Stimulation of cholinergic preganglionic axons not only increases cyclic GMP but also generates slow synaptic potentials in the bullfrog sympathetic ganglion (*6, 7*). The fact that both responses are mediated by the activation of muscarinic receptors suggests that the generation of the slow synaptic potential or potentials may be related to the increase in cyclic GMP. Compatible with this possibility, administration of dibutyryl cyclic GMP to rabbit (*23*) or bullfrog (*24*) sympathetic ganglia produces a transient hyperpolarization followed by a depolarization of the ganglia. In view of these data, it seems reasonable to hypothesize that ACh, released from presynaptic nerve terminals, activates muscarinic receptors on postsynaptic neurons, causing an increase in cyclic GMP in the neurons, and that the increase in cyclic GMP results in a depolarization of the membrane, that is, a slow excitatory postsynaptic potential. It is possible that a cyclic nucleotide is also involved in the generation of the slow inhibitory postsynaptic potential.

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11. Samples were thawed, sonicated, and centrifuged. Protein in the precipitate was determined by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)]. Cyclic GMP in the lyophilized supernatant was determined by the method of A. Steiner, C. Parker, and D. Kipnis [*ibid.* **247**, 1106 (1972)] except that the antigen-antibody complex was precipitated by the addition of 4.0 ml of a solution containing trichloroacetic acid (5 percent) and Na_2WO_4 (0.25 percent), *p*H 2.0. Analyses were performed on triplicate portions, including one with internal standard. Cyclic GMP in resting or stimulated ganglia could be completely hydrolyzed by beef heart phosphodiesterase.
12. The value of 0.82 ± 0.07 pmole per milligram of protein (mean value ± standard error) was obtained from analyses of 51 pooled samples of 12 ganglia each (ninth and tenth ganglia from six frogs). The experiments were conducted between October and May in two successive years.
13. Cyclic AMP was also increased by preganglionic stimulation; for example, stimulation for 2 minutes at 10 hertz increased the cyclic AMP content to 782 ± 85 percent ($N = 5$) of the control value.
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Electrophysiological Correlates of Meaning

Abstract. *The use of context-sensitive symbols offers an appropriate methodology for investigating the representation of meaning in the brain. This approach revealed that late components of frontal, but not occipital, evoked potentials reflect the change of meaning of a symbolic stimulus when it appears in different temporal contexts.*

Although scientists agree that the human brain is the organ of the body responsible for the elaboration of meaning, little is known of the neuropsychological mechanisms involved. We report here that neuronal activity in the frontal lobes, as evidenced by changes in the wave form of evoked potentials recorded from this area, is indicative of a change in the meaning of a stimulus.

Several experiments have suggested that the wave shape of stimulus-locked potentials may reflect a change in meaning. For example, John *et al.* (*1*) demonstrated consistent differences in the late components of visual evoked potentials (VEP's) induced by two very similar stimuli, a square and a rotated square (diamond), irrespective of the stimulus size. However, the interpretation of such experiments is difficult because the experimental procedure involves a change in the physical stimulus as well as a change in meaning. Physical attributes of a stimulus change the wave form of VEP's (*2, 3*) so, in order to avoid confounding the meaning change with the physical stimulus change, it is necessary to keep the latter constant.

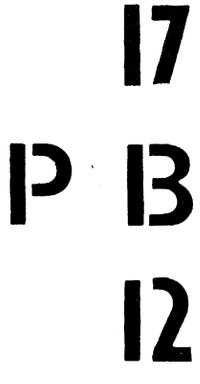

Fig. 1. Stimuli used in experimental procedures. Central stimulus is ambiguous.

It is possible to alter the meaning of a constant stimulus by adding a new association using a conditioning procedure, for example, pairing a visual stimulus with an auditory click (*4*). However, any change in the VEP as a result of this may reflect enhanced arousal or attention rather than the meaning change per se. Modification of VEP's by such variables as expectancy, affect, uncertainty, or attentional state have been demonstrated in many situations (*5*). The conditioning procedure not only brings about a change in meaning but may also have a quantitative effect on one or more of these state variables. The difficulties inherent in equating both the physical stimulus and state variables may be circumvented by the use of a symbolic stimulus that has two or more distinct meanings depending on the context in which it is presented.

The central symbol in Fig. 1 can be interpreted as "B" or "13," depending

on its context. The stimulus-locked evoked potential for this symbol was recorded when it was embedded in the temporal context of other numbers or other letters.

The subjects were seven student volunteers with normal visual acuity. They were seated in an electrically shielded, sound-deadened enclosure looking directly at a translucent Plexiglas screen. A Kodak Carousel projector, fitted with a strobe light, back-projected stimuli onto the Plexiglas screen from an adjacent room. All stimuli subtended a 2° visual angle and were presented as white figures on a black background. The nonambiguous letter stimuli were equated with the nonambiguous number stimuli in both surface area and the number of perceptual features they contained (*6*). The projector advance mechanism and strobe were under the control of a PDP8/e computer. The subject initiated a stimulus presentation by depressing a button with his left hand and, after a delay of 0.5 second to allow movement potentials to subside, the stimulus slide was projected for 10 μsec.

A session consisted of 80 stimulus presentations. The three numerals shown in the column on Fig. 1 were presented separately, in a random temporal sequence, in number sessions. During letter sessions, a similar random sequence of letters shown in the row of Fig. 1 was presented. The ambiguous stimulus occurred 40 times and the nonambiguous stimuli 20 times each within a session. Each subject participated in eight sessions (four number and four letter) on the same day. Number and letter sessions were alternated for each subject, and the nature of the first session varied among subjects. The first two were warm-up sessions and served to familiarize the subjects with all stimuli.

Subjects were instructed that the task was concerned with the speed with which they could name numbers and letters. They initiated a stimulus presentation by depressing a button with their left hand, and a voice-operated relay detected the subjects' verbal response so that reaction time was monitored throughout the experiment. Before each session, subjects were informed of the visual stimuli to be presented in that session. This also served to enhance the perceptual set for the ambiguous stimulus. Questioning after the experiment revealed that only one subject was aware that he had been calling the same stimulus by two different names according to its context. All the other subjects showed surprise when the relationship was pointed out to them.

Subjects were fitted with scalp electrodes located on the midline either 2.5 cm above the inion (occipital) or 2.5 cm above the nasion (frontal). The final subject had electrodes in both locations (*7*). Thus, four records were obtained from subjects with occipital electrodes and four from subjects with frontal electrodes. Corneoretinal potentials were reduced by providing the subject with a cross-hair fixation point, allowing self-presentation of the stimuli, and referencing the frontal electrode to the central terminal of a 40-kilohm potentiometer connected between a vertical electrooculogram electrode and the two earlobes (*8*). Before the experiment, the potentiometer was adjusted until vertical eye movements could no longer be detected on the electroencephalographic recording. Occipital electrodes were referenced to the two earlobes.

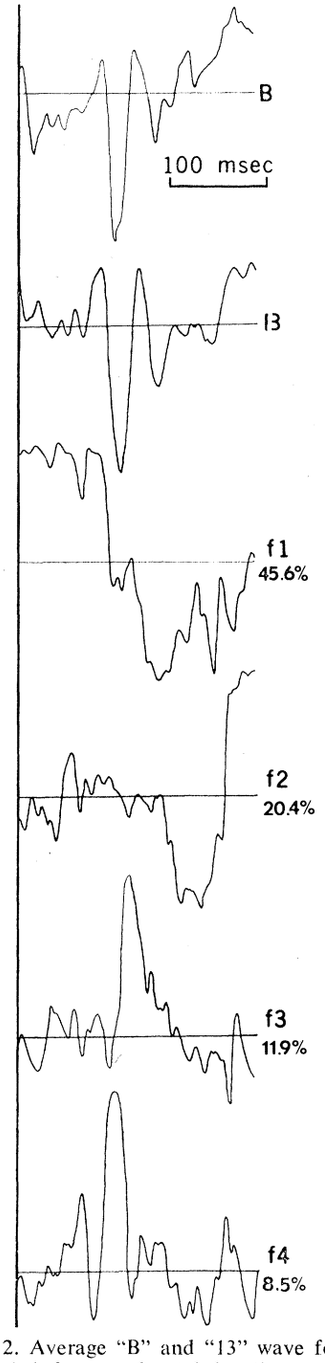

Fig. 2. Average "B" and "13" wave forms recorded from a frontal location on the same subject. First four rotated eigenvectors (**f1** to **f4**) are shown for this subject, together with the percentage of the total variance accounted for by each factor. Peaks on vectors show time period when component was most active.

Evoked potentials were amplified by Grass model 6A5 wide-band a-c amplifiers and, after being digitized at the rate of 500 points per second, they were stored in the PDP8/e computer (*7*). Each wave form, which contained 240 msec of data collected immediately after stimulus presentation, was averaged with others for the same stimulus for that subject and session. Four averaged evoked potentials, each the average of 20 stimulus presentations, were collected from each subject during each session. These wave forms were then transferred to magnetic tape for subsequent analysis on an IBM 360 computer.

Six sessions, four averaged wave forms per session, yielded a total of 24 averaged wave forms collected from the same electrode location on each subject. Of these, 12 were recorded following the presentation of the ambiguous stimulus. The data from each subject was analyzed on an IBM 360 computer by using the BMD-08M factor analysis program from the UCLA Biomedical package. Before analysis, all VEP's were adjusted to a zero mean. A principal components analysis and varimax rotation were performed on the 120 by 120 correlation matrix formed by the correlations among the 120 time points for "B" and "13." An excellent theoretical discussion of this procedure has been given by Donchin (*3*). In all cases it was found that five eigenvalues accounted for between 80 and 95 percent of the total variance in the data. The corresponding eigenvectors were cross-multiplied by the original wave forms to yield a set of component scores which indicate the extent to which each factor is represented in the original wave forms. In this way we can find which, if any, factors show a significantly different loading when the ambiguous stimulus is interpreted as "B" or as "13." This method has the advantage over cross-correlation or peak-to-peak measurement techniques in that it allows a temporal localization of differ-

ences, as well as a quantifiable assessment of statistical significance.

The analysis revealed that in the case of occipital recordings, none of the four subjects showed any significant difference related to the context of the ambiguous stimulus: that is, none of the rotated eigenvectors were differentially loaded on "B" and "13" wave forms. In the case of frontal recordings, three of the four subjects showed differential loadings on one or more factors. The subject with simultaneous recordings for occipital and frontal locations showed significantly different loadings on recordings from the frontal location and no difference in the occipital recordings.

Figure 2 shows an example of the eigenvectors extracted from frontal recordings for one subject, together with the "B" and "13" wave forms averaged over all experimental sessions. In this case, four factors accounted for more than 85 percent of the total variance. Only factor 2 (**f2**), which begins 160 msec after stimulus presentation, is differentially loaded on "B" and "13" ($U = 0$, $P < .002$). Frontal recordings for two other subjects also showed differential loading on a similar factor temporally located on this part of the wave form ($U = 0$, $P < .002$; $U = 1$, $P < .004$). This suggests that the most significant difference between "B" and "13" wave forms occurs in the late components, starting 160 msec after the stimulus. In addition, one frontal recording subject had a significantly different loading on a second factor which accounted for 5.9 percent of the variance ($U = 0$, $P < .002$). This factor (**f4**) was temporally located between 100 and 140 msec after stimulus presentation. Comparable factors in two other frontal recording subjects also showed a trend in this direction ($U = 8$, $P < .066$; $U = 7$, $P < .047$).

There was no significant difference between the reaction time to a "B" or a "13" over all subjects. This suggests that the observed differences in the wave forms cannot be accounted for by differences in the arousal level or attention of the subjects. Differences due to corneoretinal potentials are improbable since they were almost eliminated by the procedure described earlier, and it appears unlikely that differential eye movements would occur in response to a 10-μsec flash of the same stimulus in the two different contexts.

Our results demonstrate that late components of the evoked potential wave form, recorded from the frontal areas of the brain, reflect neural activity correlated with the meaning of the stimulus. No such differences can be detected from the visual cortex. Begleiter *et al.* (*9*) also reported meaning-correlated changes in VEP's recorded from the vertex but not from occipital locations. They recorded the evoked potential in response to a medium-intensity flash presented several seconds after an auditory stimulus that was predictive of a bright flash or of a dim flash. They found that late components of stimulus-locked potentials recorded from the vertex changed as a result of these two different temporal contexts. These findings suggest that the visual cortex may be concerned with the representation of the physical characteristics of the stimulus, but the frontal areas may be more involved in the subsequent representation of meaning.

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Gastric Brooding: Unique Form of Parental Care in an Australian Frog

Abstract: *The recently described leptodactylid frog* Rheobatrachus silus *of Queensland, Australia, exhibits a unique form of parental care. The female carries embryos and young in the stomach, propulsively ejecting the juveniles.*

Although most frogs play no active role in ensuring the survival of their offspring, a number have independently evolved quite elaborate forms of parental care. These range from the transport of eggs or tadpoles by one of the parents to the formation of dermal or subdermal pouches in which the embryos undergo their entire development. Because some of the life cycles are so bizarre, they have been cited widely in the popular and scientific literature. Here we report a unique type of parental care exhibited by the Australian leptodactylid frog *Rheobatrachus silus*: transport and brooding of larvae and juveniles in the stomach of the female frog.

Rheobatrachus silus is an aquatic, stream-dwelling frog recently discovered near Brisbane, Queensland, Australia (*1*). This monotypic leptodactylid genus is morphologically unlike any other in Australia, and in its general body form it resembles the African pipid *Xenopus*, maintained in laboratories throughout the world.

Evidence of the brooding habits was first obtained on 23 November 1973, when an adult frog collected 19 days previously was about to be transferred from one aquarium to another. This individual was of unusually large girth, the lateral surfaces appearing particularly distended. After rocks and other material had been removed from the original aquarium the frog swam haphazardly, seeking the sort of refuge beneath which it had normally hidden. It then rose to the surface of the water and, after compression of the lateral body muscles, propulsively ejected from the mouth six living tadpoles. Three of them were immediately preserved; one is shown in Fig. 1a.

In the new aquarium the frog spent most of its time drifting passively in a vertical position, with the arms and legs extended and the head so oriented that the eyes and nostrils remained above the surface of the water. This pose proved ideal for direct observation, for when the frog drifted near the glass, the abdominal region was