Electrical activity of the acutely isolated pons in cats

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Abstract. The cat’s pons was isolated by two brainstem transections, at the junction of medulla and pons and at the junction of pons and midbrain. In the deafferented pons the EEG activity was virtually absent, whereas the spatial density of active units and the rate of their spontaneous spike activity were at a high level. In the pons of control preparations with brainstem transected only at the ponto-midbrain junction the EEG activity was present, while the single-unit activity was such as in the isolated pons. The electrical activity of the isolated pons was similar to that previously described in the cat’s isolated midbrain. The discrepancy between EEG and single-unit activity suggests that in the deafferented pons or midbrain many neurones are asynchronously autoactive. Also, these results show that a flat EEG record is not necessarily a sign of absence of the neural activity and neural death.

Key words: deafferented pons, autoactive neurone, pontine EEG
INTRODUCTION

We have described previously the electrical activity in the cat’s midbrain isolated by two brainstem transections (Zernicki et al. 1970, 1979, Dec et al. 1978). The main result was that in the deafferented midbrain the EEG activity was greatly depressed, whereas single-unit spike activity was maintained at a high frequency. This finding was consistent with earlier records of vivid single-unit activity in the isolated midbrain (Bonvallet et al. 1956, Nisida and Okada 1960).

A problem arises whether or not a similar discrepancy between EEG and single-unit activity would exist in different isolated parts of the brainstem. It was found recently that after the brainstem transection at the junction of medulla and pons the respiration and blood circulation of cat preparations (Siegel et al. 1984, 1986, Zernicki, unpublished data) and rat preparations (Gottesmann et al. 1995) are satisfactory and thus an isolated pons preparation can be maintained in good condition for recording. We have thus proceeded to examine the electrical activity of the isolated pons in cats.

METHODS

Eleven cats were used. In six of them (C1-C6) pons was isolated by two transections: at the junction of medulla and pons and subsequently at the junction of pons and midbrain. These transections will be called, respectively, prebulbar and prepon- tine. In five control cats (C7-C11) only the prepon- tine transection was performed.

The surgery was done under ether anaesthesia which was terminated immediately after prepon- tine transection, i.e. after the transection that eliminated pain perception. The transections were performed with a spatula guided by a plate attached to a stereotactically calibrated holder. The spatula for the prepon- tine transection was Z shaped (see Slòsarska and Zernicki 1973) to allow its insertion below the tentorium after removal of the anterior cerebellum. For both transections the spatula was inclined at 30° to the vertical plane. For the prebulbar transection it was positioned to intersect the interaural-horizontal plane at P3 and for the prepon- tine transection at A4. The details of the technique of brainstem transections have been described elsewhere (Zernicki 1986).

Five cats (C1-C3, C7, C8) were used for recording pontine EEG activity. The electrodes were made from 0.3 mm diameter Nichrome wire with sharpened, uninsulated tips spaced 1 mm apart. From 4 to 14 vertical penetrations were made on the left side. The positions of tracks varied from P0.5 to P3 and from L1.5 to L5 according to Berman’s atlas (1968) and the electrodes were lowered in 1 mm steps. Thus, the EEG activity was successively recorded from the virtually whole pons. Frequencies below 1.6 c/s and above 45 c/s were filtered out.

Six cats (C4-C6, C9-C11) were used for single-unit pontine recording with tungsten microelec- trodes. Only one vertical penetration at P2.5, L3.5 was made on the left side and it was terminated at about H-6 level. This penetration was considered to be representative for pons. Spontaneous spike activity was recorded only if the wave forms of the units were typical for cell bodies (see Bishop et al. 1962) and if the spike amplitude was at least two times larger than the background noise. The activity was determined in 128 s intervals. Two small electrolytic lesions were made at selected penetration depths for further track reconstructions.

EEG activity was monitored on the right side from the frontal and occipital cortex with silver ball electrodes. On the left side cerebral EEG activity was recorded with the macroelectrode while descending to the pons. The cortical EEG activity showed typical patterns for low cereau isolé preparations (see Slòsarska and Zernicki 1973). The EKG activity was also monitored. The heart rate was somewhat different in different preparations but did not markedly vary in any given preparation. The mean values for ten samples taken during different stages of experiment varied from 126 beats per minute in C2 to 268 in C7. After transections all cats breathed spontaneously but cats used for single-unit recording were then paralyzed with Flaxedil (gallamine triethiodide, 20
In these cats the end-expiratory CO₂ was maintained at 3.5-4.0%. Body temperature was maintained at 38°C.

After the experiments preparations were killed with an overdose of Nembutal and the brains were fixed in 10% formalin. Microelectrode locations were identified on 50 μm histological sections stained by Nissl technique and their position described according to the Berman atlas (1968). Our procedures have been approved by the Animal Care Committee in the Nencki Institute.

RESULTS

Anatomical verification showed that in all cases the transections were complete and in the correct place. The prebulbar transection passed dorsally at posterior part of the middle cerebellar peduncle and ventrally just behind the pons (Fig. 1). The prepon-tine transection passed dorsally at the posterior part of the inferior colliculi and ventrally 0-2 mm in front of the pons. The microelectrodes passed brachium conjunctivum, marginal nucleus of the brachium conjunctivum, nucleus locus coeruleus and paralemniscal and gigantocellular tegmental fields. Typical location of a microelectrode is shown in Fig. 2.

In the isolated pons the EEG records were flat, representing mostly a noise of the recording system (Fig. 3, cat C2). The depression was somewhat less...
at loci below H-5, i.e. in the region of the pontine nuclei. However, even there the mean electrical activity in all preparations was extremely low, about 2 μV. In cat C1, epileptic episodes were recorded four times from various loci. They lasted from 2 to 30 s, the frequency of discharges was about 40/s and amplitude varied from 4 to 40 μV. Electrical stimulation (2-4 s, 1 ms pulses, 100/s, 0.5 mA) at one locus in the reticular formation did not influence EEG activity at a locus 2 mm away.

In control prepontine cats the desynchronized EEG activity was found in all recorded loci (Fig. 3, cat C7). The mean amplitude of EEG activity from 22 recording sites was 11 μV in C7 and from 38 recording sites 9 μV in C8. In this preparation, like in the isolated pons, electrical stimulation of the reticular formation was ineffective. EEG activity was also unaffected by tactile or noxious stimuli applied to the body (this is consistent with recent data on prepontine rats, Gottesmann et al. 1995). The spatial density of spontaneously active single-units was at a similar high level both in the isolated pons of cats and in the pons of the control cats (Fig. 4).

Discharge rates of pontine single-units was also at a similar high level in all preparations (Fig. 4). However, it varied markedly in different units: 0.08-36.2 spike/s in cat C4, 0.04-34.0 in cat C5, 0.4-
DISCUSSION

The isolation of pons in our preparations with the two transections was not complete since trigeminal input and output were preserved. However, only some sensory trigeminal fibres terminate in the pons, those mediating pain terminate in the medulla (Clarke and Bowsher 1962). In addition an output to the cerebellum was also connected with the isolated pons. These connections were presumably insignificant for our results.

Our main result is a virtual lack of EEG activity and a high (perhaps normal) level of both spatial density of active units and rate of their spontaneous activity in the isolated pons. Although data from only one microelectrode penetration were available, it is likely that this surprising discrepancy between EEG and single-unit activity was present in the whole isolated pons.

The electrical activity in the isolated pons was comparable to that in the isolated midbrain (Żernicki...
et al. 1970, 1979, Dec et al. 1978). However, in the isolated midbrain, slow waves were occasionally recorded and in the tectum EEG activity was relatively less depressed (Zernicki et al. 1970, 1979). The density of spontaneously active single units in the isolated pons was about twice higher than in the isolated midbrain. On the other hand, the rate of spontaneous unit activity was comparable to that in the isolated midbrain. It was also comparable to that found by Steriade et al. (1982) in cat’s midbrain reticular formation during synchronized sleep (mean about 13 spike/s) but somewhat lower than during wakefulness (mean about 20 spike/s).

The brainstem EEG activity was present both in the present controls isolated only rostrally, and in the preparations deafferented only caudally (Zernicki et al. 1979). In both studies, the transection in control preparations was at the junction of pons and midbrain. Thus, both rostral and caudal inputs are important for the brainstem EEG activity. In both kinds of preparations the amplitude of EEG activity was similar and probably lower than in the brainstem of intact cat having both caudal and rostral inputs. This is suggested by comparison with the EEG records from the midbrain reticular formation of intact cats (Sharpless and Jasper 1956).

The discrepancy between lack of EEG activity and good single-unit activity can be explained by two assumptions: (1) Both, in the deafferented pons and in the deafferented midbrain the spike activity of many neurones depends totally on their intrinsic electrical properties. (2) These autoactive neurones fire asynchronously, therefore their activity does not result in network oscillations. Thus, only few neurones are excited postsynaptically.

These assumptions are supported by several facts: (1) Neurones showing intrinsic electrical properties were found in several brain structures (see Llinas 1988), including the bulbar reticular formation (Limanski 1965, Segundo et al. 1967, Llinas 1988). (2) Anatomical data (see Scheibel 1984) show that direct synaptic connections between reticular neurones are not numerous. These neurones receive numerous collaterals from both afferent and efferent fibres (which are cut in the process of isolation), but short-axon Golgi type II neurones are rare in the reticular formation. (3) Both in the isolated pons and isolated midbrain (Zernicki et al. 1970), electrical stimulation of the reticular formation did not influence EEG activity at adjacent loci.

Present results confirm that a flat EEG record is not necessarily a sign of absence of neural activity and death of neural tissue and thus they have some practical significance. Such discrepancy between EEG activity and single-unit activity would be particularly important in the cerebral cortex since for clinicians the lack of the cortical EEG activity is a symptom of death of the cerebrum. However, studies on the effects of hypoxia or barbiturate anaesthesia (see Creutzfeldt 1975) and studies on the isolated cortex (Forst et al. 1966, Forst 1968, Hirsch et al. 1969, Khananashvili and Zarkeshev 1970, Bogoslovsky and Zarkeshev 1971) indicate that the disappearance of cortical EEG activity is generally associated with the disappearance of single-unit firing. Nevertheless, even in the isolated cortex some cells fire also when EEG activity becomes flat (Khananashvili and Zarkeshev 1970, Bogoslovsky and Zarkeshev 1971).

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