with pharmacologic agents. Perhaps lung or liver can be changed from being a good soil for invasive tumor spread to a poor soil without important impairment of other normal attributes.

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Relationship between Reaction Time and Electroencephalographic Alpha Phase

Abstract. Demonstrations of a relationship between human 8 to 13 per second (alpha) electroencephalographic activity and simple visual reaction time can be made at reliable levels of confidence by (i) sampling reaction times to stimuli given at phases of the alpha cycle 10 msec apart, (ii) selecting the phase with the slowest reaction times, and (iii) collecting enough reaction times to stimuli at this and some other control phase for statistical comparison.

Several investigators (*1*) have speculated on the possibility of a parallel between phasic changes in behavior and the 8 to 13 per second activity of the electroencephalogram, known as the alpha rhythm. Lansing (*2*) compared reaction times to stimuli which coincided with six different portions of the alpha cycle. He found no reliable overall differences, and to achieve statistical significance he was forced to select retrospectively the slowest and fastest groups of reaction times for comparison. He also screened 100 subjects in order to find eight with enough alpha activity for his procedure.

Our paper describes a technique that (i) allows identification of that portion of the alpha cycle most likely to be associated with slow reaction times in advance of statistical evaluation, and (ii) is applicable to about a third of an unselected adult population by virtue of requiring only moderately dominant alpha activity.

The individual being tested is seated with eyes closed in a darkened room. Alpha activity is lead off from the left occipital area and either the left ear lobe or the mid-frontal scalp. This is amplified and fed into a circuit designed to generate an electrical signal only when the phase and amplitude of the alpha rhythm correspond with a predetermined setting. This initial signal is followed by a second signal after a delay that can be varied manually from 10 to 90 msec.

The second signal flashes a strong light in the subject's face and simultaneously starts a timer. The subject responds to this light by pressing a key; his reaction stops the timer, automatically prints the reaction time, and furnishes a voltage analog of reaction time to the Y axis of an automatic X-Y plotter. By supplying the X axis of this plotter with a voltage corresponding to the delay between the two signals, one can plot each reaction time as a function of approximate alpha phase at stimulation.

If an artifact never triggered the first signal of this device, and if alpha waves were perfect sine waves of fixed frequency, then the delay between the first and second signals would specify exactly the alpha phase at the instant of visual stimulation. However, neither of these two ideals is realized. Therefore, a sweep on a monitor oscilloscope is started by the first signal and this displays the electroencephalographic (EEG) activity for 150 msec. In this way the subject's EEG activity can be visualized at the instant of each stimulation. Stimuli that fail to coincide with the desired portion of an alpha cycle are discarded from further consideration. In spite of this check, however, there is possible error of as much as 15 msec for individual stimuli.

Using this device, one can present blocks of 10 to 20 stimuli at steps of 10 msec each along the alpha cycle, and in this way accumulate a scattergraph of reaction time as a function of approximate alpha phase at stimulation. One can then select the alpha phase at which stimuli elicit the slowest responses and compare this to one other phase—either a phase associated with fast responses or one about 180 degrees away. The results of selecting phases in this way are illustrated in Table 1. Data in this figure were collected after the initial screening runs by presenting 10 to 15 stimuli at a time at one phase. Rest periods were introduced after each 20 to 40 stimuli, and the order of phase presentation was alternated to minimize the effects of learning and fatigue on differences between reaction times to stimulation at the two selected phases.

These data represent only reaction times measured after a prediction; therefore, one-tailed tests of significance could be justified. In this case, six out of the eight subjects would be found to yield significant results at the 5 percent level or better. However, to test the over-all group, two-tailed probabilities are given in the Table. Combining these by the chi-square formula, we find that differences in reaction times to stimuli presented at different alpha phases in eight independent studies such as this would have occurred by chance about one time in 500.

At the onset, we elected to disregard reaction times over 300 msec as probably representing lapses of attention. The number of reaction times disregarded in this way are also given in Table 1. This procedure biased the data against the obtained results and significantly more reaction times were excluded from the slow samples than from the fast samples.

Because of individual variability, phase at stimulation is given in milliseconds from maximum occipital positivity. Average alpha cycle duration is also indicated for each subject. In this series stimuli presented nearest to maximum occipital positivity elicited slowest reaction times. This corresponds to the

Table 1. Visual reaction times to phasic stimuli. All times in milliseconds. Stimulus phase is given in milliseconds from maximum occipital positivity. $X^2 = -2\Sigma\log_e p = 38.9$, (16 df).

Subject	Predicted slow				Approx. av. alpha period	Predicted fast				Statistics	
	Stimulus phase	Reaction time				Stimulus phase	Reaction time			t	$p <$
		Under 300		Over 300 (No.)			Under 300		Over 300 (No.)		
		Mean	No.				Mean	No.			
L. H.	0	243	47	6	100	50	231	49	3	2.22	.05
D. M.	−20	240	40	3	100	−30	226	40	3	2.21	.05
I. V.	0	249	50	10	100	20	240	50	9	2.48	.02
A. W.	0	247	107	16	110	40	241	105	15	1.68	.10
I. S.	−10	262	46	20	100	40	250	51	12	2.50	.02
J. M.	−10	239	77	5	90	20	236	79	3	0.87	.40
P. P.	0	262	49	17	100	−40	252	40	9	1.80	.10
R. D.	0	215	73	9	100	40	216	80	2	0.15	.90

segment of the alpha cycle where Lansing found stimuli associated with slowest responses.

These data indicate a more than chance relationship between visual reaction time and alpha phase at stimulation. Finer details of the relationship between alpha phase and reaction time must wait further investigation.

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3. This work was supported by contract with the Office of Naval Research, NONR 2931(00), project NR 144134, and by the California Department of Mental Hygiene, with the technical assistance of Miss Patricia Compton. Reproduction in whole or in part is permitted for any purpose of the United States Government.

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Use of Pectinase in Preparation of Mitochondria from Tobacco-Tissue Cultures

Abstract. Mitochondrial particles were isolated from tobacco cells in tissue cultures which were disintegrated with pectinase. The pectinase treatment of the tissue cultures reduced the dry weight of the particulate preparations and eliminated glutinous material, but caused little change in oxidative and phosphorylative activities. The pectinase apparently did not affect the enzymatically active sites of the particles.

Pectinase has been used to disintegrate tobacco cells in tissue cultures for the preparation of mitochondria. The hope was that pectinase would eliminate the glutinous nature of the pellet secured from centrifuging the homogenate of the tissue cultures. Thus, uniform suspensions of mitochondria could be obtained readily in the reaction medium. This approach to enzymic disintegration of tissue seemed promising because of the successful isolation of mitochondria from pigeon heart muscle and rat liver by the use of proteinase (*1*). Prior incubation of such tissues with proteinase facilitated the grinding procedure, minimized mechanical damage to the particles, and produced particles with a higher P/O ratio, greater stability, and a greater general reactivity.

The tobacco tissue used in this experiment (*2*) was a single cell clone (H196) from a stem of *Nicotiana tabacum* × *N. glutinosa,* and was grown on a modified White's agar medium (*3*). Mitochondria were prepared with pectinase as follows: 150 g of tissue, 2 weeks old, was washed by suspending it in 400 ml of 0.5*M* mannitol, and was separated from the mannitol solution by filtration on two layers of cheesecloth. The washed cells then were suspended in 400 ml of 0.5*M* mannitol to which 600 mg of pectinase (Nutritional Biochemicals Corp.) was added; the suspension was kept at 2°C for 45 min with gentle stirring with a magnetic stirrer. The suspension became more acidic with time and was adjusted to *p*H 7.2 with 0.5*M* potassium hydroxide as needed. After incubation, the suspension was homogenized in a glass homogenizer with a Teflon pestle. During grinding, the *p*H was again adjusted with potassium hydroxide. The pectinase-treated tissue was soft and was readily ground by three in-and-out movements of the pestle in the mortar as compared to five in-and-out movements with nontreated tissue suspension. The particles were separated and washed by centrifugation (*4, 5*). Oxygen consumption was measured with an oxygen electrode apparatus (*6*), and phosphate was determined by King's method (*7*).

Table 1 shows typical results of analyses of mitochondria prepared from pectinase-treated tissue as compared to mitochondria prepared from nontreated tissue. The yield of mitochondria on the basis of wet and dry weights was decreased to less than half by the pectinase treatment. At the same time, the glutinous material in the original preparation was removed, and the particles could be suspended easily in the reaction medium. The protein content in particulate preparations was little affected by the pectinase treatment; the ratio of nitrogen to dry weight was almost doubled by the treatment.

The particulate preparations from both pectinase-treated and nontreated tissues oxidized succinate, citrate, glutamate, α-ketoglutarate, reduced diphosphopyridine nucleotide (DPNH) and ascorbate. For comparison, oxidation rates for succinate, DPNH, and ascorbate are shown in Table 1. The pectinase treatment apparently caused no significant change in oxidative activities and in the relative activities of the enzymes. The P/O ratio also was unchanged by the treatment. Essentially the same results were obtained with mitochondria originally isolated from nontreated tissue but incubated with pectinase (1 mg of pectinase per milliliter of mitochondrial suspension) for 15 minutes at 2°C after isolation (see column 9, Table 1). After incubation with pectinase, the wet weight of the mitochondrial preparation decreased to less than half, and the cohesive nature of the original preparation disappeared. The oxidative and phosphorylative activities of mitochondria treated with pectinase were comparable to those of the particles prepared from either pectinase-treated or nontreated tissue cultures. Mitochondria isolated from pectinase-treated tissue cultures or mitochondria treated with pectinase after isolation responded more to adenosine diphosphate (ADP) than mitochondria isolated from nontreated tissue.

The pectinase treatment seemed to eliminate polysaccharide substances which otherwise might contaminate the pellet of mitochondria and cause them to aggregate tenaciously. This treatment,

Table 1. Comparison of mitochondrial preparations from pectinase-treated and nontreated tissue cultures of tobacco and a mitochondrial preparation treated with pectinase after isolation.

Item	Mitochondria from				Mitochondria* treated with pectinase after isolation	
	Nontreated tissue		Pectinase-treated tissue			
Wet weight	2.36 g		0.91 g		0.79†	
Dry weight	209 mg		91.5 mg			
Protein–N	2.52 mg		2.24 mg			
Protein–N/dry weight	0.0125		0.0243			
(Oxidative activities)	(mμatoms/min per mg N)	(Relative activity)	(mμatoms/min per mg N)	(Relative activity)	(mμatoms/min per mg N)	(Relative activity)
Succinate oxidation	18.7	1.0	14.1	1.0	16.6	1.0
Succinate oxidation in presence of ADP	105	5.6	102	7.3	133	8.0
DPNH oxidation	342	18.3	293	20.8	293	17.7
Ascorbate oxidation	1790	95.7	1500	106.1	1540	92.8
P/O ratio with succinate	0.85–1.0		0.84–1.0		0.87–1.0	

* Mitochondria originally isolated from nontreated tissue cultures. † Wet weight before pectinase treatment was 1.79 g.