the experiment was terminated. The addition of methylene blue had no effect, except that the methylene blue was rapidly decolorized.

In the next series of experiments an attempt was made to use a hydrocarbon as fuel. An active ethane-oxidizing culture of *Nocardia* was added to the biological half-cell, and ethane was bubbled into the solution. Apparently, no reaction occurred. On the addition of methylene blue, a slight increase in open circuit voltage was measured. However, this was later shown to be due not to the *Nocardia*-ethane system but, rather, to endogenous respiration. When glucose was substituted for ethane a definite increase in metabolic activity occurred. Additions of methylene blue to this system caused current flow eventually to reach a maximum of 2 ma (see Table 1).

Finally, a test was made of the effect of a strong oxidizing agent (potassium ferricyanide) on the system, with glucose as fuel. When 1 mg of $K_3Fe(CN)_6$ was added to the O_2 half-cell in the absence of microbial cells at the opposite electrode, only a slight increase in current was measured. However, when *Escherichia coli* cells were added to the biological half-cell the current increased markedly (from 0.1 to 1.6 ma in 30 minutes). The addition of large amounts of $K_3Fe(CN)_6$ at the O_2 electrode and of methylene blue at the biological electrode resulted in very little current flow unless metabolizing cells were also present at the biological electrode.

The experimentation described was exploratory, and no attempt was made to increase current measurements by using unit cells in series, as had been done previously by Cohen (*1*). A principal question at the outset was: Can microbes which ordinarily oxidize hydrocarbons release measurable quantities of electrical energy? The experiments performed revealed that while nocardia release measurable electrical energy in their metabolism, confirming somewhat similar experiments performed with bacteria and yeast 50 years ago by Potter (*2*), hydrocarbons were not successfully metabolized under the conditions employed.

The data clearly show that when a substitute hydrogen-acceptor for oxygen —namely, methylene blue—is successfully employed as an oxidant in endogenous respiration or glucose metabolism, by either living microbes or the enzyme glucose oxidase, a current is measurable. In fact, *Escherichia coli,* a facultative anaerobe capable of metabolizing glucose in the absence of either oxygen or methylene blue, yielded current in the experimental system in the absence of methylene blue.

Methylene blue did not serve as a successful hydrogen acceptor in the metabolism of ethane by ethane oxidizers. The simplest explanation for this is that the initial reaction of biological hydrocarbon oxidation involves a physical incorporation of molecular oxygen into the hydrocarbon molecule. This would involve an "oxygenase" enzyme, recently discussed in detail by Mason (*3*). Kallio and his associates give support to this idea in their recent work on the microbial oxidation of hydrocarbons (*4*). Since the biological half-cell must rigidly exclude molecular oxygen, no hydrocarbon oxidations will occur unless intermediate hydrogen acceptors are found. Current measurements can serve as a tool in searching for intermediate oxidants which will accept hydrogen from hydrocarbons.

Table 1. Biological electrode reactants. *E*, electromotive force; *I*, current. The load was 1000 ohms.

Condition	Glucose oxidase		*E. coli*		*Nocardia*	
	E (mv)	*E* under load (mv)	*E* (mv)	*E* under load (mv)	*E* (mv)	*I* (ma)
At equilibrium*	141	38	148	42	50	0
Added enzyme	198	50				
Added microbial cells			625	521	115	0
Ethane in *Nocardia* system					120	0
Methylene blue (0.25 mg)	280	75	625	521	192	0
Methylene blue (0.25 mg)	287†	90	625	521	245†	0.05
Glucose replacing ethane in *Nocardia* system					245	.05
Methylene blue (0.5 mg)					265	
Methylene blue (1.5 mg)					295	.45
Methylene blue (4.0 mg)					305	.60
Methylene blue (10.0 mg)					300	1.70
Methylene blue (35.0 mg)					300‡	2.00

* Glucose present in glucose-oxidase and *E. coli* systems only. † Methylene blue did not decolorize. ‡ Methylene blue was decolorizing but not completely decolorized when the experiment terminated. It was completely decolorized in all other cases.

Preliminary data of this nature can lead to only tentative conclusions. That microbial metabolism is a source of measurable electrical energy is established, but as far as we know, no one has made a serious attempt to employ this energy.

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15 January 1962

Conditioning of Induced Electroencephalographic Sleep Patterns in the Cat

Abstract. Stimulation of the basal forebrain synchronizing area of the cat induces the behavioral and electroencephalographic manifestations of sleep. By pairing such stimulation with a neutral auditory stimulus we were able to evoke electroencephalographic synchronization and sleep preparatory behavior to the presentation of a tone, and to temporal factors associated with the conditioning procedure.

In his classical investigations on diencephalic functions, Hess (*1*) distinguished two regions in the hypothalamus to which he ascribed, in a general fashion, antagonistic actions. Stimulation in caudal hypothalamic areas (ergotropic zone) resulted in a generalized behavioral activation and sympathetic discharge, whereas similar stimulation in more anterior loci (trophotropic zone) produced generalized somatomotor suppression and parasympathetic discharge. Additionally, low frequency stimulation of the massa intermedia of the thalamus resulted in the onset of behavioral sleep. It has been shown more recently that electrical stimulation of a specific basal forebrain area induces a diffuse and sustained electroencephalographic (EEG) synchronization in immobilized short-term preparations as well as in behaving cats with permanently implanted electrodes (*2*). This area has been termed the basal forebrain synchronizing area. The EEG synchronization observed in unrestrained cats is accompanied by a parallel transition from alert, waking behavior to apparent sleep, all of which typically occurs within 1 or 2 minutes

of the onset of stimulation. The question arose as to whether it would be possible to condition to an external stimulus the EEG synchronization and sleep patterns induced by basal forebrain stimulation.

Bipolar strut electrodes were placed bilaterally into the basal forebrain synchronizing area in six cats. The coordinates used in stereotaxic placement were A16, L2.5, H-4.5 with reference to the Jasper and Ajmone-Marsan atlas for the cat (*3*). Stainless steel screws were placed into the calvarium over frontal, parietal, and occipital lobes for recording EEG patterns. Leads from both the deep and surface electrodes were attached to a Winchester plug which was then fixed to the cat's head with dental cement.

After a postoperative period of 1 to 2 weeks the animals were isolated in an observation cage, and connected to a suspended multiple microdot cable. Bilateral stimulation parameters were tested and the optimal combination for the induction of sleep was determined. In general, the best stimulation parameters ranged from 1.5 to 3 volts, at 0.75 msec for frequencies of 7 and 150 impulses per second. Under these conditions, it was possible to induce sleep with 30 to 180 seconds of continuous or periodic stimulation. The behavioral pattern initiated by such stimulation included the assumption of a natural resting posture, relaxation of the head and body musculature, closing of the eyes, and maintenance of a quiescent state. This behavior was accompanied by the onset and persistence of EEG synchronization and sleep spindles.

The procedure employed in conditioning trials was as follows: A tone of 1500 cy/sec was presented for 10 seconds in advance of basal forebrain stimulation, and was continued during the first 20 seconds of an applied 30-second stimulation. The tone and stimulation thus overlapped for a period of 20 seconds. An intertrial interval of 30 seconds was employed between the termination of basal forebrain stimulation and the next onset of the tone. Trials were repeated every minute throughout the conditioning session.

The first few presentations of the tone evoked either a desynchronization of the EEG in the non-alerted cat, or no change in the flat pattern of the alerted animal. The onset of basal forebrain stimulation resulted in synchronous EEG activity (Fig. 1, trial 4). With additional trials, however, spindle bursts

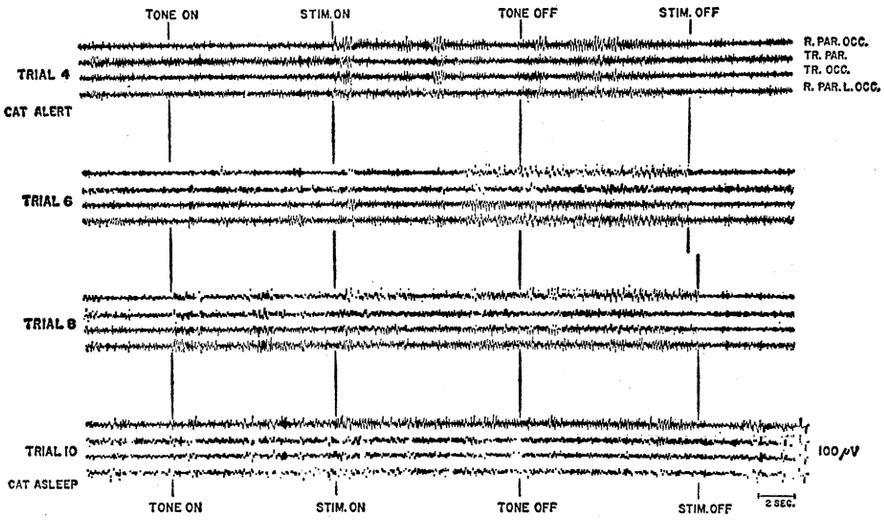

Fig. 1. Sequential change in EEG response to tone paired with basal forebrain stimulation. The stimulus was one of 3 volts, at 0.75 msec at a frequency of 7 per second, and was delivered bilaterally. Thirty seconds elapsed between trials.

appeared sporadically during the period of tone preceding stimulation, and became more prominent during basal forebrain stimulation (Fig. 1, trial 6). At the same time, the cat reclined and in later trials was observed to drop its head to its paws and close its eyes at presentation of the tone. After 6 to 10 pairings the tone specifically evoked a brief burst of synchronized slow wave activity in the EEG (Fig. 1, trial 8). At this point, the EEG pattern during the tone could not be differentiated from the pattern seen during stimulation. In general, the synchronization induced by stimulation is enhanced by the termination of the tone, suggesting some release from the excitatory aspects of the tone. In the early trials synchronization persisted until stimulation was discontinued, at which time a rebound-like after-effect was observed for several seconds. However, following the observation of conditioned spindle burst activity, the cat, to all appearances, goes to sleep and additional trials are characterized by the persistence of an EEG sleep pattern which is more or less unaffected by the various events of the conditioning sequence (Fig. 1, trial 10).

After many trials some of the animals demonstrated a consistent pattern of EEG changes during the intertrial interval. With termination of the basal forebrain stimulation a more or less pronounced desynchronization occurred in the EEG. This was gradually replaced by increasingly synchronized activity, which was climaxed by a sudden burst of high amplitude slow waves during a time interval of approximately 5 seconds preceding the next tone. In such cases the synchronization persisted frequently throughout the next conditioning trial, and was only broken up by the rebound-like desynchronization which again followed the end of stimulation. No specific changes in behavior of the animal were observed in association with these EEG patterns. The cat remained curled up in a corner of the cage and slept quietly throughout the trials. With even more repetitions the predominant EEG synchronization was replaced by a low amplitude pattern resembling the "paradoxical phase" of sleep described by Jouvet (*4*). After this time, no distinct EEG changes occurred in response to the tone or to the stimulation.

In summary, we found that pairing of electrical stimulation of the basal forebrain synchronizing area with a tone during regular intervals of time resulted in the establishment of a conditioned EEG synchronization to the tone and to temporal factors. This reflex suggests the existence of functional connections in a conditioning situation between the basal forebrain synchronizing area and the cerebral cortex.

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4 May 1962

A Law for Loudness Discrimination

Abstract. A law for loudness is proposed which implies detection and averaging in a general nonlinear device with excitation by a complex signal-noise wave form. This law provides a mechanistic explanation for subjective intensity in the general time varying case. Mathematical solutions to the general law may be obtained in certain elementary cases, the well-known power-law characteristic being an example. A modified Weber law is derived which is in good agreement with experiments at both small and moderate stimulus intensities.

Loudness. The Weber fraction $\Delta S/S$ is the just-noticeable difference in the stimulus intensity ΔS relative to total intensity S. The Weber law is: $\Delta S/S$ is independent of S. The law sometimes fails when S is large. It generally fails when S is small in the case where unavoidable noise, both external and internal to the sensory system, becomes important (*1*).

The subjective measure for stimulus intensity may be termed L, which stands for loudness but is not restricted to the auditory sense modality. Measure L is fundamental. An accurate expression for the Weber fraction depends upon the adequacy of this measure.

A modified Weber fraction may be defined as $\Delta L/L$, from which a modified Weber law may be deduced as

$$\Delta L/L \text{ is independent of } S$$

S. S. Stevens credits the idea behind this modified Weber law to Brentano (*2, 3*). Brentano's hypothesis will be further examined after a generalized expression for L is developed.

Laws for subjective intensity have been proposed by many people. Most recently S. S. Stevens (*2, 3*) has proposed a power law for subjective intensity. These proposed laws as well as the Weber fraction and Brentano's hypothesis follow a similar convention in that they employ mean-square measure for stimuli. The loudness function proposed here is *not* based on mean-square measure. The distinct break with tradition in this regard has led to the present use of the symbol L for subjective intensity instead of the commonly employed symbol ψ. The rather significant consequences of the present formulation will be commented upon later.

Let $s(t)$ be a time varying signal with mean-square value S and $n(t)$ be additive, statistically independent interference with mean-square value N. A general "loudness function" is proposed as (*4*)

$$L = \mathrm{Av} \sum_{k=0}^{k=\infty} C_k |f(t)|^{n+k} \cong \mathrm{Av}\, C_0 |f(t)|^n \quad (1)$$

where $f(t) = s(t) + n(t)$ is the gross stimulus temporal wave form, where "Av" denotes a suitable time average with an appropriate weighting function, and where C_k are coefficients which are not necessarily independent of time or other parameters (such as the frequency of the stimulus). The approximation of Eq. 1 applies when $|f(t)|$ is small. Since the exponent n need not be an integer, the proposed loudness function is more general than a Taylor series.

The definition of Eq. 1 allows time varying phenomena, such as occur in adaptation and fatigue, to be represented. For a finite averaging time and for $f(t)$ a random variable (in part), loudness in Eq. 1 is also a random variable; randomness in a subjective measure correlates with observed human behavior. Perhaps of most significance is that Eq. 1 can be modeled with practical electronic circuits. Not only does this permit L to be evaluated in cases which are not mathematically tractable, but it means that *a mechanistic interpretation for subjective intensity has been achieved.* Such a direct modeling can be quite realistic in the physiological sense if, for example, part of the circuitry uses pulse generators in the emulation of sensory cells.

If an infinite averaging time and constant coefficients C_k are assumed, mathematical solutions can be obtained for certain special stimulus wave forms, although mechanistic interpretation is thereby hindered. When $f(t)$ consists of independent Gaussian signal and noise components, a generalization of the Stevens law results as (*4*)

$$L = \sum_{k=0}^{k=\infty} C_k K_k (1 + S/N)^{(n+k)/2} \cong C_0 K_0 (1 + S/N)^{n/2} \quad (2)$$

where the $C_k K_k$ are constants and where the approximation is for the case when $|f(t)|$ is small.

When $f(t)$ consists of a sine wave imbedded in Gaussian noise, L may be expressed in terms of confluent hypergeometric functions; in spite of less familiar function notation, quantitative values remain similar to those given by the simpler Gaussian signal case. Use

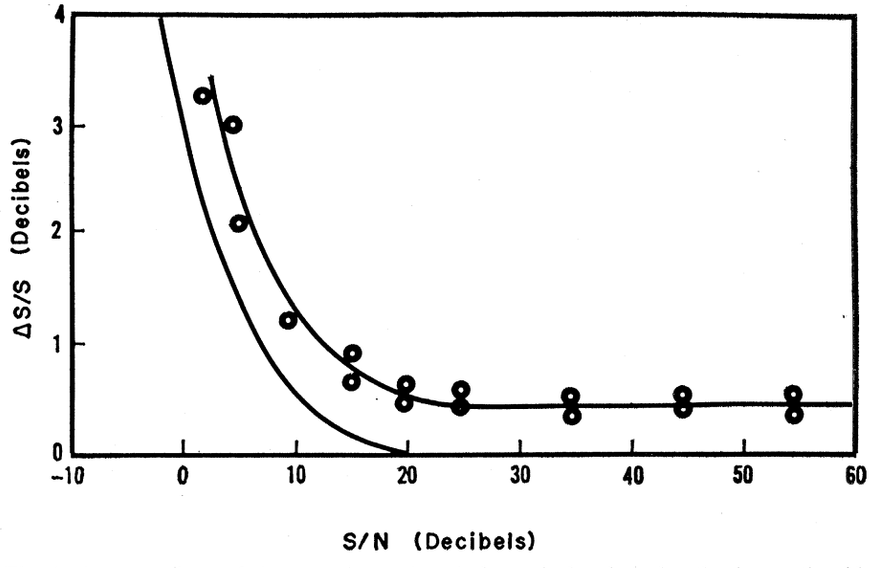

Fig. 1. A comparison of the experimental and theoretical values for the just-noticeable difference in stimulus intensity relative to total intensity (for loudness). The lower curve is plotted from Eq. 6. The circles represent Miller's experimentally determined values. The upper curve is obtained from linear translation of the lower curve.