Excitation of Slow Pyramidal Tract Cells and Their Family Neurones during Phasic and Tonic Phases of EEG Arousal

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Abstract 1. Intracellular activities of slow pyramidal tract cells and their family neurones were investigated during the EEG arousal of various intensities. 2. By assessing the intensity of midbrain reticular or natural stimuli, the arousal length of EEG and the extent of EEG stage shift, these neurones were identified as the recipient of enduring excitation (E+E) during both the phasic and tonic phases of EEG arousal, and were termed E+E cells. 3. These cells were located in all the cortical laminae. E+E responses took the form of continuous depolarization in most of the deep cortical layer cells, but separated initial and late peaks of depolarization were seen in most of the superficial layer cells. 4. The E+E response versus stimulus intensity or arousal length relationship consisted of plateau and rising limbs, which would characterize the initial and late E components, respectively. 6. The excitatory tonus was revealed in E+E cells with mean levels of resting membrane potential, being most hyperpolarized during highly synchronized EEG and gradually depolarized according to stage shift towards desynchronization.

In the preceding paper (EZURE and OSHIMA, 1981a) the phasic and tonic phases of EEG arousal were discriminated by responses of an initial disfacilitation (DF) and a late excitation (E) in fast pyramidal tract (PT) cells and their family neurones. The late E response as an index of tonic EEG arousal was demonstrated to occur in response to midbrain reticular or natural stimuli to an extent which was approximately proportional to the stimulus intensity. Other parameters such as the arousal length and the extent of stage shift in EEG were also explored to assess the physiological intensity of EEG arousal. These intensity measures will be useful in identifying the late responses of other neurone species that correspond to tonic EEG arousal.

The main purpose of the examinations in this paper is to define the nature of late responses in slow PT cells. These neurones were demonstrated to receive excitation during phasic EEG arousal (INUBUSHI et al., 1978b). The data to be

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reported will suggest that the late response of slow PT cells is also excitation. If the initial and late responses are the same in nature, their discrimination has to depend upon a quantitative analysis of the cellular responses with the full aid of the above-mentioned intensity parameters. Analysis has been made of slow PT cells, and extended further to some non-PT cells as the family neurones of the former. These non-PT cells were selected by the only criterion of responses of exactly the same nature with those of slow PT cells during both the phasic and tonic phases of EEG arousal.

METHODS

The experimental set-ups were the same as those in previous papers (Inubushi et al., 1978a, b; Ezure and Oshima, 1981a).

RESULTS

Sampling of neurones

Thirteen slow PT cells were identified by antidromic excitation with latencies of 1.4–2.9 msec from the cerebral peduncle. These slow PT cells and 27 non-PT cells were subjected to analysis in this paper. All of the cells were sampled from the encephale isolé cats (n=22) except one from a pretrigeminal cat. From these preparations 16 other cells that exhibited DF followed by E (DF+E cells) were recorded, and their dichotomic responses were used as a complementary means to assess the degree of EEG arousal in differentiating between the initial and late responses in the slow PT cell group (see below).

Response patterns as E+E and their identification

Figure 1A and B illustrates intracellular responses (3) of a slow PT cell to EEG arousal (2) induced by relatively weak (A, 1) and intense pip sounds (B, 1). These responses were the membrane depolarization with slowly rising phase, and were sustained for a longer period to the intense pip (B3 as indicated with a bar b) than to the weak pip (A3, bar a). There are no indications of response dichotomy that serves to divide the EEG arousal into phasic and tonic phases. However, the EEG arousal in B should be ranked as the most intense of all the parameters of the stimulus intensity, arousal length and the stage shift in EEG.

In Fig. 1C, a non-PT cell recorded from the same preparation shows tonic depolarization (3, bar c) to an experimenter’s disturbing voice (C1, hollow bar). This voice was the most intense acoustic stimulus throughout the experiment, in that it was given when the cat had been fully habituated to pip sound of any intensity.

These long-lasting depolarizing responses were proved to be excitation or the E response by demonstration of accompanying decreases of the effective mem-
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Fig. 1. Depolarizing responses to EEG arousal induced by acoustic stimuli. In A, weak pip sound (1.1 V in arbitrary unit, 600 Hz, thick solid bar in 1) produces EEG arousal in motor cortex (2) and a small depolarizing response (horizontal bar with arrowheads a) in intracellular potentials (3) of a slow PT cell (depth of location, 940 μm; latency of antidromic excitation from cerebral peduncle, 1.4 msec). In B, intensified pip (1.7 V) induces longer-lasting EEG arousal (2) and depolarization (bar b in 3). C shows intense EEG arousal (2) and depolarizing response (3) induced in a non-PT cell (depth, 1,015 μm) by an experimenter's noise (hollow bar in 1). Both cells are recorded from the same encéphale isole preparation. Calibrations are given in respective records in this and the following figures with units of mV and sec.

brane resistance ($R_M$). For example, hyperpolarizing pulses were injected into the slow PT cell illustrated in Fig. 1A and B, and the potential deflections thereby produced were measured throughout the period of another long-lasting episode of EEG arousal (Fig. 2A). As shown in Fig. 2B, the resting ($V_R$) and hyperpolarized membrane potentials ($V_H$) are determined for the period of applied each current step, and their difference ($ΔV$) divided by the current ($I$) serves as $R_M$. These values are plotted in Fig. 2C as the function of time measured from the onset of EEG arousal (dotted vertical lines in A and C). The depolarization in $V_R$ or $V_H$ and the concomitant $R_M$ decrease thus indicate a long-lasting E response.

For the EEG arousal occurring spontaneously, the pattern of EEG gives the intensity measures. Figure 3 illustrates in a non-PT cell three episodes of relatively weak and intense EEG arousal. In A, spontaneous EEG arousal lasts for more than 5 sec in the motor cortex (1), but it appears to be weak, because the EEG stage shift from synchronization to desynchronization occurs only in the motor EEG and a slow wave pattern remains in the visual EEG (2). In B, the extensive EEG stage shift occurs in both the cortices (1, 2), but its duration appears to be too short to involve the late tonic phase (cf. EZURE and OSHIMA, 1981a). The cellular responses in Fig. 3A and B are depolarizing (marked by bars a and b in 3) with decreases of membrane impedance, as measured with deflections produced...
Fig. 2. Demonstration of E response in a slow PT cell. Same cell as illustrated in Fig. 1A and B is subjected for spontaneous EEG arousal (A, 1: motor EEG) of a length comparable with that displayed in Fig. 1B. Membrane potentials (A, 2) are oscillated by hyperpolarizing pulses of $-1.5 \text{ nA}$ with a duration of $650 \text{ msec}$ injected every $1.5 \text{ sec}$. A horizontal interrupted line gives reference of $-80 \text{ mV}$ in membrane potential. B illustrates with schematic representation of current (upper trace) and potential records (lower trace) how to measure $V_R$, $V_H$, $\Delta V$, and $R_M$. C plots $V_R$, $V_H$, and $R_M$ throughout the arousal episode. Vertical dotted lines mark the onset of EEG arousal in A and C, and three horizontal dotted lines in C indicate the means of $V_R$, $V_H$, and $R_M$ during a control period preceding EEG arousal.

by hyperpolarizing pulse injection. Judging from the degree of EEG changes, most of these E responses would correspond only with the initial phasic phase of EEG arousal.

On the other hand, a long-lasting episode of EEG arousal in Fig. 3C perhaps involves the tonic phase. The motor EEG (1) shows enduring desynchronization, and the visual EEG also shows the arousal pattern with two separate phases of relatively slow (bar d) and fast activities (bar e). Correspondingly, the E response is well sustained for a long time, as indicated by bar c (3). $R_M$ was measured in four trials of intense and prolonged EEG arousal including the one illustrated in Fig. 3C. As is plotted in D as a function of time measured from the onset of EEG arousal (vertical broken line), $R_M$ is fluctuant during the control EEG synchronization in a wide range of $20-50 \text{ M}\Omega$, but is stabilized at low levels indicating E responses during the EEG arousal. Though there are no indications of the boundary between phasic and tonic EEG arousal in the membrane potentials (C) as well as in $R_M$ (D), these responses should include the E response to tonic EEG arousal,
as judged from the prolonged arousal length and the extensive EEG stage shift.

The initial and late E responses are termed E+E responses, and the neurones with these responses are called E+E cells. As illustrated in Figs. 1, 2, and 3, identification of E+E responses depended on the observation of many trials of the EEG arousal with the highest intensity. DF+E cells recorded from the same preparations often provided the assessment of EEG arousal with a good comparative survey of its intensity. For instance, the cell illustrated in Fig. 3 was subjected to 32 episodes of EEG arousal, and could be compared to two DF+E cells with 26 episodes of arousal recorded from the same cat, and the most intense arousal was fully assessed by using the DF+E pattern.

Of a total of 373 episodes of EEG arousal recorded from the 40 E+E cells

![Fig. 3. Different response patterns of an E+E cell. Non-PT cell at depth of 1,120 μm. Encéphale isolé cat. During three episodes of spontaneous EEG arousal (A–C) motor EEG (1), visual EEG (2) and intracellular potentials (3) are displayed. Hyperpolarizing pulses of −0.5 nA for 400 msec are applied every second. E (A, B) and E+E responses (C) are indicated with bars a–c. In C, different patterns of visual EEG (2) are marked with bars d and e. D plots RM in four long-lasting episodes of EEG arousal including the case of C, as a function of time measured from an onset of EEG arousal to a midpoint of current step. These plottings end in respective trials just before the first sign of recovery indicated by appearance of slow waves or spindle burst activities.](image-url)
sampled, 214 were considered as showing only an initial E and the remaining 159 were identified as showing E+E responses. This identification of E+E patterns would be reasonable as a whole. As illustrated in Fig. 4 in comparison with 128 trials in 16 DF+E cells recorded from the same cats, the trials of E and of E+E responses in E+E cells show the arousal length (A) and the EEG stage shift (B), roughly with the same patterns of distribution (upward histograms) as the trials of DF only \((n=71)\) and of DF+E and exclusive E (+E) responses \((n=57)\) in the DF+E cells (downward histograms). The distribution of arousal length in the motor EEG for E+E responses (open columns in A) shows a tendency of shift towards large values, compared with the distribution for DF+E and +E responses (stippled). This is perhaps the result of classifying some E+E responses into the category of E responses because of uncertainty of identification.

Identification of E+E patterns was also supported in some cells by their characteristic time course of responses and the response-stimulus relationships, as will be described below.

**Location of cells and response properties**

The location of E+E cells in depths from the cortical surface is shown in the

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Fig. 4. Distribution histograms of arousal length (A) and stage shift in motor EEG (B) obtained in E+E cells for episodes showing only E response (hatched columns) and those of E+E response (open), compared with those in DF+E cells for episodes with DF (filled) and those with DF+E and exclusive +E responses (stippled). EEG stage shifts in motor EEG in B are numbered from 1 to 6 (cf. EZURE and OSHIMA, 1981a): No. 1 is shift from high (S) to moderate synchronization \((S_d)\), No. 2 from \(S_d\) to moderate desynchronization \((D_s)\), No. 3 from \(D_s\) to high desynchronization \((D)\), No. 4 from S to \(D_s\), No. 5 from \(S_d\) to D, and No. 6 from S to D.
distribution histogram of Fig. 5A which covers all the cortical layers. Slow PT cells are located in the bottom of lamina III and laminae V and VI, as illustrated by hatched columns.

The time course of E+E responses was shown as continuing depolarization in the majority of cells, as illustrated in Figs. 1, 2, and 3. However, some E+E responses consisted of separate initial and late components with a clear trough in between, as illustrated in Fig. 5B (2) for the periods indicated with bars a and b, respectively. These separate E+E responses were observed in the cells mainly located in relatively superficial layers. In Fig. 5C, these cells are distributed at depths of 100–900 μm (stippled columns), whereas the cells with the continuous E+E response are distributed deeply at depths of 500–1,500 μm (hatched columns). These two types of E+E responses will be discussed later concerning the neuronal mechanisms of E+E responses.

To quantify E+E responses, only its continuous type could be sampled with a sufficient number of trials, and therefore the response amplitude was measured only at its maximum (cf. inset of Fig. 6A). An example of the stimulus-response relationship is illustrated in Fig. 6A for the reticular-induced EEG arousal. It appears that this relationship has a plateau limb for the range of relatively weak

![Figure 5](image-url)

**Fig. 5.** A, depth distribution of slow PT cells (hatched columns) and non-PT E+E cells (open). B, an example of E+E response (2) with separate initial (bar a) and late surges (bar b) in a non-PT cell (depth, 300 μm) during spontaneous arousal in motor EEG (1). A horizontal broken line gives a reference potential level of -66 mV. C, depth distribution of E+E cells with responses of separate (stippled columns to left) and continuous types (hatched to right). D, a three excitatory neurone-relay with downward (solid lines) and upward projecting axons (broken lines), receiving three excitatory inputs, $e_1$, $e_2$, and $e_3$. 

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Fig. 6. Response amplitude versus stimulus intensity (A) or arousal length relationship (B) in a non-PT E+E cell (depth, 800 μm). Encephale isolé cat. Each open circle represents a single trial or a calculated mean of 2-6 trials. Threshold current of reticular stimuli eliciting E responses in this cell and EEG arousal in motor cortex is 20 μA (100 Hz). Vertical bars in A indicate ranges of amplitude and those in B show standard deviations.

reticular stimuli (1–1.2 times) and a rising limb for intense stimuli (1.2–2 times). However, only four values of the intensity (eight trials altogether) limit our inspection. Therefore, the arousal length is used as the substitute for the stimulus intensity, as adopted previously in testing DF+E cells (EZURE and OSHIMA, 1981a). A total of 30 trials including reticular-induced and spontaneous EEG arousal (cf. Fig. 8) yield 12 pairs of mean response amplitude and arousal length, as plotted in Fig. 6B. The plateau limb in responses is seen for the arousal length of 4–11 sec and the rising limb corresponds to longer lengths than 11 sec. Since the positive correlation between the response and the stimulus intensity or the arousal length has been demonstrated as a property typical for the late E response in DF+E cells (EZURE and OSHIMA, 1981a), the rising limbs shown in Fig. 6A and B would perhaps reflect a property of the late E response in E+E cells to tonic EEG arousal. The plateau limbs in Fig. 6 may be a property of the initial E response to phasic EEG arousal, like that of the DF response in DF+E cells (Figs. 5 and 8 in EZURE and OSHIMA, 1981a).

The positive relationship of E+E responses to arousal length is also demonstrated in Fig. 7 for their incidence calculated from the data of Fig. 4A. The E+E incidence is very low for the range of arousal lengths from 1 to 5 sec, and grows gradually through the arousal lengths between 6 and 13 sec. For the lengths of more than 13 sec most trials show E+E responses. This relationship is similar

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Fig. 7. E+E incidence related to arousal length. Each E+E incidence is calculated in percent from 20 fractionized episodes involving trials with only initial E and trials with E+E responses lined up in increasing order of arousal length. Incidences are plotted against mean arousal lengths of the respective 20 episodes. A double circle represents 40 episodes, and a circle with asterisk 13 episodes.

to that observed for the E incidence of DF+E responses (Ezure and Oshima, 1981a).

As an important property of the late cellular responses, the excitatory tonus according to EEG stages has been demonstrated in DF+E cells (Ezure and Oshima, 1981a). A similar tonus was found in E+E cells. Figure 8A and B illustrates parts of long-lasting recording from an E+E cell that involve three episodes of EEG arousal. By examining the EEG patterns in the motor (1) and visual cortices (2), at least five different states can be differentiated as represented in Fig. 8A and B with the marks of horizontal bars a-e. The respective records are 12 sec long, and are reproduced with a faster sweep speed in the lower half of Fig. 8. The record e in the middle right figure shows the most intense arousal with sustained desynchronization in both the motor (1) and visual EEG (2). The record d (middle left) shows a pattern similar to the record e, but is a part of the EEG arousal shorter than that including e, as the original records demonstrate with a slow speed (A, B). The record b (below e) is in moderate arousal because the visual EEG shows a slow wave pattern. The record c in the lower left-hand corner shows moderate synchronization in both the motor and visual cortices. Synchronization of the highest rank is seen in the record a in the bottom right figure with well-developed slow waves and spindling in the motor (1) and visual EEG (2).

The traces of membrane potential in Fig. 8A and B (3) are shown as a train of separate peaks, between which hyperpolarizing pulses are injected. As seen in A and B, the membrane potential fluctuates considerably, but this fluctuation strictly corresponds to the EEG pattern. In C, 13 peaks for each membrane potential record in a-e (A, B) are measured and plotted with different symbols, as given for respective specimen records of EEG (a-e). The mean level of membrane potential for each of five different states is indicated with a horizontal solid line.
Fig. 8. Excitatory tonus as levels of membrane potential in accordance with EEG stages. A non-PT E+E cell in an encephale isolé cat, the same as illustrated in Fig. 6. A and B represent various stages in motor (1) and visual EEG (2) and membrane potentials (3) interrupted by hyperpolarizing pulse injections. In this particular display of membrane potentials in 3, an electrical low pass filter with a cut-off frequency of 1.1 Hz was used to reduce spontaneous synaptic noises. From A to B, records are skipped with interruption for 55 sec in between. A horizontal solid line (3) drawn through A and B gives a membrane potential level of -70 mV. In C, membrane potentials are plotted with different symbols, and their respective mean levels are indicated with solid lines corresponding to different EEG stages in a–e.

which is connected to the corresponding EEG records. These levels are in the order of EEG patterns from synchronization to desynchronization, with their lowest level associated with the highest synchronization (record a).

Most E+E cells located in the middle and deep cortical layers (laminae III–VI) demonstrated the excitatory tonus such as shown in Fig. 8, whenever a long-lasting recording was possible. The main event in these cells throughout the periods from slow to fast EEG activity patterns was the changes in excitatory bombardments onto them, as has been similarly demonstrated in the case of DF+E cells (Fig. 11 in EZURE and OSHIMA, 1981a).

DISCUSSION

The present study has been concerned with how to identify the initial and late cellular responses when they are both of the same nature. E+E responses were identified by observing as many episodes as possible showing indications of most intense EEG arousal. In some cells, the initial and late E responses were
revealed by separate surges of depolarization (Fig. 5B) or by the response versus stimulus intensity or arousal length relationship consisting of plateau and rising limbs (Fig. 6). In some cells, the excitatory tonus according to EEG stages (Fig. 8) provided other evidence to support the existence of an E component corresponding to tonic EEG arousal. All of these properties have enabled identification of 40 neurones as E+E cells.

**Neuronal mechanisms of E+E responses.** A wide range of depth distribution of E+E cells and the responses of a separate type in superficial layer cells (Fig. 5) suggest a neuronal mechanism of E+E responses. If the initial E response be caused as postulated earlier in the “arousal” circuit model of phasic EEG arousal (INUBUSHI et al., 1978b), a cascade transmission may occur, as illustrated in Fig. 5D, downwards through a relay formed by three excitatory neurones (I, II, and III with axons drawn by solid lines). The main excitatory input is e1 and is received by a superficial layer cell (I). This cascade model may explain a steep rising phase of initial E response in superficial layer cells (Fig. 5B) and initially slow development of E response in deep layer cells (Figs. 1-3) (cf. INUBUSHI et al., 1978a).

The late E response, on the other hand, develops rather later with a preceding trough in superficial layer cells (Fig. 5B), whereas it fuses at its onset into the initial E response in deep layer cells (Figs. 1-3). These different time courses suggest that a cascade transmission is processed from deep to superficial layer cells through the upward projecting axons of I–III cells in Fig. 5D drawn with broken lines. The main excitatory inputs would then be e3 or e2.

This postulated mechanism of E+E responses may not be fully analyzed without thorough knowledge of the responses of other neurone species. The results of a more extensive study in a following paper (Ezure and Oshima, 1981b) will reveal clearly a situation of E+E responses in the organized pattern of activities interwoven by all cortical neurones. Possible neuronal devices other than the excitatory neurone-relay in Fig. 5D will also be suggested.

**Functional implications.** Possible biological functions of the continuously developing E+E response in slow PT cells (Figs. 1, 2) would correspond to the functions of the DF+E response in fast PT cells which have been postulated as a general set of neuronal activities in phasic EEG arousal and a subsequent active process of estimating the stimulus intensity in tonic EEG arousal (Ezure and Oshima, 1981a). Increasing late tonic excitation of slow PT cells would lead to raised activities of many target neurones belonging to the central motor control systems (Takahashi et al., 1967; Tsukahara et al., 1968; Kitai et al., 1969; Oshima, 1969, 1979; Allen et al., 1975; Araki et al., 1976; Evarts and Fromm, 1977; Fromm and Evarts, 1977; Pilyavsky and Gokin, 1978; Fetz and Cheney, 1980). These activities together with those exerted from excited fast PT cells may account for the animal’s behaviour of investigating the environment.

Generally speaking, the E+E cells other than slow PT cells may also contribute to the investigatory response. However, details of their contribution await further
examination.

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