Arch Psychiatr Nervenkr (1981) 231:81–91

Archiv für Psychiatrie und Nervenkrankheiten
Archives of Psychiatry and Neurological Sciences

The Effects of Focal Epileptic Activity on the Somatosensory Evoked Potentials in the Rat*

H. Takahashi** and M. Straschill

Abteilung für Klinische Neurophysiologie, Klinikum Steglitz, Freie Universität Berlin

Summary. The cortical somatosensory evoked potential (SEP) of the rat, evoked by contralateral forepaw stimulation, consisted of early (P 1 and N 1) and late components (P 2 and N 2). Microelectrode recording yielded evoked unitary responses of short latencies in the range of the early components and responses of longer latencies in the range of P 2. During the development of focal epilepsy after topical application of penicillin, the late components of SEP were enhanced and the enhanced late negativity corresponded to a surface negative cortical spike. The prominent enlargement of later components was associated with prolonged, often recurrent discharges of longer latency unitary responses and with enlarged local field potentials. Early components of SEP remained relatively unaffected and so did unitary responses with short latencies.

Epileptic spike-conditioned SEPs in the cuneate nucleus, thalamic sensory relay nucleus and sensory cortex were depressed from 100 ms (cuneate nucleus) to about 300 ms (thalamus and cortex) subsequent to spike discharge. Transmission in the cuneate nucleus was least affected. Thalamic and cortical early components of SEP had similar time courses of recovery, which differed markedly from that of cortical late components. Our findings suggest that two different neuronal activities generate different components of SEP and are differentially involved in the epileptic activities, which results in the different amplitude recovery following spontaneous epileptic spike discharges.

Key words: Focal epilepsy – Penicillin focus – Somatosensory evoked potential – Rat

Zusammenfassung. Corticale, durch elektrische Vorderpfotenreizung evozierte somatosensorische Potentiale (SEP) der Ratte bestehen aus frühen (P 1 und

* This work was supported by the Deutsche Forschungsgemeinschaft (German Research Council)

** *Present address:* Department of Neurosurgery, Tokyo University Hospital Hongo, Bunkyo-Ku, Tokyo, Japan

0003-9373/81/0231/0081/$ 02.20

N 1) und späteren (P 2 und N 2) Komponenten. Mikroelektrodenableitungen ergeben unter denselben Reizbedingungen Einzelneuronenantworten kurzer und längerer Latenzen synchron mit den frühen (P 1, N 1) bzw. späten Komponenten (P 2).

Im Verlauf einer durch *topische Penicillin-Applikation* erzeugten Focalepilepsie vergrößern sich nur die Amplituden der *späten* SEP-Komponenten. Mit der Größenzunahme der späten Komponenten verlängert sich die Entladungsdauer der Einzelneuronenantworten entsprechender längerer Latenzen. Die frühen SEP-Komponenten und die entsprechenden Einzelneuronenantworten kurzer Latenz bleiben unverändert. Im Cortex und in den sensorischen Relaisstationen (N. cuneatus, spezif. Kern des Thalamus) werden von 100 (N. cuneatus) bis zu 300 ms (Thalamus und Cortex) *nach* einem epileptischen Spike die somatosensorischen Potentiale partiell oder komplett unterdrückt. Der zeitliche Verlauf der Normalisierung der Amplituden ist für die frühen Komponenten thalamischer und corticaler SEP gleich, jedoch gegenüber den späteren corticalen Komponenten initial rascher.

Unsere Befunde berechtigen zur Annahme zweier Gruppen somatosensorischer corticaler Neuronaktivität, deren Erregung frühe bzw. spätere Komponenten des SEP erzeugt und die unterschiedlich vom epileptogenen Agens beeinflußt werden.

Schlüsselwörter: Focale Epilepsie – Sensorische evozierte Potentiale – Penicillinepilepsy – Ratte

Introduction

Several studies have shown that during the development of focal epilepsy, cortical responsiveness to sensory stimulation is altered in the focus [5]. In the rat [6] and cat [12] somatosensory cortex, stimulation of afferent sensory pathways evokes negative spikes in the penicillin focus. On the other hand, spontaneous cortical epileptic spike activity is reported to depress transmission in sensory relay nuclei of the cat [14, 15].

The present study was undertaken to clarify the major factors that cause either enlargement or occasional reduction in amplitude of cortical evoked potentials recorded from an epileptic focus of the rat.

Material and Methods

Experiments were performed on 40 male Sprague-Dawley rats each weighing approx. 300 g. The animals were anesthetized IP with Nembutal (30 mg/kg) and placed in a stereotaxic apparatus, paralyzed with Flaxedil and ventilated. Body temperature was maintained at 37° C with infrared light and heating pad. All wound and pressure points were infiltrated with 1% Procaine.

Four burr holes approx. 4 mm in diameter were made over the bilateral frontoparietal and occipital cortices. Monopolar EEG, with a time constant of 0.1 s, was recorded using Ag-AgCl ball electrodes with the reference placed on the nasal bone. The electrode on the right frontoparietal cortex was placed 3–4 mm lateral to the midline and 1–2 mm rostral to the coronal suture in order to record the response from the primary cortical receiving area for the forepaw, as

described by Dawson et al. [4] and Angel et al. [2]. The percutaneous electrical stimuli, consisting of 0.1 ms rectangular pulses, varied from 5 to 20 V at intervals of 1 s and were delivered through the soft metallic electrode covered with wet cotton cloth, the negative one being wrapped around the left forearm and the positive around the digits of the same paw.

The EEG responses to the electrical stimuli on the contralateral forearm were fed into a 4-channel Nicolet 1077 averaging computer and a summation made of a sequence of 32 or 64 responses with an analysis time of 40 or occasionally 100 ms.

Cortical unitary and focal field potential responses to the contralateral electrical stimuli were obtained by means of a stainless steel microelectrode (approx. 1 MΩ resistance) from the primary receiving area simultaneously with the cortical SEP in order to clarify the origin of enlargement of SEP in the epileptic cortex. The signals were fed through a Grass-P-15 AC preamplifier and displayed on an oscilloscope and then stored on magnetic tape for later analysis.

The spike focus was induced by application of penicillin G sodium (5%). The substance was applied topically to the right frontoparietal cortex 3–4 mm lateral to the midline over the coronal suture with a piece of filter paper (3 × 3 mm) soaked in penicillin solution (after opening the dura mater); or intracortically with a Hamilton microliter syringe inserted through the intact dura mater 1 mm below the surface. The epileptic activity in the EEG was classified from stage I to III.

Stage I means occurrence of small focal sporadic spikes appearing directly after the application of the substance, stage II shows large regular interictal spikes at the frequency of 0.1–0.9 c/s and stage III indicates that spike activity is more than 1 c/s (Fig. 1). The alteration of SEP during the development of epileptic activity in the focus was analyzed in relation to the stage of epileptic activity. In another series of experiments, recovery cycles of SEP evoked by the stimuli, which were conditioned by epileptic cortical spikes, were determined in the cuneate nucleus, ventral nucleus of the thalamus [13] and primary sensory cortex.

To expose the cuneate nucleus, the neck muscles were reflected, suboccipital craniectomy was performed and the cisterna magna was opened. The cerebellar hemispheres remained intact. A monopolar stainless steel electrode (approx. 50 μ in diameter, exposed surface 0.1 mm) was introduced into the left cuneate nucleus by micromanipulation under direct visual control. The same type of electrode was inserted into the right ventral nucleus of the thalamus according to the stereotaxic atlas of Pellegrino and Cushman [10]. The test stimuli were delivered with various latencies ranging from 0–500 ms following the peak of a cortical spike. The responses from the sensory relay nuclei and the cortex were averaged and displayed in the same way as that of conventional cortical SEP.

Results

1. Effects of Focal Epileptic Activities on the Cortical SEP

The control SEP consisted of an initial positive wave (P 1) followed firstly by a negative deflection (N 1), then by a further larger positive wave (P 2) and negative wave (N 2). Figure 2 shows control SEP with average peak latencies for each component. During the development of epileptic activity in the EEG, P 2 and N 2 increased in amplitude. On the other hand, P 1 and N 1 remained relatively stable in comparison with P 2 and N 2. As described, the changes of EEG induced by the convulsant were classified from stage I to III (Fig. 1) and in each stage corresponding SEP changes were identified (Fig. 3). At stage I, the amplitude of P 2 and N 2 components became markedly enlarged with the simultaneous appearance of small spikes. When these changes occurred, stage II (regular interictal spikes at a frequency of 0.1–0.9 c/s) soon succeeded stage I. At stage II, further enlargement of these two components appeared and the enlarged N 2 corresponded to stimulus-induced spikes in the EEG. However, when spike activity increased to stage III (spiking frequency more than 1 c/s), the amplitudes of SEP became rather smaller

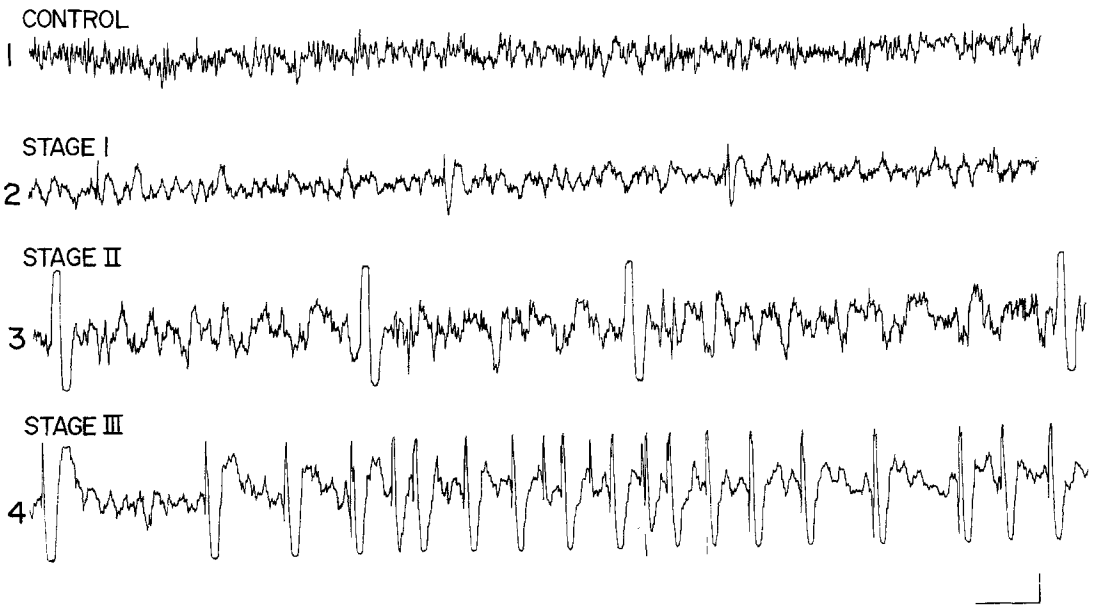


Fig. 1. Changes of the EEG recorded from the right frontoparietal cortex of a rat following the application of penicillin G sodium. 1. Control recording, 2. Stage I, 3. Stage II, 4. Stage III. Calibration: 1 s, 0.2 mV

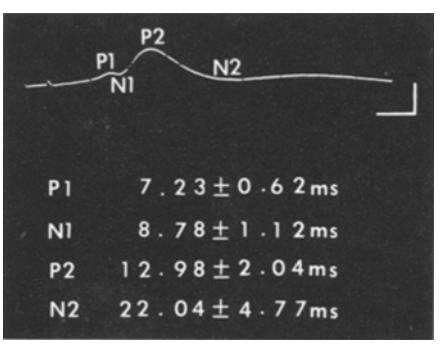


Fig. 2. SEP recorded from the primary sensory cortex of a normal rat. The mean peak latency and standard deviation of each component is also shown in the lower part of Fig. 1. Calibration: 4 ms, 0.2 mV. Summation of 64 responses

(Fig. 3). The latencies of P 2 and N 2 also proved to be longer in parallel with the development of spike activity (Fig. 3).

Figure 4 shows the changes in amplitude of P 1/N 1 and of P 2/N 2 in relation to each epileptic stage. The amplitude was measured from the peak of the positive to the bottom of the negative wave (peak to peak amplitude), i.e., from P 1 to N 1 (P 1/N 1) and from P 2 to N 2 (P 2/N 2).

At stage I, P 2/N 2 showed a tendency to enlarge ($P < 0.1$) and the enlargement of P 2/N 2 at stage II was statistically significant ($P < 0.01$). On the other hand, at

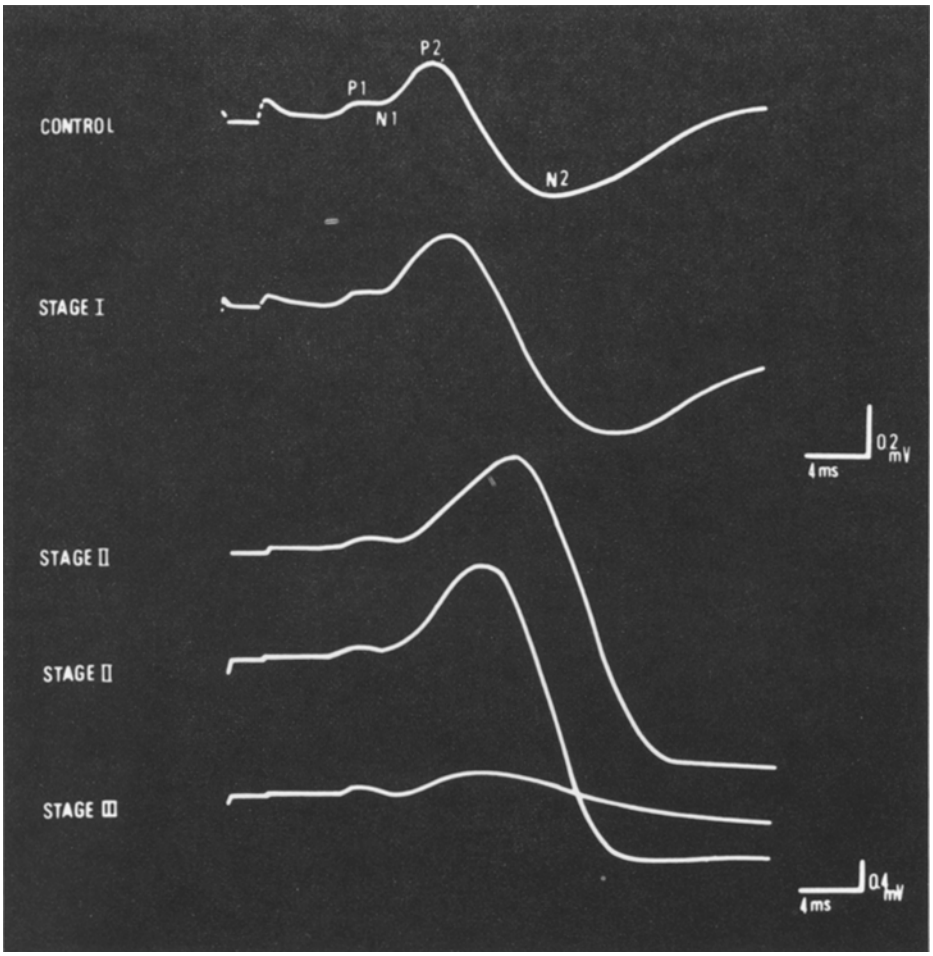
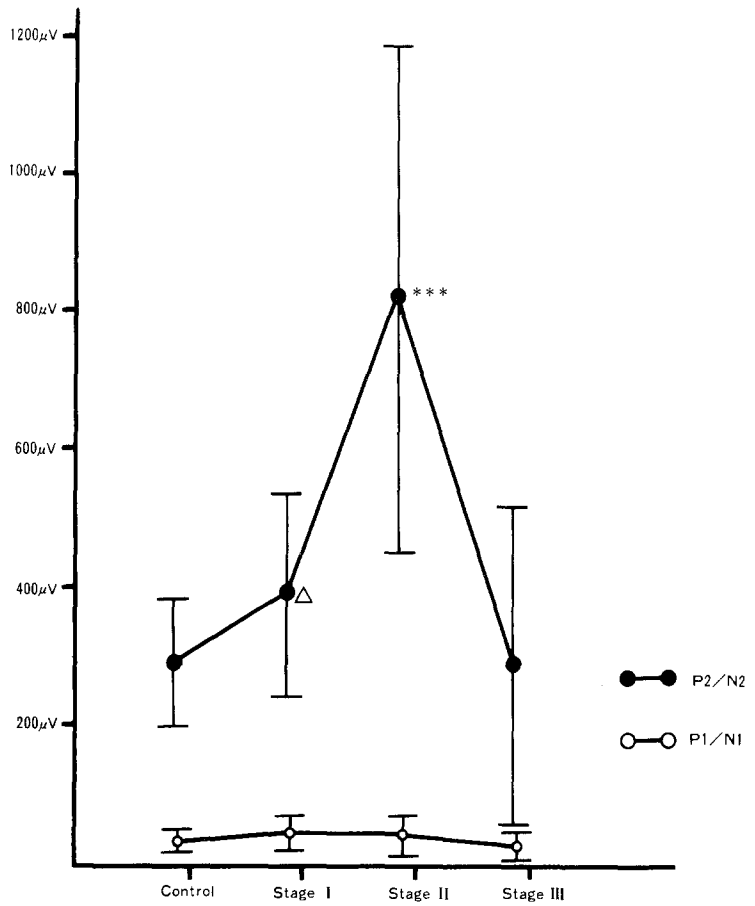


Fig. 3. Alteration of SEP during the development of focal epileptic activity from control to stage III. The amplitude calibration at stages II and III is altered because of marked enlargement of P 2/N 2 at stage II. Summation of 64 responses

stage III, P 2/N 2 became smaller when compared with other epileptic stages. The changes of P 1/N 1 also showed a similar tendency (Fig. 4) but the changes were minimal compared with those of P 2/N 2 and were not statistically significant.

2. Study of Neuronal Responses

Unitary responses to the electrical forepaw stimulation recorded from 500–800 μ below the dural surface consisted of two types of neuronal discharges; one with *short latency* (the beginning of the discharge: 4.67 ± 0.84 ms) and the other with *longer latency* (the beginning of the discharge: 8.91 ± 0.37 ms) (Fig. 5). The short latency responses appeared with the positive deflection of local field potential and with the P 1 component of cortical SEP. The responses with longer latency



	P1/N1	P2/N2
Control	30.12±16.4μV	283.8±91.4μV
Stage I	43.80±24.8μV	383.4±140.0μV
Stage II	43.20±31.4μV	810.0±388.4μV
Stage III	25.40±19.0μV	283.8±225.8μV

Fig. 4. Average changes of peak amplitude of SEP (P 1/N 1 and P 2/N 2) in relation to each epileptic stage. Ordinate value represents amplitude voltage and abscissa epileptic stage

appeared between the trough of the first negative and the onset of positive deflection of the cortical P 2 component.

When the electrode was inserted deeper than 600 μ below the surface of the primary sensory cortex, mainly unitary responses with longer latency were recorded. When the electrode was introduced 1–2 mm from the center of the receiving area, the probability of the unitary discharges with shorter latency decreased simultaneously with the reduction of the P 1 component of cortical SEP.

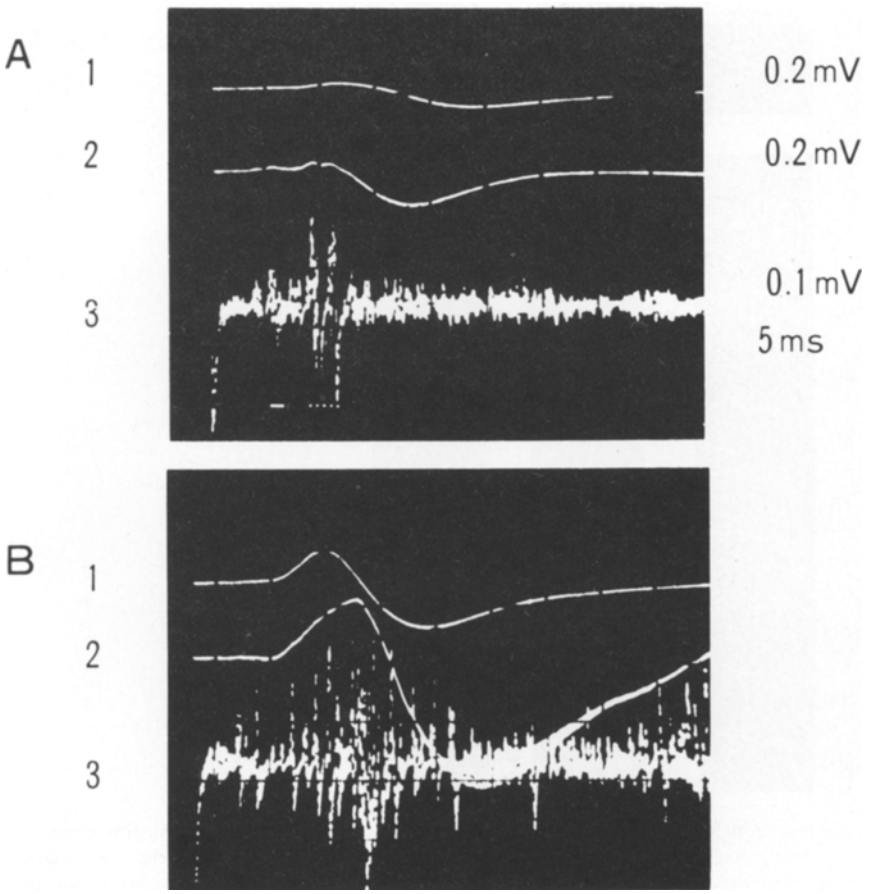


Fig. 5A, B. The effect of focal epileptic activity on cortical SEP (1), local field potential (2) and unitary response (3). **A** Control recording of single responses. There are two types of unitary responses, one with shorter latency (—) and the other with longer latency (——). **B** Epileptic responses at stage II. Parallel to the enlargement of P 2/N 2 of cortical SEP and negativity of field potential, the unitary response with longer latency shows enhanced and prolonged discharges, while the changes of the response with shorter latency remains relatively unchanged. Single responses recorded at a depth of 500 μ

During the development of focal epileptic activities in the right frontoparietal cortex, the number of discharges and the duration of the late unitary responses increased dramatically, accompanied by marked enlargement of P 2 and especially of N 2 cortical SEP, and further by marked increase in local negativity (Fig. 5).

Following the late unitary responses, periodic discharges, separated by silent intervals, were frequently observed among epileptic responses. The recurrent bursts were associated with large negativities in local field potentials and with stimulus-induced negative spikes with multiple summits in the surface EEG. Each peak corresponded to a recurrent burst.

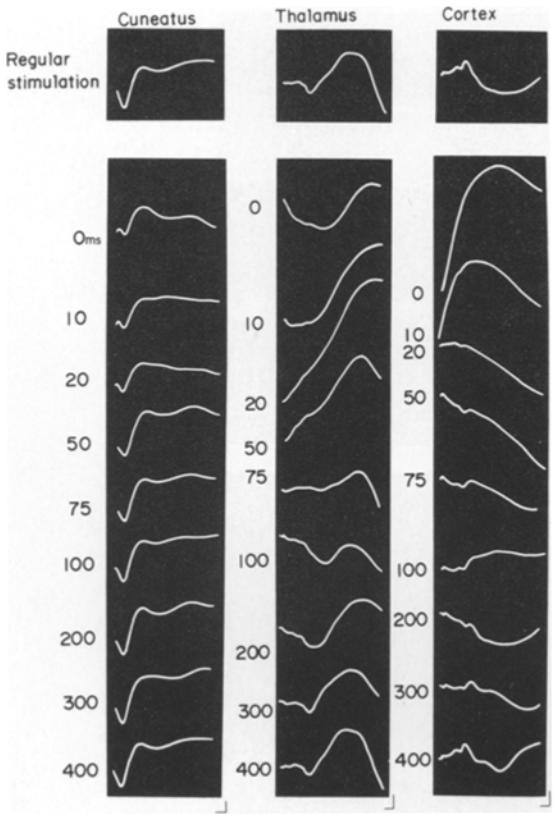


Fig. 6. Simultaneous recording of SEP from the cuneate nucleus, thalamic ventral nucleus and cortex to observe recovery cycle following cortical spikes. With each record, the time interval between the peak of cortical spikes and application of somatosensory stimuli is shown (0–400 ms). Summation of 64 responses

3. Responses to Spike-Conditioned Stimuli

The control responses from the cuneate nucleus consisted of a negative wave (average peak latency: 3.68 ± 0.44 ms) followed by a large prolonged positive wave, N- and P-waves [1, 16] (Fig. 6). The thalamic responses from the ventral nucleus showed an early positive wave (average peak latency: 5.17 ± 0.12 ms) followed by a negative inflection which was further followed by a large positive wave (Fig. 6). In the present study, N-wave from the cuneate nucleus, the early positive wave from the thalamus, and the waves of P 1/N 1 and P 2/N 2 of cortical SEP were analyzed.

The spike used as a trigger for the stimulation was an interictal one which appeared almost regularly at stage II. Epileptic-spike conditioned SEP in the cuneate nucleus, thalamic sensory relay nucleus and sensory cortex were depressed for approx. 100 ms–300 ms subsequent to spike discharge. The N-wave from cuneate nucleus showed the earliest recovery. At 0 ms, the average amplitude reduction was 16% but within 100 ms, the recovery of the amplitude was almost at control level (Figs. 6 and 7).

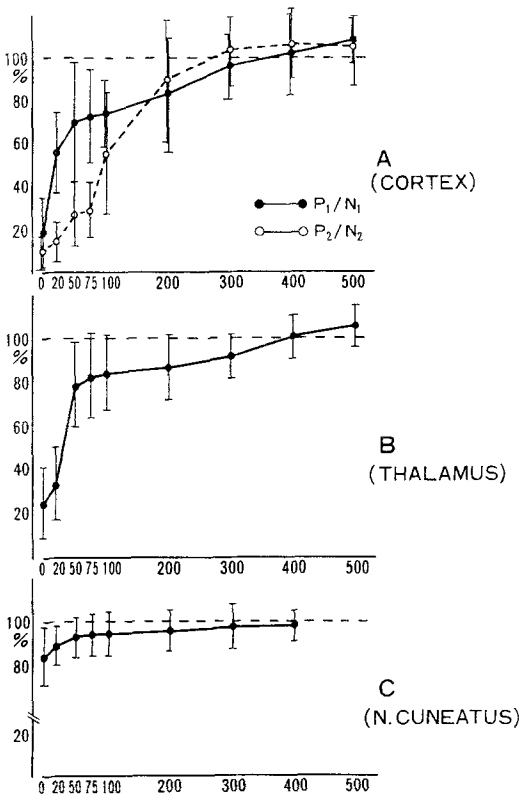


Fig. 7A–C. The amplitude recovery curves of evoked responses from the cortex, thalamus and cuneate nucleus. Ordinate represents the relative amplitudes expressed in percent of the control value which was obtained with conventional regular stimulation at same stage, and abscissa the interval between the peak of conditioning spike and spike-triggered stimuli (ms). **A** Solid line: The recovery curve of P 1/N 1 of cortical SEP. Interrupted line: The recovery curve of P 2/N 2 **B** The recovery curve of early positively from the ventral nucleus of the thalamus. **C** The recovery curve of N-wave from the cuneate nucleus

The positive wave from the central nucleus of the thalamus showed an 80% reduction in an amplitude at 0 ms following a cortical spike. However, rapid recovery occurred within 100 ms with a further gradual recovery lasting until 300–400 ms.

The recovery cycle of P 1/N 1 was similar to that of the thalamic positive wave (Fig. 7), but the dissociation of recovery cycles between P 1/N 1 and P 2/N 2 was observed within 200 ms, after which these two recovery cycles became similar. Thus, the recovery cycles showed that following a cortical spike, excitability recovered first in the cuneate nucleus, then in the thalamic relay nucleus and lastly in the cerebral cortex; in the cortex, P 1/N 1 recovered earlier than P 2/N 2.

As described above, the spike activity was followed by a relative refractory period in the cerebral cortex of about 300 ms. At stage I and III, a smaller number of the same experiments were carried out. At stage I, measurements were difficult due to the variable amplitudes of cortical spikes, but most observations showed a shorter refractory period than that of stage II. On the other hand, at stage III, the length of the refractory period was about 300–400 ms similar to that of stage II, but the inhibition of the recovery of the amplitude within this period was stronger than at stage II.

Discussion

In agreement with the observation by Holubar [6], our results demonstrated that during penicillin-induced epilepsy, the late components (P 2, N 2) of SEP in the rat were enhanced, and the late negativity (N 2) was transformed into a negative epileptic spike. On the other hand, P 1 and N 1 remained relatively unchanged. The results of simultaneous microelectrode recording of unitary responses suggested that there were two types of neuronal response to the somatosensory stimulation, i.e., responses with shorter latency and with longer latency. At stage II, discharges with longer latency appeared enhanced and prolonged. Angel and Lemon [3] also described two types of neuronal discharge responding to somatosensory stimulation in the primary receiving area of the rat. The first type had a latency between that of P 1 and N 1, and the other a longer latency. The latter probably corresponded to pyramidal cells since they responded to antidromic stimulation of the pyramidal tract [3].

We presume that the neuronal discharges with longer latency may be identical to those of pyramidal cells, since in our results the responses with longer latency are increased during the development of epileptic activity. The enhanced extracellular field potential and the cortical negative spike arising from N 2 must correspond to the *paroxysmal depolarization shift* (PDS) of intracellular events [7, 12].

The conclusions made for the neuronal elements generating stimulus-induced epileptic spikes are also valid for the origin of spontaneous epileptic spikes. On the basis of laminar exploration during penicillin epilepsy in the rabbit, Petsche et al. [11] concluded that the surface negative spike was generated by the PDS of the large pyramidal cells of layer V.

There are two possibilities regarding the origin of the responses with shorter latency. Firstly, that they are caused by the excitation of interneuronal stellate cells, and secondly that the activity of ascending fibers from the thalamus causes the responses. The fact that the responses show a relatively low amplitude, and the recovery cycle of early components is similar to that of the thalamic responses, suggests that the responses with shorter latency reflect activity of ascending fibers [8]. Our concept of different neuronal types, which generate different components of SEP, and which are differentially involved in the epileptic process, offers an explanation for the different recovery cycles of early and late components of cortical SEP conditioned by spontaneous epileptic spikes.

Prominent *hyperpolarization* usually follows PDS, as reported by Matsumoto et al. [7], and *inactivation of the sodium channel* due to excessive paroxysmal depolarization or excessive accumulation of extracellular potassium impedes synaptic transmission. These two mechanisms may contribute to the refractory period of SEP. The refractory period also accounts for the decrease in unconditioned SEP at advanced stages of epileptic activity. With increased spike frequency, the probability increases that a stimulus falls into the refractory period. The duration of the refractory period (300–400 ms) also explains why spontaneous or evoked spiking rarely exceeds the rate of 3.0–3.5 spikes per second [5].

The partial blockade of transmission in sensory subcortical relay nuclei following epileptic cortical discharges may be mediated by inhibitory collaterals of the pyramidal tract in the cuneate nucleus, and by direct cortico-thalamic

inhibitory pathways in the thalamic VPL nucleus. Antidromic activation of recurrent inhibitory collaterals by a feed-back mechanism [15] cannot be ruled out in the VPL.

Remote inhibitory effects of cortical spikes on subcortical sensory relay nuclei were previously described in the cat [14, 15]. In view of the observed differences between barbiturate and nonbarbiturate conditions the predominant inhibitory characteristic of projected effects of cortical spike discharges has recently been qualified [9].

References

1. Anderson P, Eccles JC, Schmidt RF, Yokota T (1964) Slow potential wave produced in the cuneate nucleus by cutaneous volleys and by cortical stimulation. J Neurophysiol 27:78–91
2. Angel A, Berridge DA, Unwin J (1973) The effect of anesthetic agents on primary cortical evoked responses. Br J Anaesth 45:824–836
3. Angel A, Lemon RN (1974) An experimental model of sensory myoclonus produced by 1,2-dihydroxybenzene in the anesthetized rat. In: Harris P, Mawdsley C (eds) Epilepsy. Proc. of the Hans Berger Centenary Symposium. Churchill Livingstone, Edinburgh London New York, pp 37–47
4. Dawson GD, Holmes O (1966) Cobalt applied to the sensorimotor area of the cortex cerebri of the rats. J Physiol (London) 185:455–470
5. Gastaut H, Hunter J (1950) Le phénomène de l'alternance dans les rhythmes induits par la stimulation lumineuse intermittente sur le cortex optique strychninisé. J Physiol (Paris) 42: 592–596
6. Holubár J (1966) Different modes of activation of the penicillin and mirror cortical foci in rats. In: Servit Z (ed) Comparative and cellular pathophysiology of epilepsy. Exc. Med. Int. Congr. Ser. Amsterdam
7. Matsumoto H, Ajmone-Marsan C (1964) Cortical cellular phenomena in experimental epilepsy: interictal manifestations. Exp Neurol 9:305–326
8. Mountcastle VB, Davies PM, Berman AL (1959) Response properties of neurons of cat's somatic sensory cortex to peripheral stimuli. J Neurophysiol 20:374–407
9. Noebels JL, Prince DA (1978) Development of focal seizures in cerebral cortex: Role of axon terminal bursting. J Neurophysiol 41:1267–1281
10. Pellegrino LJ, Cushman AJ (1967) A stereotaxic atlas of the brain. Appleton-Century-Crofts, New York, pp 1–81
11. Petsche H, Rappelsberger P, Frey S (1971) Intrakortikale Mechanismen bei der Entstehung der Penicillinspitzen. EEG-EMG 2:176–180
12. Prince DA (1966) Modification of focal cortical epileptogenic discharge by afferent influences. Epilepsia (Amsterdam) 7:181–201
13. Sherwood NM, Timiras PS (1970) A stereotaxic atlas of the developing rat brain. Univ. of Calif. Press, Berkeley
14. Schwarzkroin PA, van Duijn H, Prince DA (1974) Effects of projected cortical epileptiform discharge on field potentials in the cat cuneate nucleus. Exp Neurol 43:88–105
15. Schwarzkroin PA, Mutani R, Prince DA (1975) Orthodromic and antidromic effects of a cortical epileptiform focus on ventrolateral nucleus of the cat. J Neurophysiol 38:795–811
16. Therman PO (1941) Transmission of impulses through the Burdach nucleus. J Neurophysiol 4:153–166

Received January 30, 1981