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SENSORY INTERACTION IN THE SUPERIOR COLLICULUS OF FREELY MOVING RAT INDICATED BY EVOKED POTENTIALS

Jurij BRANKAČK and Fritz KLINGBERG

Department of Neurophysiology, Karl Marx University, Institute for Brain Research
Härtelstr. 16/18, 7010 Leipzig, GDR

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Abstract. Sensory monomodal and bimodal interaction was compared in the anterior medial superior colliculus (CS) of freely moving Long-Evans rats with chronically implanted electrodes using pairs of click and flash stimuli separated by intervals of 100 ms. The amplitudes and peak times of first and second evoked potentials were statistically evaluated and compared with the uninfluenced control values of visually and acoustically evoked potentials (VEP and AEP) on the background of relatively constant relaxed wakefulness. Heteromodal interactions were characterized by only very small and in most cases insignificant changes, compared with very striking depressions of component amplitudes of the second EP in monomodal paired stimulation. Significant differences of AEP and VEP amplitude and peak time changes in superficial and in deep layers of the CS indicated that the sensory interaction is different, corresponding to the functional structure of CS layers. The amplitude of the second negativity N32 in AEP 100 ms after flash is significantly influenced in the superficial layers, but not in the deeper ones. The VEP peak times are prolonged after click only in the deeper layers.

INTRODUCTION

In the physiology of the sensory systems it is generally accepted that the primary projection area of any analyzer system is rather specific. In a previously published experimental analysis we showed that acoustically evoked potentialis (AEP) can be regularly observed in the visual system of the rat (2). Large AEP were recorded in two typical forms from the superficial and from the deeper layers of the superior colliculus (CS). In a preliminary publication we discussed the question

whether both visually evoked potentials (VEP) and AEP are generated independently in primary visual structures of the rat or may influence each other (6). This problem is still unsolved. There is increasing information about monomodal non-visual, bimodal and polymodal responses of cells in the CS, but only from the deeper layers (mouse: 9; rat: 13, 24; hamster: 7, 23; guinea-pig: 14; rabbit: 11, and cat: 12, 20, 25). So far the problem of sensory and bimodal interaction was not studied with evoked potential methods in the CS of the rat, EPs, would reflect more the processes at dendrites distant from cell bodies.

A further question is whether the EP in the superficial layers and those in the deeper layers are similarly influenced or modified. This question is related to the facts that the amplitude changes of the deeper polarity-reversed VEP were similar, but not identical (as a mirror image) with the superficial (4) VEP, whereas the AEP were not found to be polarity-reversed (2).

METHODS

The experiments were performed on 18 unrestrained male hooded rats of the Long-Evans strain with chronically implanted stainless steel electrodes (300 μm thick with impedances between 5 and 10 kΩ) in different depths of the anterior

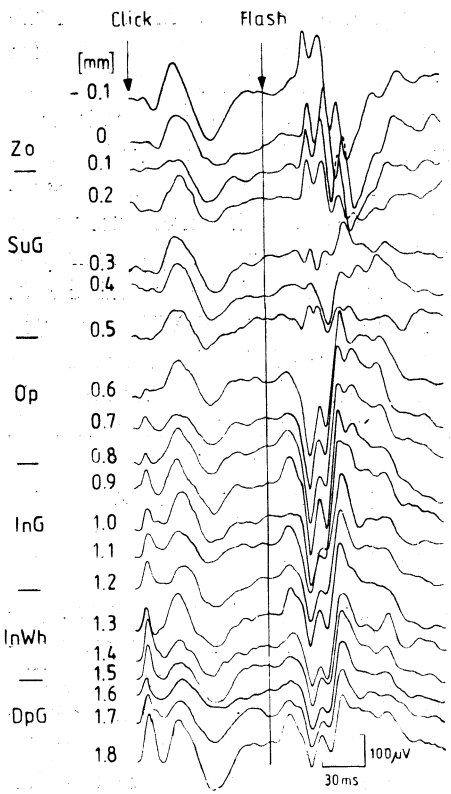

Fig. 1. Depth profiles of averaged (n = 16) AEP and VEP in the anterior medial CS recorded by a penetrating tungsten electrode in 100 μm steps with a 100 ms interval between click and flash. Zo, zonal layer; SuG, superficial grey layer, Op, optic nerve layer; InG, intermediate grey layer, InWh, intermediate white layer; DpG, deep grey layer. All samples were recorded in a rather constant state of relaxed wakefulness.

medial superior colliculus (6 mm posterior to bregma, 1 mm lateral). The optimal positions for electrode implantation in superficial and deep layers have been checked in experiments with movable tungsten electrodes (110 μm), as shown in Fig. 1. Fixed electrodes for recording from superficial layers were placed all in the dorsal part of the superficial gray layer (between steps 0 and 0.2 in Fig. 1). The fixed deep electrodes were all placed below the intermedial layers of CS (between steps 1.5 and 1.8 in Fig. 1). The EP were recorded against a reference electrode on the nasal bone (3 mm anterior bregma, 0.5 mm lateral). This point was previously checked as generating no significant AEP and VEP. The behavioral pattern of the rat was controlled by observation, by records of the respiratory potentials through an electrode in the olfactory bulb, by recording head movements with a magneto-inductive sensor on the rat's head and by the EEG control through two further epidural electrodes on the parietal and occipital neocortex. Two weeks after electrode implantation we started the recording of EEG, EP, respiration rate and movement patterns during unrestrained spontaneous behavior, which was performed in an electrically shielded mirror cage ($30 \times 25 \times 40$ cm) which guaranteed relatively constant intensity of flash light in any position of the rat. For visual stimulation we used the maximal intensity of a light flash stimulator. The flash intensity inside the mirror cage remained constant throughout all measurements, corresponding to the brightness of $6 \cdot 10^4$ cd, also during any movement of the rats (3). Single cliks from a clik generator were applied through a loudspeaker on the ceiling of the cage and corresponded to a loudness of 60 db in any position. The intervals of paired stimuli were always 100 ms and between pairs of different sensory stimuli were kept constant at 2.5 s.

Single EP were monitored by a DISA oscillocope. Averaging ($n = 20$) was performed by means of a multichannel analyzer NTA 512 B when polygraphic control, behavioral observation and the monitored single EP indicated a rather homogenous behavioral pattern. All data were compared under relatively constant conditions of relaxed wekefulness, except those presented in Fig. 6. The behavioral states were exactly classified by cortical hippocampal EEG patterns, respiration rate, recorded motor patterns and the behavior type.

The position of the recording electrodes in the superior colliculus was checked in Nissl preparations.

Statistical differences of EP components were measured by the use of the nonparametric Wilcoxon test for matched pairs.

RESULTS

In experiments with penetrating tungsten electrodes we have found that there are two regions in which the two types of VEP and AEP configurations are constantly recordable, also regarding the amplitudes and peak times of components: one

in the superficial layers and one in the deeper layers (Fig. 1). We further used a simpler paradigm with fixed electrodes in these regions. Figure 2 presents typical samples of averaged EP, evoked by monomodal and heteromodal pairs of stimuli during relatively constant relaxed wakefulness. Figure 2*A* shows EPs taken from one rat with the tip of the recording electrode in the dorsal part of the stratum griseum superficiale of the CS (Fig. 2*A*) and Fig. 2*B* — EPs taken from another rat with the tip of the recording electrode below the intermedial layers of the CS (Fig. 2*B*). The interval of the paired stimulus (100 ms) was preferred in view of the results of the foregoing experiments. in which recovery cycles were studied.

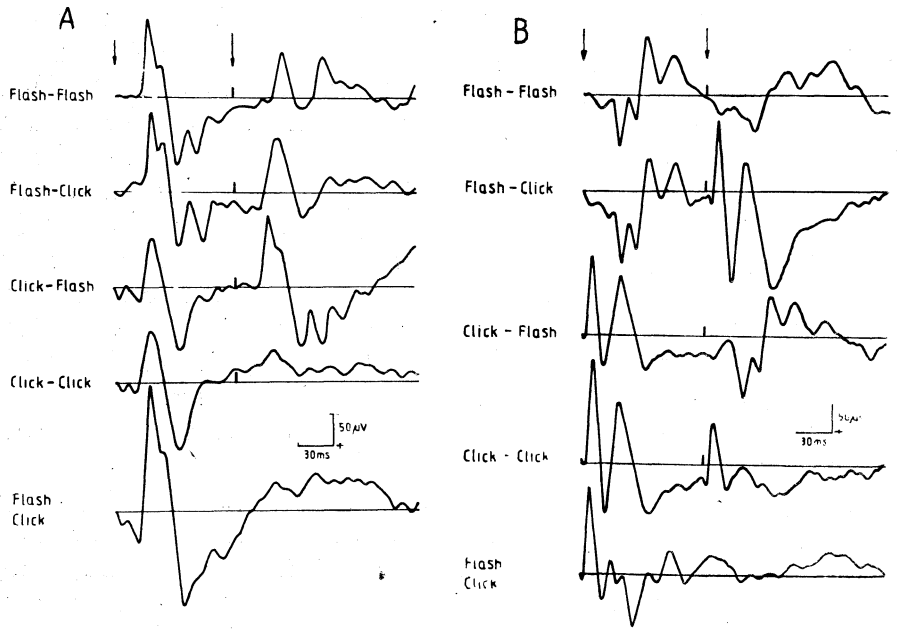

Fig. 2. Typical samples of averaged evoked potentials ($n = 20$) from superficial CS (*A*, stratum griseum superficiale, upper part) and from deep CS (*B*, below intermedial layer) in response to paired stimuli (arrows) with an interval of 100 ms (first to fourth row) and without interval (fifth row). The sequence of flash and click is indicated before the samples. All samples were recorded from one rat during relatively constant relaxed wakefulness.

In those experiments we found that heteromodal interactions were small at any interval, therefore we assumed that measurements with 100 ms interval are sufficiently representative. Using this interval, we found the most evident difference between monomodal and heteromodal interaction. Another reason was to avoid masking the components when the EPs overlap in cases of shorter intervals (Fig. 2). In the flash-flash presentation the first primary peaks (N27 of the superficial VEP and P30 of the deep VEP) were still strongly depressed after 100 ms, whereas the second peaks (N39 and P43 respectively) seem to be uninfluenced or even higher

(for identification of components compare with the peaks of uninfluenced EPs in Figs. 4 and 5). The configuration of the second VEP with its different components is extremely changed. A similarly strong influence is exerted in the monomodal acoustic stimulus pairing. In this case the depression of the second AEP is rather strong in the superficial record, whereas in the deep record it reveals a less depressed early component (N10) compared with the second negative component (N32). In all cases of heteromodal stimulus pairs, AEP and VEP configurations remained unchanged and independent of the sequence, as can be seen in Fig. 2. The relative independence of AEP and VEP from each other is also documented in the response to simulataneously applied flash and click (interval 0 ms), which is presented in the fifth row of Fig. 2. In this response the early negativity represents the algebraic sum of both, the primary components N27 of the superficial VEP and N27 of the superficial AEP and the following positivity represents the algebraic sum of VEP and AEP positivity at this time (Fig. 2*A*). The situation seems to be complicated in the case of the deep recording electrode, when VEP is and AEP is not polarity reversed (Fig. 2*B*). This relative independence of both in the coincidental heteromodal response becomes better visible in Fig. 3, where the flash response from the second row and the click response from the third row are superimposed with the EP to a simultaneous flash and click. There appears no occlusion or any kind of subtraction of components, which would be expected when coinciding flash and click stimuli would excite the same neuronal structure.

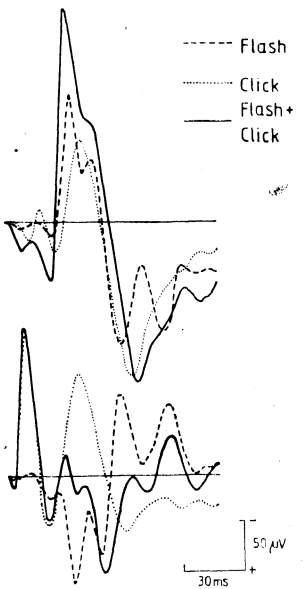

Fig. 3. The superposition of uninfluenced averaged AEP and VEP and the EP to both coinciding stimuli demonstrates that the response to simultaneous click and flash shows no occlussion or subtraction of components in the case of coinciding stimulation.

The statistical evaluation of peak times and amplitudes from a group of nine rats with superficial recording electrodes (all in the dorsal part of the stratum griseum superficiale) is shown in Fig. 4. On the left side of the figure the interindivi-

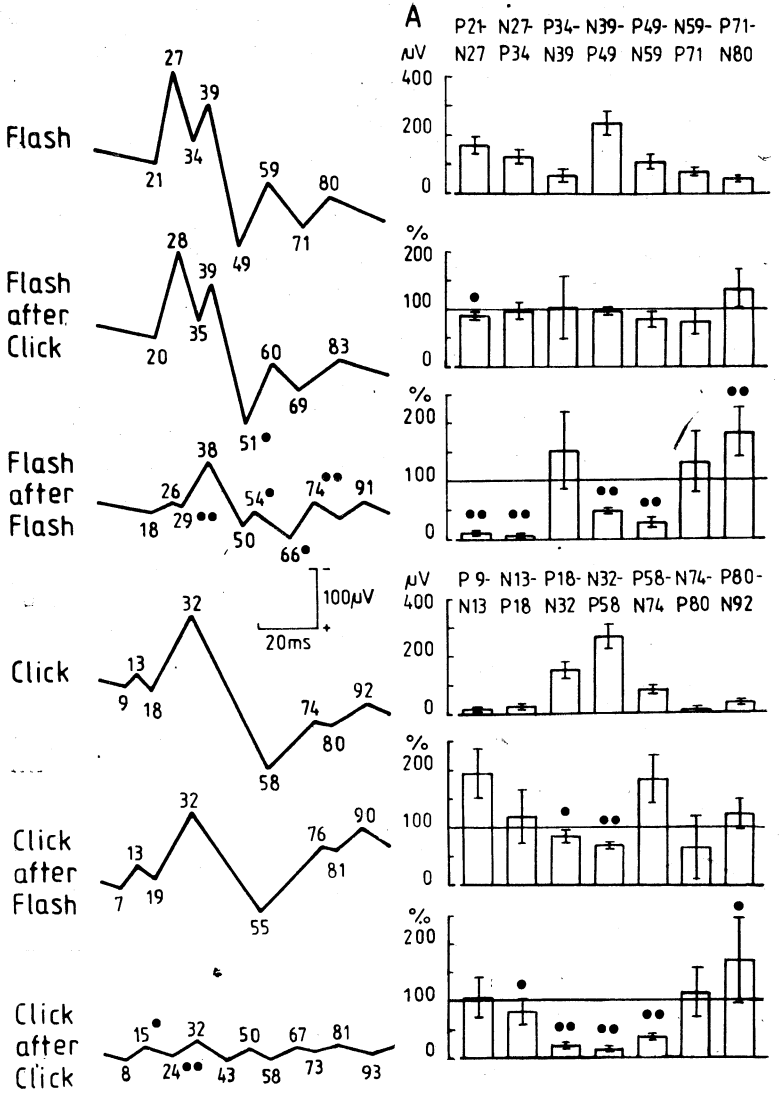

Fig. 4. Interindividual mean values of peak times and amplitudes of averaged evoked potentials to single or paired stimuli as indicated on the left side. The calculated configuration of interindividual mean EPs is demonstrated on the left side with peak times of the main components in ms. The mean amplitudes and standard errors in μV and the deviation of the second potentials in percent of control (100% was the value in the same animal when the AEP or VEP was uninfluenced) are shown on the right side. The VEP components (first to third row) and AEP components (fourth to sixth row) are shown above the columns. Results from 9 rats with electrodes in the upper part of stratum griseum superficiale. Significant differences to control are indicated by one point: $P < 0.05$ and two points: $P < 0.01$.

dual mean values of peak times and amplitudes of EP recorded in relaxed wakefulness are shown in the form of calculated EP configurations. On the right side, amplitudes from peak to peak are statistically evaluated in VEP (first row) and in AEP (fourth row) in microvolts, with their interindividual SEM when the flash or the click was unifluenced (control EPs). The corresponding components of the second stimulus response are evaluated in percent of the control EP. We compared mean values of the VEP components to a single flash with mean values of flash response before the click and with mean values of flash response before the flash to test the probable influence of the 2.5 s interval between single or paired stimuli and found no statistical difference between them. This points to the stability of the averaged VEP during relaxed wakefulness in all cases when the flash was the first stimulus. The same result was obtained when we compared click responses alone with click responses before the click and with click responses before the flash.

In the cases of heteromodal stimulus pairs, the flash response (after click) or the click response (after flash) were scarcely influenced, with the exception of a significant decrease of P21-N27 in the flash after and click P18-N32 and N32-P58. in the click after flash (Fig. 4 second and fifth row). In all cases of monomodal stimulus pairs most of the components were strongly decreased in the second EP, as shown in the third row for VEP and in the sixth row for AEP. The changes of the peak times are correspondingly small and insignificant in heteromodal stimulus pairs and greater in monomodal stimulus pairs, as can be seen in Fig. 4.

Figure 4 presents the statistical evaluation of eight rats with the tip of the recording electrode below the stratum opticum. In all these rats the VEPs were polarity reversed (compare with Fig. 4), whereas the AEPs were not reversed, but showed a much greater early negative component (N10) in comparison with the superficial response. The polarity reversed VEP is not exactly a mirror image of the superficial VEP. The peak times of the corresponding components of the VEPs from superficial and from deep layers differ significantly, mainly in the late components. This supports our previous findings (4, 5). Nevertheless, we found similar interactions in the deep layers of the CS as those in the superficial layers. There were no significant amplitude changes of the components in the second EP when heteromodal stimulus pairs were applied (except the component P-4N10 in the AEP after flash), whereas the changes of the second EP in monomodal pairings are great (Fig. 5). The monomodal suppression of the AEP components in the second AEP is relatively stronger in the deeper layers. On the other hand, we found a significant influence of the click stimulus upon the VEP, indicated by a significant prolongation of peak times (Fig. 5, second row), which has no correspondence in the superficial CS (compare with Fig. 4, second row).

These results of monomodal and heteromodal interaction between responses to acoustical and to visual stimuli are differently modified by the ongoing behavioral pattern. Figure 6 demonstrates that the responses to heteromodal paired stimuli with an interval of 100 ms are significantly changed when the behavioral pattern

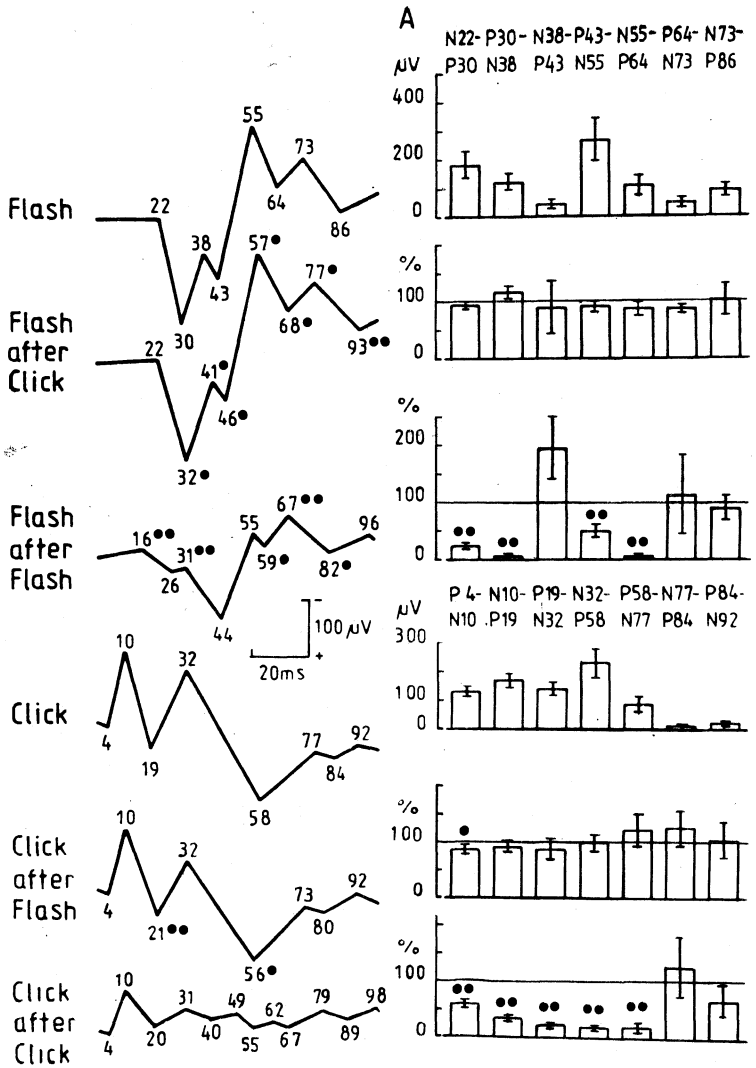

Fig. 5. Interindividual mean values of peak times and amplitudes of averaged VEP and AEP from 8 rats with electrodes in the deep layers of CS. Denotations as in Fig. 4.

is different. If one compares in Fig. 6*A* the responses during relaxed wakefulness (RW) with drowsiness (D), grooming (G) or exploratory behavior (E), one gets an impression that the proportion between amplitude changes of VEP components and those of AEP components may be different in any behavior because the heteromodal interaction may be different. This is really not the case, as the behavior-dependent changes of AEP and VEP are almost identical in both variants, click as the first stimulus and flash as the first stimulus (compare Fig. 6*A* with 6*B*). This result was reproduced in every rat.

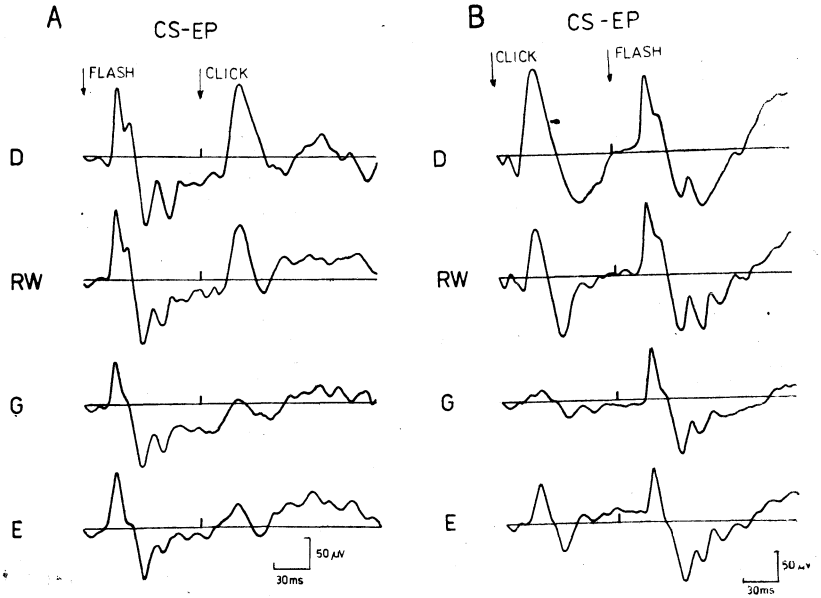

Fig. 6. Typical samples of averaged EPs to heteromodal stimulus pairs with 100 ms interval (arrows) during different behavioral patterns from the same rat. D, drowsiness; RW, relaxed wakefulness; G, grooming, E, exploratory behavior. In (A) the flash was the first stimulus, in (B) the click.

DISCUSSION

The existence of large AEPs in the CS of the rat is an indicator of a great number of neuronal elements responding to the click. Cellular responses to acoustic stimuli were recorded in intermediate and deeper layers of CS (mouse: 9; rat: 13, 24; hamster: 7, 23; guinea-pig: 14; rabbit: 11 and cat: 12, 15, 20, 25), whereas cells in the superficial layers respond only ot visual stimuli (for review see 11, 21, 23). It was reported of cats that there are acoustic fibers in the deeper layer of CS coming from the inferior colliculus (10, 18), lemniscus lateralis (10; rat: 22) and from the acoustic cortex (8, 17). There are no reports about acoustic inputs to superficial layers of CS. We have found only one paper describing AEP in the CS recorded from the deep layers (1). Those experiments were performed on restrained cats. In our first paper on AEP from the CS in freely moving rats we described two negative components of the AEP, which are difierently large at a different depth of the CS; the component N10 is largest in the deeper layers below the intermedial layers, and the component N32 can be found very large also in the most superficial layers (2). The morphological data support the hypothesis that the first negative component is more closely related to the region of cell bodies, responding to acoustic stimuli, whereas the second negativity, N32 is widely distributed and may be related dendrites responding to acoustic stimuli. In analogy to this inter-

pretation, there is evidence of a similar distribution of cells responding to somatosensory stimuli with their dendrites extending to the superficial layers of CS in the hamster (16).

In this paper we show that the visual and acoustic EP generating elements in the CS respond independently from each other to acoustic and visual stimuli, and that the heteromodal interaction of processes indicated by EP components is small. This fact supports the hypothesis that separate inputs of acoustic and visual afferents contact with different dendrites or cells in different layers and also in the superficial layer, which contains only monomodal visual cells (11, 19, 21, 23). On the contrary, many of the acoustically responding cells were bimodal, responding also to visual stimuli (7, 11, 15). With regard to this group of cells, a greater heteromodal interaction in the deeper layers would be expected, such as was found in the single cell responses of cats (15). In our EP studies we found no influence of the click stimulus upon the flash response in the superficial layers, but a significant prolongation of all peaks in the deep flash response after click. The second negativity N32 of the click response, however, was significantly stronger influenced by a preceding flash in the superficial AEP than in the deep one, whereas the large early component N10 of the AEP slightly decreased after the flash only in the deep layers. We have already reported that both these components change differently in relation to the behavioral state (2). The influence of behavioral state upon EP components was quite similar in uninfluenced EPs and in responses to paired stimuli. This underlines the fact that parameters of interactions are more likely to depend on the functional structures than on the functional state. On the other hand, these results confirm that it is necessary to compare data under constant behavioral conditions in order to avoid misinterpretations. Such an investigation can be more easely performed under conditions of carefully controlled relaxed wakefulness in freely moving animals.

The heuristic advantage of evoked mass response technique is to get more insight in the generation of field potentials from a great mass of dendrites which are widely distributed far from cell bodies and which respond to the messages in the aborization of sensory afferent terminals with primary EP components and to secondary processes in the investigated neuronal network with later EP components. This knowledge might complete and help to understand better the modulation of information processing.

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