A Statistical Analysis of Spontaneous Activity of the Temperature-sensitive Neurone in Hypothalamus of Rats

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SUMMARY

Discharges of temperature-sensitive neurones were recorded in the preoptic region of urethanized rats, with simultaneous monitoring of brain temperature in that region.   Measurement of interspike intervals and calculation of statistical variables concerning the spike train were carried out.

The results were as follows:

1) In all temperature-sensitive neurones, the standard deviation of interspike intervals increases in linear proportion to the value of the mean interval, but the changes of standard deviation with relation to mean interval is different in neurones with different distribution of interspike interval histograms.

2) Interspike interval histograms of temperature-sensitive neurones were classified into two forms. The intervals were then classified into about ten time bins; a) single valued and monotonic distribution (exponential form) and b) skewed distribution with one peak (Gamma form).

3) There was statistically no relation between the temperature quotient and the type of interval histogram of temperature-sensitive neurone.

4) Based on serial correlation coefficients (SCCs) of spike trains, in general, spontaneous activity of hypothalamic temperature-sensitive neurones with exponential distribution of interval histogram is order-independent, while these of other types of neurones depend on the order of spikes. In some neurones slow variations of SCCs were obtained regardless of the type of interval histogram of the temperature-sensitive neurones.

INTRODUCTION

It has been generally acknowledged that thermosensitive structure in

the preoptic region or the anterior hypothalamus plays an important role in body temperature regulation. Only recently have investigators undertaken to analyze, at the level of the single unit, neuronal activity in the thermo-sensitive structure for thermoregulation. However, most investigations have been carried out under association with temperature changes in various parts of the body, or under only special temperature conditions, such as fever. Very little research has been done on characteristics of the temperature-sensitive neurone under physiologically normal conditions. One of the objects in a neurophysiological study of physiological regulation is to clarify neuronal mechanism of the regulatory center and to construct a neuronal model of the center. But, at the present time, whether or how each neurone constituting the thermoregulatory center participates in the total integrative action is entirely unknown.

Murakami, Stolwijk & Hardy (1967) have found two types of tempera-ture-sensitive neurones in the preoptic region of the dog; one having a high activity, and the other a low firing rate. They proposed that the neurones having a high firing rate serve as sensors of temperature and that the neurones with a low firing rate might result from inhibitory action of the insensitive neurone pool which determines setpoint temperature, as indicated in the regulator diagram proposed by Stolwijk and Hardy (1966). Previous study of the relationship between the firing rate of the neurone and the tissue temperature around the neurone (Cunningham, Stolwijk, Murakami & Hardy, 1967) has indicated that while the greater proportion of these thermally sensitive neurones demonstrated rate changes which were closely proportional to the temperature change in their immediate environment, others were observed to undergo changes in firing rate which followed variation in brain temperature at a site other than the actual location of the unit.

The study reported here was undertaken to gain more information about firing patterns of the temperature-sensitive neurone and to specify the nature of this thermal information process.

METHODS

Healthy, male albino rats, 300 to 400g in weight, were anesthetized with urethane (100mg/100g, i. p.). The rat was mounted stereotaxically, with the head fixed according to König's (1963) stereotaxic coordinate system. Two stainless-steel tubes (1.0 mm in diameter) sealed at one end were implanted unilaterally. One was implanted in a location 2.0 mm lateral to the midline, 6.0–7.0 mm anterior to the stereotaxic zero-point and served as a thermode for thermal perfusion of the brain tissue. The other

was placed 2.0 mm lateral to the midline, 8.0–9.0 mm anterior to the stereotaxic zero-point and contained a thermister (YSI, No 511) for measurement of brain temperature. The method used for conductive heating or cooling of the brain tissue was similar to that described in an earlier paper (Murakami et al. 1967; Cunningham et al, 1967).

The impulse activity from single units in the preoptic region was recorded extracellulary by using glass-pipet microelectrodes (containing 4 M NaCl) and amplifing in a conventional manner. Unit activity was recorded predominantly in an area which extended in depth from the anterior commissure to the base of the brain and laterally to about 1.0 mm from the midline at rostral coordinates lying between 6.0 to 7.0 mm. To prevent excessive tissue damage, penetration was limited to two or three times for each animal. The thermal response curve of a neurone was determined by recording the neurone's firing rate as brain temperature was maintained at several different levels from 34 to 42°C. Thermosensitivity as measured by Q_{10}, was calculated from the thermal response curve based on two or three temperatures. In the present paper a neurone with a Q_{10} above 2.0 was defined as a temperature-sensitive neurone, while a neurone having a Q_{10} within 2.0 was the temperature-insensitive neurone. After identification of temperature-sensitive neurones, unit activity of the neurone was recorded on a four channel magnetic tape during constant level of brain temperature, with comments made by the investgator during the experiments recorded on one channel.

The time interval between consecutive impulses was measured and digitalized by electronic computer (JRA–5, JEOL) and interspike interval sequences were punched out serially in digital code on paper tape. The statistical analysis was studied from the data tape which was fed into an electronic computer, and print out results were tabulated on a teletype or x-y recorder. The JRA–5 (JEOL) computer was programed using machine language and FORTRAN.

RESULTS

Sampling of impulse sequences was made at a time when the brain temperature remained constant, i. e. during a thermally steady state. The number of impulses (sample size) in one spike train which was recorded during a thermally steady state was at least 300, and as high as 2300, depending on the brain temperature, in order to assure a stochastically significant value. Upon each spike train of the temperature-sensitive neurone with an identified thermal response curve, a quantitative measurement of times between successive spikes was made with an electronic computer.

Then a couple of statistical analysis were carried out on these interval trains.

Interspike interval histogram

First, interspike interval histograms of spike trains recorded during thermally stationary periods were studied. The interspike interval histogram of any given spike train may differ in shape for various values of $\triangle t$ ($\triangle t =$ the width of the time bins into which the intervals are classified). For some spike trains, histograms using a wide range of $\triangle t$ values did not differ
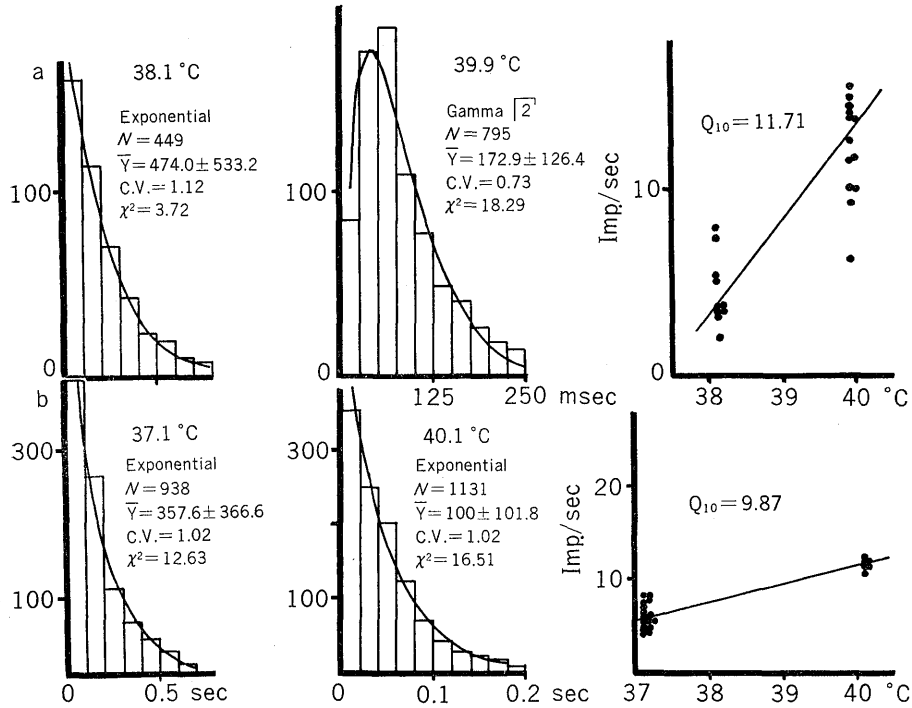

Fig. 1. Patterns of the interspike interval histogram of the temperature-sensitive neurone in the hypothalamus of rats.
Ordinate; Number of intervals.
Abscissa; time of interspike interval in msec.
Solid curves indicate the theoretical distribution fitted to obsrved values.
Thermal response curves and temperature quotients of each temperature-sensitive neurone are shown at right end of each line.
Ordinate; number of impulses per sec.
Abscissa; tissue temperature at the site where the neurone was recorded.
Results obtained at different levels of brain temperature are shown in each column.
a) Interspike interval histograms changed their pattern at different levels of brain temperature.
b) A neurone with exponential theoretical distribution.

significantly from a particular theoretical distribution. Histograms based upon some of the spike trains, however, fit a specific theoretical distribution at one value of $\triangle t$, but differed significantly when other bin widths were used. In the present paper, the number of time bins into which the intervals were classified was customarily about ten for most spike trains. In general, interspike interval histograms studied in this way were divided, in shape, into two groups; one single valued with a monotonic distribution, and the other a skewed distribution with one peak. Then a goodness of fit of a particular theoretical distribution was examined repeatedly by a χ^2 test
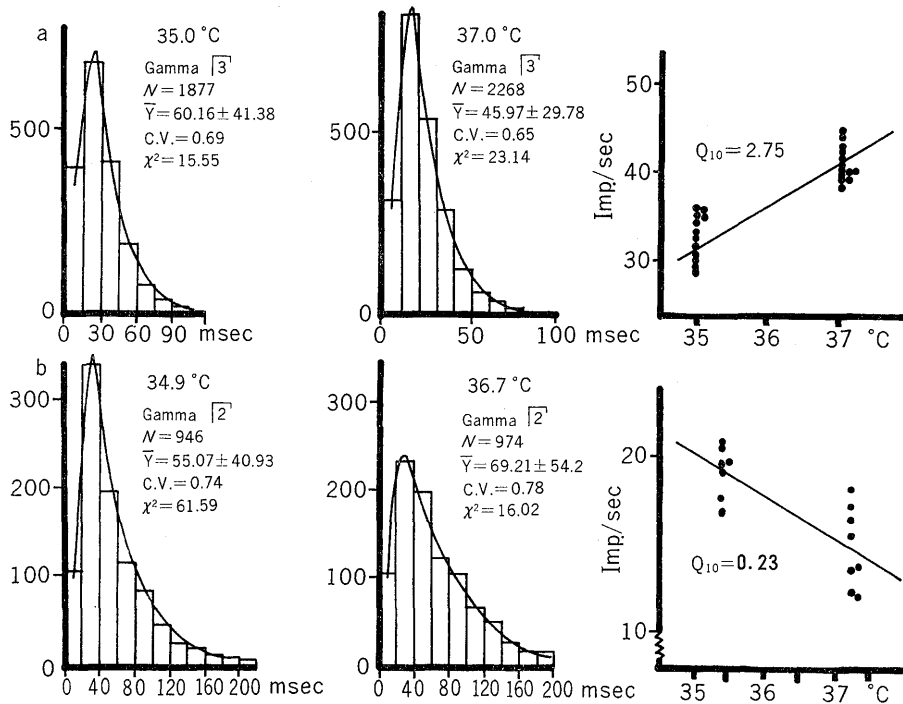

Fig. 2   Patterns of interspike interval histograms of a temperature-sensitive
neurones in hypothalamus of rats.
Ordinate; number of intervals.
Abscissa; time of interspike interval in msec.
Solid curve indicates the theoretical distribution fitted to observed values.
Thermal response curves and temerature quotients of each temperature-
sensitive neurone are shown at the right end of each line.
Ordinate; numbers of impulses per sec.
Abscissa; tissue temperature at the point where the neurone was recorded.
Results obtained at different levels of brain temperature áre shown in each
column.
a)   A neurone with Gamma theoretical distribution (order 3).
b)   A neurone with Gamma theoretical distribution (order 2).

($\alpha=0.1$) for all those interspike interval histograms. Consequently, some of interspike interval histograms showing a monotonic distribution fit well to exponential theoretical distributions with some of the histograms showing a skewed distribution fit to Gamma theoretical distribution, although goodness of fit was examined only for Erlang's Gamma theoretical distribution (Fig. 1, 2).

Twenty-eight out of 71 temperature-sensitive neurones showed a good-fit to a theoretical distribution. Of 28 neurones, 4 were Gamma of order 3 distribution, 7 were Gamma of order 2 and 17 were exponential. On the remaining 43 out of the 71, the type of histogram was inferred from the histogram which was calculated with the lowest χ^2 values. Those histograms were classified as exponential-like or Gamma-like in a table to be mentioned later. All types of interval histograms were well fitted to the theoretical distribution in one-third to one half of the cases. From this result, it was concluded that there is a continuum of histograms from exponential to Gamma, with only some of the extremes fitting the theoret-
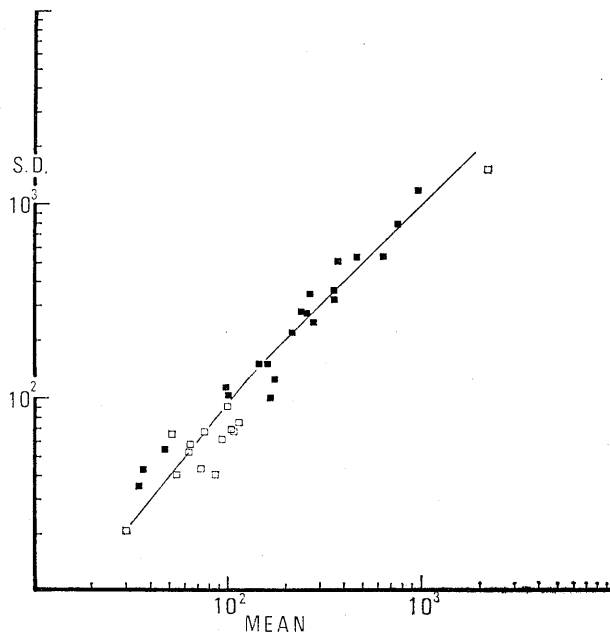

Fig. 3. Relationship between standard deviation (δ) and mean interval (x) of the
interspike intervals of the temperature-sensitive neurone.
Ordinate; standard deviation in logarithmic scale.
Abscissa; mean of the interspike interval in logarithmic scale.
Open symbols indicate neurones with Gamma distribution.
Solid symbols show neurones with exponential distribution.

ical curves and the majority forming a 'grey area' of intermediate types.

Mean, standard deviation and coefficient of variation

Mean, standard deviations of these interspike intervals were computed on each spike train of the neurone. A log-log plot of the mean interval (x) of the impulses versus the standard deviation (δ) yielded an approximately linear relationship (Fig. 3). Moreover, the slope of the curve is steeper in the range of short mean intervals in which most of the trains with Gamma and Gamma-like distribution are located, than in the low frequency range. It is clear that in all neurones δ increases with x, but the changes in δ with relation to x in neurones with exponential distributions are greater than in neurones with other distributions.

Table. 1.

The relationship between the coefficient of variation of interspike intervals and the type of distribution of the interspike interval histogram. Underlining indicates the most frequent coefficient in each column.

C. V.	Gamma	Gamma -like	Exponenetial -like	Exponential	Total
0 −0.99	8	18	11	—	37
1.0–1.99	2	—	12	15	29
2.0<	1	—	2	2	5
Total	11	18	25	17	61

Table. 2.

Relationship between temperature quotient and the type of distribution of interspike interval histogram of a temperature-sensitive neurone.

Q_{10}	Gamma	Gamma-like	Exponential- like	Exponental	Total
<2.0	—	2	4	2	8
2.0 - 3.5	3	5	5	5	18
3.5 - 5.0	3	6	4	3	16
5.0 - 6.5	—	1	2	1	4
6.5 - 8.0	—	—	2	—	2
8.0<	1	—	—	5	6
Total	7	14	17	16	54

Sixty-one neurones in which it was possible to calculate a coefficient of variation (C. V.) were categorized according to the coefficient of variation of interspike interval and to the type of the interval histogram (Table 1). The coefficient of variation in neurones with exponential distribution was more than 1.0 in most cases, while the C. V. in the group of neurones which fitted other types of theoretical distributions was less than 1.0.

A similar study was performed with special regard to the relation between the type of interval histogram and the temperature quotient, as index of thermosensitivity. However, there was statistically no difference in the temperature quotient between both types of interval histograms (Table 2).

It also became evident that the temperature quotient bore no relation to the mean value of the interspike interval in each temperature-sensitive neurone.

Order dependence

It is of considerable neurophysiological interest to determine whether or not successive interspike intervals are independent in a statistical sense i. e. whether or not a spike train can be described as a realization of a renewal process (Cox and Lewis, 1966; Perkel, Gerstin and Moore, 1967).
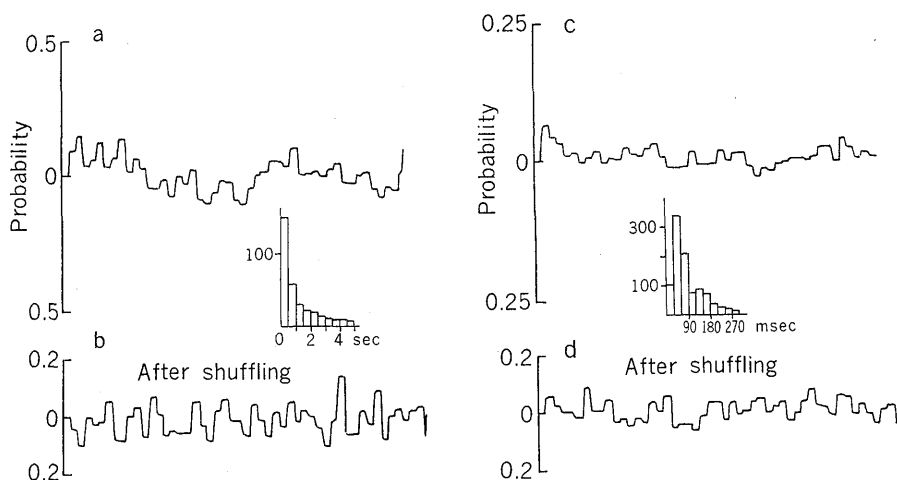

Fig. 4. The serial correlogram of interspike interval sequences in a temperature-
sensitive neurone.
a) The SCC of the neurone displayed exponential distribution.
b) The SCC after shuffling of the interval train a).
c) The SCC of the neurone displayed Gamma distribution.
d) The SCC after shuffling of the interval train c).
Ordinate; Serial correlation coefficient.
Abscissa; order.

Activity of hypothalamic temperature-sensitive neurones transmits inform-
ation about the thermal state in deeper brain tissue to the intergrative
regulation center. It is reasonable to apply this stochastic idea to the spike
train of temperature-sensitive neurones. In order to investigate the statist-
ical dependence between successive interspike intervals of the temperature-
sensitive neurone, serial correlation coefficients (SCCs) were studied. An
SCC of order 1 is a measure of the correlation between adjacent interspike
intervals Ti and Ti+1, an SCC of order 2 is that between intervals Ti and
Ti+2, and so on. The set of SCCs is called the serial correlogram (Cox and
Lewis, 1966; Perkel, Gerstin and Moore, 1967). Estimates for the SCCs of
the interval sequences of each sample were computed for orders 1 to 47 in
15 temperature-sensitive neurones. In Fig. 4 is shown the serial correlogram
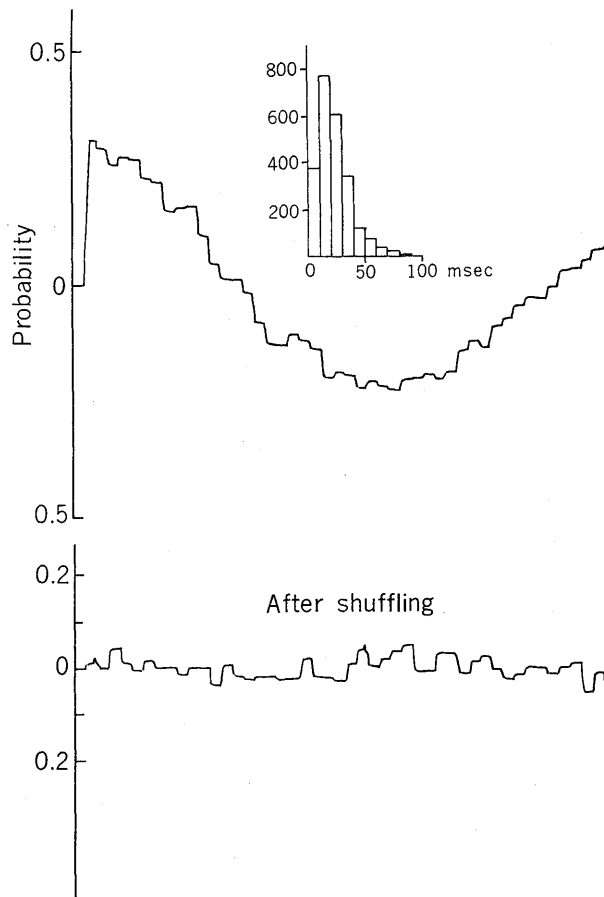of a representative temperature-sensitive neurone displaying exponential

Fig. 5. The serial correlogram of interspike interval train with a slow variation.

distribution or Gamma distribution in interspike interval histograms before
and after shuffling of interval trains. Initial positive values of the SCCs
were obtained in 4 out of 7 neurones with Gamma distribution and in 3 out
of 8 neurones with exponential distribution. In addition, there was occas-
ionally a slow variation in 6 out of 15 neurones (Fig. 5). However, it was
difficult to find any certain relation between an appearance of a slow
variation and the type of interval histogram of the neurone.

DISCUSSION

Temperature-sensitive neurones have been categorized as high disch-
arge rate or low discharge rate neurones in a previously reported paper.
Subsequently, in order to clarify neuronal connection of the preoptic region,
statistical technique was used to analyse the spike train of the temperature-
sensitive neurone.

Here, histograms of interspike intervals of hypothalamic temperature-
sensitive neurones during a thermally steady state of the brain were studied
and classified into two types of distributions (exponential and Gamma
distribution). Many of the interval histograms did not conform strictly to
any one of these theoretical distributions, although the extremes were well
fitted to the theoretical curves. It would be a more reasonable interpretation
that there exists a continuum of histograms from exponential to Gamma,
with only some of the extremes fitting the theoretical curves and the
majority forming a 'grey area' of intermediate types.

What can be learned from these interspike interval histograms about
synaptic mechanism? Braitenberg (1965) proposed that the function curve
which was inferred from comparison of the interspike interval histogram
with the distribution of levels of excitation in the input of the neurone,
assumed to be Gaussian, must suggest a variation of the threshold after
each spike. The function curve has a shape resembling that of the variation
of the threshold of a neurone as a function of the time elapsed since the
previous spike. The mechanisms by which spike sequences are generated
are essentially based on this threshold variation. In order to obtain this
function curve, a distribution of the time spent by the neurone in producing
spike intervals of various duration was obtained from an experimental
result. It is evident that each spike interval is indicative of a state of
excitation of the neurone. Here one may suppose that this distribution
depends on the input assumed to be Gaussian through the function curve.
This function curve can be made graphically in such a way as to have the
equal value of ordinate for both distributions and to establish for the
corresponding abscissae a function curve. When interpreted in this way,

our results seem to present evidence that a temperature-sensitive neurone functioning as a thermosensor is distinguishable, in terms of the interspike interval histogram, from a temperature-sensitive neurone transmitting only thermal information or processing for thermoregulation.
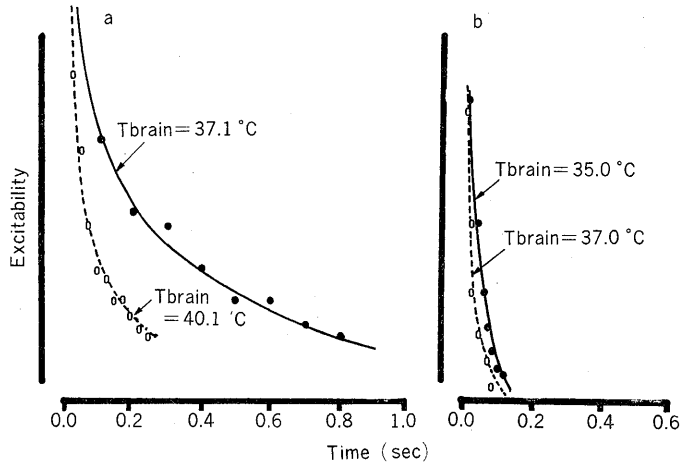

Fig. 6. The function curve of temperature-sensitive neurones which was constructed from the hypothetical Gaussian distribution of levels of excitation in time and the observed interspike interval histograms in time.
a) The function curve of the temperature-sensitive neurone presented in Fig 1 b) which has a goodness of fit of an exponential theoretical distribution. Note that there is much difference in the time constant of both functon curves obtained successively at different levels of brain temperature. Solid circle: 37.1°C Open circle: 40.1°C
b) The function curve of temperature-sensitive neurones presented in Fig. 2 a) which have a goodness of fit of Gamma theoretical distribution. Note that there is little difference in the time constant of both function curves obtained successively at different levels of brain temperature. Solid circle:35°C Open circle: 37°C

Figure 6 a) is the function curve of a temperature-sensitive neurone displaying a goodness of fit of an exponential theoretical distribution (Fig. 1 b) and was constructed from the hypothetical Gaussian distribution of levels of excitation in time and observed interspike interval histograms in time. There is much difference in the time constant of both function curves obtained successively at different levels of brain temperatures. It could be said that the recovery of excitability in the neurone depends markadly on brain temperature around the neurone and this is considered really as a thermosensor. On the other hand, a temperature-sensitive neurone (Fig. 2 a)) with a goodness of fit of Gamma theoretical distribution had a function curve as illustrated in Fig. 6 b). Even when brain temperature

was altered to different levels, the time constant changes very little. On 19 temperature-sensitive neurones with graphically made function curves, times from the last spike at which excitability was fifty percent were observed and then the differences of the times observed at different brain temperatures were calculated (Table 3). It appears that there is little difference in neurones with Gamma distribution while there is marked difference in neurones with exponential distribution.

Table. 3.

Difference of time constant of the function curve made graphically at two levels of brain temperature.

Exponential type of interval histogram

No	Neurone	brain temp. (°C)	time at which excitability is 50% (msec)	brain temp. (°C)	time at which excitability is 50% (msec)	△ brain temp. (°C)	△ time (msec)
1	1-18N-8	35.5	45	36.8	30	1.3	15
2	1-11N-1	38.1	300	39.9	62.5	1.8	138.5
3	1-26N-1	37.1	200	40.1	100	3.0	100.0
4	6- 9N-5	35.35	20	37.3	25	1.95	5
5	6- 9N-2	37.5	100	38.45	75	1.05	25
6	6- 9N-3	35.24	50	37.5	30	2.26	20
7	0- 1N-6	38.2	1200	37.0	600	1.2	600
8	6- 8N-1	35.8	300	36.45	150	0.65	150
9	0- 4N-5	38.1	400	35.6	200	2.5	200
10	1-12N-2	37.9	200	39.9	600	2.0	400
11	0- 2N-7	37.1	60	35.8	90	1.3	30
12	4-28N-3	37.5	100	34.35	200	3.15	100
13	0-1N-12	35.0	500	32.0	1500	3.0	1000
14	4-27N-4	34.25	300	36.4	500	2.15	200

Gamma type of interval histogram

No	Neurone	brain temp. (°C)	time at which excitability is 50% (msec)	brain temp. (°C)	time at which excitability is 50% (msec)	△ brain temp. (°C)	△ time (msec)
15	6- 9N-1	36.35	100	37.7	50	1.35	50
16	6- 9N-2	37.5	100	36.4	75	1.1	25
17	6- 8N-2	36.4	60	34.6	40	1.8	20
18	1-20N-2	36.85	90	38.25	40	1.4	50
19	1-20N-2B	38.3	60	39.5	40	1.2	20

These results indicate that the brain temperature did not affect the recovery of excitability in those neurones with Gamma distribution and that in neurones behaving like these, thermal information is transmitted and processed to activate the effector system for adequate thermoregulatory responses.

Oomura, Ooyama, Naka, Yamamoto, Ono and Kobayashi (1969) who have studied the hypothalamic neurones associated with appetite and feeding behaviour, reviewed the relationship between mean interval (x) and standard deviation (δ) for discharges of hypothalamus, carotid body, thalamus, skin receptor and skin, all of which were subsequently analyzed by many other investigators. It has become clear that in all systems δ increases with x, but that the patterns of change with relation to x are quite different in the different tissues studied. Consequently, Oomura et al. (1969) suggested that the hypothalamic neurones considered in their experiments transmit or process very precise information. While the value of δ used in the present experiment is much greater than that used by Oomura et al. for the same mean interval, one must recognize that more precise information processing may be performed in the central neural mechanism of thermoregulation. It has been supposed that the greater the standard deviation at a particular interval, the more precise the information processing.   This may add a likelihood to our proposal mentioned above. Moreover it should be noted that thermosensitivity in central thermodetection is almost identical at various levels of brain temperature, because the relationship between mean interval and standard deviation for a temperature-sensitive neurone is linear over the physiological range of brain temperatures.   On the order dependence of interval trains, no significant changes in serial correlation coefficient were obtained in exponential type of interval histogram of the temperature-sensitive neurone, while in Gamma distribution of interval histogram initial positive values of SCCs appeared to be significant. Implications are that spontaneous activity of hypothalamic thermodetector neurone is order-independent and that of hypothalamic temperature-sensitive interneurone is not. The fact that slow variations were observed significantly in some nourones regardless of type of interval histogram may be due to influences of a feedback mechanism upon the thermoregulatory pathway.

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