Non-acoustic Factors influencing Activity of Middle Ear Muscles in Waking Cats

THE intensity of sound transmitted from the ear drum to the inner ear can be attenuated by contraction of the middle ear muscles, the tensor tympani and stapedius. These muscles contract at the onset of sound, thereby reducing the size of cochlear microphonics as recorded at the round window. Previous explanations of middle ear muscle activity have only considered possible acoustic functions[1-4]. However, in waking, unrestrained cats, we have found middle ear muscle activity related to non-acoustic factors, for example, bodily movement and vocalization. In animals that we have experimentally deafened by crushing both eight nerves, non-acoustic contractions persist in association with bodily movement and vocalization. In these animals all acoustic middle ear muscle activity is absent, the neural component is absent from the cochlear response recorded at the round window, and they show no behavioural reaction to loud sounds.

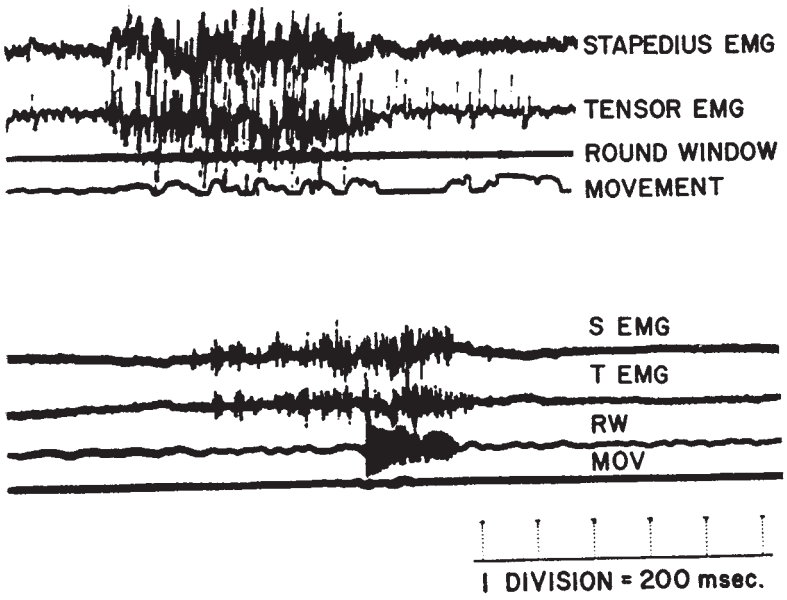

Fig. 1. Non-acoustic middle ear muscle responses. Oscilloscopic recordings during movement (top) and vocalization. The cry, as the cat hears it, is shown at the round window (bottom)

Action potentials of both intra-aural muscles were recorded in 11 cats through chronically implanted bipolar electrodes made of 36 gauge stainless steel wire. Cochlear responses were simultaneously recorded from the round window of the same ear. The electrodes were carried in a Sheatz pedestal attached over the frontal sinus, to which a cable of double-shielded wires ('Micro Dot') was attached. This set-up was virtually free of electrical noise produced by movement of the electrodes, pedestal, or cable. Responses were amplified, displayed oscilloscopically, and quantified by means of electronic averaging[5]. Small movements, such as turning of the head, were detected by a piezo-electric cartridge mounted on the cage. Limited only by a flexible cable attached to the pedestal, the cats were free to move about the cage. Sound was delivered as a free-field stimulus from a loud-speaker mounted 30 in. above the cat's head. Sound field

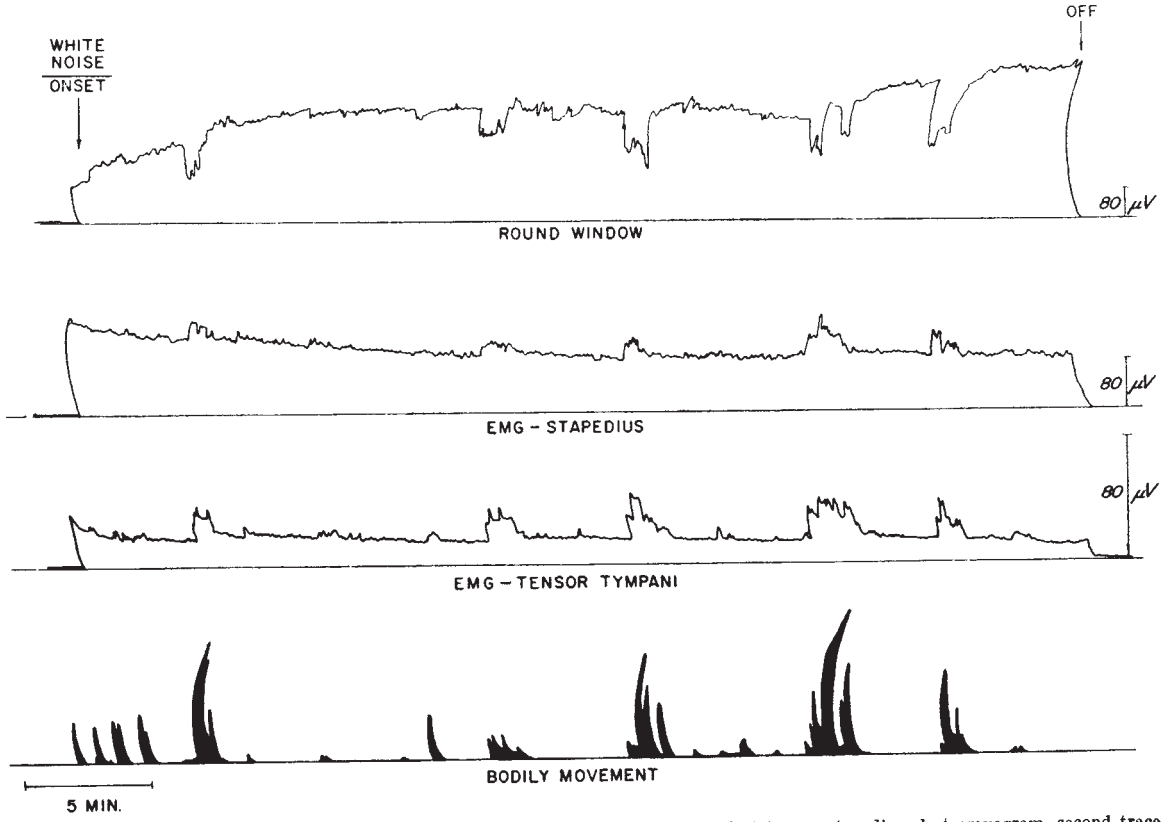

Fig. 2. Averaged responses during prolonged white noise (85 db.). Round window, first trace; stapedius electromyogram, second trace; tensor tympani electromyogram, third trace; movement, fourth trace

intensity, as measured at the corners and centre of the cage, varied only ± 3·4 db. (at 85 db. sound pressure-level).

Bodily movements are associated with activity of both tensor tympani and stapedius muscles (Fig. 1, top). This activity occurs simultaneously with or, more usually, slightly in advance of the animal's movement. Vocalization is also associated with middle ear muscle activity (Fig. 1, bottom), the muscle action potentials preceding the cochlear microphonic response by 75–500 msec. Vocalization may be considered non-acoustic in its effects on ear muscles since these muscles respond prior to the sound and since the response persists in deafened animals. Middle ear muscle activity continues during vocalization, generally stopping simultaneously with cessation of the microphonics.

Middle ear responses to continuing sound stimulation with a broad frequency noise of constant intensity (85 db. sound pressure-level) are shown in Fig. 2. Bodily movements are associated with an increase in middle ear muscle activity (first and second traces) and a corresponding decrease in cochlear activity recorded at the round window (third trace). Large, rapid bodily movements are associated with greater ear muscle activity than are slower movements or movements involving only the head or a single limb. The attenuation of round window averaged responses induced by middle ear activity ranges up to 14 db. in association with movement.

Bodily movement and vocalization may be added to other non-acoustic events associated with middle ear muscle activity, including yawning[6], swallowing[6], cutaneous stimulation of the pinna[7], defensive reaction elicited by noxious stimuli[7], and the tegmental motor reaction elicited by reticular formation stimulation[8].

Middle ear muscle activity, whether acoustic or non-acoustic in origin, results in modifications of input to the central auditory pathways. Attenuation of sound-evoked responses at cochlear nucleus[9], and round window[10] have been reported during 'attention' or 'orienting responses'. However, this attenuation may largely reflect effects of middle ear muscle contraction imposed as a consequence of bodily movement. In the absence of overt movement, animals staring at a bright flashing light show neither middle ear muscle activity nor alteration in round window response[11].

The interpretation of middle ear muscle activity as merely serving acoustic functions is too limiting. The fact that contractions occur in association with a wide variety of motor patterns suggests that middle ear muscle activity is intimately correlated with other motor-control arrays within the central nervous system. This sensory-motor integration may significantly influence experiments designed to study acoustic perception.

We thank Dr. Robert B. Livingston, in whose laboratory this work was done, for his advice.

Peter W. Carmel
Arnold Starr

Laboratory of Neurobiology,
National Institute of Mental Health,
National Institutes of Health,
Bethesda, Md.

[1] Hallpike, C. S., *J. Laryng. Otol.*, **50**, 363 (1935).
[2] Hilding, D. A., and Fletcher, J. L., *Intern. Rec. M.*, **173**, 369 (1960).
[3] Wiggers, H. C., *Amer. J. Physiol.*, **120**, 771 (1937).
[4] Simmons, F. B., and Beatty, D. L., *Science*, **138**, 590 (1962).
[5] Starr, A., and Livingston, R., *J. Neurophysiol.*, **26**, 716 (1963).
[6] Klockhoff, I., *Acta Otolaryng.* (Stockholm), Supp. 164, 32 (1961).
[7] Kato, T., *Pflüg. Arch. ges. Physiol.*, **150**, 569 (1913).
[8] Hugelin, A., Dumont, S., and Paillas, N., *Electroencephalog. Clin. Neurophysiol.*, **12**, 797 (1960).
[9] Hernandez-Peon, R., Scherrer, H., and Jouvet, M., *Science*, **123**, 331 (1956).
[10] Simmons, F. B., and Beatty, D. L., *Science*, **138**, 590 (1962).
[11] Carmel, P., and Starr, A., *J. Neurophysiol.*, **26**, 598 (1963).

Effect of Insulin on Accumulation of Radioactivity from Amino-acids by Isolated Intact Rat Diaphragm

Insulin *in vitro* enhances the incorporation of labelled amino-acid into protein of isolated rat diaphragm independent of its action in facilitating glucose transport[1,2]; neither the precise mechanism nor the exact site at which the hormone acts to stimulate protein synthesis is known.

In circumstances similar to that in which it increases amino-acid incorporation into protein, insulin will also increase accumulation of ^{14}C-α-aminoisobutyric acid, a non-utilized amino-acid, and of some, but not all, of the utilized amino-acids (cf. Table 2 for a summary and for references). This led to the postulation that accelerated amino-acid transport was responsible for the insulin mediated stimulation of amino-acid incorporation into protein; an idea with great appeal, for it provides unity to the effect of insulin on carbohydrate and protein metabolism—in both cases insulin acts, presumably, to enhance substrate transport. Despite the attractiveness of the transport hypothesis there are observations that fail to support it and lead instead to the conclusion that insulin can influence protein synthesis in muscle at a site distal to amino-acid transport, perhaps by an effect on some intracellular process[3–5]. There is, however, no experiment that excludes with certainty the possibility that insulin also stimulates amino-acid penetration into muscle; indeed, while there is no certain evidence that insulin promotes entry into muscle, it is equally true that there is no experiment that decisively rules out the possibility.

Insulin increases accumulation by isolated intact rat diaphragm of three (glycine, proline, methionine) of the thirteen natural amino-acids so far tested (Table 2). These three amino-acids do not share any chemical characteristic that distinguishes them from the others the entry of which into muscle is not increased by the hormone. We have now tested the response to insulin of six of the seven amino-acids not previously tested and can add two (threonine and hydroxyproline) to the list of amino-acids the entry of which into muscle is stimulated by insulin; in addition, one amino-acid (serine) previously considered not responsive[6] has been shown to be so.

The results are summarized in Table 1: insulin stimulated accumulation by isolated intact diaphragm of radioactivity from labelled L-threonine and L-hydroxyproline, but not from L-arginine, DL-tryptophan, L-isoleucine or DL-cystine. The hormone, nevertheless, significantly increased incorporation of radioactivity into protein from each of the amino-acids tested without regard to whether penetration into muscle was enhanced; a dichotomy between the two processes observed before[7]. Insulin also stimulated accumulation of L-serine—a finding in disagreement with another report[6]. The discrepancy cannot at present be explained, but the effect of the hormone is consistently reproducible.

There is nothing of the structure or metabolism of the six natural amino-acids and of the four model amino-acids responsive to insulin, or of the mechanism of amino-acid transport in muscle, that allows systematic sense to be made of the findings. It has been suggested[6] that insulin lowers the K_m of the amino-acid transport process without affecting the V_m, and that if the effect of the hormone is measured at an external concentration of amino-acid in excess of the K_m, then a decrease of the K_m by insulin might still leave the process saturated. This possibility was tested by measuring the effect of insulin on L-leucine accumulation at a concentration of the amino-acid of 10^{-9} M and no effect of the hormone was seen (Table 1). The actual K_m for L-leucine entry into muscle is not known, but if it is less than 10^{-9} M it can scarcely have physiological significance, for that concentration is 4–5 orders of magnitude lower than the concentration in rat plasma ($1{\cdot}5 \times 10^{-4}$ M; Scharff, R., and Wool, I. G., unpublished observation).