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FREQUENCY ANALYSIS OF EEG DURING MILK DRINKING[1]

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INTRODUCTION

The appearance of a 4–12 c/sec synchronized electrocortical activity localized in the striate cortex (posterior lateral gyrus) of cats has been reported to be associated with post-reinforcement during conditioning (Clemente *et al.* 1964; Sheer *et al.* 1966). Although the primary emphasis has been centered on this relationship, it has also been shown that conditioning was not a prerequisite for its elicitation, as the same type of activity was recorded from the striate cortex of a naive, hungry animal with the attainment of food. The slow wave synchronized EEG has also been reported to be present in the parietal cortex with an appetitive response (Clemente *et al.* 1964). This electrical response appears to be limited to a small portion of the cat brain, as recordings made from numerous other loci in both cortical and subcortical areas in approximately thirty cats during a period of several years have failed to show a similar rhythm when the cat was drinking milk (Grandstaff, unpublished data).

The present investigation was undertaken to further elucidate the details of the relationship of the 7.5 c/sec EEG activity in the striate cortex with the behavioral response of drinking milk. The question of primary interest was: Does the 4–12 c/sec activity in the visual cortex associated with drinking milk reflect in some way a change in the afferent visual input as such, or is it independent of vision and therefore affected by non-visual activating and/or inhibitory inputs to the striate cortex?

To test this question experimentally, the problem of blocking all visual input to the striate cortex without disrupting the non-visual inputs had to be met. The visually split-brain animal afforded such a possibility. By sectioning the optic chiasm plus the midline structures down to the brain-stem and visually occluding one eye with an opaque contact lens, the ipsilateral hemisphere had visual input blocked, while non-visual inputs were still supposedly intact. With the contact lens removed, the animal served as his own control; *i.e.*, the same recording loci in the striate cortex could be compared in the presence and in the absence of visual input. Such an approach should at least give some indication as to the role of visual/non-visual input relationships in striate cortex during food intake.

METHODS

Subjects and surgical procedures

A series of cats, four split-brain animals and two controls, were surgically prepared under aseptic conditions with chronically implanted bilateral, bipolar electrodes in the following loci: striate cortex (posterior lateral gyrus), auditory cortex (mid-ectosylvian gyrus) and mid-suprasylvian gyri, making a total of 12 electrode placements. All electrode placements were determined by the stereotaxic atlas (Jasper and Ajmone Marsan 1954). All electrodes were made of 300 μ insulated nichrome wire with approximately 1 mm separating the 0.5 mm exposed tips. They were placed both intracortically and

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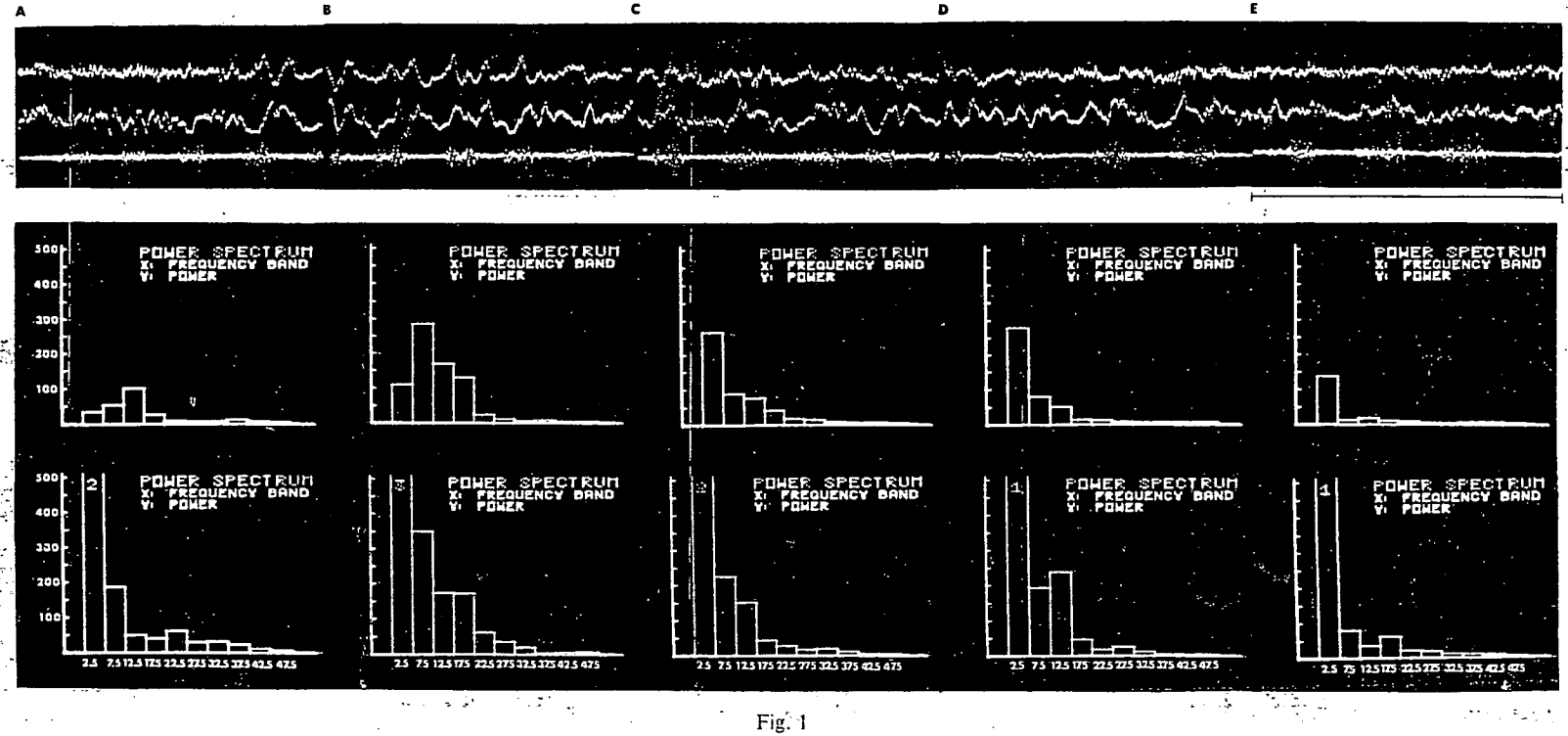

Fig. 1

EEG trace of normal cat and corresponding spectral analysis for 5 sec (*A*, *B*, *C*. *D*, *E*, respectively) of consecutive record from two different loci on the posterior lateral gyrus, showing a difference in output of the striate cortex during lapping as a function of electrode placement and time. Traces *a* and *b*: posterior lateral gyrus; trace *c*: written record of lap response. Note: an open column with a number in it indicates that the value exceeds the maximum value shown in the graph by 1, 2 or 3 times. (Calibrations: 1 sec and 50 μV).

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epidurally, the latter electrodes having small balled tips. Electrode leads were crimped to a miniature Microdot plug and cemented to the skull with dental acrylic. All subjects were allowed a minimum recovery period of 2 weeks prior to testing.

Transection of the brain in the split-brain animals included the following midline structures: optic chiasm, corpus callosum, anterior and posterior commisures, fornix, massa intermedia, and superior colliculi. Surgery was done in two separate operations several weeks prior to electrode implantation. The first operation was the sectioning of the optic chiasm through a transbuccal approach. After a 2 week recovery period, the animal underwent transection of the midline structures accomplished from a dorsal approach.

Data acquisition and analysis

All data were stored on a 7-channel magnetic tape system (Ampex, Model SR-300) for later analysis on a small general purpose digital computer (PDP-8). Six channels recorded analog EEG and the seventh was used to record event data, both the milk presentation and the lapping response made by the animal.

Data analysis was on the PDP-8 computer. In the analog to digital conversion the signal was sampled at a rate of 1000 points/sec for a period of 5 sec and stored on digital tape for further processing. A special Digital Spectral Analysis program, which was adapted for the PDP-8 computer from an original program developed by the Stanford Electronics Laboratories[1] was used for the spectral analysis. The print-out gave the power in each narrow band (5 cycles) with the center frequencies of each band given in the graphed record (see samples in Fig. 1). Only frequencies up to 50 c/sec were graphed.

Apparatus and testing procedure

The feeder system was constructed entirely of plastic and was designed to eliminate the possibility of artifacts. Actuation of a solenoid valve by a short manually controlled electrical pulse allowed a small amount of milk to be delivered into the plastic feeder. A phono-cartridge placed beneath the spring-mounted plastic feeder cup picked up the lapping movements made by the cat. The spring tension was sensitive enough to allow the cup to move with the pressure produced by the tongue touching the shallow cup thereby producing a recordable signal.

The testing session for each animal consisted of putting the animal in a sound-insulated experimental box, which had a constant background noise (80 dB) and dim illumination. Milk was presented to the animal on a random schedule for approximately 10 trials under each of the following conditions: (1) visually non-occluded, and (2) visually occluded with an opaque contact lens in one eye of each of the split-brain cats. Control trials were run with the animal eating solid cat food, and with milk lapped from a bowl.

Verification of the split-brain

Histological verification of electrode placements and surgical transection of the brain in the split-brain cats is not possible at the present time as the animals are still being tested in other studies. However, electrophysiological verification by testing for the presence of evoked potentials in the occluded hemisphere during flash stimulation was carried out. There was no evidence of an evoked potential in the visually occluded hemisphere of any animal from a total of six bipolar electrodes in each hemisphere of each animal during the flash stimulation.

RESULTS

Digitized EEG for samples of milk lapping data, with the corresponding spectral analysis for each sample, are shown in Fig. 1 and 2. Records from the striate cortex showed the peak power in the 7.5 c/sec band during lapping, whereas the data from the auditory cortex for the same period of time were variable but often had a dominant power output in the 2.5 c/sec band (Fig. 2). The EEG records from the striate cortex indicated that after the burst of 7.5 c/sec activity with lapping, there was a shift to much higher frequencies as lapping became more sporadic, and subsequently all synchrony dropped out of the record.

Lapping at a fast and highly repetitive rate

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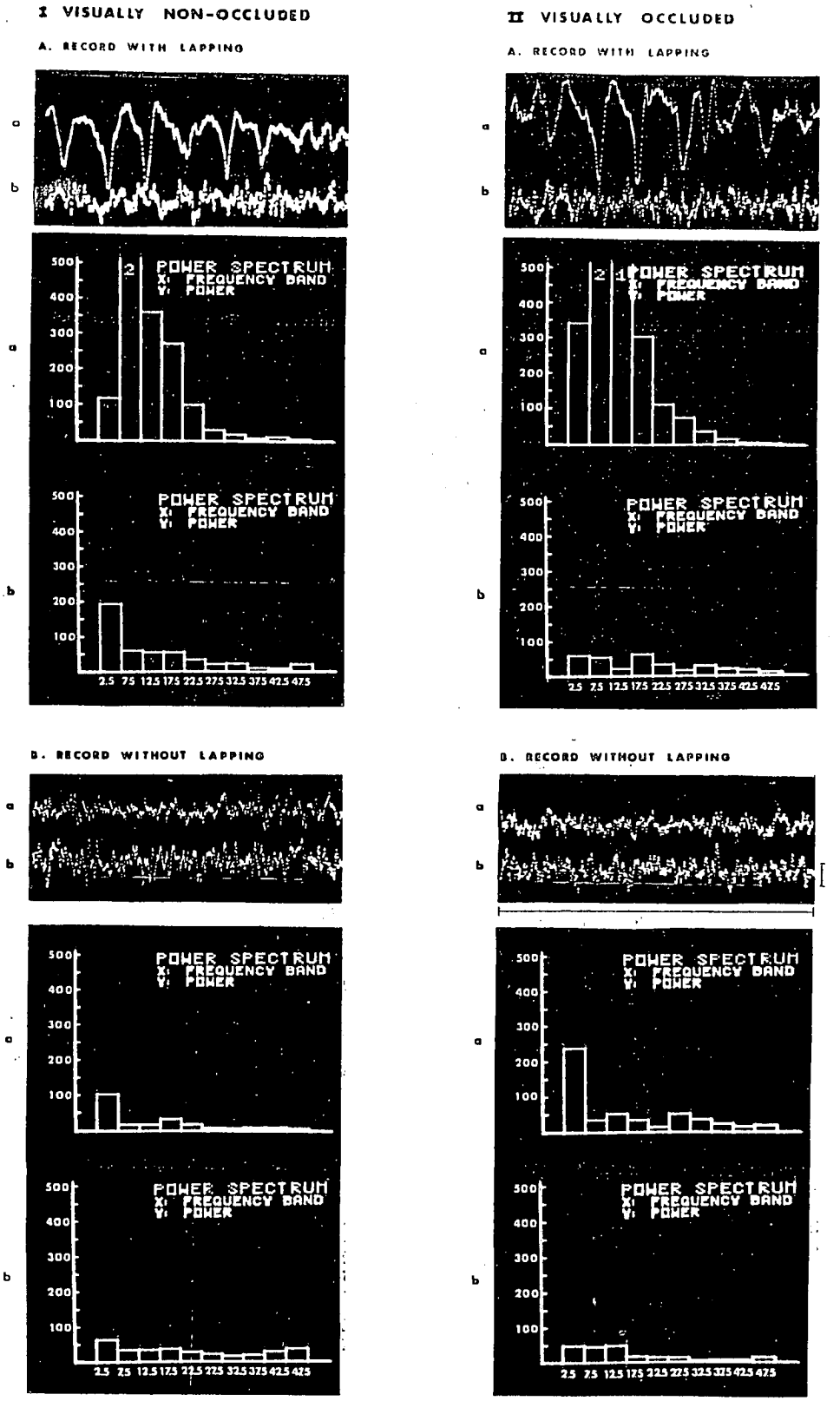

Fig. 2

Corresponding EEG trace and digital spectral analysis of split-brain cat with and without visual occlusion during lapping and not lapping. Traces *IAa*, *IBa*, *IIAa* and *IIBa* are from the same striate cortex lead and *IAb*, *IBb*, *IIAb* and *IIB* are concomitant records from the auditory cortex. Note: an open column with a number in it indicates that the value exceeds the maximum value shown in the graph by 1, 2 or 3 times. (Calibrations: 1 sec and 50 μV).

appeared to be a prerequisite for the initiation of the 7.5 c/sec activity. The EEG slow wave activity occurred approximately 300–500 msec after the onset of lapping, and then only if lapping was very repetitive; sporadic lapping simply did not produce the synchronous response. At the onset, the lapping rate was often 5 or 6 laps/sec, but after a few seconds the rate decreased and the synchronous EEG rhythm in the striate cortex evidenced a concomitant decrease. An example of the lapping response and its relationship to the EEG rhythm is shown in Fig. 1. Visual inspection of the animal during lapping, while monitoring the signal output of the phono-cartridge system designed to pick-up the tongue movements, indicated that the system produced a relatively reliable record of the lapping response.

All striate leads in all animals did not show the same proportion of synchronous EEG activity with lapping; this activity, however, was present in both epidural and intra-cortical recording sites in all animals. Electrodes having the response did so with high reliability.

An example of digitized EEG data from two striate electrodes in the same animal exhibiting different responses with lapping is shown in Fig. 1. At onset of lapping, striate electrode *b* showed a typical record of 7.5 c/sec which perseverated for several seconds. By contrast, electrode *a*, also in the posterior lateral gyrus, showed a much shorter period of oscillatory activity.

During periods when the animal was not lapping, there was a consistent shift to a more variable distribution of energy in all frequencies with a loss of all synchronous oscillatory activity in the striate cortex (Fig. 2). Auditory records taken during the same period did not exhibit a power frequency distribution that was reliably different from the records taken during lapping. The major peak of energy was often in the lowest frequency band, 2.5 c/sec, but this was not consistent from trial to trial.

A comparison of EEG data from split-brain animals with the normal cats during lapping showed that the 7.5 c/sec oscillatory activity was essentially the same in both groups of animals (Fig. 1 and 2). The major portion of energy was definitely centered in the 7.5 c/sec band.

When split-brain animals were visually occluded in one eye with an opaque contact lens and tested in the same milk lapping situation, the recordings from the ipsilateral visual cortex indicated that the peak power was distributed over the measured frequency bands in approximately the same manner as that of the non-occluded split-brain animals (Fig. 2) and the normal control animals. The same 7.5 c/sec band contained the major portion of energy. The electrical activity in the auditory cortex did not appear to vary in any systematic way from that of the normal controls or the non-occluded split-brain cats under the same behavioral conditions. Samples of analyzed records with visual occlusion of split-brain animals during periods when the animals were not lap-

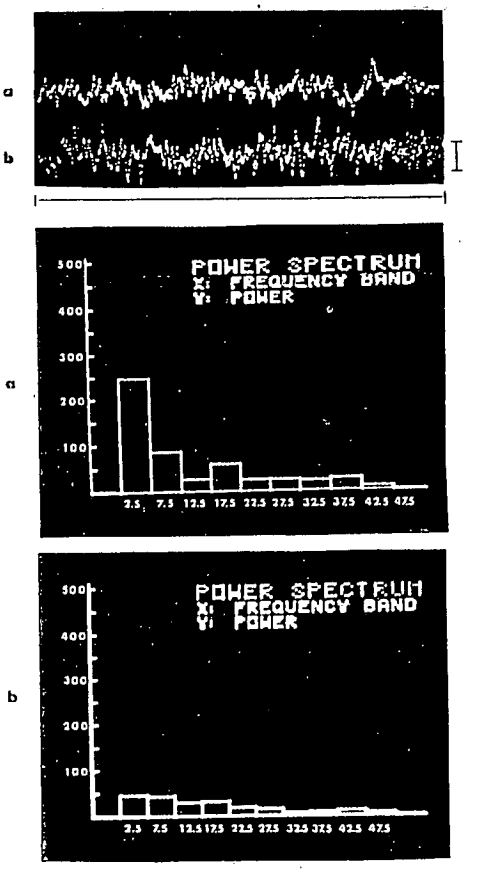

Fig. 3
EEG trace and corresponding digital spectral analysis during eating of solid cat food. Trace *a*: posterior lateral gyrus; trace *b*: mid-ectosylvian gyrus. (Calibrations: time: 1 sec and 50 μV).

ping, did not show any consistent changes when compared to similar records without occlusion (Fig. 2).

Control trials were run with the animals drinking milk from a bowl, and the frequency analysis indicated that the results were essentially the same as those with milk lapping from the feeder cup. This evidence ruled out the possibility that artifact from the feeder system somehow contaminated or was responsible for the results.

In order to ascertain whether the 7.5 c/sec oscillations were related to food intake *per se*, records were also taken with the animal eating canned cat food. Chewing and ingestion of solid food did not elicit the same electrophysiological response in the striate cortex (Fig. 3). There was no evidence of the synchronized oscillations. The major portion of power was often at 2.5 c/sec with a wider distribution over all frequencies which was continuously variable. EEG monitoring and gross observation of the animal during grooming behavior indicated that when the animal licked himself there was some evidence of a shift in the EEG to slower frequencies, however, the response was never well defined and obvious as in milk lapping. The rhythmic oscillations simply never developed.

DISCUSSION

Although previous investigations have associated the slow wave oscillatory activity in visual cortex with post-reinforcement or simply with the ingestion of food during drinking (Clemente *et al.* 1964) the results from the present research suggest that the 7 c/sec synchrony has a high correlation with the specific and highly repetitive tongue movement which occurs with lapping, and that the changes effected in the electrophysiological activity of the visual cortex seem to be a direct consequence of this complex repetitive movement.

In correlating the behavioral lapping response with the synchronized EEG response, there did not appear to be a one-to-one correspondence of the 7 c/sec activity with the motor response as such—the lapping must have been ongoing for a period of time prior to the onset of the synchronous activity occurring in the striate cortex. The latency of onset of the rhythm was quite variable. Its initiation occurred somewhere in a range of 300–500 msec or longer after the onset of vigorous lapping. The synchronous EEG activity was not invariably associated with lapping, but appeared in the record only when the animal lapped in a highly repetitive manner. If the animal lapped sporadically, the synchronous oscillatory electrical activity did not occur. Therefore, the 7 c/sec oscillatory activity does not represent a specific glossokinetic potential as such, but an electrophysiological state which is initiated and maintained as a consequence of repetitive tongue movement. If the 7 c/sec activity were a tongue potential, the potential would occur with each lick, and this certainly was not the case.

The possibility of artifactually induced electrophysiological responses should always be given serious consideration. Although artifact generated by eye movement could conceivably contribute to the 7 c/sec activity, this appears highly unlikely, as Hughes (1964) showed these potentials to be so small in the striate cortex that they were negligible when using ordinary recording techniques. If the 7 c/sec oscillations were equivalent to lambda activity in visual cortex they should disappear with visual occlusion (and they did not), as it has been shown that lambda waves disappear without patterned visual input (Hughes 1964).

The failure of previous researchers (Clemente *et al.* 1964) to elicit the same activity when a food deprived animal was given water can possibly be explained by the difference in the behavioral response; cats do not generally lap water for any prolonged period of time (and the results of Clemente *et al.* verified this), therefore the initiation of the synchronous electrical activity in the visual cortex never occurred.

Sadowski and Longo (1962) have reported "masticatory" waves in the EEG from the rabbit anterior sensori-motor cortex during eating, which were synchronized with the chewing movements. Although these researchers indicated that the "masticatory" waves spread to other cortical areas, they did not designate which areas were involved. One can only speculate that the striate cortex was one of these sites.

That the EEG synchrony in striate cortex is related to the rather global term "post-reinforce-

ment" appears fairly obvious but hardly seems descriptively adequate and is somewhat misleading. For example, when the animal is eating solid cat food, the post-reinforcement waves do not appear. To infer a causal relationship between the initiation of the synchronized EEG and a "post reinforcement" state is indeed misleading. Unfortunately, the cat does not chew in a very rhythmic manner, therefore chewing movements do not elicit a synchronous EEG pattern.

That the 7 c/sec activity can become associated with, and elicited by the conditioned stimulus during classical conditioning (Clemente *et al.* 1964) seems plausible. If indeed the repetitive tongue movement does initiate the 7 c/sec rhythm, the "intended" tongue movements which become established with conditioning in "anticipation" of the unconditioned stimulus (milk) should also initiate the 7 c/sec activity. There could be a "sub-lapping" movement which the animal makes prior to actually lapping (similar to sub-vocal movements in speech). This question can possibly be answered by recording the EMG of the tongue during a conditioning situation.

The question of why this synchronous slow EEG activity is not present in the auditory cortex is a puzzling one and does not appear solvable at the moment. However, a possible explanation might entail a consideration of the types of non-auditorys timuli which affect activity in single cells in the primary auditory cortex, and the ratio of such units to "pure acoustic units" as well as their inhibitory/activation relationship; *i.e.*, whether these non-auditory responding units are basically inhibited and/or activated. Should these relationships appear drastically different from those present in striate cortex, the distribution and coupling of activated and inhibited units present during lapping, for example, would be reflected by EEG activity which would in turn be different in terms of c/sec, amplitude, phase, etc.

In the present experiment, the split-brain visually occluded cats afforded an excellent opportunity to evaluate the relative contribution of visual input to the 7 c/sec synchrony in striate cortex. In comparing the data from the split-brain animals, both with and without visual occlusion, there did not appear to be a reliable or significant difference in the slow wave EEG synchrony in the striate cortex with visual occlusion of the ipsilateral eye during milk lapping. The conclusion seems inevitable: even though the rhythmic 7 c/sec activity was recorded in striate cortex, it was not affected by a total blocking of visual input; therefore it could not have been visually mediated.

That the striate cortex is directly involved in activity other than processing visual inputs has been amply demonstrated by the single cell research which reports unit responses to a variety of non-visual stimuli. Jung *et al.* (1963) were among the first investigators to report multisensory convergence occurring in single units of the primary visual cortex. Recent research (in our laboratory) has illustrated that unit activity exhibiting multisensory activation is probably not simply related to just a non-specific or general arousal effect, but that single cells in striate cortex of cat have a highly specific response pattern; *i.e.*, responses to tone, when frequency is varied throughout the auditory range, produce a specific pattern in individual cells (Spinelli *et al.* 1968). However, more relevant to the present study is evidence that a large percentage of single cells in striate cortex are inhibited when the animal makes a specific movement (Skrebitsky and Gapich 1967). Thus the single unit data stand as excellent corroborative evidence that the electrical activity in striate cortex need not be visually mediated.

Although it is obvious that there are numerous non-visual inputs present in such a complex act as lapping and ingesting milk, the predominant input would still most likely be that initiated by a highly repetitive movement such as lapping. The neuronal mechanisms which seem most tenable in effecting such a dramatic and well-defined electrophysiological response as the the 7 c/sec synchrony in striate cortex may be a massive inhibition of cellular activity produced by these inputs. The distribution and coupling of inputs produced by the ratio of visual/non-visual cells activated and inhibited by such a highly specific response could conceivably initiate a reliably consistent output of highly synchronous and relatively frequency specific waves in a neuronal network such as that in the striate cortex.

SUMMARY

1. When cats were presented a small amount of milk in a plastic feeder cup, their tongue movements made during lapping were recorded concomitantly with EEG records from posterior lateral gyri, suprasylvian gyri, and mid-ectosylvian gyri. Frequency analysis of the records revealed that a 7.5 c/sec activity was present only in the posterior lateral gyri during the lapping (7.5 was one of the center frequencies of the total ten 5-cycle bands of frequencies analyzed). The 7.5 c/sec activity was of relatively large amplitude and occurred only with repetitive lapping. Its initiation was approximately 300 msec or longer after the onset of lapping.

2. The synchronous oscillatory EEG activity did not occur with ingestion of solid cat food by the animals. Therefore, the fact that the slow wave EEG occurred due to the reinforcing properties of the milk hardly seems tenable. Solid cat food should be as reinforcing as milk.

3. The 7.5 c/sec synchronous rhythm occurred in both normal and split-brain animals. With visual occlusion of the split-brain cat with an opaque contact lens over one eye, and recording from the ipsilateral striate cortex, there was no reliable difference in the EEG record when compared with a record from the same animal and electrode without visual occlusion. Therefore, the 7.5 c/sec synchrony in striate cortex associated with lapping was definitely not visually mediated.

4. It is proposed that the synchronous slow wave EEG is initiated by a massive inhibition of cells in the striate cortex effected by the non-visual inputs associated with lapping. Microelectrode data showing inhibition in single cells correlated with movement and non-visual stimuli are cited as ancillary evidence for such an interpretation.

RÉSUMÉ

ANALYSE DE FRÉQUENCE DE L'EEG PENDANT L'ABSORPTION DE LAIT

1. On présente à des chats une petite quantité de lait dans une cupule de plastique; les mouvements de la langue effectués pendant le lappage sont enregistrés, concomitament aux tracés EEG des gyri postérieurs latéraux, suprasylviens et ectosylviens médians. L'analyse de fréquence de ces tracés révèle qu'une activité à 7,5 c/sec s'observe pendant le lappage, mais seulement au niveau des gyri latéraux postérieurs (7,5 est l'une des fréquences centrales des 10 bandes totales de fréquences analysées). L'activité à 7,5 c/sec est d'une amplitude relativement grande et ne survient qu'avec le lappage répétitif. Elle commence approximativement 300 msec après le début du lappage, ou même plus tardivement.

2. Cette activité EEG rythmique synchrone ne survient pas avec l'ingestion de nourriture solide par les animaux. Toutefois, que ces ondes lentes EEG surviennent à cause de propriétés de renforcement particulières au lait paraît difficilement soutenable. La nourriture solide pour chats devrait avoir les mêmes propriétés de renforcement que le lait.

3. Le rythme synchrone à 7,5 c/sec survient chez des animaux normaux aussi bien que chez des animaux à cerveau divisé (split-brain). Si chez le chat à cerveau divisé on fait une occlusion visuelle au moyen d'un verre de contact opaque sur un oeil, et que l'on enregistre le cortex strié ipsilatéral, on ne note pas de différence significative du tracé EEG d'avec le tracé du même animal et de la même électrode sans occlusion visuelle. Ainsi, il apparaît certain que la synchronie à 7,5 c/sec du cortex strié associée au lappage n'est pas médiatisée par la vue.

4. L'auteur suggère que les ondes lentes EEG synchrones sont initiées par une inhibition massive des cellules du cortex strié due aux afférences non visuelles associées au lappage. Des données micro-physiologiques montrant une inhibition au niveau de cellules isolées en corrélation avec des mouvements et des stimuli non visuels sont citées comme arguments auxiliaires d'une telle interprétation.

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