Since the composition of the cell wall is highly complex, it is very difficult to try and identify the chemical nature of the dissociable groups and their apparent dissociation constant. However, it may be recalled that, within pH 2 and 6, biologically important chemical groups such as carboxylic and phosphate dissociate.

In *S. aureus* cell wall 262 μ-equiv./g of dissociable groups have been found. Certainly this figure is less than the real number assuming that the terminal carboxylic groups and the side-carboxyl ones were free. Out of the 262 μ-equiv./g found, 28 have pK 4.70 and are certainly identifiable with the carboxylic groups. In fact pK 4.70 is very close to the acetic acid pK and the dissociation pK of carboxylic groups titrated in various proteins (e.g. ribonuclease[9]). Of the remaining 232 μ-equiv./g, it is difficult to state exactly what chemical groups are dissociable with pK 3.19. They may be either terminal α-carboxyl groups (whereas those with pK 4.56 would be side-chain-carboxyl ones[9]) or the phosphate groups of teichoic acid. The highly negative polyribitol phosphate chain certainly acts as the major cell wall polyelectrolyte. Most of the dissociable groups with pK 3.19 are probably phosphate. STROMINGER[10] states that the special structures in some strains of *S. aureus* account for about 20% of the cell wall. In this figure are included glycine polypeptides. Thus, as the *S. aureus* cell wall contains about 12% teichoic acid and, according to the structure reported by BADDILEY et al.[11], the molecular weight of the polymer structural unit is about 490, 1 g of cell wall contains about 245 μ-moles of polymer structural unit. Since each unit contains a dissociable phosphate group, this number agrees well enough with the μ-equiv./g of hydrogen ions bound with pK 3.19. On the other hand, 59.50 μ-moles of total phosphorus were measured on 1 g of *S. aureus* cell wall (strain 22 ISI). When pH is above 6.00 NH_2 groups probably dissociate ($-NH_3^+ \rightarrow NH_2 + H^+$); the pK 7.15 found is very close to the pK 7.8 of NH_2 groups for small molecules[9].

Riassunto. Il cell-wall di *Staphylococcus aureus* è capace di legare cationi. Poichè la capacità legante ioni dipende dal pH del mezzo, si è studiata la capacità legante idrogenioni di cell-wall liofilizzato isolato da *S. aureus*. Titolazioni al di sotto di pH 9.0 hanno dimostrato la presenza di 3 siti leganti con pK rispettivamente di 7,15, 4,70 e 3,19 e capacità massima legante di 2, 28 e 232 μ-equiv./g (peso secco cell-wall). Viene discussa la natura chimica dei gruppi dissociabili.

F. GALDIERO

Institute of Microbiology, The University of Naples (80138 Napoli, Italy), 20 November 1967.

[9] C. TANFORD and J. D. HAUENSTEIN, J. Am. chem. Soc. *78*, 5287 (1956).
[10] J. L. STROMINGER, in *Bacteria* (Ed. J. C. GUNSALUS and R. Y. STANIER; Academic Press, New York and London 1962), vol. 3, p. 418.
[11] J. BADDILEY, J. G. BUCHANAN, R. O. MARTIN and U. L. RAJBHANDARY, Biochem. J. *85*, 49 (1962).

Visual and Auditory Evoked Potentials: Specificity of Reticular Formation Modulating Influences

It has become commonplace to consider the brain stem reticular system as exerting diffuse, non-specific influences[1,2]. This conclusion is based largely on studies of lesions and electrical stimulation, both of which are non-selective interventions. The former abolishes all neural conduction in the immediate vicinity, and the latter induces both orthodromic and antidromic impulses in all susceptible synapses and fibres of passage, whether they are facilitatory or inhibitory[3,4]. Chemical stimulation, however, offers a possible solution to these problems since depositing suspected neurotransmitters in the vicinity of synapses may mimic the actions of endogenous neurohumors by activating or inhibiting only those neurons whose postsynaptic membranes are susceptible to that substance[5-7].

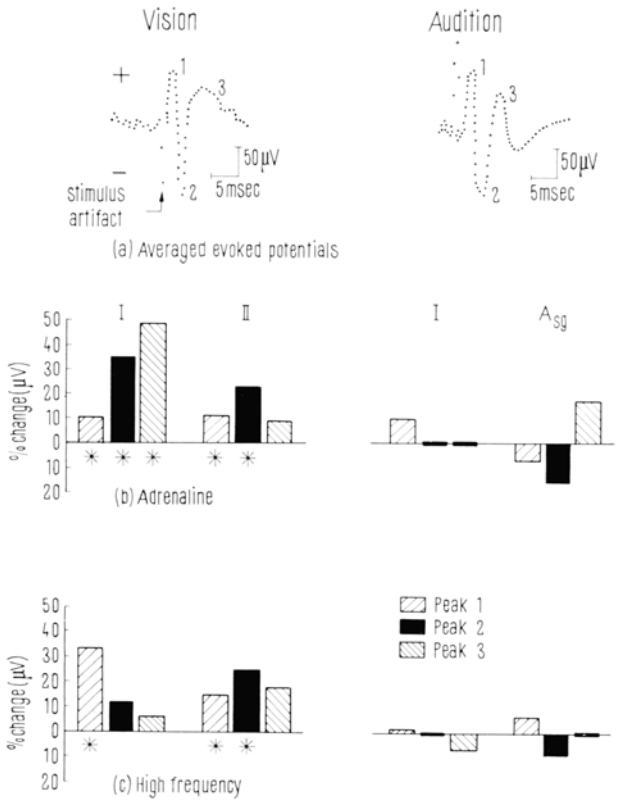
A number of investigators have demonstrated modification of sensory evoked potentials by electrical[8-10] and chemical[3,11] stimulation of the reticular formation. In the majority of cases it has been assumed that the reticular influences would have been exerted in like manner over all modalities. The present study was designed to investigate in the same animals the effects of chemical and electrical reticular activation on both visual and auditory evoked potentials.

Methods. Eleven acutely implanted cats were prepared for surgery under ether anesthesia and then maintained on artificial respiration with Flaxedil. Pressure points and incised tissues were topically treated with 2% Xylocaine[12]. Visual and auditory potentials were evoked by single shocks to the optic tract and brachium of the inferior colliculus, respectively. Evoked potentials were recorded from visual cortices I and II, and primary and association auditory cortices[13]. Drugs in solution, adjusted to pH 7.4, consisting of 20 μg of adrenaline bitartrate dissolved in 20 μl of normal saline, and 20 μg of

[1] J. D. FRENCH, in *Handbook of Physiology, Sect. I: Neurophysiology* (Eds J. FIELD, H. W. MAGOUN and V. E. HALL; American Physiological Society, Washington, D.C. 1960), vol. 2, p. 1281.
[2] D. B. LINDSLEY, in *Handbook of Physiology, Sect. I: Neurophysiology* (American Physiological Society, Washington, D.C. 1960), vol. 3, p. 1553.
[3] M. DEMETRESCU and M. DEMETRESCU, Electroenceph. clin. Neurophysiol. *14*, 602 (1962).
[4] S. P. GROSSMAN, R. PETERS, P. FREEDMAN and H. WILLER, J. comp. physiol. Psychol. *59*, 57 (1965).
[5] P. D. MACLEAN, A.M.A. Archs Neurol. Psychiatry *78*, 113 (1957).
[6] R. HERNANDEZ-PEON, G. CHAVEZ-IBARRA, P. J. MORGANE and C. TIMO-IARIA, Expl Neurol. *8*, 93 (1963).
[7] S. P. GROSSMAN, J. comp. physiol. Psychol. *61*, 42 (1966).
[8] F. BREMER and N. STOUPEL, Archs int. Physiol. Biochim. *67*, 240 (1959).
[9] S. DUMONT and P. DELL, Electroenceph. clin. Neurophysiol. *12*, 769 (1960).
[10] M. STERIADE and M. DEMETRESCU, Electroenceph. clin. Neurophysiol. *14*, 21 (1962).
[11] J. COURVILLE, J. WALSH and J. P. CORDEAU, Science *138*, 973 (1962).
[12] Supplied by Astra Pharmaceutical Products, Worcester, Mass.
[13] R. THOMPSON, R. JOHNSON and J. HOOPES, J. Neurophysiol. *26*, 343 (1963).

acetylcholine bromide dissolved in 10 μl of Tyrode solution, were injected by a microsyringe attached to a 23 gauge stainless steel tube which was inserted into a 19 gauge guide cannula. Drugs were injected into the pontine and bulbar reticular formation[14]. For high frequency stimulation of the same sites into which the drugs were injected, a 0.01 inch stainless steel wire, insulated except at the tip, was inserted into the guide for 2 sec trains of monopolar stimulation at 150/sec, 0.1 msec pulse duration, 1.2 V. At the end of each experiment, the drug and electrical stimulating sites were verified histologically[15].

The interstimulus interval was 3 sec. Stimuli to each modality were alternated in groups of 12. After habituation trials, control recordings were taken for approximately 10 min, and without interruption of the sensory stimulation, a drug cannula was inserted into the guide and the solution injected slowly over a period of 60–80 sec. The experimental data consisted of potentials recorded for approximately 5 min after the end of a drug injection. For high frequency stimulation trials, a shock to one of the sensory systems was delivered 100 msec after the end of a 2 sec train of 150/sec pulses to the reticular formation. The intertrain interval was maintained at 3 sec. Since recordings were taken immediately before a manipulation, each subject acted as its own control.



(a) Averages of 10 visual and 10 auditory evoked potentials showing the 3 peaks analyzed. (b) Mean % changes in amplitudes of the 3 components of the evoked potentials during pontine reticular formation activation by adrenalin. (c) Same as (b) except for reticular activation by high frequency electrical stimulation. The asterisks indicate a statistically significant increase of the experimental potentials compared to controls at the 5% level or better. Vision I: posterolateral gyrus, Vision II: posterior suprasylvian gyrus; Audition I: middle ectosylvian gyrus; Audition A_{sg}: auditory association area on the anterior suprasylvian gyrus.

The evoked potentials were amplified and recorded on a multichannel FM tape recorder, and averaged off-line in groups of 10 (the last 10 of each group of 12) by an average response computer, and photographed. Three components of the averaged potentials were analyzed separately for changes in amplitude from baseline. These were: (1) the largest initial positive peak; (2) the following largest negative peak; and (3) the next longer duration positive peak. These are represented in Figure a as tracings of the averages of 10 visual and 10 auditory evoked potentials.

Results. The data were analyzed statistically by multiple two-way analyses of variance with repeated measures. Each analysis was based on from 160–440 control evoked potentials and an equal number of experimental potentials. The results in Figure b compare mean % changes of visual and auditory potentials when the pontine reticular formation was activated by adrenaline. The asterisks indicate statistical significance at the 0.05 level or better (i.e., the experimental potentials were significantly different from their own controls with 95% confidence). It can be seen that visual but not auditory potentials were influenced by adrenaline injected into the pontine area. Moreover, from the Vision I site, mean % increases appear to be directly proportional to the latency, that is, the % change of peak 3 is greater than that of peak 2, and that of peak 2 is greater than that of peak 1. The same trend seems to begin in Vision II but breaks down at peak 3. Adrenaline injected into the bulbar area and acetylcholine injected into either brain stem site effected occasional increases of the visual and auditory potentials, but no consistency or pattern could be discerned. Figure c compares visual and auditory potentials when the pontine site was activated electrically. It can be seen that the adrenaline effect is essentially confirmed by high frequency stimulation.

Conclusions. A number of conclusions can be drawn from these data which indicate a fair degree of specificity of reticular control. First, there is a modality specificity of reticular influences since the visual potentials were much more strongly affected than the auditory potentials. This was true for both chemical and electrical reticular activation. Second, there is drug specificity since the adrenaline was more effective in influencing the potentials than was the acetylcholine. Finally, the site of the drug injection is an important variable since the pons was more susceptible to drug influence than the more caudal brain stem injection site[16].

Zusammenfassung. Adrenalinzufuhr wie auch hochfrequente elektrische Reizung am pontinischen Gehirnstamm führen zu Potentialverstärkung, wenn diese nicht visuell, sondern auditiv geweckt wurde. Entsprechende Versuche mit Acetylcholin führten zu keinem Erfolg, was für die Spezifität der retikularen Einflüsse spricht.

TH. D. LANGLEY[17] and J. SIEGEL

Department of Psychology, University of Delaware, Newark (Delaware 19711, USA),
16 October 1967.

[14] R. SNIDER and W. NIEMER, *A Stereotaxic Atlas of the Cat Brain* (The University of Chicago Press, Chicago 1961).

[15] J. SIEGEL, Physiol. and Behav., in press.

[16] Supported by NSF grant No. GB 4706-X to J. S. and NASA predoctoral fellowship to T.D.L.

[17] Present address: Union Carbide Corp., Linde Division, Biological Research Laboratories, Box 44, Tonawanda, New York (USA).