An Intracellular Analysis of EEG Arousal in Cat Motor Cortex

Shikio INUBUSHI,* Toshinori KOBAYASHI,* Tomokazu OSHIMA, and Shizuo TORII*

Department of Neurobiology, Tokyo Metropolitan Institute for Neurosciences, 2-6 Musashidai, Fuchu-shi, Tokyo, 183 Japan**

Abstract 1. Changes in the resting potential and the effective membrane resistance were measured in 77 cells in cat precruciate cortex during the transition from cortical slow wave phase to EEG arousal.

2. These 77 neurones were classified into the recipient cells of the following five different actions on the EEG arousal: (1) postsynaptic excitation (E cells), (2) postsynaptic inhibition (I cells), (3) disinhibition (DI cells), (4) disfacilitation (DF cells) and (5) disfacilitation followed by excitation (DF-E cells).

3. The location of E cells ranged from laminae I to V, but the majority was found in lamina II. Most I cells were located in the upper half of lamina III, and a few in lamina V. DF, DI and DF-E cells existed deeply from the lower half of lamina III to laminae V–VI.

4. Slow pyramidal tract (PT) cells (n=6) all belonged to the E cell group, whereas fast PT cells were divided into the DF (n=10) and DF-E cell groups (n=4).

5. It is postulated that the EEG arousal is initiated with a direct excitation of laminae I–II cells, followed by excitation and inhibition to the upper lamina III cells and further processed to laminae III–VI cells with indirect excitation, inhibition, disinhibition and disfacilitation. The model of four vertical transmission relays is proposed to depict the cascade pattern of information being processed through the cortex during the EEG arousal.

In a preceding paper (INUBUSHI et al., 1978) it was shown that the EEG arousal involved reactions of almost all cortical neurones toward depolarization (D-type), hyperpolarization (H-type) or both in a sequence (mixed type). The depolarizing response could be induced by bombardment of excitatory inputs (postsynaptic excitation), withdrawal of inhibitory inputs (disinhibition) or both. Likewise, the hyperpolarizing response could be produced by the postsynaptic inhibition, withdrawal of excitation (disfacilitation) or both. The present study attempts to determine which of these four actions is dominant in a cell examined during...
transition from the phase of cortical slow wave to the EEG arousal. Two methods can be adopted. First, if it is assumed that the excitatory and inhibitory postsynaptic potentials (EPSP and IPSP) are generated by increases in the ionic conductance of the cell membrane (Eccles, 1964; but see Krnjević et al., 1971), measurements of the effective membrane resistance ($R_m$) may provide a definite answer to the present question (Klee, 1966). Secondly, observation of spontaneous unitary EPSP and IPSP may reveal that their rates of occurrence change on the EEG arousal. This method would directly estimate the quantity of excitatory or inhibitory input to the cell, but this quantification needs a careful inspection. The observation of unitary PSPs in this paper will be of preliminary nature and used only to justify the assumption made on applying the former method.

These examinations have revealed that all of the above-mentioned four possible reactions actually occur in cortical neurones. A laminar distribution of the cells classified according to these four-type responses will indicate an intracortical neuronal organization in the information processing during the EEG arousal.

A part of the present study has been reported briefly (Torii et al., 1975; Oshima et al., 1977).

METHODS

The data examined in this paper were obtained from the encéphale isolé and pretrigeminal cats. Full description on the experimental procedure has been given in a preceding paper (Inubushi et al., 1978). However, an additional comment should be made on the current injection used to determine the synaptic mechanism of cellular responses to the EEG arousal. Two methods were applicable. (1) Long-lasting current steps were injected to change the level of the resting potential. For example, increases of the depolarizing response at a level more hyperpolarized than the normal resting potential would indicate involvement of the EPSP. This technique was used only for a few cells when repeated observations of the EEG arousal were possible in a consistently steady condition. In addition, observations were made when spontaneous improvement in the recording condition occurred in some cells. Their responses to the EEG arousal were then obtained at two or more different levels of the resting potential (cf. Fig. 4, Inubushi et al., 1978). These opportunities were utilized to determine the nature of cellular responses. (2) A train of relatively short pulses were intermittently injected during an episode of the EEG arousal. This method was timesaving to yield quantitative data for the rate of change in the $R_m$ for each episode. As a routine in this study, hyperpolarizing pulses with the duration of 150–650 msec were passed through the recording microelectrode every second throughout the periods from the phase of cortical slow wave and spindle burst to that of fast activity in the electrocorticogram (ECoG) recorded from the precruciate cortex. A conventional input stage
INTRACELLULAR ANALYSIS OF EEG AROUSAL

with a bridge circuit was used for this purpose (Nihon Kohden MEZ-9001). The
membrane potential record thus showed hyperpolarizing shifts intermittently with
concomitant depression of spike firing (Figs. 2–6). The intensity of applied cur-
rents ranged between $-0.5$ and $-3.0$ nA (hyperpolarizing direction being indicated
with the minus sign). Depolarizing currents up to 3.0 nA were also sometimes
used. The bridge balance in the recording system was carefully adjusted, and
monitored throughout the experiment on the screen of an oscilloscope (Tektronix
RM565) using a high speed sweep to observe changes in the membrane potential
on and off the current. Fast sweep records of the membrane potential were also
displayed during the experiment on one channel of an ink-writer (Nihon Kohden
Multipurpose Polygraph RM-85 and Mingograf 800) after processing through
a transient memory device (Kawasaki Electronica, TM-1520) (not illustrated).

The membrane potential and the ECoG were fed into a data recorder (Sony
DFR-2915) and used later for measurements of $R_m$ and spontaneous unitary PSPs
as well as for reproducing various displays (Figs. 1–6). An electronic averager
(Digitimer NL750) was sometimes used to measure the mean potential change of
the membrane (Fig. 1C, E). However, the subject of the present analysis was
essentially a dynamic fluctuation of the membrane potential on the EEG arousal
in the unananaesthetized brain. Therefore, care was taken to avoid the averaging
procedure when the pattern of potential changes was of a transient nature. To
quantify the amplitude of cellular responses, there was much difficulty as to how
to treat the spindle burst phase of the ECoG (cf. STERIADE and HOBSON, 1976).
Most cortical neurones showed considerable changes in the membrane potential
during the spindle burst. These effects will be described elsewhere, and are put
aside as far as possible from the present analysis. This treatment would be reasona-
bale because the responses in the membrane potential dealt with in this paper were
essentially independent of occurrence of the spindle burst. Repeated observations
of the EEG arousal for each cell were necessary to prove this when the spindle
burst occurred frequently. In this respect, the pretrigeminal preparation was
useful for providing the condition in which there were no spindle burst waves
during the trial of reticular stimulation (cf. Fig. 6).

RESULTS

Sampling of cells and their effective membrane resistance

The subjects of the present study were 30 D-type, 42 H-type and 5 mixed-type
cells examined by means of the current injection. The resting potential of these
cells ranged between $-30$ and $-80$ mV. Reflecting the continuously changing
bombardment of excitatory and inhibitory inputs to each cell, the effective value of
$R_m$ fluctuated considerably, but differed on the average between the phases of
the cortical slow wave and EEG arousal. This section describes the absolute value
of $R_m$ to give a rough estimate related to the size of sampled cells on the assumption

Vol. 28, No. 5, 1978
that the specific membrane resistance is the same as the first approximation in all the cortical cells (cf. Takahashi, 1965). For this purpose, 52 cells with high resting potentials from -50 to -80 mV were selected.

In Fig. 1B, two traces of the membrane potential of an H-type cell are shown on applying a current step of -1.0 nA (A) during the cortical slow wave phase. The averaged record for 8 such traces is illustrated below in C. The $R_m$ is calculated as 32 MΩ from the mean potential shift of a later steady level during the current injection ($V_s$ in C), referring to the extracellular record (Fig. 1F). Figure 1D–E shows similar records during the EEG arousal. The membrane potential is more stabilized (D) and the mean $R_m$ is calculated as 25 MΩ from the potential shift $V_a$ (E). As will be seen later, the decrease or increase of $R_m$ on the EEG arousal depends on the increase or decrease of PSPs, respectively. Therefore, the $R_m$ as the value representing the cell size should be taken at its maximum regardless of the EEG states. For example, the value of 32 MΩ is taken as $R_m$ of the cell illustrated in Fig. 1A–F. In Fig. 1G, the $R_m$ thus determined in

Jap. J. Physiol.
each cell is plotted as a function of the depth at which the cell is recorded. In
the superficial layers of 100–800 μm in depths where non-PT cells (circles) were
sampled, relatively large $R_m$ values of 12–66 MΩ were obtained. This suggests
that small-sized cells are densely located there. On the other hand, relatively small
$R_m$ values of 3–31 MΩ in the deeper layers of more than 800 μm in depths may
indicate that sampling of large neurones such as PT cells (triangles) is easier than
for small-sized non-PT cells (circles). The mean $R_m$ values of sampled PT cells
were $6.5 \pm 2.2$ MΩ (S. D.) for 11 fast cells (filled triangles) and $15.0 \pm 3.9$ MΩ for
5 slow cells (open triangles). These values were within those previously reported
(Takahashi, 1965; Oshima, 1969). Figure 1G thus shows the property of the present
sampling of cells. It is recognized that only a few small-sized cells have been
sampled in the deep laminae (V–VI).

**Cell types**

The sampled D-, H- and mixed-type cells were classified according to the
change in their $R_m$ on the EEG arousal into the following five subtypes: (1) E
cells, which were depolarized with the $R_m$ decreased, (2) DI cells, depolarized with
the $R_m$ increased, (3) I cells, hyperpolarized with the $R_m$ decreased, (4) DF cells,
hyperpolarized with the $R_m$ increased and (5) DF-E cells, initially hyperpolarized
with the $R_m$ increased and later depolarized with the $R_m$ decreased. These five
type responses on the EEG arousal and their synaptic mechanisms will be described
below. At least several repeated observations were usually necessary to identify
the response types because of a labile nature and, in some cells, a small magnitude
of the responses.

1) E cells. In Fig. 2A, the ECoG (2) shows the phase of transition from
the pattern of slow wave and spindle burst to the fast activity elicited by the reticular
(RF) stimulation (1). The simultaneously recorded membrane potential of a
D-type cell (3) shows hyperpolarizing shifts for 630 msec every second in response
to the application of current steps of -1.0 nA (4). On the EEG arousal the
membrane becomes stabilized at a level depolarized by several millivolts as com-
pared with that during the slow wave phase. The hyperpolarizing shifts also
become stabilized with concomitant decreases in their magnitude during the EEG
arousal. Thus, the $R_m$ is decreased immediately after the onset of reticular stimu-
lation, and recovers gradually during the steady state of EEG arousal. The initial
part of this EEG arousal is illustrated in Fig. 2B with a different amplification and
a different time scale to show the increase of firing rate due to the depolarizing
response.

The depolarizing response accompanying the decreased $R_m$ suggests that
EPSPs are produced in this cell on the EEG arousal. This interpretation was sub-
stantiated by observing spontaneously occurring PSPs during the transition to the
EEG arousal. In Fig. 2C the membrane potential on the reticular stimulation is
shown with a high amplification and a fast time scale. The depolarizing response

Vol. 28, No. 5, 1978
Fig. 2. E cell response to EEG arousal. Encéphale isolé cat. Non-PT cell (depth, 680 μm). In A, reticular formation is stimulated as marked in (1) for 5 sec with pulses of 0.1 msec in width, 30 μA in intensity and 100 Hz in frequency. ECoG (2) shows a typical EEG arousal pattern which outlasts period of reticular stimulation. Membrane potential (3) shows two levels due to step currents of -1.0 nA for 630 msec applied every second (4). Note that spike potentials are off the record (3). In B, same as A but with a fast recording speed showing membrane potentials during transition to EEG arousal. Horizontal broken line in (3) indicates zero level of membrane potential. C, high speed records showing unitary PSPs on reticular stimulation. Period illustrated in C is indicated with a bar (c) in B. Dots mark unitary EPSPs. Voltage and time calibrations are given in each set of A–C. For ECoG (2) a scale of 100 μV is given.

(downward arrow in (3)), initiated about 90 msec after the onset of stimulation (1), is found to be composed mainly of many unitary EPSPs (marked with small dots) which are characterized in each by the elementary configuration with a fast rising phase followed by a slow decay. Unitary IPSPs with a fast falling phase (not marked) do not participate in the membrane response at least at its initial phase.

From the observations of $R_{m}$ and spontaneous PSPs, 29 of a total 30 D-type
cells were identified as the recipient of postsynaptic excitation, being thus termed E cells. Twenty-one E cells with the resting potential higher than $-50$ mV were selected to estimate the effective $R_m$. The mean $R_m$ was calculated for each of these cells, and ranged between 6.3 and 66 M$\Omega$ (mean and S.D., $23\pm14$ M$\Omega$, $n=21$) during the slow wave phase and between 5.8 and 59 M$\Omega$ ($20\pm13$ M$\Omega$) during the EEG arousal. The effective $R_m$ thus decreased by $11\pm4.8\%$ of the control value on the EEG arousal.

2) DI cells. Only one DI cell was identified in the tested 30 D-type cells. In Fig. 3A, three episodes of spontaneous EEG arousal are marked with horizontal bars (a–c in 1). The membrane is depolarized during each episode, and the hyperpolarizing shift of the membrane in response to the current step increase in membrane potential during each episode is shown in Fig. 3B.

![Figure 3](image)

**Fig. 3.** DI cell response to EEG arousal. Encephale isolé cat. Non-PT cell (depth, 1,130 $\mu$m). A shows ECoG (1) and membrane potential hyperpolarized for 400 msec by passing current steps of $-1.0$ nA every second (2) during three episodes of spontaneous EEG arousal (a–c). B, another record of same cell without current injection. Note that spikes are off the record in A and abolished in B. C shows membrane potentials during a transition to EEG arousal (episode c in A). Horizontal broken line marks zero potential level. D and E, spontaneous PSPs during cortical slow wave phase (arrow D in B) and EEG arousal (arrow E in B), respectively. Horizontal lines, RL, mark a level of $-40$ mV for reference. Voltage calibrations: 200 $\mu$V for ECoG (1), 10 mV for membrane potential records in A–C and 5 mV for D–E. Time scales: 10 sec applies to A–B, 1 sec to C and 0.1 sec to D–E.
parallel with the process of the EEG arousal (2). The episode (c) is shown in Fig. 3C with a faster time scale, where the spike potential is inactivated due to large depolarizing responses to the EEG arousal. As the $R_m$ is elevated, a concomitant decrease of synaptic noise is observed well during the hyperpolarizing shifts due to the current injection. At a later stage of recording which included two episodes of EEG arousal (Fig. 3B, d, e) the spike potential of this cell was completely abolished. This stage without the current injection provided a good opportunity to observe the spontaneous PSPs. As seen in the slow speed record of Fig. 3B, spontaneous deflections towards membrane hyperpolarization often occur during the slow wave phase. These deflections are not always coincident with the spindle burst. A fast sweep record in Fig. 3D shows that these deflections are mainly built up of many synchronized unitary IPSPs (arrows). Most of these clustered IPSPs are depressed during the EEG arousal (Fig. 3B, E). This elimination, that is, disinhibition would play a dominant role in elevating the $R_m$ of this cell.

The DI cell thus identified showed the mean $R_m$ of $14.9\pm0.4\ \text{M} \Omega$ (S.D.) during the slow wave phase and $21.4\pm2.1\ \text{M} \Omega$ during the EEG arousal for a total of 16 episodes of spontaneous EEG arousal. The increase in $R_m$ was as large as $43.6\%$ of the value in the control slow wave phase.

3) I cells. Figure 4 illustrates an example of the I cells. When an episode of spontaneous EEG arousal is initiated by a high voltage fast wave burst in the ECoG (marked with an arrow in 1, cf. INUBUSHI et al., 1978), the membrane is hyperpolarized by a few millivolts with decreased $R_m$ (2) and firing rates (4). When the EEG arousal decays transiently within several seconds, the membrane potential, the firing rate and the $R_m$ recover the levels before the EEG arousal. These changes would indicate that IPSPs are generated on the EEG arousal. Observing the membrane potential with a high amplification and a fast speed (Fig. 4B, C), unitary IPSPs characterized with the sharp downward deflection (marked by arrows) appear to be increased during the EEG arousal (C) compared to the control phase (B). Unitary EPSPs (not marked) are not significantly changed in their rate of occurrence on the EEG arousal.

Twenty-two of the sampled 42 H-type cells were identified as the recipient of postsynaptic inhibition during the EEG arousal, and were termed I cells. The mean effective $R_m$ for the selected 13 I cells with the resting potential higher than $-50\ \text{mV}$ was $25\pm9\ \text{M} \Omega$ during the slow wave phase and $18\pm8\ \text{M} \Omega$ during the EEG arousal. The $R_m$ thus decreased on the EEG arousal by $29\pm12\%$ of the value during the control phase.

4) DF cells. A typical example for the DF cells is provided from a group of fast PT cells. In a fast PT cell illustrated in Fig. 5A, there are concomitant decreases of the firing rate, membrane hyperpolarization and an increase of the $R_m$ during the transition to the spontaneous EEG arousal marked approximately with a vertical broken line. It can be inferred, from a comparison of the membrane

*Jap. J. Physiol.*
The intracellular analysis of EEG arousal involved studying cellular responses to EEG changes. Figure 4 illustrates this with a cell response to EEG arousal. Non-PT cell (depth, 1,090 μm) showed spontaneous EEG arousal (1), changes in membrane potential (2), current steps of -1.0 nA (3) and firing rates per sec (4). In A-2, spike peaks are off the record. Calibration of membrane potential level is given to the right.

B and C, ECoG (1) and spontaneous unitary IPSPs (marked with arrows) and EPSPs (2) during control and EEG arousal, respectively, taken from periods marked with bars b and c in A (2). Reference lines (RL) in (3) mark a level of -66 mV. Scales of 2 mV and 0.1 sec apply to B and C, and 100 μV to (1) in A–C.

Potential changes during the cortical slow wave phase (Fig. 5B) and those during the arousal (C), that the increased $R_m$ may be the result of decreases in spontaneous unitary EPSPs as marked with dots.

Twenty of the sampled 42 H-type cells were thus identified as DF cells on the basis of the interpretation that inhibition of the excitatory inputs to these cells, that is, disfacilitation is the main event during the EEG arousal. Of these 20 DF cells, 10 were identified as fast PT cells by the antidromic invasion from the cerebral peduncle with the latency of 0.5–0.8 msec. The mean effective $R_m$ for the selected 14 DF cells with the resting potential of higher than -50 mV was $9.1 \pm 6.9 \text{ MΩ}$ during the slow wave phase and $10 \pm 7.4 \text{ MΩ}$ during the EEG arousal. The $R_m$ increased on the EEG arousal by 14±6.8% of the value during the slow wave phase.

5) DF-E cells. A cell illustrated in Fig. 6A shows a brief phase of membrane hyperpolarization and a subsequent depolarization (3) with corresponding changes of $R_m$ and firing rate (4) during the EEG arousal (2) elicited by the RF stimulation (1). The hyperpolarizing response accompanied increases of $R_m$ and decreases of firing rate, and the late depolarization occurred with decreases of $R_m$ and
Fig. 5. DF cell response to EEG arousal. Fast PT cell with latency of 0.5 msec from cerebral peduncle. Depth, 1,500 μm. Pretrigeminal cat. A, spontaneous EEG arousal (1), changes in membrane potential (2) and current steps of -3.0 nA (3). In A-2, horizontal broken line indicates zero potential level. B and C show spontaneous PSPs during application of a hyperpolarizing current step (B-1) during the phases of control and EEG arousal, respectively. Horizontal lines (RL) indicate a level of -68 mV for reference. These records were taken from periods indicated with bars b and c in A. Only unitary EPSPs during current injection are marked with dots. Scales of 10 mV and 0.1 sec apply to B and C.

Increases of firing rate. Figure 6B shows these changes (3–4) with a different amplification and a different time scale. According to the interpretation we have given for the four type responses mentioned above, the process in this cell would be an initial disfacilitation and a later postsynaptic excitation. The observation of spontaneous PSPs would support this interpretation. Comparing between the control phase (Fig. 6C) and the initial phase of EEG arousal (D), the membrane hyperpolarization appears to be caused by a decrease of unitary EPSPs (marked with dots) but not due to an increase of IPSPs (not marked). The membrane depolarization at a later stage of EEG arousal would be composed of increased unitary EPSPs (dots in Fig. 6E).

Five mixed-type cells sampled were all identified as DF-E cells. Four of these neurones were fast PT cells with the antidromic latencies of 0.5–0.6 msec from the cerebral peduncle. The mean effective $R_m$ was measured in the three selected cells with high resting potentials of $-65$ to $-70$ mV; it was $14 \pm 9.7$ MΩ during the slow wave phase, $15 \pm 10$ MΩ for the initial transient phase of EEG arousal and $13 \pm 8.7$ MΩ for the late steady EEG arousal. The $R_m$ thus increased initially by $5.5 \pm 2.0\%$ and decreased later by $6.6 \pm 2.0\%$ of the value during the slow wave phase.

*Jap. J. Physiol.*
Fig. 6. DF-E cell response to EEG arousal. Fast PT cell with latency of 0.5 msec from cerebral peduncle. Depth, 1,460 μm. Pretrigeminal cat. In A, reticular stimulation (40 μA, 100 Hz) (1) causes EEG arousal (2), a mixed-type response in membrane potential with hyperpolarizing shifts due to current steps of -2.0 nA every second (3) and changes in firing rates (4). B, same as A, but with a fast time scale. In B-3, horizontal broken line indicates zero potential level. Spike peaks are off the record in A and B. C-E, showing spontaneous unitary PSPs during periods indicated with bars c-e in B. Horizontal lines (RL) mark a level of -78 mV for reference. Calibration of 100 μV applies to (2) in A and B, 5 mV and 10 msec to C-E.

Response amplitude versus membrane potential relationship

The measurement of $R_m$ and spontaneous PSPs have provided evidence that all cortical neurones contribute to the EEG arousal with their specific responses. This result stimulated a difficult attempt of quantification for the response in each cell group. It was expected that the response amplitude would be related to the membrane potential level in the same manner as is the usual EPSP or IPSP to its specific equilibrium potential. In the preceding section, the rate of changes in the $R_m$ was generally smaller for E, DF, or DF-E cells than for I or DI cells. Less sensitivity of the EPSP than the IPSP to the alteration in membrane potential around the resting level may be expected from the difference in their equilibrium potentials. This factor would contribute mainly to the difference in the rate of $R_m$ changes for these subtype cells.

The maximum response amplitude was measured during each episode of EEG arousal. Several different levels of the resting potential were obtained as the
control values during the cortical slow wave phase. These control levels were fractionized with the bin of 5 mV. With the procedure of injecting currents with different intensities or by utilizing spontaneous improvements of the membrane potential, more than three fractions of the control resting potential were obtained in each cell. The response amplitude was averaged for several trials performed in each fraction of the resting potentials. Paired values of the response amplitude and the resting potential thus obtained are plotted for each representative case of E, I, DI and DF cells in Fig. 7A and B. The response amplitude shows a considerable variation, but clearly indicates its dependence on the membrane potential

Fig. 7. Relationship between depolarizing or hyperpolarizing response and membrane potential. In A, depolarizing responses (ordinate) in an E cell (depth, 450 μm) are plotted with open circles as a function of membrane potential during control slow wave phase (abscissa). Each circle represents a mean value of several trials (2-11 trials in this cell) for a corresponding membrane potential obtained with or without passing current pulses of different intensities. Filled circles in A represent similar plots in a DF cell (depth, 1,330 μm). Hyperpolarizing responses are indicated in minus scale of ordinate. B, similar plots for a DI cell (depth, 1,130 μm) and an I cell (depth, 660 μm) with open and filled circles, respectively. Broken straight lines (a-d) are regression lines calculated for respective cells.

level in the manner specific to each cell group. In the E (a in Fig. 7A) and DF cells (b in A) the response amplitude is smaller in lower resting potentials. The equilibrium potentials for these responses are found to be close to the zero potential level with the extrapolation method (straight broken lines, a and b). These results would indicate a dominant contribution of EPSP genesis on these two type cells. In the DI (c in Fig. 7B) and I cells (d in B) the responses are greater in lower resting potentials, and a dominant contribution of IPSP genesis is indicated by the values of their equilibrium potential of −80 to −90 mV.

Altogether, 24 E cells, one DI cell, 18 I cells, 14 DF cells and three DF-E cells were examined with reasonable accuracy. The mean equilibrium potential of each cell type was −6±23 mV for the E cells, −84 mV for the DI cell (Fig. 7B-c),

Jap. J. Physiol.
INTRACELLULAR ANALYSIS OF EEG AROUSAL

$-82 \pm 12$ mV for the I cells and $-5 \pm 30$ mV ($n=17$) for the disfacilitation of the DF and DF-E cells. The large values of S.D. in these measurements may show a labile nature of the responses, but the quantification of average values would provide further support for the interpretations given for different responses in the preceding section.

**Cell location**

Basically four different actions, that is, excitation, inhibition, disfacilitation and disinhibition, have all been found in cortical cells in response to the EEG arousal. The measurements of response amplitude, $R_m$ and unitary PSPs have indicated a dominant role of either of these four actions in a cell group tested. Together with the mixed type of DF-E cells, five subtypes were classified. The coexistence of these different type cells suggests a highly complicated nature of a functional state of the cerebral cortex. A key parameter for detecting an organized pattern of these complicated responses will be a laminar distribution of these subtype cells, as has been partly suggested in a previous paper (Inubushi et al., 1978).

Figure 8A–E summarizes the depth distribution of the five type cells. The laminar boundaries are given with the guide of anatomical studies (cf. Strick and Sterling, 1974). The E cells are distributed widely through laminae I–V (A), and include slow PT cells at the maximal depths in laminae III and V (hatched in A). However, the majority of E cells are concentrated in lamina II. On the other hand, most I cells are located in lamina III, but a few also in lamina V (B). The cells which receive indirect influences such as disfacilitation (DF and DF-E cells) and disinhibition (DI cells) are located in deeper layers (laminae III–VI) (C–E). The majority of DF and DF-E cells were fast PT cells (hatched in D–E).

![Fig. 8. Depth distribution of five subtype cells. PT cells are indicated with hatched, and non-PT cells with hollow columns. Further explanation in text.](image-url)
From these results, three layers of a-c, as shown to the right of Fig. 8, could be divided from the physiological aspect in accord with the main location of the E, I and DF or DI cells. Since the present sampling of cells is small and has perhaps some bias (Fig. 1), we should not overestimate the differences in the number of recorded five type cells. Thus, the pattern of distribution may be characterized as follows: Laminae I–II cells receive only the excitatory action, the cells in the upper lamina III receive both the excitatory and inhibitory actions, though the latter being the majority. The cells located more deeply receive four type actions, that is, excitation, inhibition, disinhibition and disfacilitation.

DISCUSSION

A dynamic as well as slowly developing neuronal event such as the EEG arousal has long been one of the most difficult subjects for the intracellular study. The present experiment is perhaps the first systematic intracellular analysis of the EEG arousal. It is revealed that on the EEG arousal the membrane potential becomes stabilized at a certain level in each neurone of all the cortical layers by four basically different synaptic mechanisms. These findings suggest a functional reorganization of the cortex during the EEG arousal (JASPER, 1958), but it is so complicated that we need a theoretical model to summarize all the patterns of cellular response. In this section we will first propose a model circuit, and with the guide of this circuit discuss the cerebral state of EEG arousal.

‘Arousal’ circuit model. The analysis in this paper has yielded a different laminar distribution of five subtype cortical neurones (Fig. 8). Taking into account the previous finding that the neuronal transmission on the EEG arousal is directed from the superficial to deep cortical layers (INUBUSHI et al., 1978), a simple model is proposed in Fig. 9 to explain all the response patterns observed. Four parallel three-neurone relays (A–D, open or filled thick lines) are arranged in this figure vertically through three layers a–c. A neurone with the axon spreading in the horizontal direction is illustrated only in the lamina I according to the anatomical finding (see below). Four vertical columns (A–D), each consisting of three layers (a–c), give 12 compartments. Different symbols are used to mark E (circles), I (squares), DI (rhombuses), DF and DF-E cells (triangles). The E cells occupy 7 compartments (open), the I cells 3 compartments (hatched), the DI cells one (stippled) and the DF and DF-E cells share one compartment (densely hatched). The vertical relays include all possible combinations of the synaptic connexion between excitatory (open symbols) and inhibitory neurones (filled symbols) in the layers a and b, thus conveying the four different actions to the layer c cells. Non-specific afferent (nsA) inputs to the cortex could be either excitatory (e) to the E cell compartments or inhibitory (i) to the I cell compartments. The excitatory input (e) may play a predominant role to initiate the state of EEG arousal, since it produces the earliest excitation of the laminae I–II cells. With these vertical relays,
either (e) or (i) afferent input brings about in a cascade fashion finally the four-type responses in the layer c cells. The specific afferent fibres from the thalamus (sA) would give excitatory inputs (s) to the layers b and c. The cortical excitatory outputs which carry the motor command signals would be originated from the E, DF and DF-E cells in the layer c. The I and DI cells in the layer c could be either excitatory (open) or inhibitory (filled) in nature.

Additional connexions are drawn in Fig. 9 with thin lines to explain late sustained activities of cells which develop gradually and often outlast the period of reticular or natural stimulation. Of these lines, the late excitation of the DF-E cells may be relayed from the E cells in the layers b and c. The remaining vertical or horizontal lines may intensify the basic cascade pattern of cellular responses. In particular, the upward excitatory or inhibitory lines from the E and DI cells in the layer c to the E or I cell compartments of the layers a and b may serve as the positive feedback system.

On the basis of the framework of the neuronal circuit represented above, the cerebral state of EEG arousal could be depicted with reality in a Sherringtonian style (Sherrington, 1940): Imagine excitation shown by spots of light and inhibition by shadow on the cortical neurones laid out in one vertical plane. While this cortical plane shows some irregular or critically poised pattern of light and shadow, the EEG arousal is initiated by a stream of light moving up to the cortex. Instantly a strong beam of light spreads over horizontally in laminae I and II, from which stripes of narrow light and wide shadow flow down to lamina III and further cascade with much finer stripes to laminae V and VI. Then, some stripes of light in the deep layers emit sprinkled beams upwards or horizontally, resulting in cycling of the cascade pattern through all the layers. These activities further
lead to a gradual growing of light stripes in the deep layers to become a strong stationary beam. Now the cerebral cortex is ready to be driven for the next functional state.

**Fast PT cells on transient arousal.** The behaviour of fast PT cells on the EEG arousal may be of particular interest because of their role of carrying the main motor command (Evarts, 1965, 1966, 1968; Fetz and Baker, 1973; Fetz and Finocchio, 1975). The initial depression of fast PT cells in our experiments corresponds well to that found during the transient phase of arousal in the chronic monkeys (Evarts, 1965; Steriade et al., 1974). Disfacilitation within the cerebral cortex has been postulated to explain the synaptic mechanism responsible for the depression of fast PT cells (Steriade and Hobson, 1976). If the phenomenon be essentially the same in our transected brains of the cat, disfacilitation would have fully been confirmed with the intracellular examinations.

The transient disfacilitation of the fast PT cells could be interpreted as the "reset" of its activity without being subjected to a powerful decrease of excitability due to the postsynaptic inhibition. The fast PT cells whose activity has been reset may be in a state of readiness suitable for excitation. Firstly, elevation of the $R_m$ on the arousal would be effective to build up a greater depolarization to a next coming excitatory drive. Secondly, elimination of the excitatory synaptic noise would improve the signal to noise ratio. This interpretation is favourable to the functional concept of the orienting response (Pavlov, 1927; Fangel and Kaada, 1960; Sokolov, 1963; Floru, 1975; Steriade and Hobson, 1976), as well as an earlier concept of the EEG activation (Moruzzi and Magoun, 1949).

**Relevant cytoarchitecture of cortex.** The model circuit illustrated in Fig. 9 is constructed on the basis of the present physiological observations. It is difficult to find the counterpart of this circuit in the actual cytoarchitecture represented from anatomical studies. However, there is some similarity between the circuit of Fig. 9 and the diagram illustrated by Lorente de Nó (1949) as the elementary cortical pattern. Therefore, we can speculate that some schemes so far demonstrated by anatomical and physiological studies are relevant to the EEG arousal. For instance, (1) the vertical cellular relay from the superficial to deep layers has been demonstrated since Ramón y Cajal (1892). (2) The fibres widely spreading in lamina I with a tangential direction (Ramón y Cajal, 1892; Sholl, 1956) could at least partly mediate generalized excitatory responses of laminae I–II cells. (3) A nonspecific afferent projection with a pattern of extreme divergence to laminae I and II has been demonstrated (Scheibel and Scheibel, 1958, 1970; quoted also by Chow and Leiman, 1970). (4) The positive feedback signal may be carried by ascending axons of the deeply located neurones such as Martinotti cells as well as by some collateral branches of pyramidal cells (Sholl, 1956; Colonnier, 1966; Scheibel and Scheibel, 1970; Szentágothai, 1972, 1975, 1978). (5) The excitatory collateral action of slow PT onto fast PT cells (Takahashi et al., 1967) could be partly relevant to the late excitation of some DF-E type fast PT cells as

*Jap. J. Physiol.*
marked with a thin horizontal line from the E to DF-E cells in the layer c (Fig. 9).

It should be noted that a popular scheme of the recurrent inhibition in the motor cortex (PHILLIPS, 1956, 1959; STEFANIS and JASPER, 1964) does not participate in our model circuit of the EEG arousal. It has been shown that the presumed interneurones which mediate the recurrent and thalamic afferent inhibition are depressed on the arousal (STERIADE and DESCHÊNES, 1974; STERIADE et al., 1974). These neurones were characterized by their high-frequency burst discharges in response to stimulation of the cerebral peduncle or the thalamus. Unfortunately, I or DF cells which showed such burst discharges have not been sampled in our experiments. Functional significance of the recurrent inhibition has been discussed in relation to the lateral inhibition which may contribute to the selective action of a functional column (COLONNIER, 1966; BROOKS, 1969; OSHIMA, 1969; Szentágothai, 1969). It is conceivable that the recurrent inhibition participates less in the nonspecific states such as both the EEG arousal and the slow wave phase (but see STERIADE and HOBSON, 1976; STERIADE, 1978). To solve this problem, sampling of these Renshaw type interneurones should be attempted, though its opportunity has been rare for the intracellular studies when compared with the recording of presumed interneurones of the E or I type in laminae I–III.

Limitations of 'arousal' circuit model. The model of the motor cortex in Fig. 9 could show an example of the integrative aspects of the intracortical neuronal circuit. This model should be taken as a working hypothesis in a double meaning. First, the interneuronal connexions illustrated in this figure await substantiation hopefully with anatomical identification of the projection and local circuit neurones. The vertical transmission from superficial to deep layers was postulated from the laminar distribution of response latencies to the reticular stimulation (INUBUSHI et al., 1978). These latencies were generally long because only weak, near-threshold stimulation was used. The slow development of PSPs to the reticular stimulation (cf. Fig. 2) would explain slow transmission even through a mono- or oligosynaptic neuronal relays. However, this presumed mode of transmission should be corroborated.

Secondly, the 'arousal' circuit model should be related with other mechanisms so far postulated for the genesis of EEG arousal. For example, the increased rate of release of acetylcholine (PHILLIS, 1968) and its inhibitory or excitatory action upon cortical neurones (PHILLIS and YORK, 1968; KRNJEVIĆ et al., 1971) would contribute to the EEG arousal. These actions should be understood in relation with the intracortical neuronal circuits. Another problem is how extracortical structures contribute to the EEG arousal. In particular, the thalamic nuclei may switch the neuronal devices in the cerebral cortex on or off according to their own activities or those in the brainstem structures (AKIMOTO and SAITO, 1966; PURPURA et al., 1966; MANCIA et al., 1974; SASAKI et al., 1976; STERIADE and HOBSON, 1976). These actions would be reflected indirectly in the activities of cortical neurones, but could hardly be examined at present.
The ‘arousal’ circuit model should be tested with the experimental examinations related to the above-mentioned problems. At the present stage of investigation this model is useful to provide a general aspect of how different cortical neurones are integrated for the functional state which has long been called the EEG activation or arousal.

The authors thank Miss Takako Sato for assistance in histology and typing the manuscript. This work was partly supported by a grant for scientific research from the Ministry of Education, Science and Culture of Japan.

REFERENCES


*S. INUBUSHI, T. KOYASHI, T. OSHIMA, and S. TORII*


J. Physiol.