almost as small as those of prey-deprived controls; these males had failed to copulate and were the ones having ejaculatory ducts unusually small in diameter.

Since the corpora allata might control the onset of sexual behavior indirectly, through development of reproductive organs, the ejaculatory ducts and testes of newly emerged males were removed through incisions in the abdomen; these males were then allowed ten *Musca*, which were restored daily. Six days after emergence only one male survived, which then attempted to copulate with females; one testis had not been removed from this specimen. Of ten males deprived of ejaculatory ducts and both testes after sexual maturity, six continued to attempt copulation for as long as 1 day later, when all had died.

These experiments hinted that the corpora allata hormone acts independently both on development of reproductive organs and on sexual behavior. This suggestion was substantiated by implantation of two retrocerebral complexes (containing corpora allata, corpora cardiaca, and hypocerebral ganglia) of sexually mature and well-fed males into the thorax of each of seven newly emerged males. By 11 days after emergence, all three surviving prey-deprived recipients of implants displayed sexual behavior, while none of the six unoperated prey-deprived controls did. None of the recipients of implants had ejaculatory ducts nearly as large as those that normal prey-supplied males have at the onset of sexual maturity, and insemination was not achieved; but the mean duct diameter of the seven recipients (including those dying 5 to 7 days before the mating test) was greater than that of the controls (t, 2.95; d.f., 10; $P < .01$; one-tailed test), and the differences in length of testis showed a similar relation (t, 1.87; d.f., 10; $P < .05$; one-tailed test).

Possibly the act of feeding stimulates production of corpora allata hormone in *Scatophaga* males, but such stimulation has not been found in female insects of other species. Studies of the influence of deprivation of prey in *Scatophaga* males showed that hunger is manifested through both increase in prey killed relative to time and increase in tissue consumed per unit of prey. Thus, when males were allowed to prey on only three *Musca* daily from emergence, they consumed more tissue from each prey, so that development of reproductive organs and onset of sexual behavior were only slightly more retarded than in males allowed ten *Musca* daily. This finding suggests that the amount of complex diet consumed, rather than the number of prey killed, controls the rate of sexual maturation.

It is also unlikely that gut expansion triggers corpora allata activation in insects that feed frequently (*7*): In *Scatophaga,* imbibition of sucrose solutions has no effect on sexual maturation even though the abdomen becomes distended by the engorged diverticulum; the tissues of consumed prey move straight to the midgut, but do not cause it to become greatly enlarged. It appears rather that food in the gut or metabolites in the hemolymph act indirectly on the corpora allata through the central nervous system [as Johansson (*7*) has proposed for the female milkweed bug *Oncopeltus fasciatus*], or that metabolites in the hemolymph act directly on the corpora allata, as Strangways-Dixon (*8*) has proposed for the female blow fly *Calliphora erythrocephala.*

Thus it appears that *Scatophaga* males are nutritionally anautogenous in the full physiological sense ascribed to many female insects, whereas, in most male insects so far studied, the males are autogenous because the corpora allata are activated spontaneously at emergence or are unnecessary for sexual maturation. The corpora allata hormone may control a switch mechanism allowing sexual responses to females, and associated sexual behavior. Although the hormone could act by blocking a nervous inhibitor of the behavior, the whole nervous mechanism would lie within the thoracic ganglion, for decapitated mature males respond sexually to females on contact, whereas decapitated immature males do not.

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11 September 1967

Responses of Human Somatosensory Cortex to Stimuli below Threshold for Conscious Sensation

Abstract. *Averaged evoked responses of somatosensory cortex, recorded subdurally, appeared with stimuli (skin, ventral posterolateral nucleus, cortex) which were subthreshold for sensation. Such responses were deficient in late components. Subthreshold stimuli could elicit sensation with suitable repetition. The primary evoked response was not sufficient for sensation. These facts bear on the problems of neurophysiological correlates of conscious and unconscious experience, and of "subliminal perception."*

Previous studies have indicated that the first appearance of any evoked potential in sensory cortex, elicited by a stimulus to skin or sensory nerve, coincides with the threshold for some report of subjective sensation by the human subject (*1*). A similar relationship was reported for the threshold of sensory discrimination in the cat, upon stimulation of a cutaneous nerve (*2*). Such conclusions were based upon recordings made with electrodes on the scalp or situated epidurally. This provides a relatively diffuse lead from unresponsive as well as responsive cortex (see, for example, *3*). It has been demonstrated in monkeys that localized responses recorded with cortical surface electrodes may be essentially indetectable with scalp electrodes (*4*); we have found this to be true in man (see also *3*). In the present work, the recording electrode is placed subdurally, directly on the pia-arachnoid surface of somatosensory cortex (postcentral gyrus). In addition, the stimulus to the skin or to ventral posterolateral (VPL) nucleus of thalamus is so located as to elicit a sensation within the same somatic area as that in which the sensation was subjectively felt when the recording site on somatosensory cortex was stimulated directly. With such relatively precise localization it has be-

come quite evident that at least some components of the evoked potential are recordable in somatosensory cortex with stimulus levels which are distinctly below those required to elicit any conscious sensory experience. This was true whether stimuli were applied to the skin, the specific projection relay nucleus in the thalamus (VPL nucleus), or directly to somatosensory cortex.

Subjects were patients undergoing stereotaxic neurosurgical therapy for motor dyskinesias or intractable pain, who volunteered some study time during the operative stage in which they had to remain unanesthetized for purposes of therapy (*5*). Local anesthetic was injected into the scalp but generally no premedications were administered. Conditions of such experiments and criteria for conscious sensory experience have been described earlier (*6, 7*). The subject was alerted to attend to the stimulus and was asked to report (i) whether he subjectively experienced or "felt" a sensation even if it was very weak, (ii) whether he felt none at all, or (iii) if he was uncertain about having felt a sensation. With single-pulse stimuli the range of stimulus intensity of which the subject was uncertain was usually small (less than 5 percent for skin); false positive responses almost never occurred. (Throughout this report, the terms *threshold* or *subthreshold* refer exclusively to the ability or inability of stimuli to elicit conscious sensory experience, rather than to their ability to elicit electrophysiological responses. In order to keep this distinction clear to the reader, the term *threshold-c* will be used to describe threshold stimuli that can elicit conscious experience. We are not implying that a subthreshold stimulus as determined under the present conditions would necessarily remain subthreshold under all conditions of testing, for example, with extensive training of the subject.) All recordings were unipolar (*8*), with the three skull contacts of the stereotaxic frame generally serving as the reference or indifferent lead (except for d-c recordings). The stimuli were constant-current electrical pulses applied to the skin through a 5-mm disc, or to VPL by means of a coaxial needle electrode. To elicit direct cortical responses, stimuli were applied within 1 mm of the recording site by a twisted pair of metal wires.

Averaged evoked potentials elicited in somatosensory cortex by various strengths of skin stimuli are shown for two representative subjects in Fig. 1 (*9*). Threshold-c level (*T* in Fig. 1) refers to bare threshold intensity at which not all the stimuli in the series elicited a subjective sensory experience. All the components which are visible at much higher stimulus intensities (for example, at twice threshold-c) may already be present at threshold-c, though with smaller amplitudes. Intensity of the subthreshold-c stimulus (designated *SubT* in Fig. 1) was such that none of the stimuli gave rise to conscious sensation. Nevertheless, a distinct evoked potential was visible with such subthreshold-c stimuli. It should be noted that when a scalp lead was placed so as to lie over the postcentral gyrus, and was recording simultaneously with the subdural lead, it did not exhibit an evoked potential with subthreshold-c stimuli or generally even with threshold-c stimuli. This latter negative result was apparently a function of the small area of skin stimulated; when the median nerve was stimulated at the wrist, appearance of an evoked potential coincided roughly with threshold-c.

Although skin stimuli which were completely subthreshold-c could elicit distinct evoked potentials, recorded subdurally, the responses showed some differences from those obtained with threshold-c or stronger stimuli. The initial positive component and even more so the succeeding negative one were smaller than at threshold-c level, while the still-later positive and negative waves (which are smaller and longer lasting) were not detectable at all.

It might be argued that subthreshold-c skin stimuli which elicit evoked potentials are exciting a class of afferent fibers which can never elicit conscious sensory experience; indeed Swett and Bourassa (*2*) have found that cats were unable to discriminate afferent volleys in larger nerve fibers of deep nerves, even though certain of these (in the deep radial nerve) could evoke a large cortical response. In our studies, however, subthreshold-c stimulus pulses which produce an evoked potential could elicit a conscious sensory experience, that is, they could become threshold-c if delivered repetitively at higher frequencies (20 to 60 pulses per second) for a brief period. In fact, the minimum intensity of single-pulse stimuli which could evoke some detectable cortical potential appeared to coincide roughly with the minimum threshold-c current required by a train of such pulses to elicit a sensation. The minimum threshold-c current was achieved by trains with durations longer than a certain minimum, 0.05 to 0.1

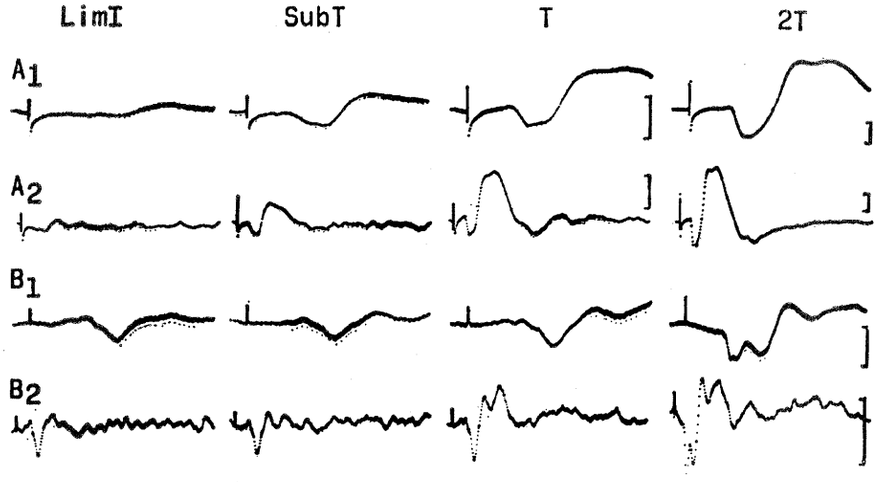

Fig. 1. Averaged evoked potentials of somatosensory cortex in relation to threshold stimuli at skin. Each tracing is the average of 500 responses at 1.8 per second. Total trace length is 125 msec in A_1 and B_1 and 500 msec in A_2 and B_2; beginning of stimulus artifact has been made visible near start of each tracing. *A* and *B*, separate subjects, both parkinsonian patients. Vertical column *T*: threshold stimuli, subject reporting no feeling of some of the 500 stimuli. Column *2T*: stimuli at twice threshold current; all stimuli felt distinctly. Column *SubT*: subthreshold stimuli, none felt by subject; current about 15 percent below *T* in subject A, 25 percent below *T* in B. Column *LimI*: subthreshold stimuli at "liminal intensity" (see text), about 25 percent below *T* in subject A, about 35 to 40 percent below *T* in B. Polarity, positive downward in all figures. Vertical bars in *A*, under *T*, indicate 50 μvolt in A_1 and A_2 respectively, except for those in *2T* as shown; for B_1 and B_2, 20-μvolt bars. (Calibration obtained by summating 500 sweeps of calibrating signal.)

second for stimulus pulses to skin. This minimum threshold-c current for such trains is referred to as the *liminal I* (see *6, 7*). Liminal I was generally about 15 to 20 percent below the intensity required for threshold single pulses at the skin; it was also usually below the first completely subthreshold-c level for single pulses, as in subjects A and B in Fig. 1. The same afferent nerve fibers which were excited by subthreshold-c single pulses undoubtedly included those responsible for eliciting some conscious sensory experience when the subthreshold-c pulses were delivered at a suitable repetition rate; it is highly unlikely that afferent nerve fibers which are only excited by single pulses at threshold-c strength are recruited by temporal summation with subthreshold-c stimuli when pulse frequencies as low as 20 to 60 pulses per second are employed. That several cutaneous afferent nerve fibers from the finger must be excited to elicit a conscious experience, when single-pulse stimuli are used, has been found by Buchthal and Rosenfalck (*10*).

The ability of subthreshold-c stimuli to elicit some components of the evoked potentials in sensory cortex is even more striking when localized stimuli are applied to the specific projection pathway in the thalamus, that is, to nucleus VPL. In VPL we have found that single stimulus pulses (or pulses repeated at 2 pulses per second) were completely inadequate to elicit a conscious sensory experience (or a motor response), even with peak currents which were as much as 20 times liminal I [liminal I being the minimum threshold-c current, with a train of pulses, usually at 60 pulses per second, lasting for 0.5 second or more, when stimulating VPL or somatosensory cortex directly; see Libet *et al.* (*6*)]. For example, in Fig. 2 the VPL stimulus pulses at 1.8 pulses per second and with a strength six times liminal I did not elicit any conscious experience. The primary (initial positive) evoked potential was nevertheless recorded from somatosensory cortex. To elicit a similar amplitude of primary evoked response to skin stimuli, the latter had to be raised to twice threshold-c current (*S* in Fig. 2). The difference between evoked responses to VPL and skin stimuli arises in components following the primary one. Ervin and Mark (*11*) have reported that the types of evoked potentials they recorded with scalp leads, in response to stimulation with a thalamic electrode, could also appear without any awareness of the stimulus by the patient.

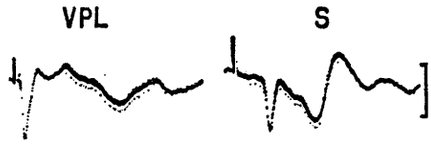

Fig. 2. Evoked potentials of somatosensory cortex in response to thalamic (*VPL*) and skin stimuli in the same subject (patient with heredofamilial tremor). Each tracing is the average of 250 responses at 1.8 per second; total trace length, 125 msec. *VPL*: stimuli in ventral posterolateral nucleus of thalamus; subject reported not feeling any of these stimuli, though current was 6 times liminal I for *VPL* electrode (liminal I being minimum current to elicit sensation with 60 pulses per second train of stimuli). *S*: stimuli at skin; current at twice threshold; all stimuli felt. Vertical bar, 50 μvolt. Note the shorter latency of the primary (positive) evoked response to *VPL* stimulus.

Direct cortical responses (DCR's) to stimuli applied nearby directly on sensory cortex can also be elicited by stimuli which produce no conscious sensory experience. These responses are large enough to be clearly visible above the level of background activity without averaging. Here too, single-pulse stimuli (0.5 msec pulse duration) are relatively ineffective for eliciting sensation, the first response with a sufficiently strong single pulse being a muscular twitch (*6, 7*). Thus, strong single-pulse stimuli at intensities up to several times the liminal I can elicit large DCR's without any subjective experience (Fig. 3). Single pulses with strengths which are below even liminal I can still elicit DCR's (Fig. 3A). If a train of liminal I pulses is cut short below 0.5 second, one can observe the abbreviated train of relatively large DCR's, again with no conscious experience accompanying it. Indeed, no distinctive change in DCR responses other than in amplitude or in duration of repetition period could be observed to accompany the transition between stimuli which were inadequate and those which were adequate for eliciting a sensory experience.

It appears evident, then, that neither the primary component of a single evoked potential nor a single DCR response complex represents or leads to the adequate cerebral condition which is associated with a conscious sensory experience, even in the awake, attentive human subject. Although the primary positive component of the evoked response was larger with threshold-c as opposed to subthreshold-c stimuli to skin, mere increase in amplitude of this response does not appear to provide the crucial difference for adequacy. This conclusion follows from the inability of strong single-pulse stimuli in VPL to elicit any sensation, even though these stimuli produced larger primary responses at the cortex than did threshold-c stimuli at skin. The evidence does not rule out some role for the primary response in sensation. However, the appearance of later components of the evoked potentials elicited by skin stimuli seem to be even better correlated with sensory awareness and may be equally if not more significant for conscious processes than the primary response (*6, 7*; see also *12*). The requirement of a minimum train duration of about 0.5 second for cortical or VPL stimuli at liminal I (minimum threshold-c current) may represent an activation period which substitutes for the normally occurring late components of the evoked potential elicited by single skin stimuli at threshold-c (*6, 7*). The further hypothesis has been proposed (*7*) that one physiological difference between conscious and unconscious experience in the awake and alert individual may lie in the duration of neuronal activation.

In contrast to earlier indications our results demonstrate that, when suitably recorded, cortical evoked potentials are detectable with sensory inputs below the adequate level for conscious

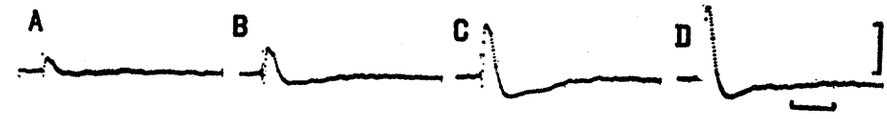

Fig. 3. Direct cortical responses (DCR) evoked in somatosensory cortex by adjacent direct stimuli (0.3-msec pulses). Each tracing is the average of 18 responses at 0.5 per second; horizontal bar in *D* indicates 100 msec. Subject is a parkinsonian patient. *A*, stimulus current 0.3 ma; *B*, 0.8 ma (equal to liminal I for trains of 20 pulses per second to elicit sensation in this subject); *C*, 1.7 ma; *D*, 5.0 ma (4 ma gave a similar response). Subject reported not feeling any of these stimuli, in *A* to *D*. Vertical bar, 200 μvolt.

sensation, even when the attention of the subject is directed to the stimulus. This fact may be taken to indicate that a possible physiological basis could exist for so-called "subliminal perception," at least under the conditions of stimulation employed (in contrast to the conclusion of Schwartz and Shagass, *13*), but this inference requires some clarification. Stimuli which were subthreshold-c, but could still elicit some evoked potential, could be made adequate for conscious sensation by simple repetition at a suitable frequency. It remains to be seen, then, whether responses evoked by subthreshold-c stimuli which are nonrepetitive or of low frequency can play any role in unconscious experience or in behavioral responses to sensory stimuli of which the subject is not aware.

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7 November 1967

Actinomycin D Blocks Formation of Memory of Shock-Avoidance in Goldfish

Abstract. *When 2 micrograms of antinomycin D was injected intracranially into goldfish immediately after a training session, the formation of long-term memory of a shock-avoidance was blocked. The results are discussed in relation to similar findings with acetoxycycloheximide and puromycin in the goldfish and with apparently conflicting results in the mouse.*

Goldfish can be trained to swim over a barrier upon a light signal to avoid mild electrical shock administered through the water in a shuttlebox (*1*). They show a significantly increasing probability of avoidance-responding during 20 training trials administered over a 40-minute session, and, on ten retraining trials 3 days later, they demonstrate further improvement in avoidance scores, indicating memory of the training (*2*). Using large numbers of fish and observing responses in six shuttleboxes, we found this paradigm useful in studies on formation of memory. Either of two inhibitors of protein synthesis, puromycin dihydrochloride (170 μg) or acetoxycycloheximide (0.1 to 0.2 μg), blocked memory-formation when injected intracranially immediately after training, but not when injected 1 or more hours later (*3*). We also learned that the decrease of susceptibility to these agents during the period after training (fixation) could be suppressed by manipulating the external environment (*4, 5*). Puromycin injected before training did not affect acquisition and short-term memory but nevertheless blocked formation of long-term memory (*6*). By retraining groups of fish at various times after an immediate injection of puromycin following the trials, short-term memory of the avoidance task was observed to decay over a 2-day interval (*4*). The results suggest that there are two stages of memory in the goldfish: a short-term variety which is not susceptible to puromycin, and a long-term form which also is not susceptible, but whose formation is susceptible to the drug. A relation of the block in memory-formation to the block in protein synthesis is supported by a number of findings. Both puromycin and acetoxycycloheximide block incorporation of leucine-^{3}H into protein in goldfish brain (*7*) at doses that do not depress uridine-^{3}H incorporation into RNA (*8*). Puromycin aminonucleoside and methyl tyrosine, two moieties of puromycin that are not known to block protein synthesis, have no effect on memory-formation (*3*). Also, the vast differences in the doses of puromycin and of acetoxycycloheximide required to block protein synthesis in the goldfish brain extensively are the approximate doses required to block memory-formation. While we believe that these two agents block memory-formation in the goldfish by their roles as inhibitors of protein synthesis, an important question is whether metabolic blocks, other than that of protein synthesis, also block memory. Since we had previously observed that 2 μg of actinomycin D does not markedly block protein synthesis for hours after intracranial injection (*7*), and since actinomycin D is known to block RNA formation selectively (*9*), the effects of

Table 1. Effect of acetoxycycloheximide, injected at various times after training, on the performance on day 4. Acetoxycycloheximide (0.2 μg) in 10 μl of 0.15*M* NaCl was injected intracranially at various intervals after training on day 1. *N*, number of fish; A–P, achieved minus predicted.

Day 4 score	*N*	Hours between training session and injection		
		0	1	3
Achieved	26	1.88	3.54	4.56
Predicted*	27	4.87	5.19	5.18
Retention (A–P)	28	−2.99†	−1.65†	−0.62

* See reference *11*. † $P < .01$.

Table 2. Effect of actinomycin D injected at various times after training on the performance on day 4. Actinomycin D (2 μg) in 10 μl of 0.15*M* NaCl injected at various times after training on day 1. Groups of fish were retrained on day 4 and memory loss was determined as for Table 1. *N*, number of fish; A–P, achieved minus predicted.

Day 4 score	*N*	Hours between training session and injection		
		0	1	3
Achieved	24	2.31	4.07	4.82
Predicted*	26	4.31	4.83	4.67
Retention (A–P)	23	−2.00†	−0.76‡	+0.15

* See reference *11*. † $P < .01$. ‡ $P < .05$.