ELECTROPHYSIOLOGICAL PROPERTIES OF

NEURONS AT SELF-STIMULATION SITES IN

THE MEDIAL FOREBRAIN BUNDLE OF THE RAT

© Ivan Kiss

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ABSTRACT

ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS AT SELF-STIMULATION SITES IN THE MEDIAL FOREBRAIN BUNDLE OF THE RAT

Ivan Kiss

Recent psychophysical experiments have characterized the medial forebrain bundle (MFB) elements that comprise the directly stimulated stage of the neural circuit responsible for brain stimulation reward (BSR). These inferred characteristics, which include recovery from refractoriness that begins at about 0.4 msec. and usually ends by 1.5 msec. and conduction velocities of between 1.8 and 8.0 m/sec., may play an important role in determining which neurons are directly responsible for BSR.

Field potential recordings were obtained to directly assess the plausibility of the behaviourally derived inferences. Stimulating and recording electrodes were aimed at MFB targets that support self-stimulation (SS). In this manner, electro-

physiological estimates of refractory periods were collected. Recovery began at a median value of .0.4 msec. and ended at a median value of 1.5 msec. Estimated conduction velocities ranged between 2.7 and 13.1 m/sec. The close correspondence between the behavioural and electrophysiological estimates provides firm support for the plausibility of the psychophysical inferences.

Auxilliary experiments were performed to test the fidelity of the recording apparatus, to distinguish field potential records from artifacts and to see whether the recorded potentials had the properties of compound action potentials. The test for fidelity consisted of replicating classic studies of hippocampal field potentials. The records contained all of the main features of the classic potentials. To distinguish the field potential responses from artifacts, I demonstrated that reversal of stimulus polarity causes reversal of artifact polarity, although field potential polarity is not affected. Furthermore, it was demonstrated that, in a dying subject, the component of the record that we referred to as the compound action potential gradually waned while the artifact remained. Finally, the field potentials

were of invariant latency, followed high frequency

stimulation and were triphasic in shape. These are

known characteristics of compound action potentials

and suggest that the responses were not trans-

synaptically driven.

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INTRODUCTION

Physiological psychology is a reductionistic discipline that attributes organized behaviour to the activities of specific neural mechanisms. In order to study this link between neurophysiology and behaviour, it would be useful to electrically stimulate a particular neural circuit and observe the behavioural consequences. However, the electrical activation of functionally specific circuits in the central nervous system can be difficult, since the neural substrates for a number of different behaviours are often in close proximity to each other. One requires a means by which the substrate for a particular behaviour may be distinguished from the many neural circuits that may be activated by the stimulating probe. Psychophysical methods have been used to characterize the neural circuitry mediating stimulation-elicited behaviours so that a particular substrate may be identified by means of electrophysiological and neuroanatomical experiments. These psychophysical methods are inferential in nature. The purpose of the present study is to use electrophysiological recordings to directly assess the plausibility of such inferences.

Brain Stimulation Reward

Most psychophysical studies that incorporate electrical stimulation of the brain have been carried out in the SS paradigm. The reasons for this are both

substantive and practical. It has been argued that
studies of BSR may shed light on the neural bases of
motivational and learning processes (Gallistel, 1975).
Subjects actively seek out brain stimulation and, when
given a choice, may prefer it to conventional rewards,
such as food and water (Routtenberg and Lindy, 1965).
Furthermore, they retain a record of the magnitude of
previous brain stimulations, as reflected in subsequent
responding (Gallistel, Stellar and Bubis, 1974). One
can also summon support for the contention that BSR and
conventional rewards share a common neural basis.
Hoebel (1968) has shown that gastric loading and food
deprivation can influence SS responding.

From the practical point of view, it can be seen
that the BSR phenomenon lends itself readily to
psychophysical methods. Stable responding at high
operant rates is obtained at many SS sites. Further-
more, responding is resistant to satiation (Olds, 1958)
and subjects show good threshold discrimination
(Gallistel, 1974).

The Linkage Problem

The MFB is well known for its anatomical
complexity. It is actually a collection of bundles
with as many as twenty subdivisions (Nieuwenhuys,
Vening and Geeraedts, 1981).

Therefore it is not surprising that stimulation of the
MFB produces a wide variety of effects including locomotion
(Rompré and Miliaressis, 1980), motor twitches (Matthews,
1977), changes in endocrine function (Harris, 1948) heart
rate and blood pressure (Perez-Cruet, McIntyre and
Pliskoff, 1965), predation (Flynn, Vanegas, Foote and
Edwards, 1970) escape behaviour (Bower and Miller, 1958;
Roberts, 1958) and nociception (Rose 1974). In fact,
multiple behaviours can result from stimulation by
means of a single electrode (Matthews, 1977; Bower and
Miller, 1958; Roberts, 1958; Rompré and Miliaressis, 1980).
Thus, difficulties arise in linking a given effect of
stimulation with the activation of a particular substrate
located near the electrode tip.

The Psychophysical Approach

As a means of dealing with the linkage problem,
Deutsch (1969) was the first to employ a standard psychophysical
technique for estimating the refractory periods of the
directly stimulated neurons responsible for BSR. These
estimates were to be used to interpret electrophysiological
studies of neurons directly driven by electrodes supporting
SS. Only those neural elements whose electro-

physiological estimates matched the psychophysically derived ones could be considered candidates for the first stage of the neural circuitry underlying BSR. It was hoped that, by using a large number of psychophysically derived characteristics, the possibility of a false identification could be reduced.

The psychophysical approach draws inferences from behavioural data regarding the directly stimulated neurons that support BSR. How is it that neural stages intervening between the directly activated stage and this electrically elicited behaviour can be disregarded?

Behavioural output has been shown to be a monotonic function of stimulus input for BSR (Edmonds, Stellar and Gallistel, 1974). For each discrete stimulus input (eg. current level) there is one and only one output (eg. running speed) over a reasonably wide working range. This monotonic relationship must hold for all intervening stages. Unlike transformations such as inversion or exponentiation, compensatory changes in later stages cannot remove non-monotonicity; once introduced, its effects propagate through all subsequent stages (Gallistel, Shizgal and Yeomans, 1981).

Psychophysical experiments employing trade-off designs are dependant upon this monotonic relationship between stimulus input and behavioural output. If pairs of stimulus parameters are traded off so that behavioural output remains constant, the existence of this monotonicity dictates that the outputs of all interposed stages are held constant. Therefore, regardless of the number of intervening stages, trade-off experiments may, in principle, be used to characterize the directly stimulated stage by determining which combinations of parameters result in constant behavioural output.

Relevant Psychophysical Results

Yeomans (1975) has modified and improved the method developed by Deutsch to estimate refractory periods from behavioural data. The resultant equivalent stimuli procedures involve the trading-off of the number of pulse pairs per stimulation train and the pulse pair interval (C-T interval). As this interval is shortened, the second or test (T) pulse has a progressively diminished effect probably due to the refractory state of the directly stimulated stage. The action potentials lost due to refractoriness must be replaced by increasing the number of pulse pairs in order to

hold behavioural output constant. The magnitude of the refractory effect can be estimated from the size of the required increase in the number of pulse pairs.

If the conditioning (C) pulse and the T pulse are applied to MFB sites that support SS by means of separate, ipsilateral electrodes, one may again observe that at short C-T intervals, the maintenance of constant behavioural output requires an increase in the number of pulse pairs. This reduced paired pulse effectiveness is thought to be due to collision between antidromic and orthodromic action potentials travelling along the same fibres, so that at short C-T intervals one pulse of each pair has little effect on behaviour (Shizgal et al., 1980). The abrupt, step-like rise in the effectiveness of the T pulse as C-T interval is increased thus suggests that there is a direct axonal connection between the two sites of stimulus application. Since the time at which the step occurs is the sum of the conduction time between stimulation sites and the refractory period of the neurons undergoing collision, a conduction velocity can be calculated from the results of these pulse pair experiments.

The implications of the one and two electrode

pulse pair experiments outlined above can be
summarized as follows: a) The directly stimulated
elements responsible for SS recover from refract-
oriness over an interval of approximately 0.4 and
1.5 msec. b) There are direct anatomical
connections between SS sites extending 3.0 mm
along the MFB. c) These neurons exhibit conduction
velocities between 1.8 and 8.0 m/sec. (Shizgal et
al., 1980). d) A number of investigators have
suggested that catecholamine (CA) - containing
neurons constitute the first stage of the BSR
circuit (German and Bowden, 1974; Wise and Stein,
1969). While there is substantial evidence
implicating these neurons in BSR (Fouriezos and
Wise, 1976; Franklin, 1978), several of their
properties are incompatible with the notion that
CA elements constitute the directly activated
subpopulation. CA neurons have appreciably
longer refractory periods and lower conduction
velocities than the behaviourally characterized
elements (Yim and Mogenson, 1980; Maeda and
Mogenson, 1980; Deniau, Thierry and Feger, 1980;
German, Dalsass and Kiser, 1980).

Field Potential Recording: Rationale

The geometric implications of the collision

experiment lead one to expect that stimulation
within a 3 mm segment of the MFB would elicit a
propagating wave of action potentials that could
be recorded elsewhere in that segment and perhaps
beyond. This would allow for electrophysiological
estimation of refractory periods and conduction
velocities in neurons driven by stimulation at SS
sites. The electrophysiological estimates could
then be used to assess the plausibility of the
behaviourally derived inferences that are the
basis for rejecting CA neurons as the first stage
elements subserving BSR.

Macroelectrode field potential records,
which represent summed action potentials, could be
expected to contain contributions from a sizeable
fraction of the axons synchronously activated by
MFB stimulation. Since the relative impacts of
neural subpopulations upon these records are a
direct function of the number of elements as well
as their diameters (Lliñas and Nicholson, 1974),
even small fibres could make a sizeable impact
upon extracellular field potentials. In contrast,
unit recording methods preferentially register
cell body action potentials which result in
larger currents than axonal spikes. One difficulty

associated with field potential methods concerns
the interpretation of records that can reflect the
sum of different types of neural signals arising
from multiple sources. To facilitate interpretation
of the records obtained in this study, a series of
control experiments was performed.

Field Potential Recording: Interpretation

For theoretical and empirical reasons
(Tasaki, Polley and Orrego, 1958; Lorente de No,
1947) one would expect that extracellular field
potential records of action potentials generated by
a synchronously fired, homogeneous bundle of fibres
would have a triphasic appearance (Figure 1).
This pattern can be explained with reference to the
electrochemical events underlying the propagation of
an action potential in a single fibre.

To a monopolar recording electrode located
sufficiently near an excited axon, the propagating
action potential can appear as a series of
voltage changes that are roughly proportional to
the transmembrane current (Lorente de No, 1947).
A suprathreshold stimulus results in the
propagation of a perturbation corresponding to
sodium influx and appearing, from the extra-
cellular viewpoint, as a negative-going potential

FIGURE 1

Five superimposed oscilloscope sweeps. The initial
spike-like perturbation is the stimulus artifact.
Stimulation site: lateral hypothalamus
Recording site: ventral tegmental area
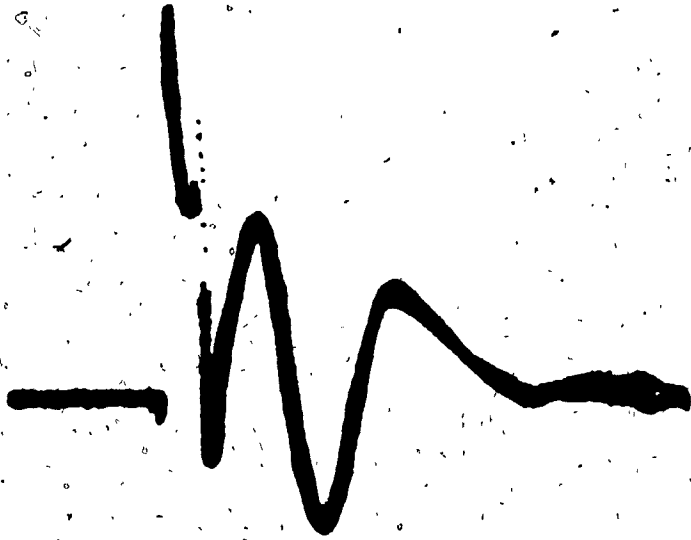
or current sink.   Its arrival at the recording
electrode is preceded by a positive-going
potential, a current source. By definition,
current flows from sources to sinks. Therefore
this outward-directed current must flow to
complete the extracellular limb of the local
circuit initiated by the current entering at
the previous node(s). A second source follows
the sink and can be attributed to the repolarizing
current that in many cells is due to potassium
efflux (Ranck, 1979; Llinas and Nicholson, 1974).

If field potential recordings were made
from a homogeneous axonal population, they would
reflect a weighted sum of individual field
potentials whose contributions would be proportional
to their distances from the recording electrode
(Nicholson, 1979).   The time course of the
voltage changes in the multiple element record
would be identical to that observed to arise from
a single element, although the absolute magnitudes
of the various phases in the compound record would
be expected to be larger. On the other hand, if
the activated neuronal population in the vicinity
of the recording probe were heterogeneous with
regard to fibre diameter, individual neurons

would exhibit different conduction velocities and their action potentials would arrive at the recording electrode out of phase. The degree of this dispersion over time is a direct function of the distance of axonal conduction between the point of action potential initiation and the recording locus (Erlanger and Gasser, 1937).

Field Potential Recording: Experimental Scheme

If the inferences drawn from the psycho-physical data are correct, they define the properties that neurons driven by rewarding stimulation must possess in order to be considered as candidates for membership in the directly stimulated substrate responsible for BSR. The experiments that follow investigated the plausibility of these inferences by recording field potentials at MFB loci through which the behaviourally characterized substrate is thought to pass. These potentials were evoked by stimulating at other MFB sites thought to contain the axonal extensions of this neural subpopulation. Electrophysiological refractory period and conduction velocity estimates were obtained and compared to those attributed to the first stage elements responsible for BSR on the basis of

psychophysical data. Auxilliary experiments were
performed to test the fidelity of the recording
apparatus, to distinguish neural responses from
stimulus artifacts and to test the hypothesis that
the potentials were mostly due to directly driven
axonal responses.

MATERIALS AND METHODS

Subjects

Eleven male hooded (Royal Victoria) and eight male albino (Sprague-Dawley) rats obtained from Canadian Breeding Farms and weighing 350-600g at the time of recording served as subjects. They were individually housed in hanging wire-mesh cages. Rat chow and tap water were available ad libitum and lighting in the animal colony was programmed to provide a 12 hour light/dark cycle.

Electrode Construction

Twisted bipolar electrodes fashioned from 178 um Dimel insulated nichrome wire were used for stimulation and recording, except for subjects MFB 1 and MFB 2 in which twisted tripolar electrodes were used. The square-cut tips of the bipolar electrodes were separated about 0.75 mm in the dorsal-ventral plane. Approximately 0.25 mm of insulation was removed from the longer pole, whether the electrode was to be used for stimulation or recording. The shorter pole was bared of insulation to the same extent if it was part of a recording electrode and about 2.0 mm was removed if the electrode was to be used for stimulation. In animals implanted with tripolar electrodes the shortest of the three wires remained unused. The two poles that were used were cut

square and insulation was removed to the same extent as the previously described bipolar electrodes.

Surgical Preparation

Animals were anaesthesized by means of intra-peritoneal injections of urethane (ethyl carbamate) at a dosage of 1.2 g/kg body weight. A portion of the skull and the underlying dura was removed. The exposed cortical surface was kept moist by means of repeated applications of isotonic saline. An uninsulated stainless steel wire was wrapped around several stainless steel jeweller's screws which were embedded in the occipital skull plate.

Electrical Stimulation and Recording

Rats were placed in a #1204 stereotaxic instrument (David Kopf) with the incisor bar elevated to 5 mm above the interaural line. With the exception of one subject, electrodes were lowered toward at least two of the following MFB loci in each subject:

1) lateral preoptic area (POA):

    2.0 mm anterior to bregma

    2.2 mm lateral to the midsagittal suture

    8.0 mm below dura

2) lateral hypothalamus (LH):

    0.4 mm posterior to bregma

    1.7 mm lateral to the midsagittal suture

    8.0 mm below dura

3) ventral tegmental area (VTA):

    3.3 mm posterior to bregma

    0.7 mm lateral to the midsagittal suture

    8.0 mm below dura

    In HPC 1, stimulating and recording electrodes were aimed at the perforant path region of the entorhinal cortex and dentate granule cell layer respectively. The following level skull coordinates were obtained from Douglas and Goddard (1975):

1) perforant path:

    7.2 mm posterior to bregma

    4.1 mm lateral to the midsagittal suture

    0.3 mm above the interaural plane

2) granule cell layer:

    2.0 mm posterior to bregma

    1.0 mm lateral to the midsagittal suture

    2.0 mm below the interaural plane

    Monopolar stimulation was applied by means of Grass S4 stimulators, Grass SIU4 stimulus isolation units and Grass CCU1 constant current units. The

stimuli consisted of constant current rectangular

pulses or pulse pairs delivered at 2 to 5 cps; the

polarity of the pulses was usually cathodal. Current

return was accomplished via jeweller's screws embedded in

the skull. The preparation was grounded via the shorter

wire at one of the stimulating electrodes. Stimuli were

monitored on a Tectronix 502 oscilloscope by reading the

voltage drop across a precision 10 Kohm resistor in

series with the preparation. Electrodes not used for

stimulation were used to obtain field potential records.

The tip of the longer pole of the recording electrode was

to record both the stimulus artifact and compound action

potential while the shorter wire was to record only the

artifact. The signals from the two wires were conditioned

in an attempt to equate the stimulus artifacts; the

conditioned signal obtained at the shorter wire was then

subtracted from its counterpart obtained at the longer

wire in an attempt to remove the artifact from the

final records. To accomplish this each pole of the

recording electrode was led to an input of a head stage,

consisting of a dual FET input operational amplifier

(Texas TL082CP) configured as a voltage follower and

located within 3 cm of the rat's skull. The two inputs

from the head stage were led into a dual preamplifier

designed to improve common mode rejection at the

differential amplification stage that combined pre-
amplifier outputs. Because of the low likelihood that
the two recording elecrodes were at isopotential loci,
the preamplifier was designed to provide independantly
variable gain for the two input signals. Since brain
tissue attenuates high frequency signals and because the
two recording electrode tips were not necessarily
equidistant from the stimulating electrode, independant
low pass filtering of the two headstage outputs was
provided to compensate for differences in the shape of
the artifact recorded from the two tips. Gain and
filter controls were adjusted to achieve the smallest
sized artifact of the same shape as the stimulating
pulses. Preamplifier outputs were led to a Tektronix
3A9 differential amplifier with high and low pass
filters set at 10 cps and 10 Kcps respectively.
Differential signals were monitored on a Tektronix 565
oscilloscope and stored on either a Hughes 110 storage
oscilloscope or a Nicolet Explorer III digital storage
oscilloscope. Oscilloscope displays were photographed on
Polaroid type 47 film with a Tektronix C27 camera.

Histology

Upon completion of recording sessions electro-
lytic lesions (500 uA DC, 10 sec.) were made through
all electrodes with the longest pole serving as the

anode and a skull screw as the cathode. Subsequently,
animals were given lethal doses of pentobarbital (IP)
and perfused intracardially using 100 ml of 0.9%
saline followed by 100 ml of 10% formaldehyde saturated
with potassium ferrocyanate. Reaction of the ferro-
cyanide with iron ions expelled from the anode produced
a blue reaction product at lesion sites. 40 um
coronal sections of the frozen brains were cut, and, in
the vicinity of the electrode tip loci, every second
section was mounted on glass slides. Unstained sections
were moistened with water and then examined under a
projection microscope to localize electrode tips.

PROCEDURE

During recording sessions, animals remained fixed in the stereotaxic instrument and a sufficient degree of anaesthesia was maintained by means of 0.5-1.0 ml boosters of urethane (120 mg/ml) or 0.5 ml of diazepam (5 mg/ml). Electrodes were positioned in the brain using #1460 electrode manipulators and #1260 electrode carriers (David Kopf).

The longest pole of the recording electrode was lowered to 0.5 mm dorsal to its target. With the recording electrode stationary, the first stimulating electrode was advanced into the brain and the previously described stimuli were applied at an initial current of 500 to 1000 uA. Stimulation-evoked field potentials were monitored as the longest pole of the stimulating electrode approached its target. When potentials with the characteristic triphasic shape of extracellular recorded action potentials (Lorenté de No, 1947) and an amplitude of at least 100 uV peak to trough were encountered within 1 mm of the target, the first stimulating electrode was left in place. This procedure was repeated with the second stimulating electrode. Finally, I attempted to optimally position all three electrodes. If stable potentials of appropriate shape and amplitude could not be evoked by a given stimulating

electrode within 1 mm of its stereotaxic target using
currents as high as 2000 uA, the electrode was usually
repositioned in the anterior-posterior planes and/or
medial-lateral planes (deviations from the original
coordinates did not exceed 0.5 mm) and then driven
again into the brain.

Conduction Velocity Estimates

Conduction velocity estimates were obtained by
dividing the interelectrode distance by the response
latency (see Appendix 1). The interelectrode distance,
the distance between the longest poles of the
stimulating and recording electrodes, was measured
directly upon careful removal of the electrodes after
recording sessions and/or calculated from the stereo-
taxic coordinates. The latency was calculated from the
field potential records as the time interval between
stimulus artifact onset and the vertex of the trough, or
second phase, of the field potential. This point in the
field potential record was chosen for latency measurement
since it corresponds to the apex of the spike, or
depolarizing phase, of an intracellularly recorded action
potential (Lorenté de No, 1948; Tasaki, Polley and
Orrego, 1958) which is traditionally used in intra-
cellular response latency measures.

Refractory Period Estimates

Pairs of equal amplitude 0.1 msec. duration pulses were applied to a single stimulation site at inter-pulse pair frequencies not exceeding 5 cps. The interval between C pulse onset and T pulse onset (C-T interval) was gradually incremented beginning with intervals as short as 0.2 msec. and terminating at up to 3.0 msec. The size of increments varied between 0.1 and 0.5 msec. The beginning of recovery from refractoriness was taken to be the shortest C-T interval at which a stable response to the T pulse could just be observed. If the record produced at the next shortest C-T interval showed no evidence of a T pulse response, the estimate for the beginning of recovery was the midpoint between these two inter-pulse intervals (see Table 1). The estimate for the completion of recovery from refractoriness was taken to be the shortest C-T interval at which the response to the T pulse was observed to be identical in shape and amplitude to the response evoked by single pulse stimulation. If the preceeding C-T interval produced a field potential response to the second pulse that was less than about 75% of this amplitude from the first peak to the trough and/or was markedly different in shape, the

TABLES 1A, 1B and 1C

Data sets for all nineteen subjects. Table 1A
lists those experiments in which the VTA was the
recording site. Tables 1B and 1C list those in
which the recording electrodes were aimed at the LH
and the POA respectively. The first column of
conduction velocities was based on measured inter-
electrode distances, the second column was based
on calculated interelectrode distances (see Procedures).
Conduction velocity figures are in m/sec. and
refractory period estimates are in msec. The data
upon which conduction velocity estimates were based
appear in Appendix 1. Asterisks refer to the fact
that collision was observed in that subject.

Table 1A

Recording electrodes aimed at the VTA

Subject	Stimulation Target	Current	Conduction Velocity		Refractory Period Begin	End
IM 1	LH	1.0		8.6	0.3-0.5	1.3-1.5
IM 2	LH	1.0		9.1	0.8	1.6
IM 3	LH	0.8		6.9	0.5	1.3-1.5
MFB 1*	POA	1.0		9.8	0.6-0.8	1.6
	LH	0.6		6.5	0.6	1.2-1.6
MFB 2*	POA	2.0	7.8	8.4	0.4-0.5	1.2-1.6
	LH	2.0	7.3	6.5	0.4-0.5	1.2-1.6
MFB 4	LH	1.0	5.4	5.4	0.4-0.8	1.6-2.0
MFB 9	LH	0.2	6.3	6.3	0.6-0.7	1.5-2.0

Table 1A (continued)

Subject	Stimulation Target	Current	Conduction Velocity		Refractory Period Begin	End
MFB 11*	POA	2.0	6.7	7.5	0.8-1.0	1.6-1.8
	LH	1.0	6.1	6.1	0.5-0.6	1.1-1.3
MFB 13	POA	2.0	5.2	6.2	0.6-0.8	1.8-2.0
	LH	0.8	3.8	4.6	0.5-0.7	1.2-1.4
MFB 14	LH	1.0		9.4	0.3-0.4	1.3-1.5
PI 1*	POA	0.8		7.0	0.8-1.0	2.2-2.5
	LH	0.8		11.0	0.6-0.8	2.2-2.5
PI 2*	POA	1.6		8.2	0.6	1.5
	LH	1.0		7.8	0.5	1.2
PI 3*	POA	1.6		9.7	0.8	2.0
	LH	0.4		7.8	0.6-0.7	1.6
R 1	POA	0.8			0.6-1.0	2.0-2.5

Table 1B

Recording electrodes aimed at the LH

Subject	Stimulation Target	Current	Conduction Velocity		Refractory Period Begin End	
MFB 3	VTA	2.0	6.8	6.2	0.3	1.2-1.6
MFB 5	VTA	0.6	7.7	9.3	0.5-0.6	1.0-1.2
MFB 6	VTA	1.0	3.5	2.7	0.5-0.6	1.0-1.5
MFB 8	VTA	2.0		9.7	0.4-0.5	0.7-0.8

Table 1C

Recording electrodes aimed at the POA

Subject	Stimulation Target	Conduction Current	Velocity	Refractory Period Begin	End
IM 4	LH	1.6	6.3	0.4	1.2
	VTA	1.0	13.1	0.4	1.2

estimate of the end of recovery from refractoriness was
taken to occur between the C-T interval at which complete
recovery was observed and this next shortest C-T interval.

Collision Tests

This experiment required that both stimulating
electrodes evoke criterial responses at the recording
site. Pulse pairs were applied and the C-T interval was
varied, as in the refractory period experiments except
that the first pulse of each pair (the C pulse) was
applied to the site proximal to the recording electrode,
whereas the second (the T pulse) was applied to the
distal site.

Auxilliary Experiments

1) Stimulus Artifact Recording

Stimulating and recording electrodes were placed in
a 50-ml capacity beaker of 0.9% saline. Pairs of 100 msec.
duration pulses were applied at 1000 uA current strength.
The C pulse was anodal and the T pulse was cathodal. C-T
intervals of 50 and 100 usec. were employed. The
artifacts were displayed on a storage oscilloscope and
photographed.

2) Dying Rat Record

After injection of a lethal dose of pentobarbital,
a succession of responses to 100 usec. cathodal pulses

were applied to the LH region of MFB 14. The current intensity was 800 uA and the stimulation frequency was 5·cps. Recordings of evoked activity were made by means of an electrode aimed at the VTA region at approximately 20 sec. intervals until cardiac failure was noted. A final record was made 2 min. post mortem.

3) Hippocampal Recording

The previously described bipolar stimulating and recording electrodes were aimed at the perforant path and dentate gyrus granule cell layer respectively. The stimuli were 100 usec. cathodal pulses of 525 uA intensity delivered at a frequency of 2 cps. The response was visualized on a storage oscilloscope and photographed.

4) Anodal/Cathodal Comparisons

Bipolar electrodes aimed at MFB targets were used to apply the same stimuli as in the other single-pulse recording experiments except that anodal as well as cathodal stimuli were used. Recordings of responses to anodal and cathodal stimuli of the same current strength were recorded on the same photograph to facilitate comparison.

5) Intensity Series

The usual electrodes and cathodal stimulating pulses were employed to evoke a series of MFB responses to pulses ranging in intensity from threshold level (little

or no visible differences in the amplitudes of the three
response phases between the last and next to last records).
Each series was recorded on the same photograph.

RESULTS

Superimposed Single Pulse Records

Figure 1 represents five superimposed single pulse records following the stimulus artifact which resembles a large, positive-going spike. Note the invariant latency and constant shape of the triphasic potential. Beyond this triphasic portion, less stable deviations from baseline are evident.

Conduction Velocity Estimates

Tables 1A, 1B and 1C include conduction velocity estimates for the four different stimulation/recording configurations. In some cases, interelectrode distances were estimated rather than measured because the electrodes were bent during removal from the brain. In cases where both measurement and calculation could be used, the resultant conduction velocity estimates were fairly similar. Nonetheless, the highest conduction velocities are found in instances when only estimated (calculated) interelectrode distances were available. Figure 2 indicates an approximately normal distribution of combined conduction velocities with a high degree of overlap between velocity figures based on estimated (calculated) and directly measured interelectrode distances. Velocities based on

FIGURES 2A, 2B and 2C

Histograms of conduction velocity estimate distributions
based on calculated interelectrode distances (2A)
measured interelectrode distances (2B) and combined
calculated and measured interelectrode distances (2C).
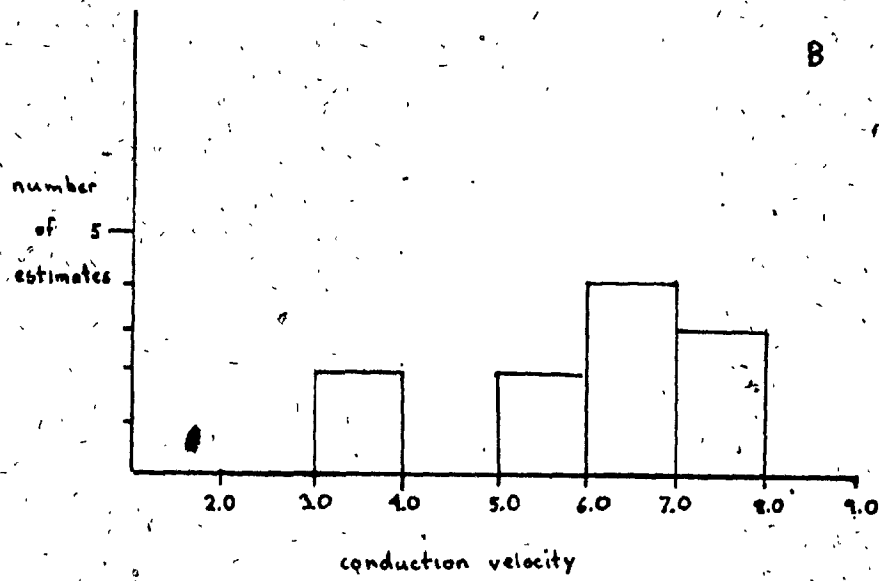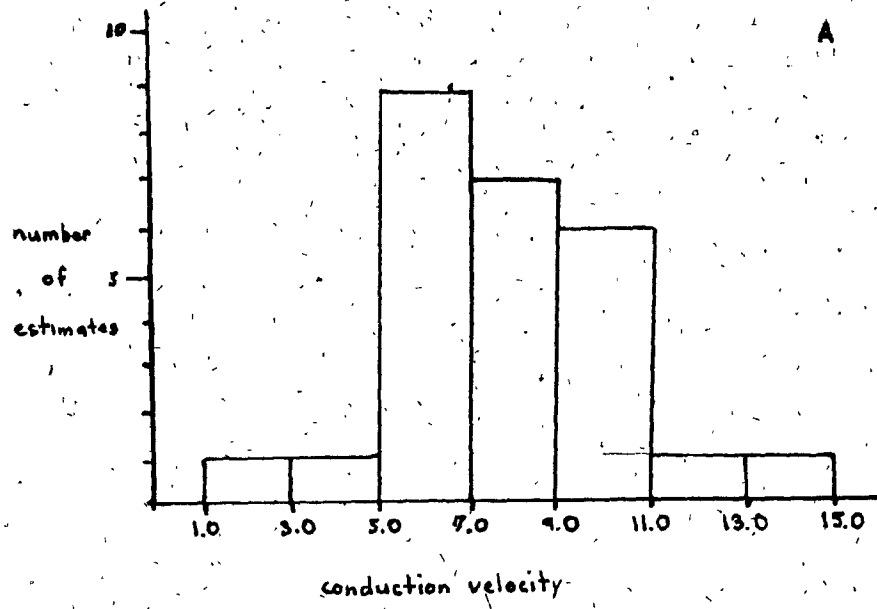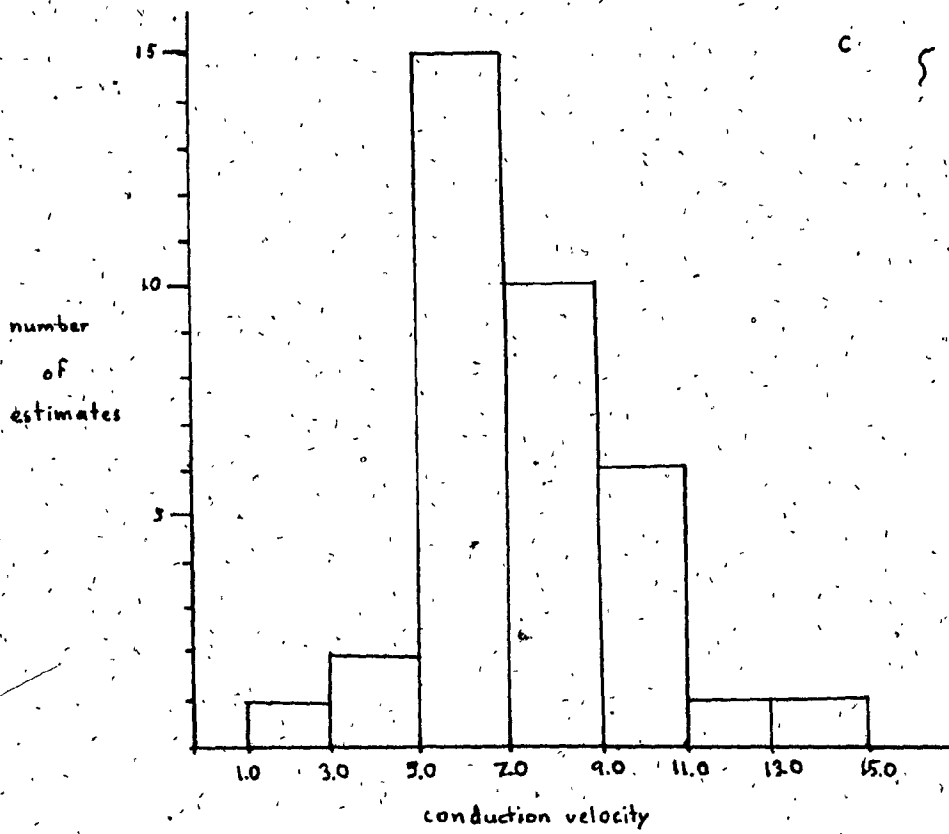
measurement of interelectrode distance range from 3.5 to
7.8 m/sec. with a median of 6.3 m/sec. while the
velocities arrived at by employing calculated distances
exhibit a range from 2.7 to 13.1 m/sec. with a median
of 7.8 m/sec. (see Figure 2). In animals for which
direct distance measurements were available and both
POA and LH electrodes evoked criterial responses (MFB 2,
MFB 11 and MFB 13), the estimated conduction velocity
between the POA and VTA placements is greater than
that between the LH and VTA.

Refractory Period Estimates

The means (±standard error of the mean) of the
estimates for the beginning and end of recovery from
refractoriness for the VTA, LH and POA stimulation
sites are 0.45 (±.05) and 1.14 (±.11), 0.55 (±.03)
and 1.47 (±.08) and .73 (±.05) and 1.84 (±.13) msec.
respectively. Examination of Figure 3 reveals that in
the LH stimulation case, the beginning and end of
recovery from refractoriness appears less variable
than in the other two cases. Note the strong, negative
skew in the end of recovery portion of this histogram.
In the other two histograms, both the beginning and end
of recovery portions seem to exhibit a rectangular
distribution, although this may be due to the limited
sample sizes. The widest scatter of refractory period

FIGURES 3A, 3B and 3C

Histograms of the beginning and end of recovery from
refractoriness for neurons activated by LH (3A),
VTA (3B) and POA (3C) stimulation. Cross hatching
indicates the end of recovery estimates.

A

B

number of estimates

C

C-T Interval
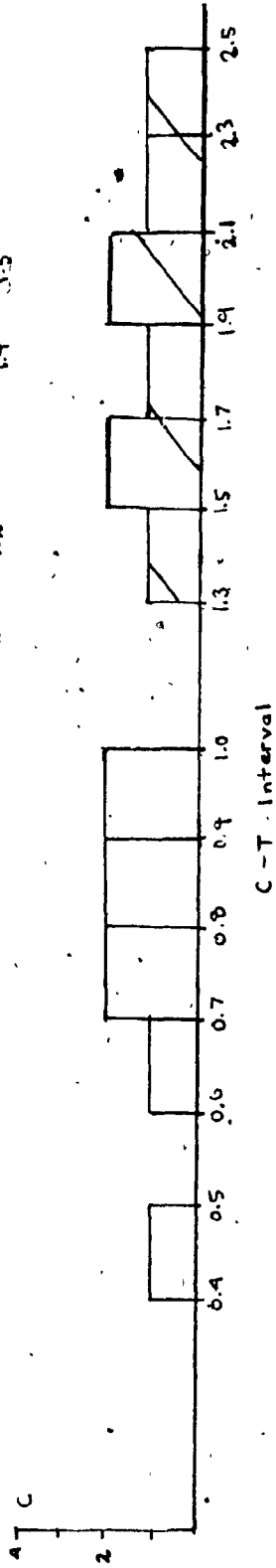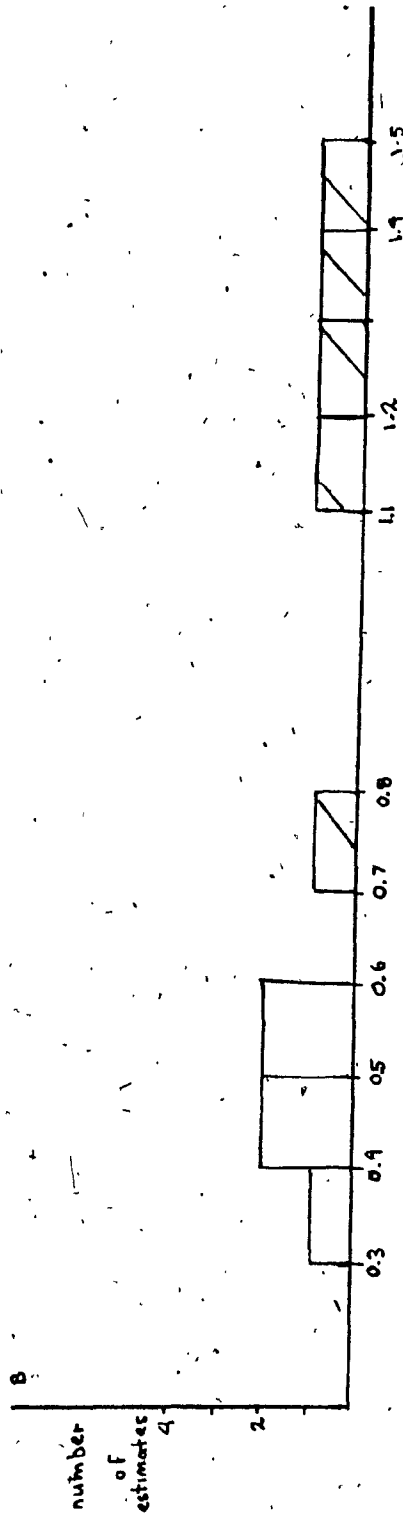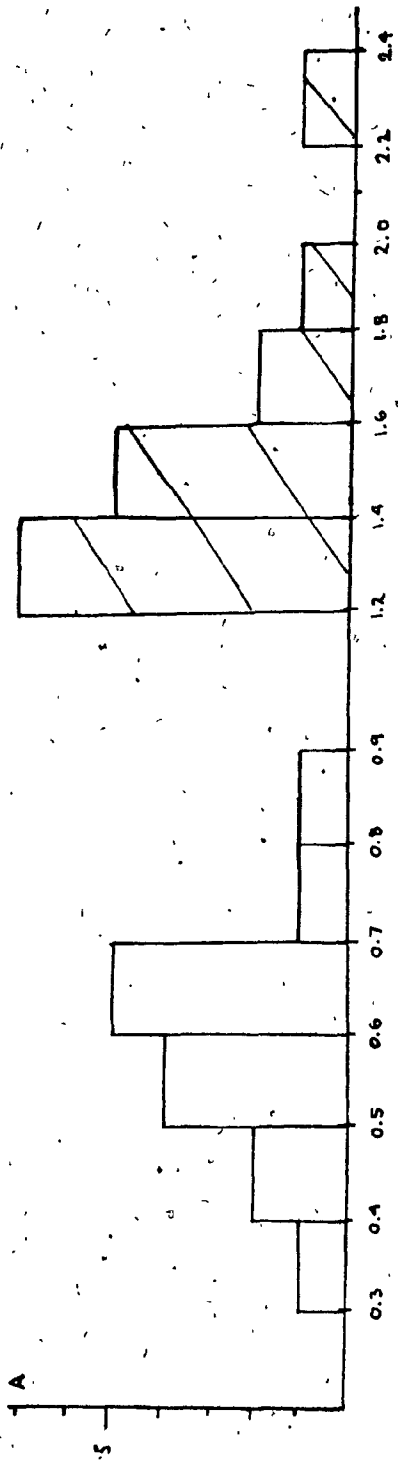
estimates is observed in the POA stimulation data. It was often difficult to obtain useful field potential records by stimulating the POA even after multiple penetrations. The paucity of VTA refractory period estimates is mainly due to the fact that this site was by far the most reliable recording electrode target and therefore was rarely used for stimulation.

Figures 4A, 4B, 5 and 6 are typical examples of single sweep field potential records evoked by stimulation of LH, POA, and VTA respectively. The paired pulse record for the shortest C-T intervals resembles the C pulse only records except for the presence of a second artifact. In contrast, records obtained at the longer C-T intervals include two responses, one following each artifact. As the C-T interval is increased, the T pulse response region of the record grows increasingly similar to the single pulse responses. Two examples of LH records are included since the majority of refactory period records were of this type.

Collision Tests

Evidence for collision phenomena was observed in six animals (see Tables 1A and 1B). The collision interval (the time span over which T pulse effectiveness goes from minimum to maximum) was not estimated due to
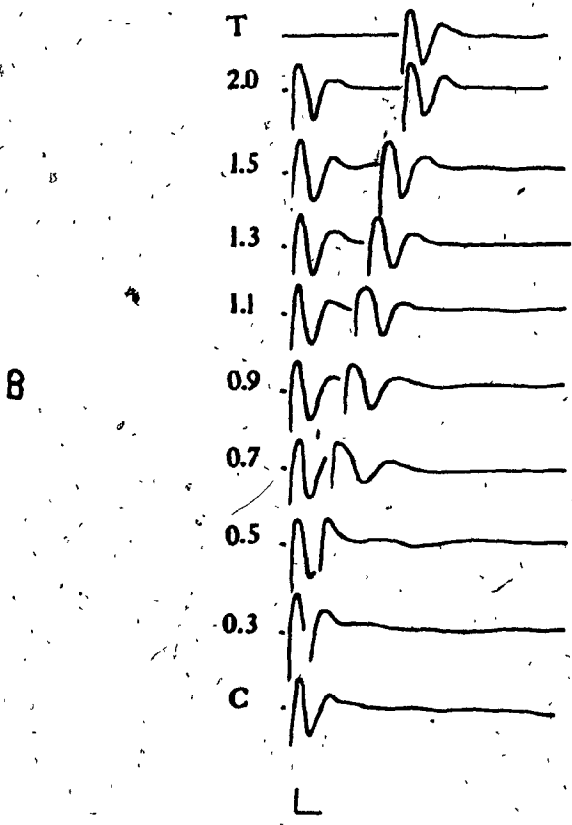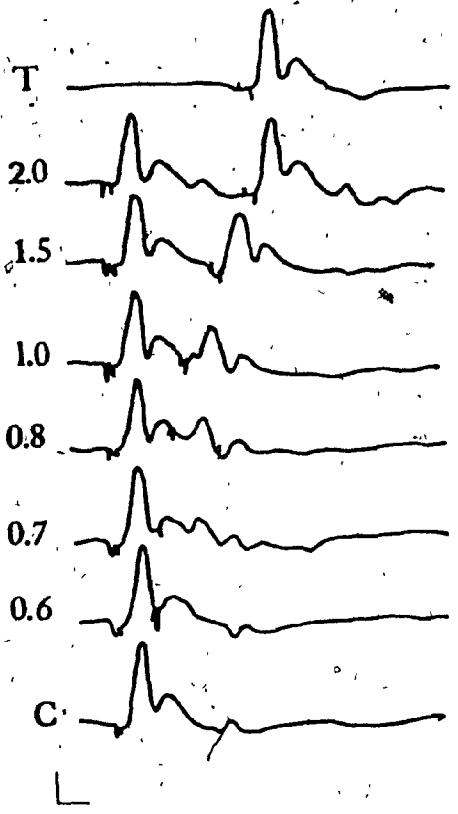
FIGURES 4A and 4B

Refractory period effects in neurons driven by LH
stimulation. In both figures, the top trace records
the response to a single T pulse, the bottom trace
records the response to a single C pulse and
remaining traces are records of responses to single
pairs of pulses. The numerals to the left of
traces indicate the corresponding C-T intervals. The
recording electrode was aimed at the VTA.

Subjects: MFB 9 (Figure 4A)

MFB 11 (Figure 4B)

Calibration marks: vertical: 400μV

horizontal: 0.5 msec.

FIGURE 5

Refractory period effects in neurons driven by POA
stimulation. The top trace records the response to a
single T pulse and the bottom trace records the
response to a single C pulse. The remaining traces
record responses to single pairs of pulses. The
numerals to the left of these traces indicate the
corresponding C-T intervals. The recording electrode
was aimed at the VTA.

Subject: MFB 11

Calibration marks: vertical: 100uV

horizontal: 0.5 msec.

T

2.0

1.8

1.6

1.4

1.2

1.0

0.8

C.

FIGURE 6

Refractory period effects in neurons driven by VTA
stimulation. The top trace records the response to a
single T pulse and the bottom trace records the
response to a single C pulse. The remaining traces
record responses to single pairs of pulses. The
numerals to the left of these traces indicate the
corresponding C-T intervals. The recording electrode
was aimed at the LH.

Subject: MFB 6
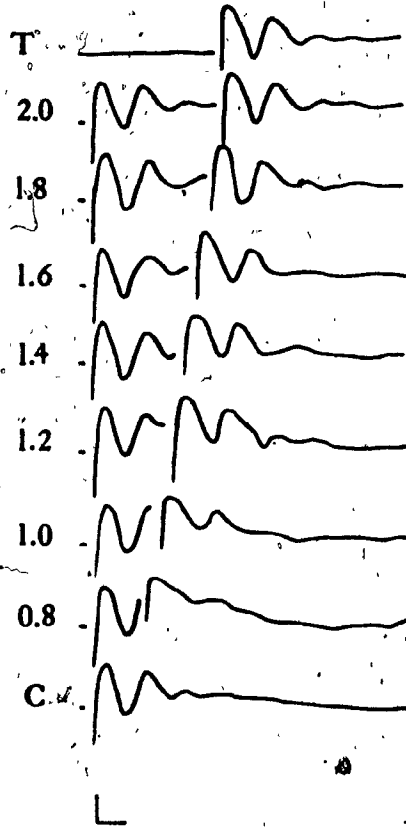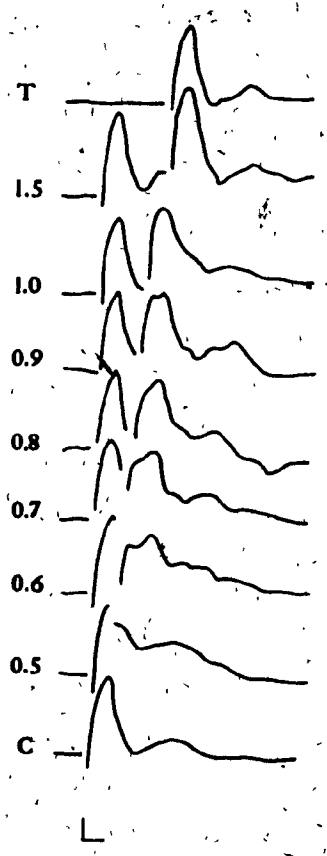
Calibration marks: vertical: 100uV

horizontal: 0.5 msec.

T

1.5

1.0

0.9

0.8

0.7

0.6

0.5

C

L

difficulties in separating late events due to C pulse
stimulation from those due to T pulse stimulation in
the paired pulse records. This problem was more
severe in collision records than in the refractory
period traces because the responses to the C and T
pulses were usually very different in amplitude in the
former case. The clearest collision series is
presented in Figure 7. As the C-T interval is
decreased, the response to the T pulse in the C-T
record becomes progressively diminished in relation
to the T only response (uppermost trace). In this
case, the two compound potentials are separable
because of the length of the collision interval relative
to the durations of the responses.

Results of Auxilliary Experiments

1) Dying Rat Records

Figure 8 indicates that the field potential decays
as the depth of anaesthesia approaches lethal levels.
The bottom-most record, taken two minutes post-mortem,
is a nearly pure artifact. It can be seen that the
field potential record in the living animal was
superimposed upon this artifact.

2) Stimulus Artifact Recording

Figures 9A and 9B indicate that artifact polarity
is determined by stimulus polarity. Furthermore,

FIGURE 7

Collision effects between neurons directly connecting
the LH, the site of C pulse application and the POA,
the site of T pulse application. The top trace is a
recorded response to T pulse only stimulation. The
numerals to the left of single pulse pair traces
indicate the corresponding C-T intervals. No C pulse
only response is presented since it is the T pulse
that is blocked during collision and the collision
interval is sufficiently long so that the C pulse
response is always visible. The recording electrode
was aimed at the VTA.

Subject: PI 1

Time marker: 5 Kcps.

T

2.4

2.0

1.6

1.2
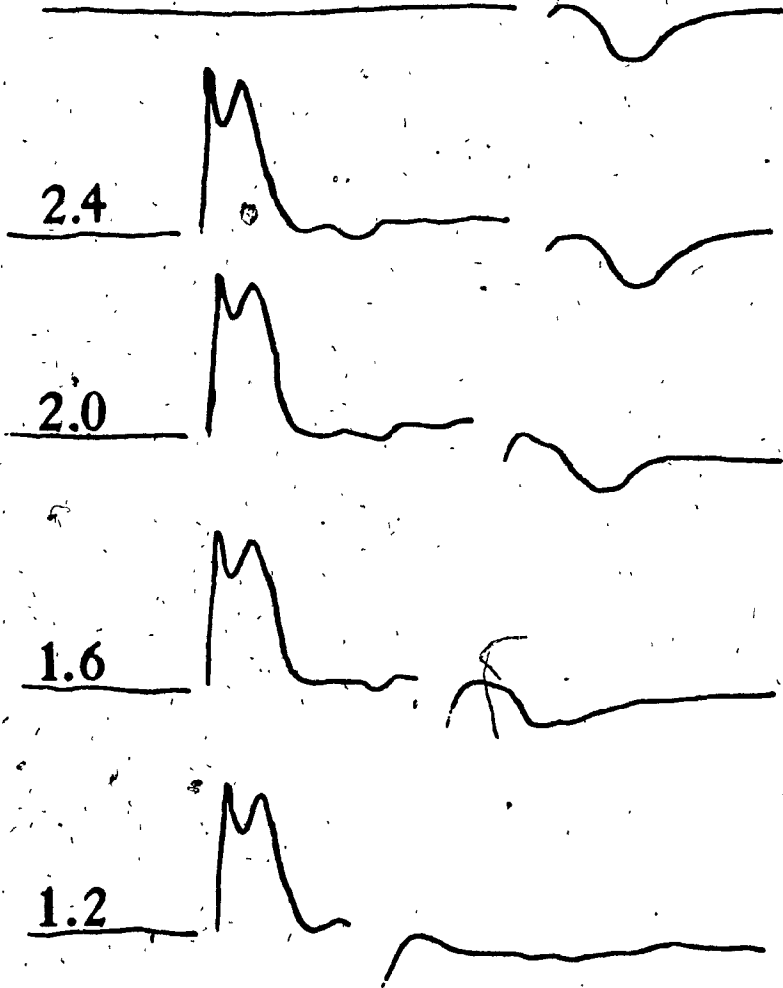
FIGURE 8

Field potential records from a dying subject. The
stimulation target was the LH and recordings were
taken from the VTA site. Records taken every twenty
seconds are presented in descending order as death
approached. The bottom trace was taken two minutes
post mortem.

Subject: MFB 14

Calibration works: vertical: 50uV

                          horizontal: 0.1 msec.
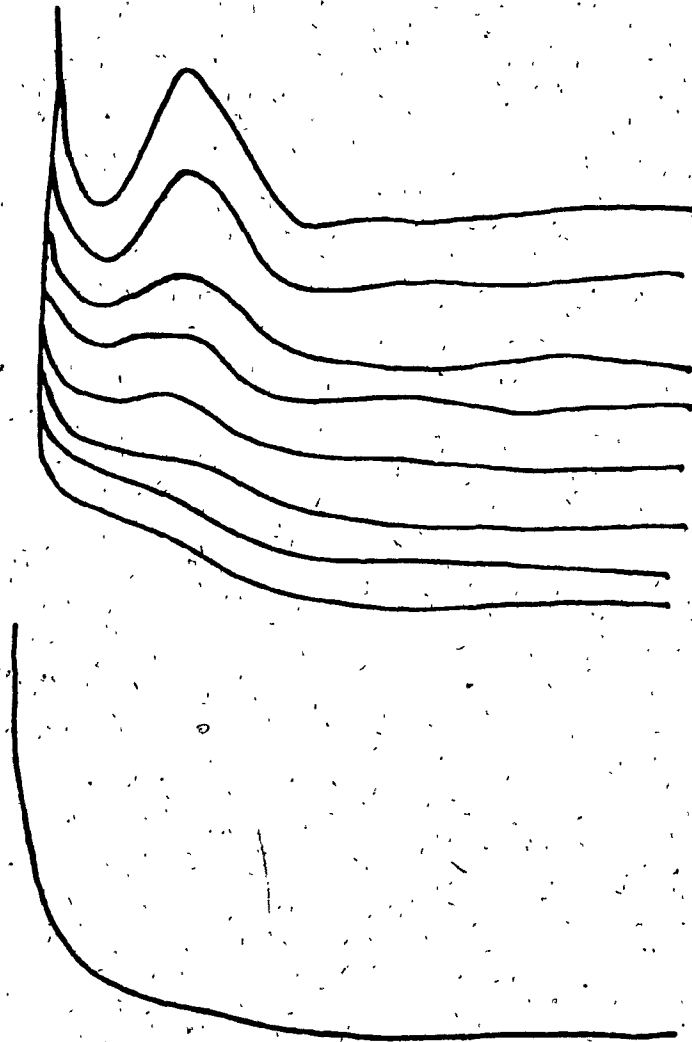
FIGURES 9A and 9B

Stimulus artifacts recorded in 0.9% saline. A pair
of 100 usec. pulses were applied. The C pulse was
anodal and the T pulse was cathodal.

Figure 9A: C-T interval: 100 usec.

Figure 9B: C-T interval: 50 usec.

Calibration marks: vertical: 100uV

                            horizontal: 50 usec.

A

B

when the C-T interval is shorter than the duration of
the first artifactual response, the two artifacts
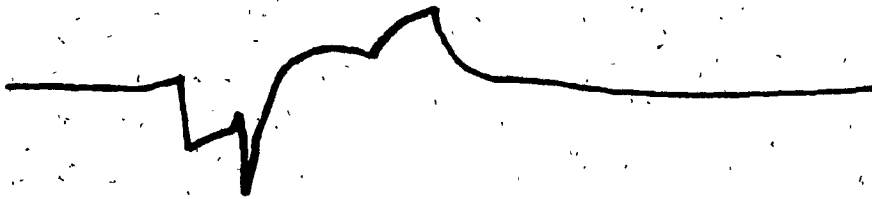summate. When the C-T interval is about half the
duration of the artifact (Figure 9B), about half of
each artifact remains virtually unaltered and the
overlapping halves sum.

3) Anodal/Cathodal Comparisons

Figure 10 indicates that a reversal of the polarity
of the stimulation used to evoke a field potential
does not result in the reversal of the polarity of
the recorded response. However, the anodal response
amplitude undergoes attenuation of all three phases.
Note also the reduction in response latency despite
the absence of changes in the time courses of the
three phases in the anodal record.

4) Intensity Series

As intensity is increased, overall response
amplitude in Figure 11 increases, except for the
highest intensity record. This response has the
same amplitude as the next highest intensity record
except that it includes additional, late components.
The latency of the triphasic response is invariant.
A small positive-going, late component of about
1.6 msec. latency emerges at 200 uA and beyond;
a second late component occurs at 800 uA.

FIGURE 10

Equal intensity responses to anodal and cathodal
stimuli. Cathodal (upper trace) and anodal (lower
trace) stimuli were used to activate the LH region.
Single sweep responses were recorded from the VTA
region.

Subject: MFB 9

Calibration marks: vertical: 400uV

horizontal: 0.5 msec.

A

B

FIGURE 11

Effect on field potential components of stimuli of
progressively greater intensity applied to the LH.
The decimals adjacent to traces indicate the current
(mA) used to elicit the corresponding potential.
Single sweep responses were recorded from the VTA
region.

Subject: MFB 9

Calibration marks: vertical: 400uV

horizontal: 0.5 msec.

.05

.1

.2

.4

.8
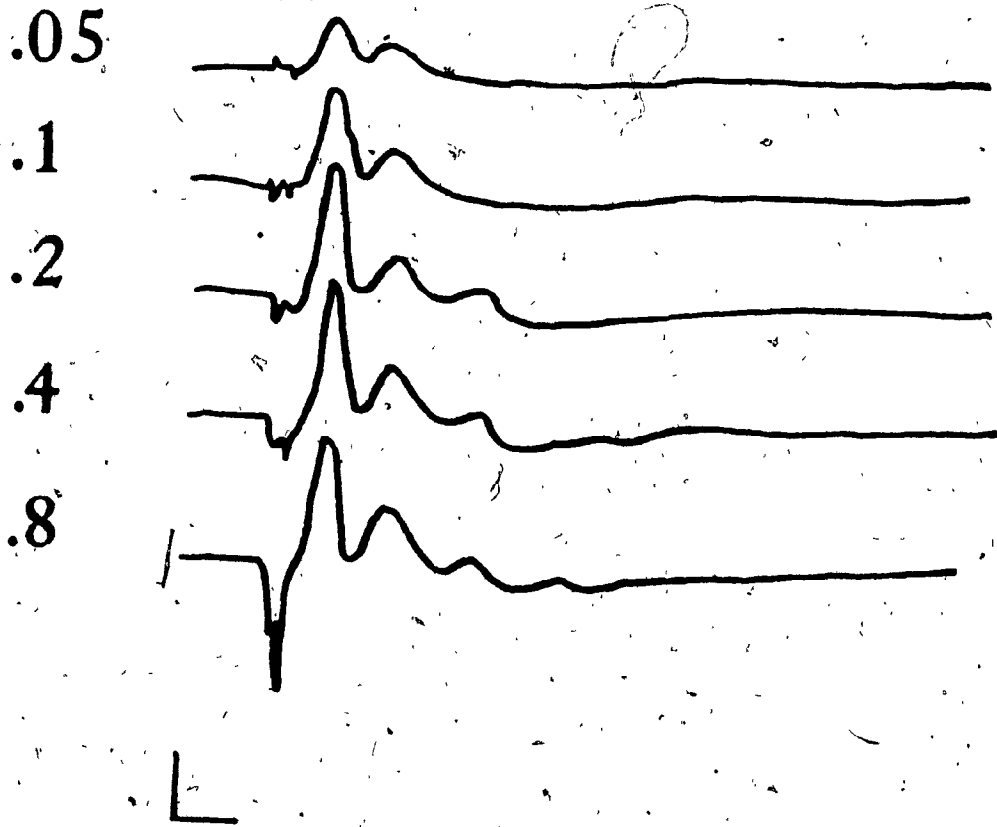
5) Hippocampal Recording

Figure 12 contains all the components observed in classic hippocampal records. Just beyond the artifact a small positive-going deflection is observed. This is followed by a large, slow event which is also positively going, upon which a negative-going potential appears to be superimposed.

Histology

Figure 13 presents the locations of the electrode tips that were successfully identified. Due to the unavoidable disruption of the cerebral vascular network, multiple electrode penetrations and human error, difficulties in perfusion and microtomy precluded localization of all electrode tips. In particular, the tips of only a few of the recording electrodes aimed at the VTA were found. Of the six that were found, the VTA placement for animal R 1 was situated in substantia nigra and the most posterior sites for IM 2, MFB 5 and MFB 6 were in or near the periaqueductal gray. Of the POA and LH placements that could be localized, many were near their intended targets, excepting the POA site for MFB 11 which was found to just dorso-lateral to the anterior commisure. Twenty-five of a possible fifty-three tips were located.

FIGURE 12

Field potential response to perforant path stimulation
recorded at the dentate gyrus granule cell layer.

Subject: HPC 1

Calibration marks: vertical: 500uV

horizontal: 2 msec.
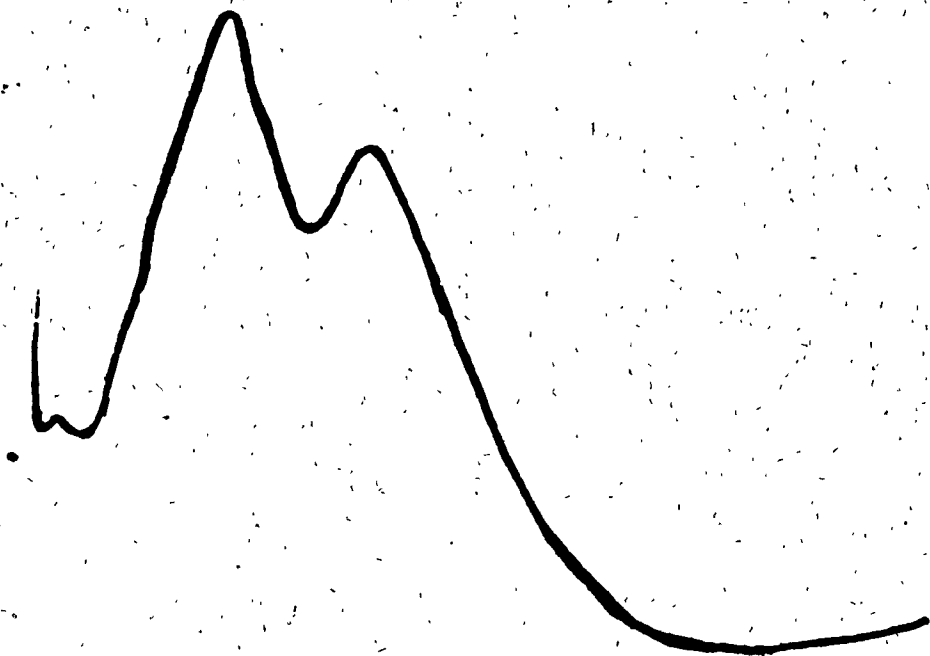
FIGURE 13

Partial tracings of sections from Pellegrino et al. (1979). The symbols indicate the location of an electrode tip for the subject whose alphanumeric appears adjacent to the appropriate symbol to the right of the tracing. The numbers to the left of the tracings refer to the distance (mm) of sections from bregma.

• mfb 13
• pi 1

2.2

• mfb 5
• mfb 14

2.0

• mfb 6

1.8

• mfb 11
• mfb 12
▪ pi 2

1.6

• mfb 9
• pi 1

1.4

• mfb 2

0.2

• r 1

0.0

• pi 1

-0.4

• pi 3

-0.8
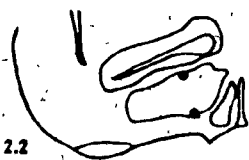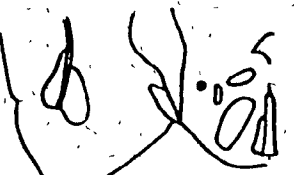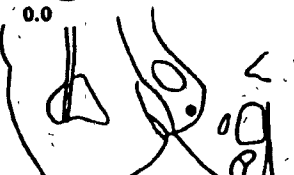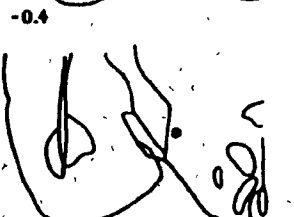
• mfb 1
• mfb 9
▪ mfb 11
▲ im 2

-1.0

• mfb 14

-1.2

• im 2

-2.4

• mfb 5
• mfb 6
▪ mfb 9
▲ mfb 13
○ r 1

-3.2

DISCUSSION

The purpose of the present study was to examine the plausibility of inferences drawn from psychophysical studies of the substrate for BSR. These inferences include the refractory periods and conduction velocities of the directly stimulated stage. Before recording studies could be employed toward this end, preliminary experiments were carried out to examine the fidelity of the recording apparatus, to distinguish field potentials from artifacts and to establish that compound action potentials were the major contributors to our recordings.
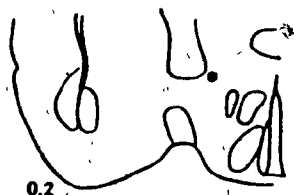
Auxilliary Experiments

Comparative analysis of hippocampal responses produced by perforant path stimulation attests to the high fidelity of the system. Figure 12 strongly resembles responses observed by a number of investigators (Lomo, 1971; Douglas and Goddard, 1975) using similiar electrode placements. The small positive-going deflection immediately following the artifact is interpreted as a compound action potential propagating along the perforant path fibres. The large positive-going potential following this is seen as the EPSP produced by the perforant path volley at the granule cell dendrites. The negative deflection superimposed on the larger

positive perturbation is attributed to the synchronous
firing of granule cells and is known as a population
spike (Lomo, 1971). This experiment demonstrates that
the electrophysiological apparatus can produce high
fidelity recordings across the bandwidth required to
record compound action potentials.

Several lines of evidence attest to the non-
artifactual nature of the record component we refer to
as a neural response. The triphasic shape of this
component (see Figure 1) is consistent with a compound
action potential that propagates past the recording site
(Lorenté de No, 1947). In recordings from a dying
subject (Figure 8), the feature we refer to as the
response diminishes and eventually disappears while
the capacitive discharge-like artifact remains. We
also demonstrate that stimulus artifact polarity
reverses when stimulus polarity is reversed
(Figure 9A). Stimulus polarity does not affect the
polarity of our responses (Figure 10). Furthermore,
these responses have refractory periods at C-T
intervals at which artifacts sum (Figure 9B).

Several features of these responses lead us to
suggest that they are compound action potentials apart
from their characteristic shape. These potentials

exhibit latencies that are too short to be easily explained with reference to transynaptic potentials. Also, their latencies are invariant (see Figure 1) and they follow high frequencies well. These properties have been attributed to direct axonal activation. (Tasaki, Polley and Orrego, 1958).

Characteristics of the Directly Driven Fibres

The fibres that carry the compound action potential begin to recover from refractoriness at a mean value of about 0.5 msec. and recovery is complete at a mean value of 1.5 msec. Their conduction velocity estimates range between 2.7 and 13.1 m/sec. Figures 2 and 3 indicate that there is a considerable amount of dispersion of the conduction velocity and refractory period estimates across subjects. In-accuracies in the determination of interelectrode distances may have contributed to the dispersion of conduction velocities. The point at which stimulation effectively activates neurons may be some distance from the electrode tip, particularly at high currents, so that interelectrode distance values overestimated the actual distance of conduction between the site of action potential initiation and the recording site to varying degrees. Also, recall

that in a number of cases, interelectrode distances
could not be directly measured but had to be
calculated (see Tables 1 and 2).

Since, in some instances, there are uncertainties
as to whether electrodes were located in the MFB due
to histological difficulties, both refractory period
and conduction velocity estimates may include
extreme values partly due to sampling of directly
driven neurons outside the region of interest.

Some of this dispersion may also be due to
neuronal heterogeneity within the MFB. Evidence for
multiple subpopulation contributions to field
potential records comes not only from this between
subject dispersion of neurophysiological property
estimates but from examination of some individual
records as well. Figure 4A and Figure 11 both show,
small but stable late occurring responses, perhaps
suggesting the presence of finer, slower conducting
axons. Note that in the bottom-most trace in
Figure 11, an additional, late component comes in at
the highest current. However, the possibility that
these late components are due to transynaptic
connections between the stimulating and recording
fields cannot be ruled out.

Comparisons with Psychophysical Results

Nearly the entire range of behaviourally based
conduction velocity and refractory period estimates
has been duplicated by the present electrophysiological
results. Only the low end of the behaviourally
derived conduction velocities (below 2.7 m/sec.) is
not included in electrophysiological results.
However, long latency components such as those
observed in Figures 4A and 11 were not used in
conduction velocity calculations because their
presence was infrequent and often ephemeral. It is
likely that, due to random noise, and the predominance
of larger, faster conducting fibres, these small
amplitude responses were often obscured.

The dispersion of electrophysiological estimates
and the occasional presence of multiple components
supports the notion that the directly driven
contributors to field potential records are hetero-
geneous. Psychophysical experiments that attempt
to reduce the relative refractory phase still find a
relatively gradual rise in recovery from refractoriness.
This is seen as evidence for the existence of multiple
subpopulations with different absolute refractory
periods within the first stage of the circuit

responsible for BSR (Bielajew et al., in press). Note
that in the physiological data as well, the recovery
from refractoriness is rarely abrupt (eg., see Figure
4A).

The electrophysiological experiments firmly
support the plausibility of behaviourally-based
inferences regarding the conduction velocities and
refractory periods as well as the heterogeneity of
the directly stimulated population supporting SS.
As a result, they aid in the preclusion of CA neurons
as members of this directly stimulated stage. In
addition, the action potential durations of at least
the dopaminergic subset of CA neurons exceed any
observed in the present experiments by at least a
factor of two (Yim and Mogenson, 1980; Guyenet and
Aghajanin, 1978).

Future Experiments and Technical Modifications

As the present study progressed, it became evident
that certain technical modifications would allow more
thorough testing of the psychophysically based
inferences. A microcomputer could provide signal
digitization and subsequent digital subtraction of one
record from another, permitting estimation of collision

intervals from electrophysiological data and improving
refractory period estimation. The collision interval
was difficult to estimate from our records since late
occurring events due to the C pulse obscure the
recovery process. Subtraction of C pulse records from
combined C-T records would permit accurate assessment of
the contribution of the T pulse to combined records
from both collision and refractory period data. The
digital averaging capability that a microcomputer can
provide would be useful in several ways. It would
permit study of more subtle features in the record and
possibly allow separation of absolute and relative
refractory periods and examination of supernormal and
subnormal periods. Furthermore, averaging would
permit the use of a statistical rule of thumb, such
as 95% confidence intervals, in the evaluation of
differences between responses.

Another difficulty could be alleviated by the
addition of a high-pass filter stage to each channel of
the recording circuit. The existing differential
amplification and low-pass filtering cannot eliminate
many artifacts because of the differences in the low
end of the power spectra of the artifactural signals
induced in the two poles of the recording electrode,
which in turn are likely due to differences in tip

shape and location. These differences prevent the
use of gain adjustments to make the two artifactural
signals sufficiently similiar to allow for their
adequate differential removal, since such adjustments
often exacerbate low frequency components. High-pass
filtering is expected to deal with this difficulty.
The importance of this improvement would be twofold:
1) The obscuring of response components (see Figure 8)
would be less frequent and less severe. 2) A high
degree of artifact rejection would permit strength-
duration trade off experiments to be conducted
electrophysiologically. The pulse widths these
experiments require often exceed the sum of the
response latency and the response duration and good
artifact rejection would allow visualization of the
superimposed response.

The utility of the field potential recording
technique in the assessment of the plausibility of
psychophysically based inferences could be greatly
increased through alterations of a non-technical
nature. Recall that the recording methods do not
have the filtering capabilities of the behavioural
methods; that recordings include not only the
contributions from the first stage elements underlying
BSR but those of all activated elements within range

of the recording electrode. Therefore, it is important to attempt to maximize the contributions of directly activated behaviourally relevant neurons. If one were to record through the same electrodes that yield behaviourally derived collision effects, one would be more confident that members of the directly stimulated substrate for BSR contributed to the records. To be certain, one would have to establish that the area sampled by the recording electrode was at least as large as the stimulation field produced when that electrode is used in the behavioural collision experiment. Comparing these records to ones made through electrodes that fail to support SS could contribute to the assessment of the uniqueness of the psychophysical portrait of BSR. If it turned out that there were key differences between the two sets of records, that a particular set of electrophysiologically determinable features were unique to the first stage elements, the region in which these elements must reside could be circumscribed by moving recording electrodes until the key features are maximized. One could then move electrodes along the trajectory of candidate pathways in attempts to trace the first stage of the BSR circuit. Correlating these results with anatomical data may allow unit recording methods to

locate the somata of the directly stimulated elements
responsible for BSR and permit the characterization of
individual elements. The eventual impalement of one
such neuron with a glass microelectrode could result
in intracellular records and the injection of a dye,
permitting the localization of an individual element
and the direct identification of dendritic regions
and terminal zones. It is likely that identification
of the second stage in the circuit would not be far
behind.

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Latencies and Interelectrode Distances

Recording electrodes aimed at the VTA

Subject	Stimulation Target	Latency	Interelectrode Distance Measured	Calculated
IM 1	LH	0.36		3.1
IM 2	LH	0.34*		3.1
IM 3	LH	0.45		3.1
MFB 1	POA	0.56		5.5
	LH	0.48		3.1
MFB 2	POA	0.65	5.1	5.8
	LH	0.40	2.9	2.6
MFB 4	LH	0.54	2.9	2.9
MFB 9	LH	0.49	3.1	3.1
MFB 11	POA	0.75	5.0	5.6
	LH	0.51	3.1	3.1
MFB 13	POA	0.92	4.8	5.7
	LH	0.68	2.6	3.1
MFB 14	LH	0.33*		3.1
PI 1	POA	0.80		5.6
	LH	0.30		3.3
PI 2	POA	0.68		5.6
	LH	0.40		3.1
PI 3	POA	0.58*		5.6
	LH	0.40		3.1
R 1	POA	0.80		

Appendix 1 (continued)

Recording electrodes aimed at the LH

Subject	Stimulation Target	Latency	Interelectrode Distance	
			Measured	Calculated
MFB 3	VTA	0.50	3.4	3.1
MFB 5	VTA	0.44	3.4	4.1
MFB 6	VTA	1.20	4.2	3.2
MFB 8	VTA	0.33*		3.2

Recording electrode aimed at the POA

Subject	Stimulation Target	Latency	Interelectrode Distance	
			Measured	Calculated
IM 4	LH	0.40		2.5
	VTA	0.42		5.5

* first phase of response obscured by artifact