TEMPERATURE INFLUENCE ON THE DEVELOPMENT OF ELECTRICAL ACTIVITIES IN MAMMALIAN BRAIN SLICE DURING INCUBATION

Takanori Fujii and Kazuo Yoshizaki

Department of Physiology, Kyoto Prefectural University of Medicine, Kyoto 602, Japan

Abstract Thin slices, 500 μm in thickness, prepared from the guinea pig olfactory cortex and maintained in vitro, generate presynaptic and postsynaptic potentials following electrical stimulation of the lateral olfactory tract. These responses, however, cannot be elicited immediately after slice preparation; they start to develop gradually as the incubation continues. It was found that there was a remarkable time lag for the development between the pre- and the postsynaptic potentials. The presynaptic potential appeared early and developed its maximum height within 10 to 20 min from the onset of incubation. This time course was only slightly influenced by low temperature. Development of the postsynaptic potential in incubation was delayed and the time required to obtain the maximum height was about 30 min at 37°C and 70 min at 22°C. Upon step-like warming from 24°C to 37°C, there was a rapid increase in the amplitude of the response. In the glucose-deficient solution the presynaptic potential maintained its amplitude longer than the postsynaptic potential. Frequency potentiation of the postsynaptic potential was studied by applying a train of six stimuli. The maximum augmented ratio among six responses was the highest in early incubation and reached a steady at 50 min. The ratio of the post-tetanic potentiation of olfactory neurons was the highest at 5 min incubation and reached a steady at 20 min.

Thin slices prepared from brain tissue, if properly incubated, are known to generate electrical activity, either spontaneously or in response to stimulation. However, it is noted that the slices develop electrical activity gradually as the incubation continues; it is very insignificant at the early stages of incubation.

It is known that at early stages of incubation neurons of a brain slice are more or less damaged not only mechanically but also biochemically. For development
of electrical activity under the incubated condition, several recovery processes must proceed; the incised processes of neurons must be sealed and the production of energy-rich substances must re-start to allow for the synthesis of transmitter substances and the restoration of the membrane potential of neurons. These processes are all dependent on temperature.

In the experiments reported here, slices from the olfactory cortex were incubated under various conditions and responses to olfactory tract stimulation were recorded as has been described in previous works (YAMAMOTO and MCILWAIN, 1966; RICHARDS and SERCOMBE, 1968; FUJII et al., 1970; RICHARDS, 1972). By making a comparison between the pre- and postsynaptic components of the evoked potential with regard to the time course of development during incubation, we attempted to clarify the metabolic activities responsible for the generation of each component.

METHODS

Guinea pigs were used. The techniques of slice preparation and methods of stimulation and recordings were the same as those described in previous reports (FUJII, 1970; FUJII et al., 1974).

The slices from the olfactory cortex including the lateral olfactory tract were incubated by the “gas-blow and medium-flow method” which allowed continuous stable recordings of electrical activity during the perfusion of the medium (FUJII et al., 1970). The tract was stimulated by means of a pair of silver ball electrodes set on the rostral cut end. Supramaximal shocks were used. The evoked responses were recorded with a monopolar silver ball electrode from the olfactory cortex, medial and close to the tract. Incubation temperatures, measured with a thermistor at the lateral edge of the brain slice, were 37°, 32°, 27°, 24°, and 22°C. The incubation medium was Krebs-Ringer solution with bicarbonate buffer (NaCl, 120 mM; KCl, 4.8 mM; KH₂PO₄, 1.2 mM; MgSO₄, 1.2 mM; CaCl₂, 2.6 mM; NaHCO₃, 25.5 mM; Glucose, 10 mM) at a pH of 7.4. When necessary, metabolic inhibitors such as monoiodoacetate, 2,4-dinitrophenol and Antimycin A were added to the Krebs-Ringer solution. The concentrations of these inhibitors were determined in reference to YAMAMOTO and KUROKAWA’s description (1970) of the reduction of ATP production in brain tissues.

RESULTS

The field potential, elicited by stimulation of the lateral olfactory tract and recorded from the olfactory cortex in sliced preparations, consists of an initial spike potential (IS), a negative (N) and a positive (P) potential. The IS potential is a conducted action potential of the lateral olfactory tract and the remaining two components are of postsynaptic origin (YAMAMOTO and MCILWAIN, 1966;
Changes in amplitudes of these potential components were followed after starting the incubation.

**IS and N potentials and their development during incubation**

In Fig. 1A are records which were obtained from a brain slice incubated at 37°C. In Record a, which was obtained after 5 min incubation, only the IS potential could be elicited without any sign of the N potential. The IS potential continued to develop until 10 to 20 min after the start of incubation (Records b and c), but thereafter it remained constant. The N potential was first recorded at 10 min (Record b). Then it increased very rapidly as the incubation continued, though there was a tendency for the N potential to decrease slightly toward 40 min (Record e). The same observations as in Fig. 1A were made at 22°C, 24°C, 27°C, 32°C and 37°C using more than 3 preparations for each temperature. The data showing the development of the N potential are summarized graphically in Fig. 1B.

![Fig. 1. A: records showing IS and N potentials from a brain slice incubated at 37°C. a, b, c, d, e, and f are the responses at 5, 10, 15, 30, 40, and 60 min from onset of incubation, respectively. Negativity, upward in records of all Figures. Voltage calibration, 1 mV. Time, 5 msec. B: relation between amplitudes of N potential and incubation time at various temperatures. Each plot is an average from preparations whose number is shown in parentheses.](image)

The time course of development of the N potential was sigmoidal. The time at which the development ceases, the rate of development, and the maximum amplitude differed according to the incubation temperatures. Generally the N potential was of an extremely low amplitude, and in some preparations it was not detectable after 5 min. In the incubation at 37°C the amplitude of the N potential started to increase in height after passing through a time of extraordinary low amplitude. The amplitude was two thirds of the maximum size at 15 min, and
five sixths at 20 min, and then finally attained the maximum at about 30 min. After reaching the maximum the N potential began to decline gradually and diminished by 2% at 60 min. A gradual decline of the N potential after long incubation was generally seen, independent of the incubation temperature. When the slice was maintained at 32°C, the N potential was one third of the maximum at 15 min, and the fully developed potential was gained after 70 min incubation; it was noted in this case that the potential increased continuously for 70 min. The fully developed amplitude at 32°C was the highest one of all the maximum potentials obtained at other temperatures. The highest N potential gained during incubation at 27°C was close to the maximum obtained at 37°C, but the rate of increase was slower at 27°C than at 37°C. The N potential showed the greatest height at 70 min during incubation at 24°C. The development of the N potential was extremely slow in incubation at 22°C. At this temperature the fully developed height was attained at 70 min and it was the lowest stationary value of the N potential. The averages of the half time for development were 16 min at 37°C, 19 min at 32°C, 20 min at 27°C and 28 min at 22°C. The maximum rates of development were 0.57–0.15 mV/min at 37°C, 0.31–0.21 mV/min at 32°C, 0.16–0.09 mV/min at 27°C, 0.21–0.06 mV/min at 24°C and 0.10–0.04 mV/min at 22°C.

**Abrupt increase of the potentials caused by a step-like warming**

Figure 2 illustrates the amplitude changes of the IS and the N potentials from a preparation which was maintained at 24°C for 90 min after slice-making and then abruptly warmed up to 37°C at a rate of 2.6°C/min. The IS potential had attained
the full amplitude by 20 min and maintained its height up to 90 min at 24°C. Then, there was an increase from 1.3 mV to 1.8 mV upon changing the temperature from 24°C to 37°C. The N potential started to appear after 10 min. It had a slow rate of increase and reached a steady amplitude of 1.9 mV through 70 to 80 min. In response to a step-like warming from 24°C to 37°C, the amplitude of the N potential increased from 1.9 mV to 3.5 mV. These results are interpreted as follows: since the slice had been incubated at 24°C long enough for the maximal development of the N potential, its neurons were supposed to have recovered from damages due to preparation. When its ambient temperature was raised, the neurons were ready to exhibit their activity at a new temperature.

**Development of evoked response under suppressed production of ATP**

The effects of a glucose-deficient solution were studied in order to clarify the role of energy-rich substances in the development of the IS and N potentials during incubation at 37°C. Sample data are shown in Fig. 3A. The IS potential appear-

![Figure 3](attachment:image.png)

**Fig. 3.** A: developments of the potential in glucose-deficient solution. Open circle, IS potentials. Solid circle, N potentials. Incubation temperature, 37°C. B: brain slice perfused with 10^{-4} M monoiiodoacetate. a, b, and c, responses at 10, 20, 45 min, respectively, after beginning of incubation. d, a response after 50 min washing with normal solution. Incubation temperature, 37°C. C: effects of 4 \times 10^{-4} M 2, 4-dinitrophenol and 0.5 μg/ml Antimycin A on development of potentials. a, b, and c, responses at 10, 30, 40 min, respectively. d, a response after 30 min washing with normal solution. Incubation temperature, 37°C. Voltage, 1 mV; time, 5 msec for B and C.
ed 5 min after the start of incubation in the absence of glucose. Thereafter it increased slightly and maintained its height until 40 min without great variations. Then, it began to decrease and disappeared at around 60 min. Upon replacing the glucose-deficient solution with a normal solution, the IS potential reappeared and gradually developed. Finally, it reached the maximum after a 20 min perfusion. The N potential continued to increase in amplitude from the beginning of perfusion with a glucose-deficient solution until 20 min, and then began to decrease. The reduction of the potential size was rapid for about 30 min and finally disappeared at 60 min. Replacing a glucose-deficient solution with the normal solution, the N potential reappeared and gained its amplitude rapidly, and attained the maximum size at 30 min.

Records in Fig. 3B show effects of monoiodoacetic acid. When a brain slice was perfused at 37°C with Krebs-Ringer solution containing 10^-4 M monoiodoacetate, the IS potential had a tendency to increase in amplitude and did not diminish through a 45 min incubation. The test solution was replaced with normal Krebs-Ringer solution and the amplitude was slightly augmented after 40 min washing with the normal solution. In contrast to this, the N potential showed the maximum height at 20 min and declined to one half of the maximum at 45 min. After the test solution was replaced with the normal one, the N potential again increased in size; after 50 min washing, its size recovered to 80% of that which was reached at 20 min of incubation with the test solution. Effects of incubation with a solution containing both 4 × 10^-5 M 2, 4-dinitrophenol and 0.5 µg/ml Antimycin A are shown in Fig. 3C. The IS potential was not affected considerably, and the N potential exhibited the fully developed amplitude at 40 min. But the maximal size of potentials was not equal to that seen in perfusion with the normal solution at 37°C (Fig. 1); the amplitudes of the IS and N potentials, suppressed partially by metabolic inhibitors, were clearly seen to increase in size after 30 min perfusion with the normal solution.

Development of synaptic activity during incubation

In the experiment shown in Fig. 4A, a slice was incubated at 37°C and 6 pulses at 50 Hz were applied to the olfactory tract at various times after incubation. The IS potential was neither facilitated nor suppressed by applying repetitive shocks. This was the same throughout the whole incubation period. However, in the same way as in Fig. 2, as incubation continued, the IS potential increased in size and finally reached the stationary value. The amplitude of the IS potential was 0.6 mV at 5 min (Record a), and 1.3 mV at 10 min (Record b). Thereafter, there were no changes in the amplitude of the IS potential.

With regard to the N potential the continuation of incubation had effect in two ways: 1) Until 20 min of incubation the N potential continued to increase in amplitude. 2) At any instant of incubation, there was a temporal facilitation of the N potential to repetitive shocks. The number of shocks required for obtain-
ing the maximal facilitation became less and less as the incubation continued.
For example, the maximal facilitation of the N potential occurred with the 6th
shock at 5 min, with the 4th shock at 10 min and with the 3rd shock at 20 and 50
min. At the same time the maximum ratio of frequency potentiation, defined as
the amplitude ratio between the largest one among the six responses and the
response to the 1st shock, continued to decrease during incubation: 7 at 5 min,
1.7 at 15 min and finally only 1.2 at 50 min.

![Figure 4](image.png)

**Fig. 4.** A: frequency potentiation of N potential by repetitive stimulation at 50 Hz. a, b,
c, d, and e, responses at 5, 10, 15, 20, and 50 min after start of incubation at 37°C, respec-
tively. Voltage, 1 mV. B: upper panel illustrates schematically that testing N potential
(T) is recorded 20 sec after termination of tetanic stimulation for 20 sec at 100 Hz.
C, control N potential before tetanic stimulation. Lower panel: potentiation ratio of N
potential plotted against incubation time.

In Fig. 4B data are shown for post-tetanic potentiation. In this experiment
the olfactory tract was stimulated at 100 Hz for 20 sec. The amplitude of the N
potential decreased rapidly and disappeared during this high-frequency repetitive
stimulation. Application of a test stimulus to the tract was made 20 sec after the
termination of the 100 Hz stimulation, and the amplitude of the test N potential
was increased in comparison with that of the control. This procedure was repeated
once every five or ten minutes during the course of incubation. The potentiation
ratio, defined as the amplitude ratio between the control and test responses, changed
during the incubation period along a curve depicted in Fig. 4B. The potentiation
ratio was as high as 3.4 at 5 min of incubation. It decreased promptly as the
incubation continued, and reached a steady value of 1.2 after 20 min.

**DISCUSSION**

The IS potential is the compound action potential of the lateral olfactory
tract. The N potential is interpreted as representing the EPSPs generated in post-synaptic neurons (Richards, 1972). The different time courses of development of these local field responses in incubated slices may be explained as due to the differences in the processes of restoration from damage in the presynaptic and postsynaptic neurons.

Initial mechanical damages due to slice preparation are incision of the rostral portion of the lateral olfactory tract and axonal processes of pyramidal cells at the cutting face (Richards and Sercombe, 1968; Haberly, 1973). When postsynaptic neurons of the olfactory cortex were injured at their somata, they could not survive. Only neurons in which cutting occurred at axonal processes remote from the cell bodies may have had a chance to restore their activity if the cut axons were sealed by coagulation of the intracellular material in the presence of calcium ion (Déleze, 1970; McIlwain and Bachelard, 1971). However, until this is accomplished, a severe ionic leakage may occur, resulting in depolarization of the membrane potential, an increase of intracellular sodium ion concentration and a decrease of intracellular potassium ion concentration. Subsequently a process of restoration may occur. Measurements of the intracellular ionic concentrations in incubated slices of adult rat brain revealed that in postsynaptic neurons there was a gradual restoration of concentrations of sodium and potassium ions to the initial levels (Piccoli et al., 1971). This restoration of ionic composition in neurons would be brought about by the active transport of ions, associated with utilization of ATP. The level of brain tissue ATP was restored to 75% of the normal content after a 10 min respiration with glucose (McIlwain and Bachelard, 1971) and reached values similar to those reported for brain in vivo within 20 to 30 min. The same tendency was seen for the intracellular concentration of ATP in incubated slices of rat brain; 0.39 at 0 min, 2.27 at 30 min and 2.22 μmol/ml at 120 min (Piccoli et al., 1971). Therefore, it seems reasonable to suppose that to replenish the shortage of ATP and to generate the membrane potential, postsynaptic neurons require a longer time and these sequences would also be affected greatly by temperature.

On the other hand, the ionic leakages from the olfactory tract would be less and a small amount of energy-rich substances would be sufficient to restore the ionic composition to the initial level. The presynaptic potential in the glucose-deficient solution could maintain its size longer than the postsynaptic potential, because the small amount of ATP already stored would be enough to keep the axonal tract active. Then, the generation of the membrane potential in presynaptic nerve fibers would result in an early appearance of the presynaptic potential in the incubated slices. These results support the idea that the IS potential is presynaptic and the N potential is transsynaptic in origin.

In addition to generation of the membrane potentials, the transsynaptic activities are restored when the transmitter substances are produced and mobilized in presynaptic nerve endings during incubation. According to the model of the presynaptic terminals in olfactory brain slice described by Richards (1972), the total
presynaptic store of transmitter is divided into three parts: a) immediately available transmitter, which is ready for release by nerve impulse at all times, b) conditionally available transmitter, which requires a conditioning nerve volley for release by a subsequent nerve volley and c) main depot transmitter. Augmentation of the postsynaptic response by repetitive stimulation would be proportional to the amount of the conditionally available transmitter. The present results, where the frequency potentiation rate is higher at an early incubation than at a later one, suggest that the conditionally available transmitter was mobilized and stored rapidly from the synthesized main depot transmitter at an early incubation time and continued to increase in amount. Decrease and abolition of the postsynaptic response caused by high-frequency repetitive stimulation is interpreted as due to depletion of the available transmitter at presynaptic nerve endings. The amplitude of response elicited by a single test stimulus after a train of conditioning stimuli would be dependent on the amount of immediately available transmitter replenished during the 20 sec after the end of tetanic stimulation. High values of the potentiation ratio at the early incubation stage would mean rapid restoration of immediately available transmitter. In conclusion, mobilization of the transmitter substance and the amount of immediately and conditionally available transmitter substances are restored rapidly at first, then reach a stationary level after a while.

A reserve of energy-rich substances was proved to be stored in sliced tissue by studying the effects of glucose-deficient solution and metabolic inhibitors such as monoiodoacetate, 2, 4-dinitrophenol and Antimycin A. It was able to bring about the development of the evoked responses during the early incubation stage. This fact indicates that a living tissue can keep itself active for a while under a severe

Fig. 5. Relation between relative rates of development of N potential (ordinate) and reciprocals of absolute temperature of incubation (abscissa). N pot., N potential.
The amplitude of the potential which was developed during incubation of the brain slice was dependent on the incubation temperature. It was noted that the steady N potential was largest at 32°C and decreased at 37°C. An explanation for such behavior of the postsynaptic N potential has already been given, being based upon the finding that the presynaptic IS potential is maximal around temperatures slightly lower than the normal body temperature (Fujii et al., 1974).

It is evident that many biochemical reactions are involved in the development of electrical activity in incubated brain slices. The speed of these biochemical reactions determines how fast the electrical activity develops as incubation continues, and it is a function of incubation temperature. As a measure for the speed of the biochemical reactions occurring in incubated brain slices, maximal rates of development of the N potential were calculated as percentages of the end value of development for individual data of the curves of Fig. 1A. The percentage rates of development so calculated were highest for 37°C and steadily decreased with decreasing incubation temperature. As seen in Fig. 5 it is most interesting that the percentage rates of development are linearly related to reciprocals of the absolute temperature of incubation. In analogy with Arrhenius’ equation, one can calculate the activation energy from the curve of Fig. 5: it is 11 kcal/response/slice. Further consideration of the implications of this result cannot be warranted without direct measurements of the biochemical reactions taking place in incubated brain slices.

REFERENCES


Richards, C. D. and Sercombe, R. (1968) Electrical activity observed in guinea-pig olfactory
