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SUPRACHIASMATIC NUCLEI AND CIRCADIAN RHYTHMS.
THE ROLE OF SUPRACHIASMATIC NUCLEI ON RHYTHMIC ACTIVITY OF NEURONS
IN THE LATERAL HYPOTHALAMIC AREA, VENTROMEDIAN NUCLEI AND PINEAL
GLAND

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16. Abstract Unit activity of lateral hypothalamic area (LHA) and Ventromedian nuclei (VMN) was recorded in urethane anesthetized male rats. A 5 to 10 sec. a 3-5 min and a circadian rhythmicity were observed. In about 15% of all neurons, spontaneous activity of LHA and VMN showed reciprocal relationships. Subthreshold stimuli applied at a slow rate in the septum and the suprachiasmatic nuclei (SCN) suppressed the rhythms without changing firing rates. On the other hand, stimulation of the optic nerve at a rate of 5 to 10/sec increased firing rates in 1/3 of neurons of SCN. Iontophoretically applied acetylcholine increased 80% of tested neurons of SCN, whereas norepinephrine, dopamine and 5 HT inhibited 64, 60 and 75% of SCN neurons respectively. These inhibitions were much stronger in neurons, the activity of which was increased by optic nerve stimulatio. Stimulation of the SCN inhibited the tonic activity in cervical sympathetic nerves.

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THE ROLE OF SUPRACHIASMATIC NUCLEI ON RHYTHMIC ACTIVITY OF NEURONS IN THE LATERAL HYPOTHALAMIC AREA, VENTROMEDIAM NUCLEI AND PINEAL GLAND

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Summary

Unit activity of lateral hypothalamic area (LHA) and of /941* ventromedian nuclei (VMN) were recorded in male rats anesthetized with urethane or chloraolose-urethane. Unit activities of 5-10 seconds, 3-5 minutes and a circadian rhythm throughout 24 hours were observed. The discharge frequency and rhythmic fluctuation of LHA neurons were greater in the day than at night. Neurons of 5 out of 24 groups (14%) exhibited reciprocal fluctuation frequently while 10 groups (29%) exhibited such fluctuation occasionally. This reciprocal fluctuation was greatest when the LHA neuron activity rose or fell. Reciprocity due to spontaneous discharge was evident upon external stimulation (Spl. N, Cortex, SEPT stimulation). SEPT, SCN high frequency stimulation suppressed LHA neuron discharge but weak stimulation of 0.2-1cps, 0.2-0.4 msec, 2-4V had no effect on discharge frequency but altered only the rhythmic fluctuation. Optic nerve stimulation increased the firing rates in 50 out of 129 SCN neurons (40%) with suppression in 33 neurons (25%). Conversely, SCN stimulation suppressed cervical sympathetic nervous firing. Iontophoretically applied ACh increased 26 neurons out of 158 SCN neurons (80%) while NA suppressed 96 out of 149 neurons (64%). DA suppressed 59 out of 98 neurons (60%) and 5 HT suppressed 93 out of 126 neurons (75%). Observation of the relation between optic nerve stimulation and drug susceptibility indicated that DA and 5 HT suppressed 6 out of 9 and 6 out of 8 neruons respectively which had been accelerated by optic nervous stimulation. However,

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*Numbers in the margin indicate pagination in the foreign text.

DA and 5HT suppression of neurons which had been inhibited by optic nervous stimulation was slight. This data indicates that SCN is significant in controlling VMN and LHA neuron activity within the hypothalamus and the pineal body.

Introduction

Individual cells and organisms have biorhythms. Daily rhythms are evident in our daily physical activities involving hormone secretions from glands, autonomic nervous activity, ingestion and movement and the daily fluctuations of hypo-thalamic and pineal activity are especially prominent. Daily rhythms have been studied in detail in invertebrates [1-3] but there are few such reports concerning mammals and many unknown points regarding the mechanism. Lateral hypothalamic area (LHA) and ventromedian nuclei (VMN) cell activities exhibit reciprocal activities and they seem to regulate ingestion and satiation functionally [4-6]. The neuron activity in these areas exhibits fluctuations over 24 hours [7]. It has already been reported that the frontal lobe cortex, septum (SEPT) and hypothalamic suprachiasmatic neuclei (SCN) affect LHA neuron activity [8] and recent reports have indicated that SCN plays an important role in the regulation of pineal activity via the cervical sympathetic nerves with input from the optic nerves [9-11]. However, the properties and functional significance of SCN cells have not been detailed. Accordingly, we have analyzed the reciprocal changes in LHA and VMN neurons in white mice, the effect of SCN therein and have examined the role of SCN neurons in the system involving "optic nerve-SCN-cervical sympathetic nerve-pineal body."

Experimental Method

1. Recording of LHA and VMN Neuron Activity

Tracheotomies were conducted on male mice (200-400 g) under urethane sedation (1.2-1.5 g/kg i.p.) and brain adjustment devices were fixed. The splanchnic nerve (Spl) was separated

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for stimulation. A cuff electrode was affixed to the central
cut tip. Cannulation was conducted on one jugular vein for
drug administration. A portion of the cranial bone was removed
and dipolar electrodes were inserted in the frontal lobe cortex
(area 10), SEPT and SCN following electroencephalogram results
(Pellegrino and Cushman, 1967) and stimulation was conducted.
Tungsten electrodes were used for recording from single neurons
but these electrodes were contained within glass capillary
tubes. The regions of exposure of their tips were adjusted to
10-15μ under microscopes. Since LHA and VMN are close, one of
the two recording electrodes was inserted in LHA while the other
was inserted in VMN (Fig. 1A). The signal was introduced by
oscilloscope, counter (Nicolet 1070) and polygraph (Grass 7A)
through a pre-amplifier (5A, WP Instruments) and a high
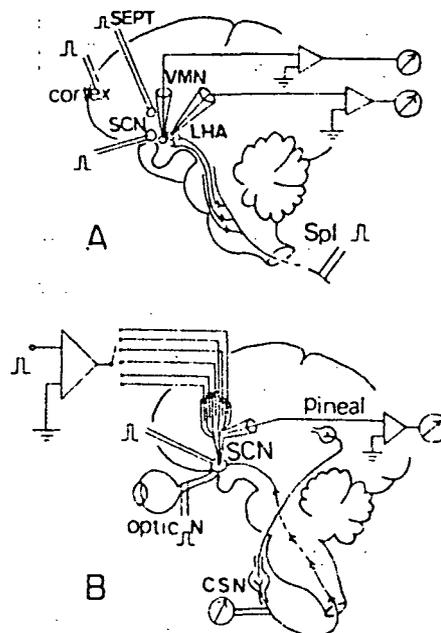sensitivity amplifier (502 A Tektronix). Once a spike discharge

Fig. 1. A: Recording the unit activities in lateral hypothalamic area (LHA) and ventromedial nucleus (VMN). LHA electrode was inserted into one side, VMN electrode into the other side of the brain. Stimulating electrodes were placed on the afferent splanchnic nerve (Spl), prefrontal cortex (Cortex), septum (SEPT) and suprachiasmatic nuclei (SCN). B: Recording the unit activity and iontophoretical application of chemicals in SCN. Drug and recording electrodes consisted of side-by-side assemblies. Stimulation was applied to optic nerves and SCN. Tonic activity in cervical sympathetic trunk was also recorded.

is achieved from a single neuron, the discharge frequency is
counted every 1-2 seconds through a window discriminator and
recording is conducted via oscilloscope and X-Y recorder. All
signals are recorded on tape for subsequent computer analysis.
The brain waves were recorded from the suboccipital cortex in all
cases. The rectal temperature was maintained at 37-38.5°C
throughout the experiments. After the experiments were over,
the regions of electrode insertion were destroyed by DC current
(3-20mA, 30 sec); the brains were removed and fixed in a 10%
formalin solution. 50 μ frozen sections were prepared, stained
with thionin or cresyl violet and the positions of electrodes
were confirmed under microscopes.

2. Recording of SCN Neuron Activity

Under urethane or chloraloseurethane sedation (40-70 mg,
400-700 mg/kg i.p.), the animals were fixed in the prone   /943
position. After tracheotomy, cannulation was conducted in one
femoral vein and carotid artery. Installation of Ringer solution
and measurement of blood pressure were conducted. Cuff electrodes
were installed after nuclear exposure of both eyes for electrical
stimulation of the optic nerve. Light stimulation involved
opening both eyes and shining light directly in front of the
eyes. The optic chiasma was exposed from the fundus by medial
incision and steel electrodes were inserted for SCN stimulation.
SCN neuron activity recording was conducted in the same manner
as 1. with the insertion of glass micro-electrodes (10-30 MΩ).
Cervical sympathetic nervous discharge was conducted by
oscilloscope and polygraph through a pre-amplifier (Tektronix
122, 0.8-1 KC) and silver dipolar electrodes involving
incision directly before the superior cervical nervous plexus
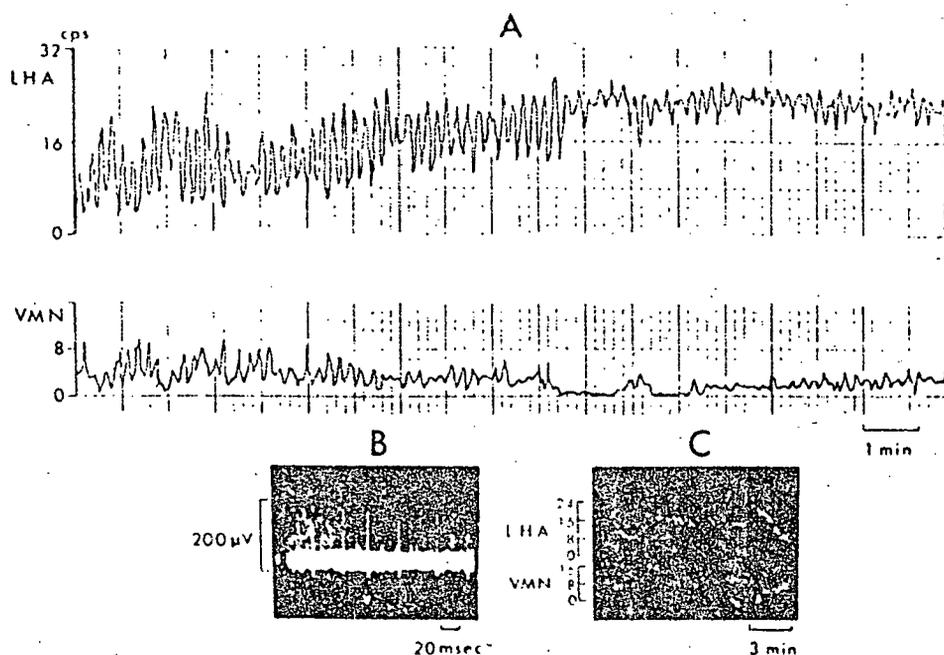and exposure of the nervous sheath.

Fig. 2. Simultaneous recordings of firing rates both in LHA and VMN neurons displayed on an X-Y plotter (A) and oscilloscope (C). A 5 to 10 sec and 3 to 5 min rhythm was observed. Note tendencies toward reciprocal action between units of LHA and VMN in both A and C.

3. Iontophoresis Experiments in SCN

Five glass tube microelectrodes were packed with 2M sodium glutamate (Glut), 1M acetylcholine HCl (ACh), 1M noradrenaline HCl (NA) 1M dopamine HCl (DA) and 2×10^{-2}M 5 hydroxytryptamine creatine sulfate (5HT) respectively. Current was circulated (10-80 nA) by constant current device [12] and changes in SCN neuron discharge frequency were studies (Fig. 1B). The recording electrode involved the 5 electrodes stuck to conventional glass microelectrodes but in this case, the tips of the recording electrodes were positioned so as to protrude several μ away from the drug electrodes. The amount of current varied considerably with the electrode. In general, 10-30 nA was used with Glut and ACh, 30-60 nA was used with NA and DA while 30-80 was used with 5HT.

Experimental Results

I. LHA and VMN Neuron Activity

1. Reciprocity of LHA and VMN Neuron Activity

Simultaneous recording of unit discharge of LHA and VMN neurons was possible in 35 out of 75 neurons. Among these 35 gourps, reciprocal activity was exhibited in 5 gourps (14%). Fig. 2B shows LHA neuron discharge; A shows recording on X-Y recorder while C shows the discharge frequency counted on an oscillograph. In general, LHA neurons exhibit higher discharge frequency than VMN neurons and a greater rhythmic fluctuation is exhibited. Rhythm fluctuations every 5-10 seconds faster and fluctuations 3-5 minutes slower are observed. /944 These exhibit mutually reciprocal fluctuations (Fig. 2A).

Various types of external stimulii were imposed on the neuron groups exhibiting reciprocal action in spontaneous discharge. Spl N 20 cps, 0.3 msec, 10 V stimulation accelerated LHA neuron discharge and inhibited VMN neuron discharge but cortex 5 cps 0.2 msec, 10 V stimulation inhibited LHA neuron discharge and accelerated VMN neuron discharge in contrast to Spl N stimulation. Conversely, venous administration of 20% glucose inhibited LHA neuron discharge and accelerated VMN neuron discharge (Fig. 3). Ten groups among the 35 groups of neurons occasionally exhibited reciprocal fluctuations while reciprocal relations were unclear in the remaining 20 groups (58%).

2. Daily Fluctuation in LHA and VMN Neurons

LHA neuron discharge frequency and rhythmic fluctuation are greater than those of VMN neurons but long term recordings of day and night were conducted for a more detailed examination. LHA neuron activity exhibits one period of acceleration around noon. It is generally high in the afternoon but exhibits a temporary phase of decline in the evening (16-18:00). Before
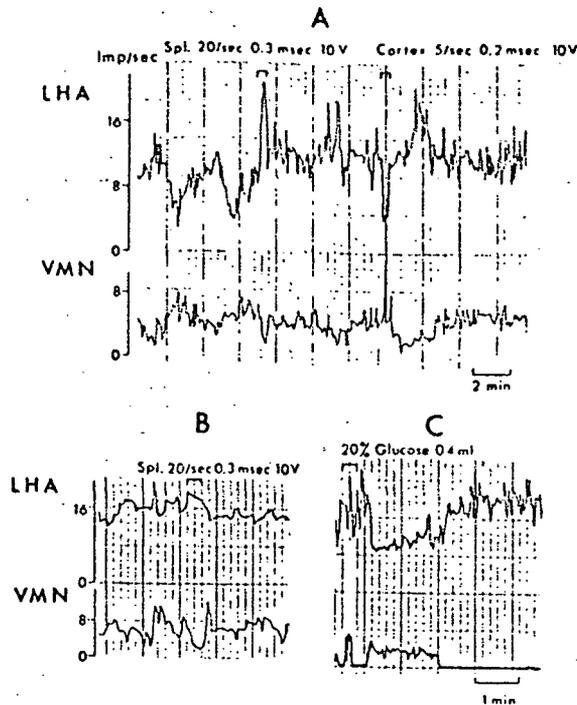
Fig. 3. Simultaneous effects of stimuli on units of LHA and VMN. A and B show effects of stimulation of prefrontal cortex and of splanchnic afferents. C: Effects with an intravenous injection of glucose. Note reciprocal effects.

reaching the period of low activity at night, a second period of accelerated activity is exhibited (Fig. 4).

Conversely, the discharge frequency and rhythmic fluctuation of VMN neurons are both low in the day but the frequency and rhythm both increase symmetrically to LHA neurons after 19:00. The reciprocal changes in LHA and Vmn neuron activity are found most often in those periods when LHA neuron activity in the day and evening changes from low frequency to high frequency and conversely, from high frequency to low frequency (indicated by bands of black in Fig. 4). These changes are evident in the time periods shown by oblique lines also to a lesser degree.

The lower half of Fig. 4 illustrates autocorrelation and reciprocal correlation in a typical case which exhibits correlation acitvity. In comparison to VMN, the rhythmic changes of LHA neurons are great and the variety is found from autocorrelations graphs. A downward peak of about 0.4 is evident
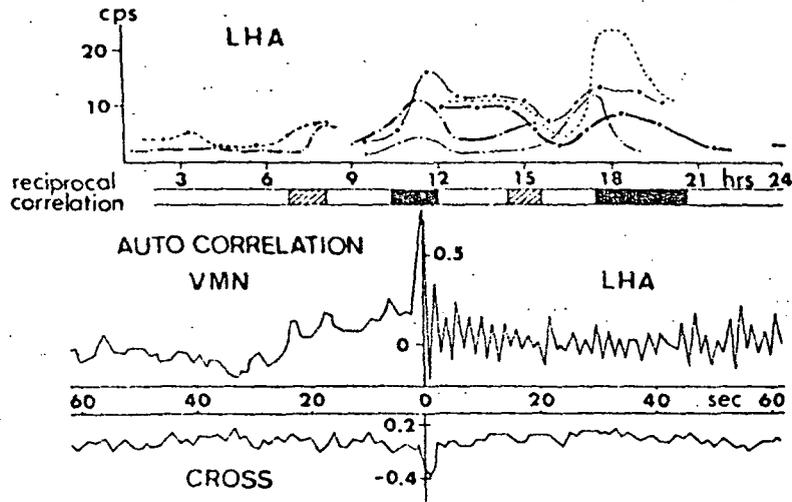
Fig. 4. Firing rate in LHA is rather low in the a.m. but increases around noon. In the p.m. it is generally high, increases especially in late afternoon before going into a decline at night. Reciprocal correlation in firing rate in LHA and VMN was observed most often during the time indicated by black squares and then in hatched squares. Auto-correlation function shows there are considerably fast rhythms in LHA but slow rhythms in VMN neuron. A negative sharp peak (−0.4) at 0∼1 sec means that firing rate of neurons in LHA and VMN have reciprocal correlation every 0∼3 sec.
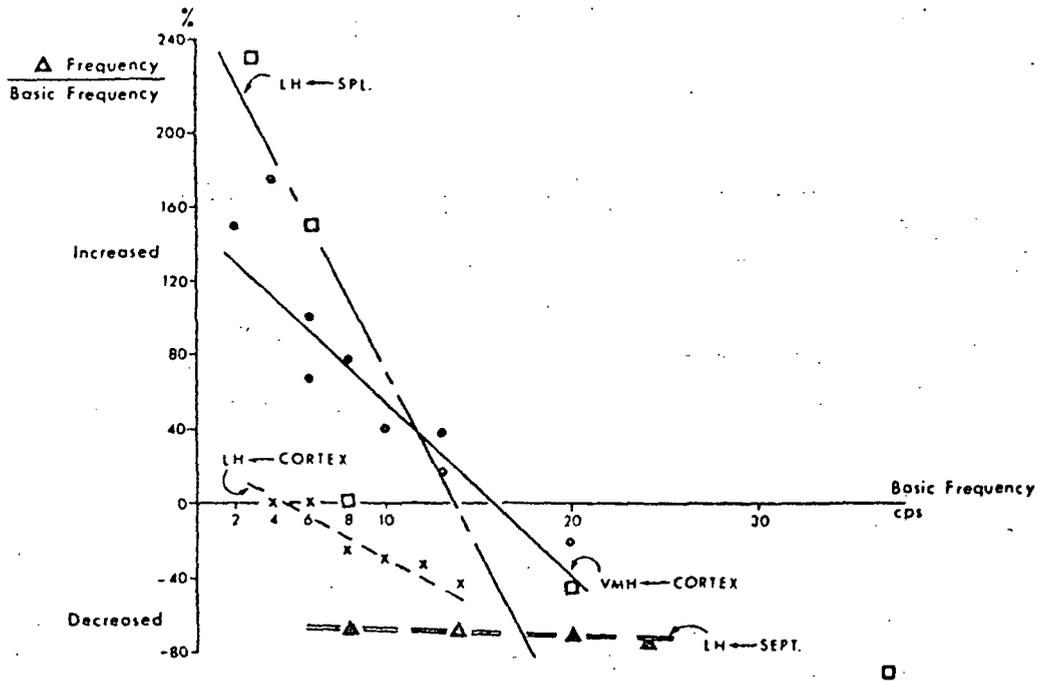

Fig. 5. Effect of stimulations in splanchnic nerve (□), prefrontal cortex (x) and septum (▲) on firings in LHA (LH) neurons. And that of cortex stimulation on VMN (VMH) firing (●) is also plotted. The ratio of changed frequency is plotted against basic firing frequency. The effects of stimuli depend on basic firing frequency.

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at 0-1 sec. in the reciprocal correlation graph but in this experiment, the discharge frequency is measured reciprocally each second in LHA and VMN so that an inverse correlation every 0-3 seconds is indicated.

This experiment was conducted from March to July. While seasonal fluctuations must also be considered, that is deferred /946 to later studies.

3. Reactivity to External Stimulation

As indicated in 1, Spl N stimulation generally accelerates LHA neurons and inhibits VMN neurons but the reactivity varies as seen in the results of studies over various time periods. Specifically, LHA neurons are inhibited under the same Spl N stimulation in neurons (above 20 cps) and in periods of extreme acceleration of LHA activity. Similarly, VMN neurons of high discharge frequency are inhibited by cortex stimulation. The rate of discharge frequency change achieved by stimulation is affected by the spontaneous discharge frequency (Fig. 5).

4. Effect of SEPT and SCN on LHA Neuron Discharge Rhythms

Since the daytime discharge frequency fluctuation of LHA neurons is more pronounced than that of VMN, the effect of external factors on rhythmic fluctuation was studied primarily on LHA neurons. Experiments were conducted involving blocking external input by isolating LHA from peripheral tissue but isolation was difficult while preserving unit discharge recording. Consequently, an additional measure involved frontal plane incision from the crebral cortex to the base of the brain only on the side of LHA discharge recording at a site 2mm away from the recording electrodes at the head and tail sides. After incision at the tail side, the LHA discharge rhythm was barely affected but 30 minutes after incision at

the head side, the discharge rhythm fluctuation vanished and
there were also cases in which the discharge frequency declined.
There is a dense fiber connection between SEPT and the hypo-
thalamus and the discharge frequency is affected in response
to light stimulation [24] so that the effect of SEPT stimulation
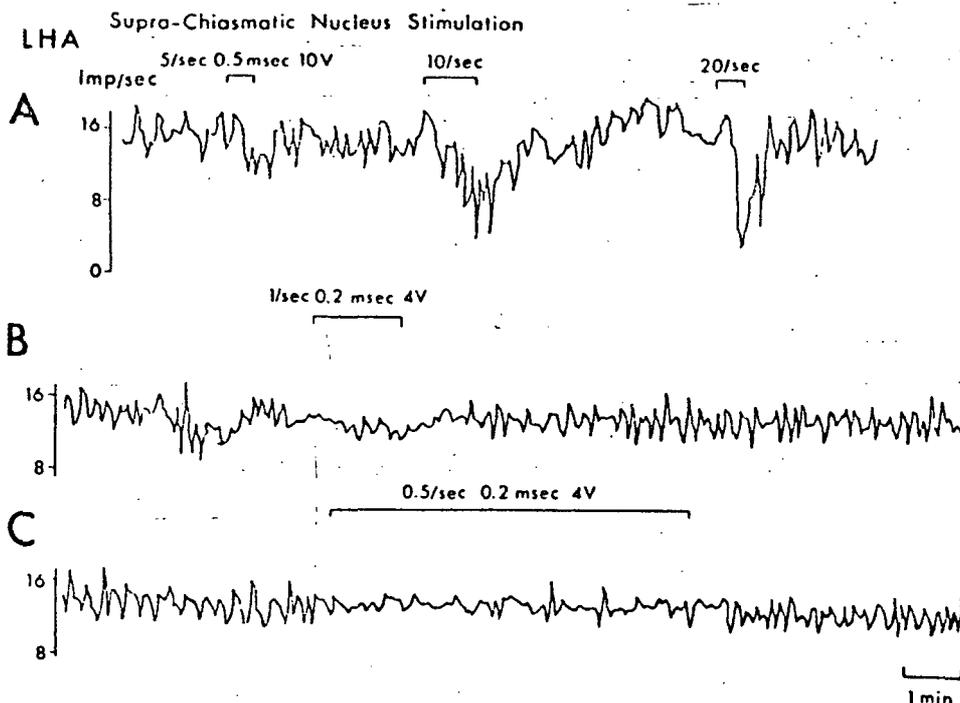on LHA activity was studied first.

Fig. 6. Effect of stimulation in suprachiasmatic nucleus on firing of LHA neuron. 5 cps, 10 cps and 20 cps (0.5 msec, 10 V) stimuli decrease LHA firing (A). Very low frequency and weak stimuli (0.5~1 cps, 0.2 msec 4 V) suppress the rhythms without changing firing rates (B and C).

SCN is present in the same hypothalamus as LHA and recent
impressions indicate that it plays a significant role in the
"pineal activity regualtion system" via cervical sympathetic
nerves with input from the optic nerve [9-11]. The effect
of SCN stimulation on LHA neuron activity was studied. Intense
SEPT stimulation of 15 V, 1 msec induced activity potential
4 msec after LHA stimulation after which spontaneous discharge
was suppressed.

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The effect was different from the case of alteration of
stimulation frequency. Specifically, 1-5 cps stimulation
increased LHA neuron frequency while stimulation above 10 cps
suppressed it.   When the stimulation frequency and intensity
were gradually reduced (0.2-0.5 cps, 2=4V) until the induction
potential resulting suppression were not evident at all, no
effect on spontaneous discharge was evident but change developed
only in the rhythmic fluctuation. SCN stimulation did not give
rise to induced activity potential in LHA as in the case of
SEPT stimulation but field potential of different confirurations
were induced in different sites within LHA. However, when the
stimulation frequency rose (5-10 cps), LHA neurons were
suppressed. The degree of suppression was also determined by
the degree of spontaneous discharge but the suppression effect
was greater as the stimulation intensity increased. When the
frequency and degree of stimulation were sufficiently reduced
(0.2-1 cps, 2-4 V), there was no effect on neurons as in the
case of weak SEPT stimulation. There was only change in the
rhythmic fluctuation (Fig. 6).

II. Recording of Cervical Sympathetic Nervous Activity
        and of SCN Neuron Activity
        1. Effect of Optic Nerve Input on SCN neuron Activity
    SCN neuron discharge frequency was generally lower than
the neuron discharge of other hypothalamic nervous nucleii,
(0-8 cps). However, there was occasional exhibition of high
frequency discharge around 15 cps.   Virtually no rhythmic
fluctuation was evident when the discharge frequency was low.
When the discharge frequency increased, delayed rhythmic
fluctuation with a 3-5 minute cycle appeared. Optic nerve
stimulation of 10 cps, 0.7 msec, 10 V increased SCN neuron
discharge. Prolonged periods of time were required from
commencement of stimulation until appearance of stimulation effect
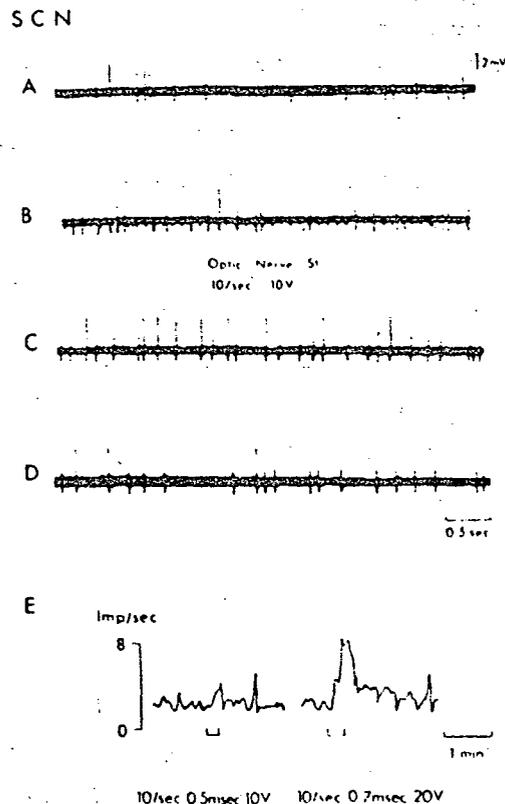and from the cessation of stimulation until reversion to the

SCN

Fig. 7. Effect of optic nerve stimulation on firings of SCN neuron. A: Control. B: During stimulation (10 cps, 0.7 msec, 10 V). Unit firings start to increase a few seconds after the stimulus. C: Soon after the cessation of the stimulus. Note the augmenting effect of the stimulus is still present. D: 10 sec later. E: Effects of optic nerve stimuli of different strengths.

original discharge frequency level. Periods of several seconds to 10 seconds were not unusual (Fig. 7). Increases in discharge frequency due to optic nervous stimulation developed in 50 out of 129 studied n eurons (40%). Suppression developed in 33 neurons (25%) and the rest indicated no effect. The optimum stimulation frequency was 5-10 cps. The greatest effect was evident at 10 cps while the effect was slight when stimulation exceeded 20 cps. There was virtually no effect with stimulation below 2 cps. There was no development of induced potential or change in discharge frequency with single stimulation or 3-5 groups of high frequency stimulation. There were few cases of light stimulation but acceleration occured in 10 out of 16 neurons with suppression in 2 neurons and no effect in 4 neurons.

2. Effect of Optic Nerve Input on Cervical Sympathetic Nervous Discharge

Optic nerve stimulation regulates pineal activity through the cervical sympathetic nerves. This optic nervous stimulation suppresses pineal activity while cervical sympathetic nervous stimulation accelerates pineal activity [13]. Thus, a study must be made of the action of optic nervous input on cervical sympathetic nervous discharge and the effect of light and optic nervous electrical stimulation was studied. The cervical sympathetic nerves were isolated from surrounding tissue at the beginning of the experiment and were cut before the ganglion. The nervous sheaths were removed immediately before recording and silver dipolar electrodes were implanted in a mineral oil pool. The spontaneous discharges were then recorded. There was considerable variation in potential since the potential involved grouped rather than single nervous fiber discharge. However, groups of 3-5 discharges were frequently observed. Fig. 8A illustrates spontaneous discharge; B illustrates the discharge upon 10 cps, 0.5 msec, 15 V optic nervous stimulation while C illustrates the discharge of 20 seconds after termination of stimulaton. Suppression developed several seconds (1-3 seconds) after commencement of stimulation. The effect continued for 10 seconds after the termination of stimulation. Light stimulation induced the same effect but the degree was less than with electrical stimulation. Chloralose-urethane was used in a small number of cases but under this sedation, intense induced potential was brought about in the cervical nerves also perhaps because of excitation of the entire autonomic nervous system.

3. Effect of SCN Stimulation on Cervical Sympathetic Discharge

The effect of SCN stimulation on sympathetic nervous discharge is clarified below.

Fig. 9A shows the effect of 20 cps, 0.5 msec, 15 V stimulation

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CERV. SYMP. N.

]100 µV

A

B

Optic Nerve St. 10/sec 15V

C

1 sec
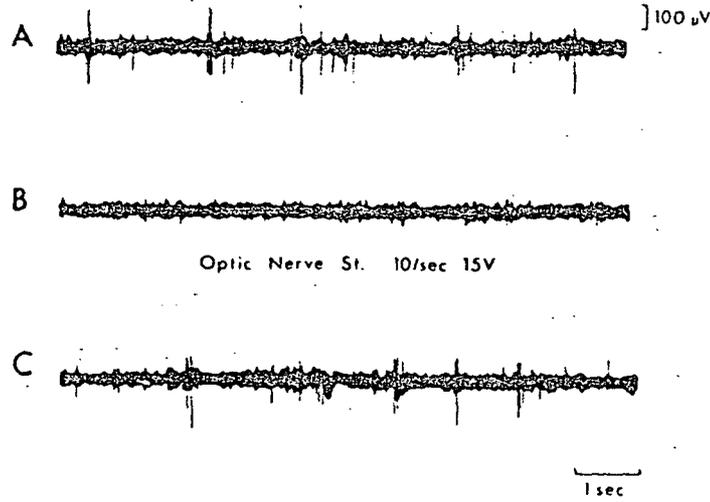
Fig. 8. Recordings of tonic activities of cervical sympathetic trunk. A: Control. B: During optic nerve stimulation (10 cps, 0.5 msec, 15 V). C: After control.

mm Hg   A

100

0

CERV.
SYMP. N.

S C N St. 20/sec 15V

mm Hg   B

100

0

S C N St. 10/sec 15V

mm Hg   C

100

0

L H St. 20/sec 15 V

1 & 5 sec

Fig. 9. Polygraph recordings which show the effect of stimulating suprachiasmatic nuclei. Top tracings are systemic blood pressure, lower tracings are electrical activities in the cervical sympathetic trunk. Stimulus at 20 cps (A) is more effective than 10 cps (B) in inhibiting sympathetic activity. C: Stimulus of the same strength (20 cps, 0.5 msec, 15 V) in LHA had no inhibiting effect on sympathetic activity.

while B shows 10 cps, 0.5 msec, 15 V stimulation effect. The
suppression with 20 cps stimulation is greater than with 10 cps
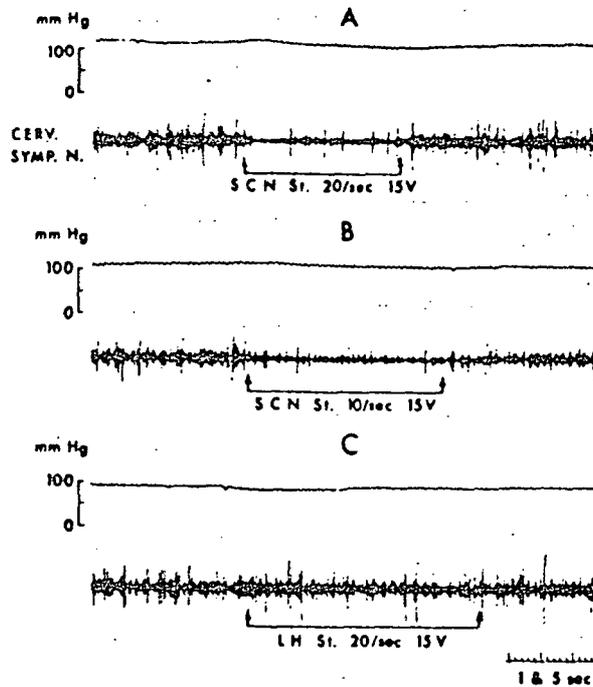stimulation. Single stimulation had no effect in this system.
Central nervous stimulation, especially of the hypothalamus,
affects the sympathetic nervous activity so that the possibility
cannot be denied that this SCN stimulation effect is based on
stimulation of other regions due to the influence of current.
However, this effect is not evident if the same stimulation
electrodes are used to stimulate other hypothalamic nuclei such
as LGA or VMN under the same conditions. Conversely, since
there was no change in the total blood pressure which was
recorded due to stimulation, this suppression effect seems to
be an effect solely of SCN stimulation.

III. Chemical Susceptibility of SCN neurons

Based on the idea of Oomura et al. [14], 5 micro electrodes
were packed with Glut, Ach, NA, DA and 5 HT. Adhesion was
conducted under a microscope with a separately prepared glass
electrode of 3M KCl. The recording electrodes were prepared so
as to protrude several μ from the drug packed electrodes. The
drug electrodes generally exhibited high resistance (above
100 MΩ).

In addition, since resistance continually fluctuated
while current circulated, a feed-back circuit was built in and
electricity was circulated by a constant current device prepared
so as to provide a constant flow of current. The amount of
current varied with the inidvidual electrodes and with the
drugs ih each electrode but a minimum current was provided so
that the effect could be determined. Negative current was
circulated through the Glut electrode while positive current
was circulated through the ACh, NA, DA and 5 HT electrodes.
The drugs were applied iontophoretically near the recorded cells
and the effect on discharge frequency was studied. The raphe

nuclei of the mesencephalon contained large amounts of 5 HT. Dorsal and ventral raphe N had fiber connections with hippocampus and frontal lobe fundic sections. The fluctuation of 5 HT amounts determined the activity of these regions and thus of the entire brain [15-19]. In particular, cats have been reported to have a significant SCN suppression action [20]. Table 1 collects the reactivity to optic nervous stimulation, the reactivity to various drugs and the reactivity in response to raphe N stimulation. ACh accelerated 126 neurons out of 158 neurons studies (80%) and suppressed 18 neurons (14%). NA accelerated 30 neurons out of 149 (20%) and suppressed 96 neurons (64%). DA exhibited results similar to those of NA, with acceleration in 11 out of 98 neurons (11%) and suppression in 59 neurons (60%). Conversely, 5 HT accelerated 21 out of 126 neurons (16% but suppressed 93 neurons (74%). Glut is used as a nervous activator. The distance between cells and drug electrodes is surmised by studying its effect. It is used to alter and correct the position of electrodes. However, 11 neurons out of 115 neurons (9%) were suppressed. Raphe nuclei stimulation was studied in 43 neurons and acceleration, suppression and no effect were each evident in about 1/3 of the cases. Intense suppressive effects were not evident under any
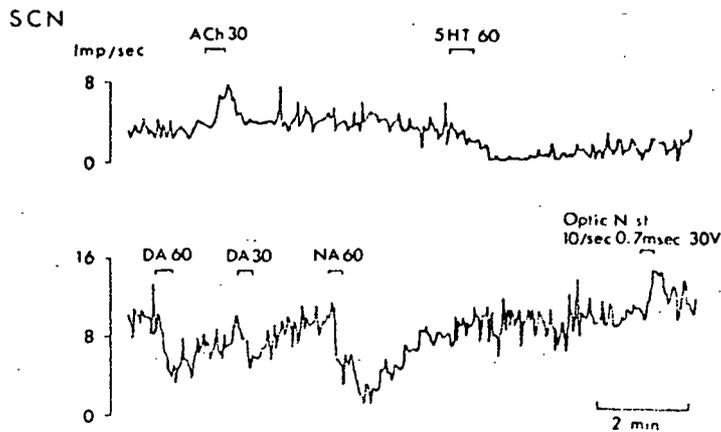

Fig. 10. Effects of chemicals and optic nerve stimulation on firing of a SCN neuron. Numbers after each chemical symbol indicate current strength applied (in nA). Note long lasting effect of serotonin.

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Table 1. Effects of stimulating optic nerve, raphe nucleus and of iontophoretical application of chemicals.

		Optic			Ach			NA			DA			5HT			Glut			Raphe N			Total
		+	0	−	+	0	−	+	0	−−	+	0	−	+	0	−−	+	0	−	+	0	−	
Optic N St	+				7	1	3	2	1	9	1	2	6	2		6	5	3		1		1	50
	0				10		1	2	3	7	1	1	4		1	7	6	1	1		1		46
	−				6		3	4	1	5	1	3		1	1	2	5	1					33
Ach	+	7	10	6				7	5	17		4	11	5	3	18	13	7	3	3	4	3	126
	0	1		3						4	1	1				1	3						14
	−	3	1							3	1		2			4	3			1			18
NA	+	2	2	4	7							4		3	1	1	2	2		1		1	30
	0	1	3	1	5							1	1	1	1	4	3	1			1		23
	−	9	7	5	17	4	3				3	3	11			14	13	3	1	1	1	1	96
DA	+	1	1	1		1	1			3				1	2								11
	0	2	1	3	4	1		4	1	3				2	1	4	1					1	28
	−	6	4		11		2		1	11				1		10	7	2	1	1	1	1	59
5HT	+	2		1	5			3	1		2	1					2	1		1	1	1	21
	0		1	1	3			1	1			1					2			1		1	12
	−	6	7	2	18	1	4	1	4	14	1	4	10				9	2	3	2	3	2	93
Glutamate	+	5	6	5	13	3	3	2	3	13	2	1	7	2	2	9				2	1	1	80
	0	3	1	1	7			2	1	3		2			2						1		24
	−−		1		3					1		1			3					1	1		11
Raphe N St	+	1			3		1	1		1		1	1	1	1	2	2						14
	0		1		4			1		1		1	1		3	1	1	1					15
	−	1			3			1		1	1	1	1	1	2	1		1					14

+: augmentation, 0: no change, −: reduction in firing rate of SCN neuron.

stimulation conditions (0.2-20 cps, 0.5-1 msec, 5-30 V). Fig. 10 is a most typical reaction pattern. The discharge is accelerated by optic nerve stimulation.

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Consideration

I. Rhythmic Fluctuation of LHA and VMN Neuron Discharge

Simultaneous unit discharge was achieved fro LHA and VMN neurons. In order to continue recording for the longest possible duration, various devices were applied and holding brain activity to the minimum accompanied by respiration and heart beat is most important. The scope of cranial bone excision was small and the surface was covered with gelatin after insertion of electrodes. The abdomen of the animal was released from the fixation table and the effect of abdominal movement due to respiratory movement was minimized. Particular attention was paid to the recording electrodes. Glass tube micro electrodes, tungsten electrodes and stainless steel electrodes in various

shapes were tried and finally, tungsten electrodes covered
by glass with a tungsten wire protruding 10-15µ was selected for <u>/951</u>
continuous recordings of discharge over prolonged periods.
Since the tip of glass mocro electrodes is sharp, it must be
very close to the cell so that there is frequent escape of
discharge which is recorded due to slight vibration and
movement.

Whether the unit discharge is due to a single neuron discharge
or a number of neurons is determined by the size, duration and
configuration of the discharge coming from a trigger in acitvity
potential with a rapid oscilloscope sweep.

Simultaneous recording of LHA and VMN neuron activity
was possible in 5 out of 35 groups of neurons (14%). A clear
reciprocal fluctuation was exhibited there. Adding the 10
cases (29%) in which occasional reciprocal action was evident
indicates more than 40% of neurons in which spontaneous discharge
or reciprocal fluctuation to external stimulation was evident.
From LHA, there was no union of direct duppression of VMN.
There are reports of suppressive interposed neurons from without
VMN [21]. The report of Oomura et al. [6] indicates LHA stimulation
suppresses VMN discharge while VMN stimulation is believed by
some to suppress LHA discharge but wherher this is direct or
indirect is irrelevant since these both are closely related.
In the case of long term observation, there are few cases of
exhibition of reciprocal fluctuation with VMN neurons when LHA
neuron discharge frequency is low at night or at those times
when the activity exceeds 20 cps. However, the most frequent
reciprocal fluctuation is evident when there is a shift from
low to high values of discharge frequency at noon and at night
as well as when the frequency falls from high to low values.
LHA and VMN neurons seem to have the greatest reciprocity when
the discharge frequency is actively altered by changes in
hypothalamic input. LHA neurons exhibit a daily fluctuation

involving low discharge frequencies in the morning, rise before noon, generally high frequency in the afternoon followed by a second period of activity acceleration in the evening (18-20:00) after which comes a stable period at night. In addition, in comparison to VMN neurons, the rhythmic fluctuations in the day are greater 5-10 second and 3-5 minute small rhythms are evident in the 24 hour daily fluctuations. Since LHA neurons have close fiber connections with inner frontal lobe fascicles, there are fiber connections with the frontal structure of the olefactory bulb, septum and with the posterior structures of the pons, tegment, and black substance. -Due to close connection with the thalamus via the lower thalamic peduncle, extensive input from a peripheral and central regions are collected. The phenomenon of a great rhythmic fluctuation in the day seems to be based on the unification of these inputs. Since there is greater ingestion at night in nocturnal animals such as the rat, LHA neuron activity would tend to be greater at night but the ex-perimental results are the opposite of this. Similar results have been reported in cats not under sedation [22]. An explanation cannot be derived from the single behavior of ingestion activity. The short cycle rhythmic fluctuation evident in this experiment may be related to hormonal secretory activity from the moderate potential fluctuating rhythms [23] measured from the cerebral surface of rabbits and from the hypothalamus or to the rhythmic activity [22] of "non-specific cells" reported in chronic experiments with cats which resembles the fluctuation.

In a study of daily fluctuation over a 24 hour period, the most important factor is the sedation. It must be determined whether the changes recorded are daily fluctuations or whether they are the result of influences of changes in the degree of sedation. In order to maintain the most stable degree of sedation, appropriate supplements of sedative sould be conducted. Brain waves should be recorded from the suboccipital cortex and

the state of sedation should be constantly monitored. The daily rhythms observed in this experiment were determined at the beginning of sedation and even when the time of surgery was changed, these rhythms were observed indicating that they are not based on changes in the degree of sedation. They seem to be rhythms dependent on the body. Spl N, cortex and SEPT stimulation generally accelerate (in the first case) and suppress (in the latter two cases)LHA neuron activity but these effects are not constant. Changes in effect due to stimulation conditions are evident. Above and below a certain spontaneous discharge frequency, the effect reverses and complexity in the mechanism regulating hypothalamic neuron activity is surmised.

It is not known whether the daily rhythm over prolonged periods and the small rhythms noted above are due to the hypothalamus itself or whether they are due to an external factor. If they are due to an external factor, an investigation must be conducted regarding its influence. While recording a discharge, a frontal plane section was made on one side of the recorded sides 2 mm from the electrode. Behind the section, there was little influence on LHA neuron rhythms but in the front of the section, effect of sectioning remained at 30 minutes after it was conducted while low amplitude brain waves and rapid pulse developed. The LHA neuron rhythm was regular, but upon development of the stable period after the passage of 30 minutes, the discharge frequency and rhythmic fluctuation both were suppressed. There was virtually no effect behind the section perhaps because of sectioning of only one side of the hemispehre with input form the other side not excluded. However, although the same conditions prevailed in front of the section, a more intense influence was received which may indicate that LHA neurons are more greatly affected by the frontal structure. SEPT has the closest relation in terms of a frontal structure with fiber connections to the hypothalamus. Since

there are reports [24] of SEPT neurons reacting to light, the
influence of SEPT stimulation was investigated first. Conversely,
SCN receives input from the optic nerve and exists in the
system which regulates pineal activity. SCN destruction
affects pineal activity and suprarenal cortex hormone levels
simultaneously. There are also reports [25] of relation to
the anterior lobe of the pituitary. With single stimulation of
SEPT, active potential develops after a latent period of about
4 msec. Thereafter, a suppression phase is evident. This
suppression phase intensifies as the frequency of stimulation
rises and LHA neurons are suppressed. However, stimulation with
no effect on discharge frequency, not giving rise to activity
potential and reducing both the stimulation frequency and
intensity clearly altered only small rhythmic fluctuations.
SCN stimulation also suppressed LHA neuron discharge above
3 cps but low frequency weak stimulation of 0.2-0.3 cps,
0.2-0.3 msec, 2-4 V specifically altered the slight rhythms of
LHA neuron discharge in the same manner as SEPT weak stimulation.

There have been reports recently [26] involving horseradish
peroxidase which has a direct fiber connection involving the
frontal lobe cortex and the hypothalamus without the medium of
the thalamus. In this experimental also, cortex stimulation
accelerated VMN neurons and suppressed LHA neurons but the
degree was determined by the spontaneous discharge frequency.
When the frequency was high, the VMN neurons also were suppressed.
However, rhythmic changes in LHA neuron discharge which appeared
due to weak SEPT, SCN stimulation were not induced by weak
cortex stimulation.

II. Functional Significance of SCN Neurons and Chemical
Susceptibility

Nervous ganglion cells within the retina undergo firbrous
projection bilaterally in SCN [27] and receive input from the
optic nerves. SCN has a significant role in the system regulating

the pineal body involving the inner forebrain fascicle and the
sympathetic nerves. The daily fluctuation of suprarenal cortex
steroids in rats raised in darkness remained with some phase
shifts. However, destruciton of SCN on both sides resulted in
the disappearance of this daily fluctuation [25]. In addition,
the fact that SCN neurons contain neurophysin and vasoperessin
[28,29] signifies that SCN not only is present in the system
regulating pineal activity but that it occupies a significant
position in the cycle of regulation of the anterior and
posterior lobes of the pituitary. Observation from the aspect
of fabrous connection indicates that SCN neurons receive
fine fibrous projections bilaterally from the nervous ganglion
cells and the presence of dendro-dendritic synapses [30].
However, the efferent side fron SCN [31] has not been completely
clarified. Conversely, there are no reports concerning
chemical susceptibility and electro-physiological properties of
SCN neurons. Consequently, the next stage of this experiment
is the consideration of the functional significance of SCN and
the chemical susceptibility of it.

Fourty percent of SCN neurons were accelerated by optic
nervous stimulation and 35% exhibited no reaction. However,
there are optimum conditions in stimulation and low frequency
stimulation of 1-3 cps has no effect and there is little result
due to high frequency stimulation above 20 cps. The effect
is greatest with 5-10 cps. In addition, the fact that several
seconds elapse from stimulation until manifestation of effect
seems to indicate that the input from optic nerve to SCN is
connected along very fine fibers which corresponds to
histological results. However, some consideration must be given
to the 25% of the neurons which are suppressed by optic nervous
stimulation. In fact, there are cases in which the accelerated
neurons and the suppressed neurons are within 100μ of each other
which relates the functional complexity of SCN neurons.

Iontophoretically administered ACh accelerated 126 out of
158 examined neurons (80%) while NA, DA and 5 HT suppressed
respectively 96 out of 149 neurons (64%), 59 out of 98 neurons
(60%) and 93 out of 126 neurons (74%). Iontophoresis involved
the same methods as used by Krnjević [32,33] and Oomura et al.
[14]. Differentiation of effects due to drugs and those due
to electric flow was made on the basis of latent period from
current flow to manifestation of effect, period from termination
of current flow to disappearance of effect and on polarity of the
current [34]. In this experiment, when the discharge frequency
was accelerated or suppressed due to drugs, a judgement of
significant change was rendered only when the change exceeded
30% of the original spontaneous discharge. Investigation of
combinations involving susceptibility to drugs involved the
susceptibility to NA in 126 neurons in which there was acceleration
due to ACh. Among 29 neurons studied, there was acceleration
in 7 (24%) and suppression in 17 (57%). DA resulted in
suppression in 11 out of 15 neurons (73%). 5 HT resulted in
suppression in 18 out of 26 neurons (70%). Conversely, raphe N
stimulation showed 1/3 each of acceleration, suppression and no
effect. No special effect was evident.

Investigation of susceptibility among autonomic nervous
amines indicated NA suppression in 96 out of 149 neurons (64%).
However, examination of the susceptibility to DA and 5 HT among
the neurons suppressed by NA indicated that DA accelerated 3
out of 17 neurons (18%) and suppressed 11 neurons (65%). 5 HT
suppressed all 14 of the neurons studied. Investigation of
the relation between DA and 5 HT indicates that 5 HT suppressed
10 out of 11 neurons (90%) suppressed by DA.

Conversely, 50 out of 129 neurons (40%) were accelerated
due to optic nervous stimulation while 46 neurons (35%) exhibited
no change. Thirty-three neurons (25%) were suppressed but

examination of the relation of chemical susceptibility to
reactivity toward this optic nervous stimulation indicates that
NA suppressed 9 out of 12 neurons (75%) in the group of neurons
accelerated by optic nervous stimulation while DA suppressed
6 out of 9 neurons (66%) and 5 HT suppressed 6 out of 8 neurons
(75%). Similar tendencies were recognized in the group of
neurons in which there was no reaction to optic nervous stimu-
lation but in the group of neurons which were suppressed by
optic nervous stimulation, NA suppressed 5 out of 10 neurons
(50%) while 5 HT suppressed 2 out of 4 neurons (50%). DA was
not recognized to have induced suppression in any of 4 neurons.
Conversely, raphe nuclei stimulation did not exhibit special
effects on the three groups of acceleration, suppression and
no reactivity due to optic nervous stimulation or susceptibility
to ACh or 5 HT.

SCN stimulation suppresses cervical sympathetic nervous
discharge. This stimulation effect seems to be solely a result
of SCN stimulation since it was not eveident in stimulation of
other regions of the hypothalamus. Conversely, optic nervous
stimulation also suppressed cervical sympathetic nervous discharge.
Light suppresses pineal activity [13,35] and consideration also
of the fact [13] that cervical sympathetic nervous stimulation
accelerates pineal activity indicates that SCN receives input
from the optic nerve and is accelerated. This rise in SCN
neuron activity suppresses cervical sympathetic nervous activity.
Thus, the decline in cervical sympathetic nervous activity
seems to be a suppression of pineal activity. The fact that SCN
neurons in this system are greatly suppressed by NA, DA and
especially by 5 HT indicates in conjunction with the fact that
SCN contains large amounts of 5 HT, that their activity is
regulated by these autonomic nervous amines.

REFERENCES

1. Lickey, M.E., S. Zack and P. Birrell, _Biochronometry,_ _Nat'l. Acad. Sci._, p.549 (1971).

2. Strumwasser, F., _Physiologist_ 16/9 (1973).

3. Strumwasser, F.,"The Neurosciences," p. 459, MIT Press 1974.

4. Anand, B.K. and J.R. Brobeck, _Yale J. Biol. Med_ 24, 123 (1951).

5. Anand, B.K., G.S. Chhina, K.N. Sharma, S. Dua and B. Singh, _Am J. Physiol_ 207, 1146 (1964).

6. Oomura, Y., H. Ooyama, T. Yamamoto and F. Naka, _Physiol. Behav._ 2, 97 (1967).

7. Schmidt, M., _Am. J. Physiol._ 225, 1089 (1973).

8. Nishino, H. and K. Koizumi, _Fedn Proc._ 34, 424 (1975).

9. Wurtman, R.J., J. Axelrod and D.E. Kelly, "The Pineal," p. 199, Acad. Press, 1968.

10. Moore, R.Y. and D.C. Klein, _Brain Res._ 71, 17 (1974).

11. Moore, R.Y. and N.J. Lenn, _J. comp. Neurol._ 146, 1 (1972).

12. Oomura, Y., H. Ooyama, T. Ono, M. Kamimoto and K. Yoneda, _Igaku no Ayumi_ 70, 314 (1969).

13. Brooks, C. McC., T. Ishikawa and K. Koizumi, _Brain Res._ 81, 181, (1975).

14. Oomura, Y., T. Ono, H. Ooyama and M.J. Wayner, _Nature_ 222, 282 (1969).

15. Dahlström, A. and K. Fuxe, _Acta. physiol. scand._ 62/Suppl 232, 1 (1964).

16. Anden, N.E., Dahlström, A., K. Fuxe, K. Larsson, L. Olson and U. Ungerstedt, _Acta. physiol. scand._ 67, 313 (1966)

17. Aghajanian, G.K., J.A. Rosecrans and M.H. Sheard, _Science_ 156, 402 (1967).

18. Jouvet, M., _Physiol Rev._ 47, 117 (1967).

19. Gumulka, W., R. Samanin, S. Garattini and L. Valzelli, _Europ. J. Pharmacol._ 8, 380 (1969).

20. Bloom, F.E., B.J. Hoffer, G.R. Siggins, J.L. Barker and R.A. Nicoll, Fedn. Proc. 31, 97 (1972).

21. Murphy, J.T. and L.P. Renaud, J. Neurophysiol. 32, 85 (1969).

22. Oomura, Y., H. Ooyama, F. Naka, T. Yamamoto, T. Ono and H. Kobayashi, Ann. N.Y. Acad Sci. 157, 666 (1969).

23. Aladjalova, N.A., "Slow electrical processes in the brain," Prog. Brain Res. p. 7 (1964).

24. Yamaoka, L., and N. Hagino, Brain Res. 67, 147 (1974).

25. Moore, R.Y. and V.B. Eichler, Brain Res. 42, 201 (1972).

26. Kievit, J. and H.G.J.M. Kuypers, Science, 187, 660 (1975).

27. Moore, R.Y. and J.L. Nicholas, J. comp. Neurol. 146, 1 (1972).

28. Vandesande, F., J. De Mey, and K. Dierickx [sic], Cell Tiss. Res. 151, 187 (1974).

29. Vandesande, F., K. Dierickx [sic] and J. De Mey, Cell Tiss. Res. 156, 377 (1974).

30. Güldner, F.-H. and J.R. Wolff, J. Neurocytol. 3, 245 (1974).

31. Swanson, L.W. and Cowan, W.M., J comp. Neurol., 160 (1975).

32. Krnjević, K, J.F. Mitchell and J.C. Szerb, J. Physiol. 165, 421 (1963).

33. Krnjević, K, and V.P. Whittaker, J. Physiol. 179, 298 (1965).

34. Curtis, D.R. and Koizumi, K., J. Neurophysiol. 24, 80 (1961). /954

35. Newman, T.A. and R.W. Wilson, Experientia 26, 267 (1970).