

USE OF AN OPERATIONAL AMPLIFIER SIGNAL DIFFERENTIATOR REVEALS THAT OCTOPAMINE INCREASES THE RATE OF DEVELOPMENT OF NEURALLY EVOKED TENSION IN INSECT MUSCLE

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Octopamine is a biogenic amine that is widespread in the insect nervous system (Evans, 1978; Dymond & Evans, 1979). In the locust, it potentiates the amplitude and increases the rate of relaxation of tension, in the extensor tibiae muscle of a hindleg, induced by the stimulation of the slow excitatory motoneurone (SETi) (Evans & O'Shea, 1977; O'Shea & Evans, 1979). O'Shea & Evans (1979) determined relaxation rates by graphical analysis of the data; a process which proved to be very time consuming and subject to error in the measurement at high relaxation rates. The device we describe here allows the continuous measurement of contraction and relaxation rates, and any changes in these parameters, during the course of an experiment. It was developed for use in a comparative pharmacological study of the octopamine receptors producing the above effects (Evans, 1980; P. D. Evans, in preparation).

The electrical output of the tension transducer which is attached to the muscle tendon to monitor tension almost isometrically (see O'Shea & Evans, 1979), is first differentiated and then separated into the two components representing the contraction and relaxation rates. These two signals can be displayed separately and the voltage of each is proportional to the maximum rate of increase or decrease of tension respectively.

This operation is performed automatically by the circuit shown in Fig. 1. Amplifier 1 provides a low impedance source for the differentiator (Amplifier 2) which is designed to deal with low frequency signals. Amplifiers 3 and 4 are half wave precision rectifiers which separate the positive and negative halves of the differentiated signal. Amplifier 5 inverts the rectified output of Amplifier 4 providing a positive going signal for ease of comparison with the output of Amplifier 3.

The performance of the differentiator agrees closely with the theoretical calculated performance shown in Fig. 2 A where f_1 (0.08 Hz) is the characteristic frequency of the differentiator, f_2 (140 Hz) is the first pole frequency of the close loop circuit, and f_3 (560 Hz) is the second pole frequency of the closed loop circuit. Between the frequencies of 0.08 Hz (f_1) and 140 Hz (f_2) the circuit acts as a differentiator, whilst above 140 Hz the circuit becomes a straightforward amplifier of gain 32 dB, rolling off at 20 dB/decade (6 dB/Octave) above 560 Hz. There is a linear relationship be-

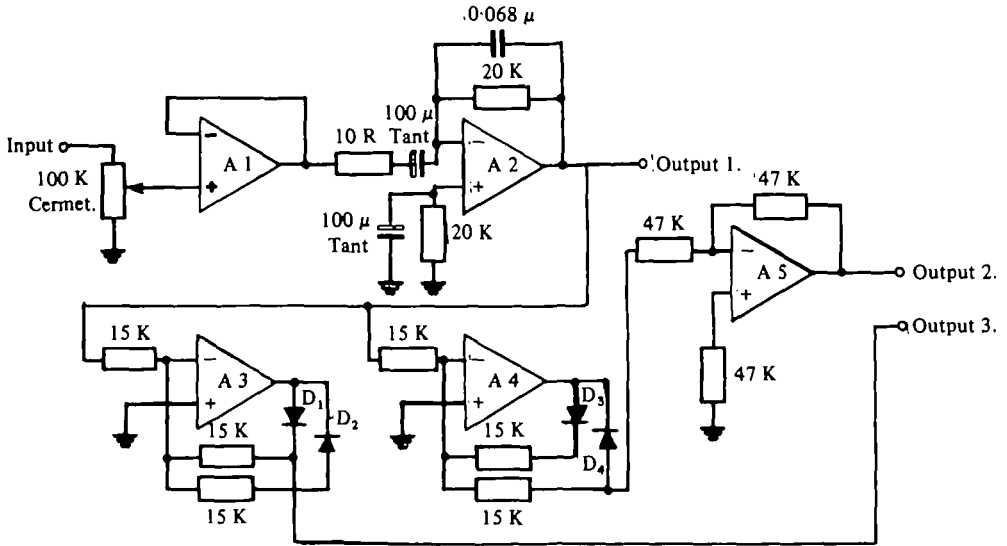


Fig. 1. Circuit diagram of signal differentiator and half-wave rectifiers (see text). Amplifiers A_1 - A_5 , Texas 741CP. Diodes D_1 - D_4 1SJ150 matched. Resistors Carbon Film C25. The 15 K resistors associated with A_3 and A_4 are matched, as also are the 47 K resistors associated with A_5 . Supply voltage ± 15 volts. The 100 K Cermet variable resistance at the input provides a means of dealing with a range of input voltage amplitudes. Once set for a series of experiments this control must be locked in position. If absolute values of rise-time are required account must be taken of the attenuation of the signal by this control.

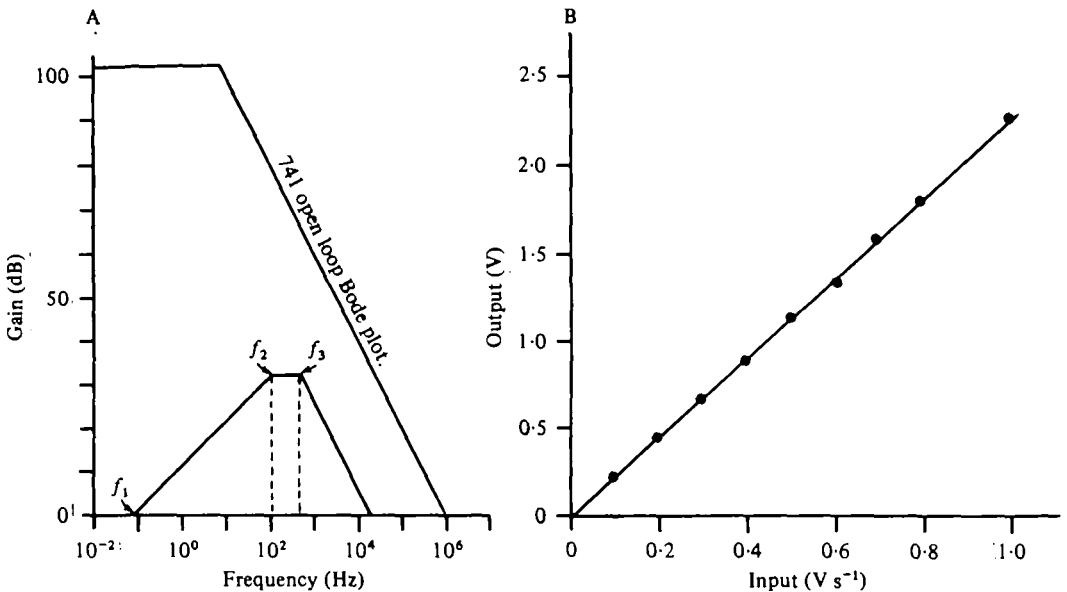


Fig. 2. Electrical characteristics of circuit. (A) Shows the typical open-loop Bode plot for a 741 Operational Amplifier. The closed-loop characteristics of the amplifier when used as a differentiator is shown within the open-loop plot. Linear differentiation of the input signal takes place between f_1 and f_3 . (B) Output of the differentiator plotted against a ramp waveform input voltage of constant amplitude, but variable period. (The re-set transient of the ramp was ignored.) The particular rates of rise were chosen to span the rates found in the biological experiment.

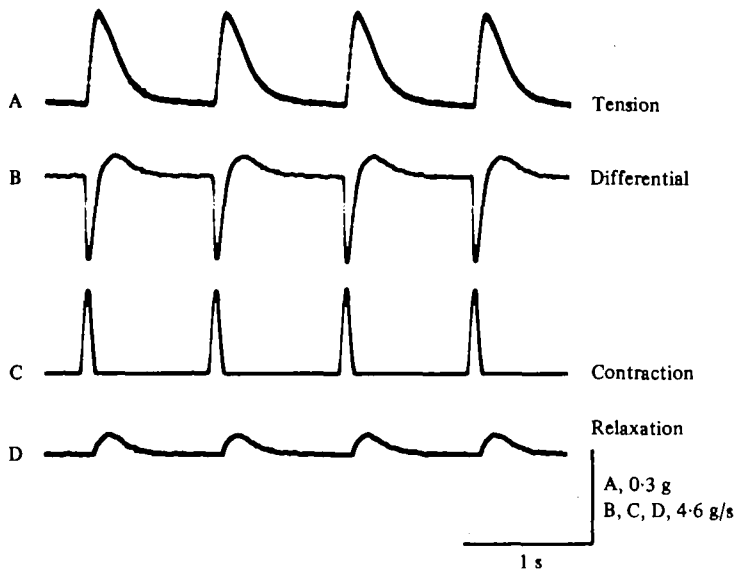


Fig. 3. Effect of Operational Amplifier signal differentiator on tension transients recorded from extensor tibiae muscle whilst stimulating the SETi motoneurone at a frequency of 1 Hz. Trace A shows the tension transients. Trace B is the differential of Trace A. Traces C and D represent the separated differential components representing the contraction and relaxation rates respectively.

tween the rate of change of voltage input to the differentiator and the output voltage over the range of the maximum rate of change of tension (0–10 g/s) produced in the muscle by stimulation of SETi (Fig. 2B).

The output of the Operational Amplifier circuit is illustrated in Fig. 3 for the individual tension transients elicited in the extensor tibiae muscle of the hindleg of the locust, *Schistocerca americana gregaria*, by repetitive stimulation of SETi (see O'Shea & Evans, 1979 for experimental details). Fig. 3A shows the output of the tension transducer obtained by stimulating SETi at 1 Hz. The differentiated form of the signal is shown in Fig. 3B (corresponds output 1 in Fig. 1) Figs. 3C and D show the differentials corresponding to the contraction and relaxation rates respectively (correspond outputs 2 and 3 in Fig. 1 respectively). The differential responses obtained by this method closely match those obtained by the determination of the tangents at the points of maximum rate of increase and decrease of tension.

An example of the use of the circuit to examine the effect of a 30 s pulse of 10 μ M DL-octopamine on the SETi-induced tension in the extensor muscle is shown in Fig. 4. Fig. 4A shows that the pulse of octopamine potentiates the evoked tension by 21%. Fig. 4B shows that the rate of contraction is also increased by 21% and that it follows the same time course. Fig. 4C shows the more marked effect on the rate of relaxation of tension. The relaxation rate is increased by 159% at the peak of the response. As previously described (O'Shea & Evans, 1979) the relaxation response also has a shorter latency and peaks sooner than the effect on the peak amplitude of tension.

The mechanism by which octopamine produces its potentiating effect on SETi-

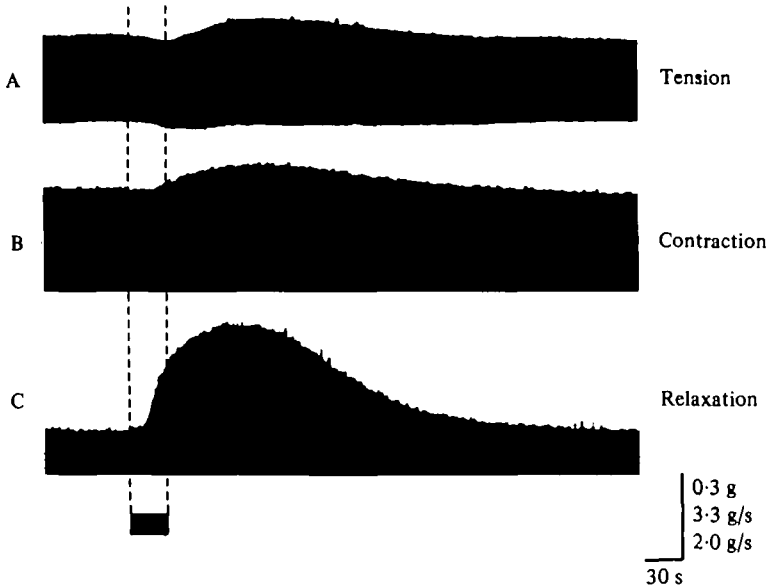


Fig. 4. Effect of a 30 s pulse of $10 \mu\text{M}$ DL-octopamine (black bar) on twitch tension responses elicited in extensor tibiae muscle whilst stimulating the SETi motoneurone at a frequency of 1 Hz. Trace A shows the response of twitch tension. Traces B and C represent the effects on contraction and relaxation rates respectively. All traces displayed at a slower time base than in Fig. 3.

evoked tension remains unknown. O'Shea & Evans (1979) suggested that at least a part of the potentiation could be the result of the interaction of octopamine with presynaptic receptors on the terminals of the SETi neurone. The novel finding presented in the present paper, that octopamine increases the rate of tension development and that this response has a similar time course to that of the potentiation of evoked tension, is also consistent with the suggestion that the latter effect may have a presynaptic origin. The fact that this effect of octopamine on contraction rates was not observed in a previous study (O'Shea & Evans, 1979) emphasizes the difficulties involved in the determination of small changes in the rate of development of tension at high speeds of muscle contraction unless a circuit such as we described here is used.

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