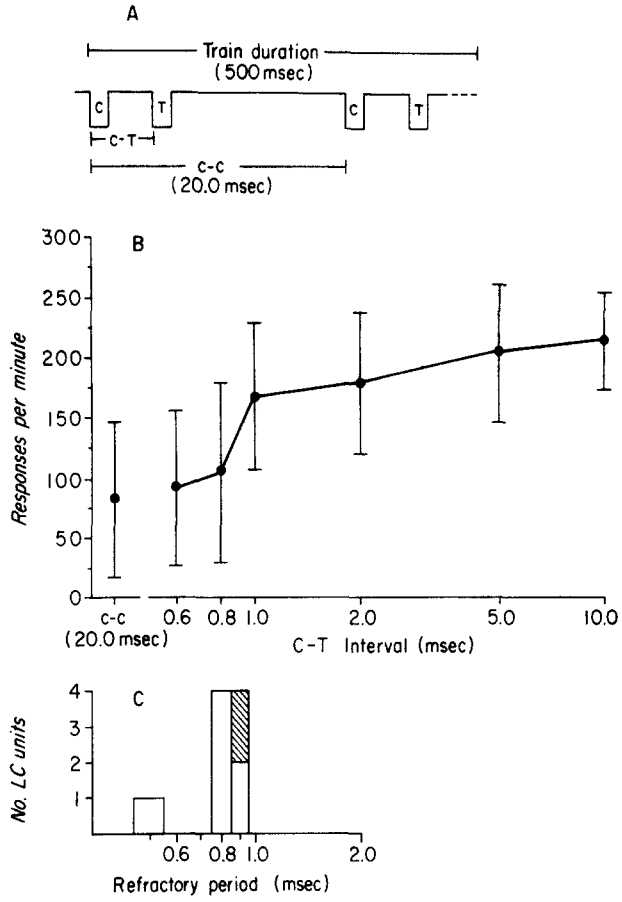

Fig. 4. A: stimulus parameters used to determine refractory period of ICSS consisted of a 500-msec train of 0.1 msec cathodal pulse pairs. The first pulse in each pair was the conditioning (C) pulse, and the second, the test (T) pulse. Current intensity (0.6 mA) and C–C interval (20 msec) were held constant. B: mean number of ICSS key press responses per minute (± S.D.) as a function of C–T interval. Each value is based on 9 trials. C: electrophysiologically determined refractory period of C units. Cross-hatching indicates units that followed two shocks at 1 kHz, but were not tested at shorter C–T intervals.

was doubled, the mean ICSS rate also doubled. Although under all C–T conditions the net current was double that under the C–C condition, the ICSS rate depended upon the C–T interval. The ICSS rate was significantly higher for the 1.0-msec C–T interval than for 0.8-msec ($P < 0.05$), 0.6 msec ($P < 0.02$), or C–C shocks ($P < 0.01$), whereas there was no significant difference between the ICSS rates for C–T shocks at 0.8 msec or 0.6 msec compared with C–C shocks.

Unit responses

Fig. 5A shows a coronal brain section through the LC at plane P-1.2 (ref. 14). As the microelectrode was lowered through the brain, a clear and repeatable sequence of unit response characteristics was usually observed. At the level of the superior

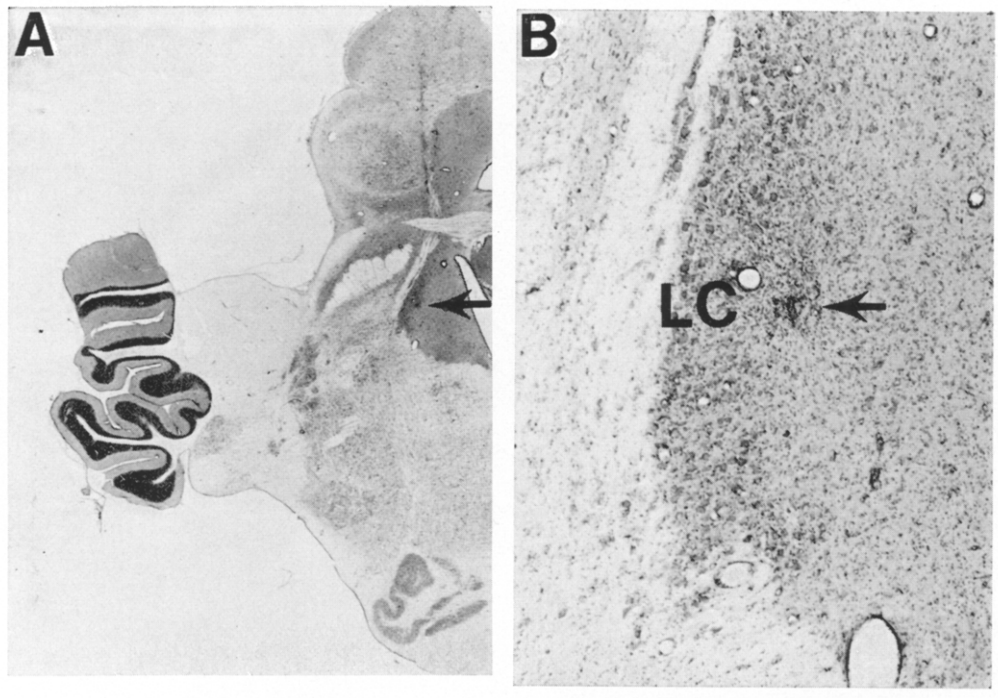

Fig. 5. A: coronal section through the left LC region, at approximately P-1.2, illustrating a microelectrode track through the superior and inferior colliculi, and a microlesion (arrow) at the location of unit 21.2 in monkey 'B'. B: a higher power magnification of the area containing the microlesion (arrow) shown in A.

colliculus, units responded to visual stimuli such as moving objects. Lower in the track, presumably in the inferior colliculus, units responded to auditory stimuli (*e.g.*, hand clap). The units encountered next were active with downward eye movements, suggesting trochlear nerve recording. Below this level, each isolatable unit was tested with single shocks through each stimulating electrode and/or observed during the performance of the operant response. In a single track up to 8 units responded to shocks through one or more ICSS electrodes; histological reconstruction indicated that these units were in the vicinity of the LC. Neighboring units had similar action potential shapes and firing patterns, but could not be activated by any ICSS electrode. Below this depth units sometimes fired synchronously with chewing, suggesting recordings from trigeminal motor neurons.

A total of 64 units were recorded in the LC and SC region (46 in monkey 'A' and 18 in monkey 'B'). In monkey 'A', all units on the right and 16 on the left were considered to be antidromically activated on the basis of their response to two shocks at 500 Hz with invariant latency (Fig. 6A), and/or collision testing (Fig. 6B). Table I summarizes the conduction time and response threshold data for the antidromically activated units. The conduction velocity estimate assumes a conduction distance equal to the distance between the stimulating and recording electrodes.

Ipsilateral activation of units on the right, excited by R-SEP and R-MFB shocks,

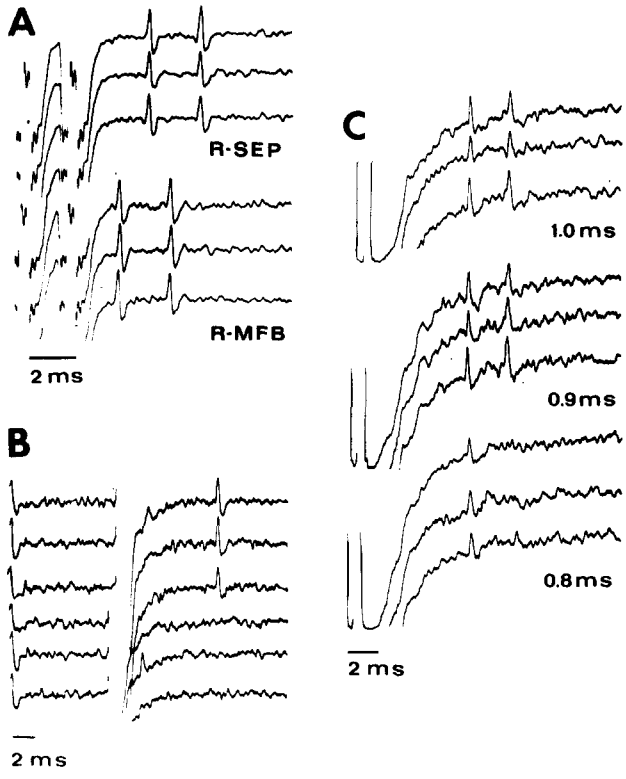

Fig. 6. Antidromic responses of C units. A: unit in the right SC region which responded to two biphasic shocks (500 Hz) at R-SEP and R-MFB electrodes. B: collision between spontaneous orthodromic action potential in the left LC region (at beginning of each sweep) and antidromic action potential evoked by L-MFB stimulation (shock artifact at center). Stimulation of L-MFB 10 msec after the spontaneous spike (top 3 traces) evoked an antidromic spike; for 9-msec intervals (bottom 3 traces), the antidromic and orthodromic spikes collided. C: same unit as in B (track 42) illustrating the neural refractory period. Two suprathreshold shocks separated by 1.0 and 0.9 msec evoked two action potentials; at 0.8 msec the second action potential was not evoked.

occurred with a mean conduction velocity of about 4 m/sec and two units were activated by shocks through both electrodes (*e.g.*, Fig. 6A). One unit responded to R-VTA shocks with a much slower conduction velocity, 1.7 m/sec. In the left LC region, 4 units were antidromically activated by L-MFB shocks, and the conduction velocity for these axons averaged 1.3 m/sec. Several units in monkey 'B' were antidromically activated by non-ICSS electrodes at high current intensities. Their estimated conduction velocities averaged less than 1 m/sec.

Twelve units in the left LC region were antidromically activated by shocks through contralateral ICSS electrodes. Six responded to R-MFB shocks with a conduction velocity of about 6 m/sec, and generally with a higher current threshold than for ipsilateral activation. Six units were activated by contralateral R-VTA shocks; the mean conduction velocity was 2 m/sec, and the threshold averaged 4.0 mA. No units responded to stimulation of contralateral R-SEP, nor non-ICSS electrodes. Three

TABLE I

ANTIDROMICALLY ACTIVATED C UNITS IN MONKEY 'A'; LATENCY, ESTIMATED CONDUCTION VELOCITY AND THRESHOLD

Unit location	*Stimulation site*	*Latency (msec) mean ± S.D.*		*N*	*Estimated conduction velocity (m/sec) mean ± S.D.*		*Threshold (mA) mean ± S.D.*		*N*
Right SC	R-SEP	5.03	0.95	3	3.76	0.72	0.55	0.07	2
	R-MFB	2.71	0.86	21	5.03	1.45	0.73	0.64	6
	R-VTA	5.00	—	1	1.70	—	—	—	0
Left SC	R-SEP	—	—	0	—	—	—	—	0
	R-MFB	2.43	0.64	6	6.34	1.78	3.28	2.84	5
	R-VTA	7.58	2.62	6	1.64	0.94	3.92	1.74	6
	L-MFB	10.50	1.29	4	1.30	0.16	1.87	1.86	3

units on the right and 5 on the left were considered to be orthodromically activated because they did not respond to double shocks with invariant latency (2–40 msec).

The neural refractory period was determined for several units, both ipsi- and contralaterally activated (Fig. 4C). Two suprathreshold shocks were delivered at various C–T intervals, and the interval at which T shocks failed to elicit an action potential was considered to be the absolute refractory period of the axon. Often when the C–T interval was less than 2.0 msec, the second action potential occurred at a longer latency (*cf.* ref. 12) but did not fail to occur until the C–T interval was reduced to 0.8 msec (Fig. 6C).

Thirty-one C units were examined for changes in firing pattern while the monkey performed the operant response for apple-sauce reinforcement and/or sat quietly. In monkey 'A', 11 units in the right SC region and two units in the left LC region were recorded while the monkey performed the continuously reinforced elbow movement response and/or sat quietly. In monkey 'B', 18 units in the LC region were recorded while the monkey performed the fixed ratio-10 key press response for apple-sauce and/or sat quietly. For the LC and SC units recorded under both conditions, there was no significant difference in firing frequency between operant responding and quiet resting (Table II). The mean firing rate was 15 ± 2 Hz (S.D.) for SC (monkey 'A') and 5 ± 3 Hz for LC (monkeys 'A' and 'B'). Time histograms of unit activity triggered from the apple-sauce feeder discharge showed no change in unit activity following the delivery of the apple-sauce or the feeder tone. Fig. 7 illustrates a SC unit whose activity was not affected by arm movement, apple-sauce delivery or the tone paired with the apple-sauce delivery.

Histology

Histological sections revealed microelectrode tracks on the right side of the brain in monkey 'A' within the lateral portions of the superior and inferior colliculi

TABLE II

FIRING RATES OF C UNITS DURING INACTIVITY AND OPERANT RESPONDING

Unit		*Sitting quietly (imp./sec)*		*Operant responding (imp./sec)*	
		*Mean**	± *S.D.*	*Mean* ± *S.D.*	
Monkey 'A' SC Units					
3		—	—	14.1	1.4
11		—	—	15.8	4.5
17		16.6	1.7	—	—
21.1		18.7	4.6	17.7	3.6
21.2		13.6	2.2	13.5	1.8
23		15.1	2.8	—	—
24		14.9	1.1	—	—
26.1		16.6	1.3	—	—
28.2		14.5	1.4	14.3	1.7
30.1		16.4	0.9	17.5	0.6
31.2		11.8	2.7	—	—
	Mean	15.4		15.5	
	S.D.	2.0		1.8	
	N	9		6	
Monkey 'A' LC Units					
42.1		10.3	1.1	—	—
42.4		8.4	1.6	—	—
	Mean	9.4			
	S.D.	1.3			
	N	2			
Monkey 'B' LC Units					
8.1		8.2	1.6	9.2	2.8
10.1		1.7	0.3	—	—
11.1		1.9	1.7	1.4	0.3
11.2		1.0	0.3	—	—
13.1		5.7	1.6	5.7	1.4
21.1		1.6	0.8	1.2	0.2
21.2		5.2	1.3	5.6	0.3
22.1		7.5	2.2	8.6	1.8
27.1		9.9	0.8	—	—
27.2		1.7	0.2	1.3	0.7
29.1		6.6	1.4	4.4	1.7
29.2		2.4	0.8	2.1	1.2
29.3		3.9	0.6	—	—
37.1		2.6	0.1	2.3	0.1
39.1		7.9	0.5	—	—
39.2		7.9	1.9	—	—
40.2		4.4	0.6	—	—
40.3		2.1	0.2	2.2	0.1
	Mean	4.6		4.0	
	S.D.	2.8		2.9	
	N	18		11	

* Each mean is based upon an average of 90 sec of unit activity averaged over 10-sec intervals (range 30–150 sec).

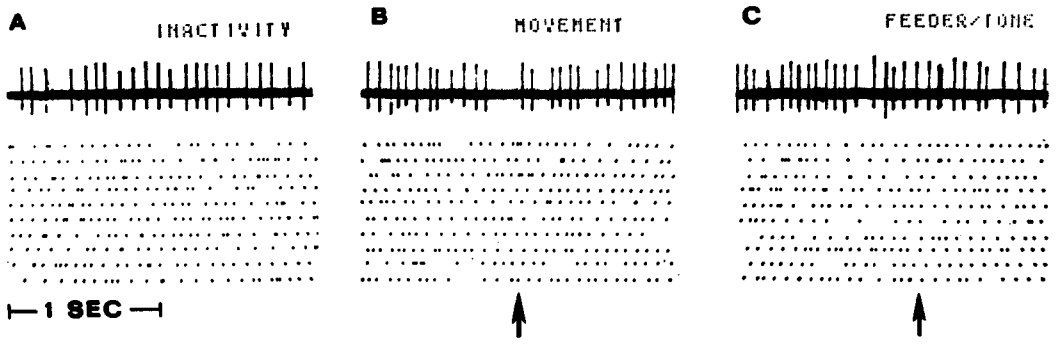

Fig. 7. Two-second samples of unit activity (top) summarized in dot raster form (bottom). This unit (21.2) was recorded in monkey 'A' in the right SC region while sitting quietly and inactive (A), and while flexing and extending the elbow for apple-sauce reinforcement (B and C). Responses are aligned at onset of elbow movement in B, and onset of tone and apple-sauce feeder discharge in C (at arrows). This unit responded to R-MFB shocks at a latency of 2.0 msec with a threshold of 0.3 mA.

and down into the lateral portions of the LC, and in the medial parabrachial nucleus. This latter region contains some of the catecholamine-containing cells of nucleus. SC[14]. Two microlesions were located below the right SC region, and one below the left LC region near the trochlear nerve. Allowing for tissue shrinkage, and considering the distance between unit recording sites and microlesion sites, the units recorded on the right were in SC and those on the left were in LC. In monkey 'B', microelectrode tracks on the right side of the brain went through the medial portions of the superior and inferior colliculi and into the right LC region. Microelectrode tracks on the left extended into the left LC region. Fig. 5B shows a microlesion made near a presumed LC unit encountered 0.75 mm below a unit excited by jaw opening. Fig. 5B confirms cells of the mesencephalic tract of the trigeminal nerve just dorsal to LC. A second microlesion was located in the caudal tip of LC, corresponding to nucleus A4. These microlesions indicate that LC units were recorded in monkey 'B'.

DISCUSSION

By recording activity of LC and SC cells in monkeys trained to operantly respond for food and intracranial reinforcement, we documented the responses of coeruleus cells to electrical stimulation at reinforcing sites and under natural behavioral conditions. Parameters of intracranial stimulation effective for sustaining ICSS behavior were similar to those which antidromically activated coeruleus cells. In particular, the following parallels between the behavioral and electrophysiological effects of intracranial stimulation were noted: (1) stimulus sites which were most effective for sustaining ICSS also activated the greatest proportion of C cells; (2) stimulus intensities which sustained highest ICSS rates were comparable to those which elicited antidromic responses in C cells; and (3) with paired stimuli the refractory period measured behaviorally by ICSS rates was similar to that measured physiologically by antidromic responses of C cells. As discussed below, these results are

consistent with the hypothesis that activation of C cells may mediate ICSS behavior. During operant responding for food reward, the activity of most C cells was relatively unaffected by delivery of food or conditioned reinforcers. This suggests that neural mechanisms mediating natural reinforcement may involve other cells or more subtle mechanisms.

The fact that many C cells were antidromically activated from shocks through electrodes in the MFB and septal regions is consistent with anatomical findings that LC axons project to these regions[15]. Many cells interspersed with antidromically activated cells had similar firing patterns, but were not themselves antidromically activated; this is consistent with evidence that some C cells do not send axons through the MFB[26,44] and the possibility that some C axons in the MFB may not have been stimulated. The fact that ICSS could be sustained by stimulation at these sites, as well as by stimulation at LC and SC[7,35,36] further supports the possibility that stimulation of these cells mediates the reinforcing effects of ICSS.

Based upon the anatomy of the catecholamine neuron systems[26,44] we did not expect stimulation at R-VTA to antidromically activate cells in the vicinity of LC; however, high current intensities did antidromically activate some cells. The R-VTA electrode was aimed at the dopaminergic cell bodies of nucleus A10 which lies dorsal and lateral to the interpeduncular nucleus (*i.e.*, nucleus paranigralis). Activation of this mesolimbic dopamine system may also support ICSS behavior[6,13]. The histology showed the electrode tip to be about 2 mm above nucleus A10 which could explain the low ICSS rate. Assuming that ICSS sustained by stimulating a dopaminergic system is independent of the activation of the noradrenergic LC neurons, one would not expect LC cells to be antidromically activated by VTA stimuli. In fact, only one cell out of 25 was influenced by ipsilateral stimulation, and the units that were contralaterally activated all had high current thresholds. Similarly, in the rat more brain stem units were reported to be activated by shocks through MFB ICSS electrodes than by shocks at nucleus accumbens ICSS loci (a region containing nucleus A10 dopamine terminals)[39]. More recently, cells in the vicinity of LC were antidromically activated by MFB ICSS shocks but not by shocks at nucleus accumbens ICSS loci[5]. Interestingly, as the current intensity was increased, VTA shocks antidromically activated more contralateral LC cells, and also sustained higher ICSS rates. Since this electrode was close to the decussating LC axons in the region of the decussation of the superior cerebellar peduncle[15], it may be that ICSS rates for 1.0 mA current intensity reflect activation of the mesolimbic dopamine neurons, whereas ICSS rates for current above 4.0 mA reflect the recruitment of the dorsally situated LC axons.

This appears to be the first electrophysiological confirmation of the anatomical observation[15,41,44] that LC neurons send contralateral projections. LC axons ascend ipsilaterally in the MFB to innervate diencephalic and cortical areas, and axon collaterals cross the midline and ascend in the contralateral MFB. Consequently, unilateral stimulation of LC axons should produce responses in both contra- and ipsilateral fibers. The greater estimated conduction velocity of the contralateral axons compared to the ipsilateral axons is not readily explainable.

The stimulus intensity sustaining the highest ICSS rate was correlated with the mean ipsilateral antidromic response threshold for C neurons. Maximal ICSS rates required higher current intensities at the L-MFB electrode (2.0 mA) than at the R-MFB electrode (0.6 mA). Similarly, the mean threshold for antidromic responses of ipsilateral C units was higher for L-MFB (1.9 mA) than for R-MFB (0.7 mA). The lower antidromic threshold suggests that the R-MFB electrode was closer to the C axons than the L-MFB electrode. Indeed, recent anatomical evidence suggests that the LC axons are located more dorsal to the site of the L-MFB electrode than the R-MFB electrode[15].

The ICSS rate was also related to the number of C cells antidromically activated. The highest ICSS rate was sustained by the MFB electrodes, which ipsilaterally activated 25 of 41 responsive units (61%). Stimulation of R-SEP sustained lower ICSS rates, and activated fewer ipsilateral C cells (3/25 = 12%). This is consistent with the possibility that the reward value of the stimulation at a given site is proportional to the number of C cells activated; however, it must be remembered that the entire volume of LC and SC was not sampled homogeneously, and that dopamine fibers also ascend in the MFB and have been implicated in ICSS[6,13].

An inverse relation between axonal conduction velocity and refractory period has been demonstrated in peripheral nerve[17] and in central fiber pathways[34]. This relationship is based upon axonal diameter and various membrane properties[20]. In peripheral nerves, the fast-conducting myelinated A-delta fibers, which range in size from about 2 to 6 μm in diameter, have refractory periods between 1.1 and 0.5 msec (see ref. 17); the more slowly conducting unmyelinated C fibers, which are less than 1.4 μm in diameter, have refractory periods of about 2.0 msec (ref. 18). A refractory period of 0.8 msec has been documented behaviorally and electrophysiologically for neurons excited at MFB ICSS loci[8,11,16,38,43]; this would correspond to a peripheral A-delta fiber of about 2 μm in diameter. However, Szabo *et al.*[43] recently reported that most myelinated axons near MFB ICSS electrodes have diameters between 0.6 and 1.3 μm. Moreover, the unmyelinated, fine diameter coeruleus axons are structurally similar to peripheral C fibers[21]. The fact that we found a 0.8 msec refractory period for these axons suggests that the refractory periods of coeruleus axons may be much shorter than those of comparably sized peripheral fibers.

The 0.8-msec refractory period of C axons corresponds well with the behaviorally measured refractory period for ICSS at the R-MFB electrode. In the rat similar refractory periods have been measured behaviorally for ICSS sustained by MFB stimulation[8,11,16,43], and physiologically for antidromic responses of units in the vicinity of LC[5]. The present experiment allows direct comparison between the behavioral refractory period of MFB ICSS and the refractory period of C axons in the same animal from the same stimulation site. The close correlation further supports the hypothesis that stimulation of C axons is important for sustaining ICSS, and suggests that the refractory period of C axons could account for the behavioral ICSS refractory period of 0.8 msec. (A similar approach has been recently employed in comparing psychophysical and electrophysiological refractory periods of fibers in primate anterolateral spinal cord quadrant subserving pain[28,34].)

Both histological and electrophysiological data suggest that we recorded two cell populations which we would attribute to LC and SC. Histological reconstruction of electrode tracks indicates that cells recorded in monkey 'B' and on the left side of monkey 'A' were in LC, while cells recorded on the right side in monkey 'A' were in SC. The LC cells responded antidromically with longer latencies to ipsilateral stimulation (10.5 $\pm$ 1.3 msec) than the SC cells (2.7 $\pm$ 0.9) msec. Moreover, SC cells had higher firing rates (15 $\pm$ 2 Hz) than LC cells (5 $\pm$ 3 Hz).

During operant responding, no change in C unit activity was found related to food reinforcement. The spontaneous activity of the C cells while the monkey sat quietly was relatively slow and comparable to that observed in the cat during attentive wakefulness[3,4,19]. In some LC cells, a slight increase in unit firing rate was observed while the monkey ate raisins, banana pellets or apple-sauce. This activity did not seem correlated with any specific component of food consumption. For example, the gustatory stimuli did not drive C cells as they clearly do in the pontine taste neurons[30], and chewing did not evoke correlated bursts as seen in nearby mesencephalic tract of the trigeminal neurons. Moreover, these units were not modulated during active or passive arm movements, and were unaffected by auditory stimuli paired with apple-sauce delivery. Since only a small number of SC units were examined during operant responding for food, the conclusion that these units are not related to natural positive reinforcers remains tentative. Because there is both anatomical[26,44] and physiological [4,19] heterogeneity within the LC, more data are desirable to determine whether subgroups of LC cells may be related to operant responding for a natural reward.

ACKNOWLEDGEMENTS

This research was supported by Grants RR00166, NS05082 and NS11027 from the National Institutes of Health, U.S. Public Health Service.

We thank Dr. Douglas Bowden for helpful discussion in the preparation of this manuscript, and Ms. K. Schmitt for editorial assistance.

REFERENCES

1 ANLEZARK, G. M., CROW, T. J., AND GREENWAY, A. P., Impaired learning and decreased cortical norepinephrine after bilateral locus coeruleus lesions, *Science*, 181 (1973) 682–684.
2 BOYD, E. S., AND CELSO, M. B., Effect of some brain lesions on septal intracranial self-stimulation in the rat, *Amer. J. Physiol.*, 219 (1970) 734–741.
3 CHU, N.-S., AND BLOOM, F. E., Norepinephrine-containing neurons: changes in spontaneous discharge patterns during sleeping and waking, *Science*, 179 (1973) 908–910.
4 CHU, N.-S., AND BLOOM, F. E., Activity patterns of catecholamine-containing pontine neurons in the dorso-lateral tegmentum of unrestrained cats, *J. Neurobiol.*, 5 (1974) 527–544.
5 COOPER, S. J., AND ROLLS, E. T., Relation of activation of neurons in the pons and medulla to brain-stimulation reward, *Exp. Brain Res.*, 20 (1974) 207–222.
6 CROW, T. J., Catecholamine-containing neurons and electrical self-stimulation: 1. A review of some data, *Psychol. Med.*, 2 (1972) 414–421.
7 CROW, T. J., SPEAR, P. J., AND ARBUTHNOTT, G. W., Intracranial self-stimulation with electrodes in the region of the locus coeruleus, *Brain Research*, 36 (1972) 275–287.
8 DEUTSCH, J. A., Behavioral measurement of the neural refractory period and its application to intracranial self-stimulation, *J. comp. physiol. Psychol.*, 58 (1964) 1–9.

9 FUXE, K., GOLDSTEIN, M., HÖKFELT, T., AND HYUB, J. T., Immunohistochemical localization of dopamine-β-hydroxylase in the peripheral and central nervous system, *Res. Commun. chem. path. Pharmacol.*, 1 (1970) 627–636.
10 FUXE, K., HÖKFELT, T., AND UNGERSTEDT, U., Morphological and functional aspects of central monoamine neurons, *Int. Rev. Neurobiol.*, 13 (1970) 93–126.
11 GALLISTEL, C. R., ROLLS, E. T., AND GREENE, D., Neuron function inferred from behavioral and electrophysiological estimates of refractory period, *Science*, 166 (1969) 1028–1030.
12 GASSER, H. S., AND ERLANGER, J., The nature of conduction of an impulse in the relatively refractory period, *Amer. J. Physiol.*, 73 (1925) 613–635.
13 GERMAN, D. C., AND BOWDEN, D. M., Catecholamine systems as the neural substrate for intracranial self-stimulation: a hypothesis, *Brain Research*, 73 (1974) 381–419.
14 GERMAN, D. C., AND BOWDEN, D. M., Locus ceruleus in rhesus monkey (*Macaca mulatta*): a combined histochemical fluorescence, Nissl and silver study, *J. comp. Neurol.*, 161 (1975) 19–30.
15 GERMAN, D. C., AND BOWDEN, D. M., An autoradiographic study of locus ceruleus projections in the rhesus monkey, *Neurosci. Abstr.*, 1 (1975) 324.
16 GERMAN, D. C., AND HOLLOWAY, F. A., Behaviorally determined neurophysiological properties of MFB-self-stimulation fibers, *Physiol. Behav.*, 9 (1972) 823–829.
17 GRUNDFEST, H., Bioelectric potentials, *Ann. Rev. Physiol.*, 2 (1940) 213–242.
18 GRUNDFEST, H., AND GASSER, H. S., Properties of mammalian nerve fibers of slowest conduction, *Amer. J. Physiol.*, 123 (1938) 307–318.
19 HOBSON, J. A., MCCARLEY, R. W., AND WYZINSKI, P. W., Sleep cycle oscillations: reciprocal discharge by two brainstem neuronal groups, *Science*, 189 (1975) 55–58.
20 HODGKIN, A. L., AND HUXLEY, A. F., A quantitative description of membrane current and its application to conduction and excitation in nerve, *J. Physiol. (Lond.)*, 117 (1952) 500–544.
21 HOFFER, B. J., SIGGINS, G. R., OLIVER, A. P., AND BLOOM, F. E., Activation of the pathway from locus coeruleus to rat cerebellar purkinje neurons: pharmacological evidence of noradrenergic central inhibition, *J. pharmacol. exp. Ther.*, 184 (1973) 553–569.
22 HUBBARD, J. E., AND DICARLO, V., Fluorescence histochemistry of monoamine-containing cell bodies in the brain stem of the squirrel monkey (*Saimiri sciureus*). I. The locus coeruleus, *J. comp. Neurol.*, 147 (1973) 553–566.
23 JOUVET, M., Monoaminergic regulation of the sleep–waking cycle in the cat. In F. O. SCHMITT AND F. G. WORDEN (Eds.), *The Neurosciences, Third Study Program*, MIT Press, Cambridge, Mass., 1974, pp. 499–508.
24 KORF, J., AGHAJANIAN, G. K., AND ROTH, R. H., Stimulation and destruction of the locus coeruleus: opposite effects on 3-methoxy-4-hydroxyphenylglycol sulfate levels in the rat cerebral cortex, *Europ. J. Pharmacol.*, 21 (1973) 305–310.
25 KUHAR, M., ROTH, R. H., AND AGHAJANIAN, G. K., Synthesis of catecholamines in the locus coeruleus from H^3-tyrosine *in vivo*, *Biochem. Pharmacol.*, 21 (1972) 2280–2282.
26 LINDVALL, O., AND BJÖRKLUND, A., The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method, *Acta physiol. scand.*, Suppl. 412 (1974) 1–48.
27 MAEDA, T., ET SHIMIZU, N., Projections ascendantes du locus coeruleus et d'autres neurons aminergiques pontiques au niveau du prosencéphale du rat, *Brain Research*, 36 (1972) 19–35.
28 MAYER, D. J., PRICE, D. D., AND BECHER, D. P., Neurophysiological characterization of the anterolateral spinal cord neurons contributing to pain perception in man, *Pain*, 1 (1975) 51–59.
29 NOBIN, A., AND BJÖRKLUND, A., Topography of the monoamine neuron systems in the human brain as revealed in fetuses, *Acta physiol. scand.*, Suppl. 388 (1973) 1–40.
30 NORGREN, R., AND LEONARD, C. M., Ascending central gustatory pathways, *J. comp. Neurol.*, 150 (1973) 217–238.
31 OLDS, M. E., AND OLDS, J., Effects of lesions in medial forebrain bundle on self-stimulation behavior, *Amer. J. Physiol.*, 217 (1969) 1253–1264.
32 OLSON, L., AND FUXE, K., Further mapping out of central noradrenaline neuron systems: projections of the subcoeruleus area, *Brain Research*, 43 (1972) 289–295.
33 PICKEL, V. M., SEGAL, M., AND BLOOM, F. E., A radioautographic study of the efferent pathways of the nucleus locus coeruleus, *J. comp. Neurol.*, 155 (1974) 15–42.
34 PRICE, D. D., AND MAYER, D. J., A neurophysiological characterization of anterolateral quadrant neurons subserving pain in *M. mulatta*, *Pain*, 1 (1975) 60–73.
35 RITTER, S., AND STEIN, L., Self-stimulation of noradrenergic cell group (A6) in locus coeruleus of rats, *J. comp. physiol. Psychol.*, 85 (1973) 443–452.

36 RITTER, S., AND STEIN, L., Self-stimulation in the mesencephalic trajectory of the ventral noradrenergic bundle, *Brain Research*, 81 (1974) 145–157.
37 ROBINSON, B. W., Head-mounted electrode platform: construction and error analysis, *Physiol. Behav.*, 2 (1967) 455–457.
38 ROLLS, E. T., Involvement of brainstem units in medial forebrain bundle self-stimulation, *Physiol. Behav.*, 7 (1971) 297–310.
39 ROUTTENBERG, A., AND HUANG, Y. H., Reticular formation and brainstem unitary activity: effects of posterior hypothalamic and septal-limbic stimulation at reward loci, *Physiol. Behav.*,3 (1968) 611–617.
40 SCHIFF, B. B., The effects of tegmental lesions on the reward properties of septal stimulation, *Psychon. Sci.*, 1 (1964) 397–398.
41 SHIMIZU, N., OHNISHI, S., TOHYAMA, M., AND MAEDA, T., Demonstration by degeneration silver method of the ascending projections from locus ceruleus, *Exp. Brain Res.*, 21 (1974) 181–192.
42 STEIN, L., Chemistry of reward and punishment. In D. H. EFRON (Ed.), *Psychopharmacology: A Review of Progress, 1957–1967*, Public Health Service No. 1836, Government Printing Office, Washington, D.C., 1968, pp. 105–123.
43 SZABO, I., LENARD, L., AND KOSARAS, B., Drive decay theory of self-stimulation: refractory periods and axon diameters in hypothalamic reward loci, *Physiol. Behav.*, 12 (1974) 329–343.
44 UNGERSTEDT, U., Stereotaxic mapping of monoamine pathways in the rat brain, *Acta physiol. scand.*, 82, Suppl. 367 (1971) 1–48.