Spectral Analysis of Variations in Force during a Bar-Pressing Time Discrimination

Abstract. *The ability of rats to perform correctly a bar-pressing response that meets a specific duration criterion is related through spectral analysis to systematic variations in response force. It is suggested that these oscillations are representative of sensory feedback which aids in making the required temporal discrimination.*

It has been theorized that response feedback of kinesthetic and cutaneous origin is involved in the development and maintenance of temporal discrimination (*1*). Data reported by Notterman and Mintz (*2*) lend support to this idea. Rats learned to press an isometric, force-sensing manipulandum to obtain food pellets upon termination of responses lasting 1.6 seconds or more. Although the force requirement was a negligible 2.5 g, these rats eventually emitted unnecessarily high forces, of the order of 50 g. In addition, examination of strip chart recordings revealed striking force variations within individual responses. These two observations suggested that the rats were supplying themselves with neuromuscular sensory feedback that provided a basis for making the required temporal discrimination, and that the force oscillations, in particular, might represent a type of kinesthetic "scanning." Should this indeed be the case, then one would expect that the extent and regularity of the force oscillations should be correlated with the accuracy of the required temporal discrimination. Our research is addressed to this hypothesis.

The aforementioned experiment was repeated, but with the added feature that the force wave forms were recorded in a manner suitable for spectral analysis, so that a quantitative description could be made of the force oscillations within samples of separate responses. The spectra were, in turn, compared with the proportion of each rat's responses actually meeting the 1.6-second criterion, this ratio being taken as the index of accuracy of temporal discrimination.

The apparatus for measuring and recording responses has been described (*3*). Any pressure on the isometric manipulandum above 2.5 g (threshold) was recorded as a response. Reinforcement delivery accompanied termination of criterion responses only. Accordingly, receipt of a pellet did not serve as an external cue indicative of elapsed time of response, because the pellet was withheld until the response force again fell below the 2.5-g threshold.

Six male Sprague-Dawley rats, maintained on a 22-hour hunger rhythm, were shaped (trained by the method of successive approximations) to paw-press the manipulandum, which was located outside the cage; bar-biting behavior was thereby precluded. After shaping, the subjects received 20 daily sessions of training on an initial criterion of 0.8-second duration and then an additional 20 daily sessions on a criterion of 1.6-second duration. A session ended when the subject had procured a total of 50 45-mg food pellets.

A Sanborn recorder (model 60-1300, attenuation less than 1 percent at 25 hz) was used to obtain wave forms of two separate responses for each subject on training session 20 with the 1.6-second criterion. These two responses were arbitrarily designated as the responses immediately following reinforced responses 20 and 40. The longer of these two wave forms was hand-quantized at a time spacing of 0.02 second (*4*). The numerical records were then analyzed by means of a digital computer; the spectral analysis techniques outlined by Jenkins and Watts (*5*) were used.

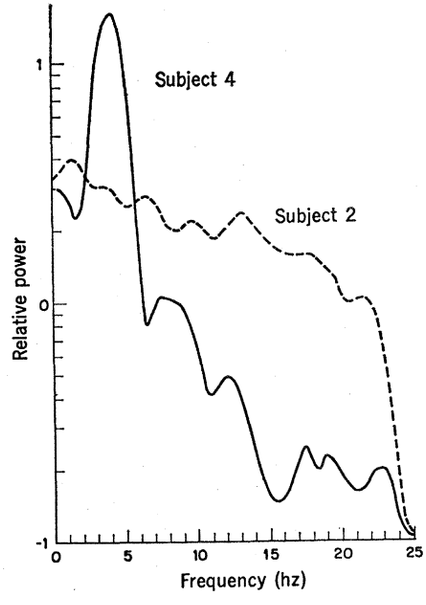

Fig. 1. Spectral density functions estimated from single responses for the best and worst subjects in a task requiring bar-pressing temporal discrimination.

The results of these analyses are summarized in Fig. 1. These estimated spectral density functions show the two extreme cases. Subject 4 displayed highly regular force oscillations of about 4 hz; this effect completely dominates its spectrum. In contrast, subject 2 exhibited practically no tendency toward consistency in force variations; well-defined peaks are absent.

If the spectral effects do indicate some type of self-cueing, kinesthetically mediated process, then one would expect that relative spectral power differences in the region of 4 hz should be correlated positively with relative frequency of success in meeting the duration requirement, and this is the case. The relative frequency of criterion responses correlates (rho $= +0.886$, Spearman rank correlation, $P < .05$) with the relative contribution to the power in the bandwidth 2.0 to 5.0 hz, the range in which the most obvious cyclic effects were observed. The relative frequencies of successful responses for the different subjects were 66.8, 27.8, 60.7, 75.8, 49.3, and 69.8 percent. In the same order of subjects, the relative power values in the bandwidth 2.0 to 5.0 were 33.0, 17.6, 39.4, 67.9, 17.5, and 42.2. Very similar results were obtained with a pilot group of animals ($N = 5$). However, the rank correlation of $+0.700$ fell short of significance; this was probably due to insufficient training, in that the subjects were immediately exposed to 20 sessions at the 1.6-second criterion, without any prior training of 20 sessions at the 0.8-second criterion. Their mean relative frequency of successful discriminations was 40 percent; the same statistic for the experimental group reported here was 58 percent.

At this stage of the research, any attempt to separate the hypothesized scanning phenomenon into components representative of a combination of conditioned and unconditioned behaviors would be quite speculative. The data do demonstrate, however, some relationship between cyclic variations in force (with assumed attendant variations in feedback) and intact motor performance that appears to demand such information.

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References and Notes

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2. J. M. Notterman and D. E. Mintz, *Dynamics of Response* (Wiley, New York, 1965), pp. 55, 66–67.
3. R. D. L. Filion, S. C. Fowler, J. M. Notterman, *J. Comp. Physiol. Psychol.* 73, 2 (1970).
4. The longer wave form was chosen because of the relative improvement it produced in the stability of the spectral estimates. Spectra for the shorter responses were calculated and were found to be essentially similar to those based on the longer records. The quantizing procedure limits the maximum detectable frequency to 25 hz.
5. G. M. Jenkins and D. G. Watts, *Spectral Analysis and Its Applications* (Holden-Day, San Francisco, 1968).

6. We thank Jeanette F. Koffler for laboratory assistance. Supported by ONR contract NONR 1858(19) (N00014-67-A-0151-0015) and by PHS grant MH 18189. Use was made of computer facilities funded in part by NSF grants NSF-GJ-34 and NSF-GU-3157.

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Calcium Transient in Presynaptic Terminal of Squid Giant Synapse: Detection with Aequorin

Abstract. *Microinjection of aequorin, a bioluminescent protein sensitive to calcium, into the presynaptic terminal of the squid giant synapse demonstrated an increase in intracellular calcium ion concentration during repetitive synaptic transmission. Although no light flashes synchronous with individual presynaptic action potentials were detected, the results are considered consistent with the hypothesis that entry of calcium into the presynaptic terminal triggers release of the synaptic transmitter substance.*

One of the prevailing hypotheses concerning the mechanism by which depolarization of a presynaptic terminal results in the release of a synaptic transmitter ("depolarization release coupling") incorporates the assumption that calcium flux into the terminal is the triggering factor for release (*1*). In the squid giant synapse, the existence of a membrane potential–dependent increase in calcium conductance at the presynaptic terminal has been demonstrated; a calcium action potential may be recorded at the presynaptic terminal after simultaneous reduction of the peak sodium and potassium currents with tetrodotoxin and tetraethylammonium ions, respectively (*2*). Under these conditions synaptic transmitter is released, as is indicated by a prolonged postsynaptic potential change in the giant axon after this calcium-dependent presynaptic spike.

The question remains whether, as a result of the action potential, calcium actually enters the axoplasm of the presynaptic terminal and is available there as a free ion, or whether it simply moves from one "membrane compartment" to another without changing the axoplasmic level. That the latter might be the case has been considered because the ionophoretic application of calcium inside the presynaptic nerve terminal is without effect on the release of transmitter (*3*). A second possibility suggested to explain these negative results is the existence of an avid intracellular calcium sequestering system that would prevent the injected calcium ions from triggering synaptic release (*3*).

We attempted to ascertain whether the intracellular calcium concentration, $[Ca^{2+}]_i$, changes during the activation of the presynaptic nerve terminal. Changes in the concentration of this ion were detected by microinjection of aequorin, a bioluminescent protein sensitive to calcium (*4*), into the presynaptic terminal. This substance emits light when exposed to very low concentrations of ionized calcium, and has been used to demonstrate calcium entry into the giant axon of the squid under various conditions (*5*). Our results demonstrate that concomitant with repetitive synaptic transmission there is an increase in $[Ca^{2+}]_i$ at the presynaptic level. The time course of this increase is consistent with the existence of a strong intracellular calcium buffering system.

Experiments were performed in the stellate ganglion of *Loligo pealii*. The stellate ganglion, together with its pre- and postsynaptic axons, was removed from the mantle and mounted in a transparent acrylic plastic chamber (*6*). The synapse was bathed with flowing, oxygenated, artificial seawater (NaCl, 466 m*M*; KCl, 10 m*M*; $CaCl_2$, 11 m*M*; $MgCl_2$, 54 m*M*; $NaHCO_3$, 3 m*M*) (*2*), and the chamber was kept at 10°C by means of a thermoelectric (Peltier effect) cooling system (Fig. 1). The presynaptic axon was activated by means of external silver–silver chloride elec-

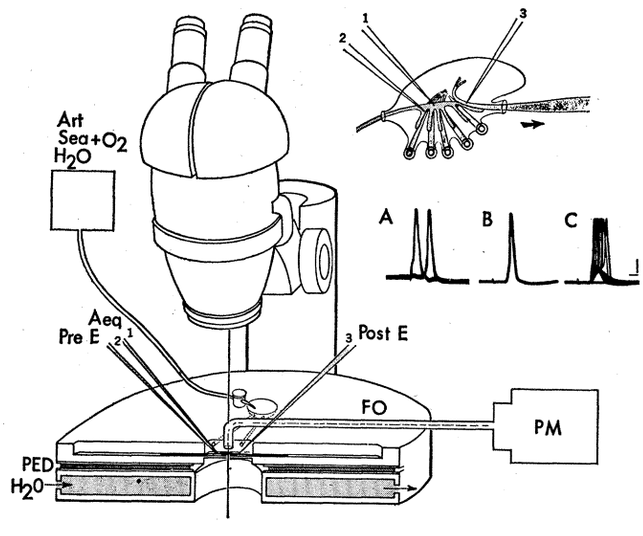

Fig. 1. Diagram of experimental arrangement and electrophysiological recordings. The chamber was kept cool by means of a Peltier effect disc (*PED*) with an underlying water-cooled heat sink. The temperature was automatically controlled from a thermistor in the chamber. The synapse was superfused with oxygenated artificial seawater throughout the experiment (*Art. sea* $H_2O + O_2$). Aequorin was injected presynaptically through a micropipette (*Aeq*) (upper right schematic diagram). The injection was performed and observed under a dissecting microscope. Electrical activity in pre- and postsynaptic fibers was recorded with electrodes *2* and *3*, respectively (details shown in upper right diagram). The presynaptic fiber was activated by means of external electrodes. *A*, simultaneous records from electrodes *2* and *3* (baselines superimposed), illustrating typical action potentials of pre- and post-synaptic fibers and synaptic delay. *B* and *C*, superimposed trains of postsynaptic responses (from electrode *3* only) evoked at 10 per second stimulation in *B* and 100 per second in *C*; those in *B* are of uniform configuration, while those in *C* show progressive diminution and failure of the EPSP. The light emission after aequorin injection was measured with a fiber-optic system (*FO*) located immediately above the synapse and in contact with the seawater. The collected light was detected with a photomultiplier (*PM*). In records *A* to *C*, voltage and time calibration are 20 mv and 1 msec, respectively.