occurring octopamine has not been previously determined. Esparmer's "assumption" (*6*) that the levo form was present in the octopus was based on blood pressure studies. However, his proof did not distinguish between the levo and dextro isomers. The isomer in animals is yet to be determined. Octopamine has not been previously isolated and identified from plants. Gjessing and Armstrong (*7*) reported finding in oranges "small amounts of a substance which has chromatographic properties of octopamine." However, these workers offered no further explanation.

This amine was first extracted from the salivary glands of octopus, from which the name was derived (*6*). Later it was reported in human and animal urine (*8*). Pisano *et al.* (*9*) have pointed out that the significance of synephrine and octopamine in animals is not known, but that these compounds are fairly active pharmacologic agents. Furthermore, they are readily transformed into adrenaline and noradrenaline, respectively, by enzymatic action or ultraviolet radiation (*6, 10*). The presence of these physiologically active amines in plants will make it more difficult to determine if these compounds and their metabolites in animals are from dietary or metabolic sources.

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Pacemaker Neurons: Effects of Regularly Spaced Synaptic Input

Abstract. *The consequences of inhibitory or excitatory synaptic input between pacemaker neurons were predicted mathematically and through digital-computer simulations, and the predicted behavior was found to occur in abdominal ganglia of* Aplysia *and in stretch receptors of* Procambarus. *Discharge patterns under conditions that do not involve interneuronal feedback are characteristic and self-stabilizing. Paradoxically, increased arrival rates of inhibitory input can increase firing rates, and increased excitatory input rates can decrease firing rates.*

A neuron is known as a pacemaker when each impulse it generates is followed first by a repolarization and then by a depolarizing drift of the membrane potential, which again reaches threshold, thereby spontaneously producing a regular sequence of impulses. Such a neuron may also receive input (Fig. 1) in the form of inhibitory (IPSP) or excitatory (EPSP) postsynaptic potentials (*1*). Since an EPSP generally reduces the time required to reach threshold level and an IPSP prolongs this time, intervals containing EPSP's are shorter than the "natural" intervals (those with no postsynaptic potentials), and those containing IPSP's are longer (Fig. 1*a*). Mathematical descriptions, extended by digital-computer simulations, predict distinct and patterned sequences of impulses in a pacemaker cell subjected to certain regularly spaced IPSP's or EPSP's of constant magnitude, such as would be generated by other pacemaker cells. These predictions were confirmed by experiments in the sea slug (*Aplysia californica*) and crayfish (*Procambarus clarkii*).

The basic condition for the production of such responses is the functional dependence of the delay (that is, the amount by which the natural interspike interval is modified by a postsynaptic potential) upon the latency of the postsynaptic potential (that is, the time elapsed between the previous spike and the arrival of the next postsynaptic potential). Experimental delay-function curves were similar for the two species; Fig. 2 shows typical examples for an IPSP. The interspike interval as modified by a single postsynaptic potential is given by

$$s = \sigma + f(\phi), \qquad (1)$$

where σ is the "natural" interspike interval of the pacemaker, ϕ the latency, and $f(\phi)$ the delay caused by a particular postsynaptic potential. If the interval τ between successive postsynaptic potentials from the same source is constant and sufficiently long, so that at most one postsynaptic potential arrives between successive pacemaker firings, then an arrival at latency ϕ_1 will be followed, after the next spike, by an arrival at a latency

$$\phi_{i+1} = \phi_i + \tau - \sigma - f(\phi_i). \qquad (2)$$

The sequence of latencies $\phi_1, \phi_2, \ldots$, converges to a limiting stable value ϕ_∞ if the derivative of the delay function $df(\phi)/d\phi$ is positive and less than 2 (*2*). The approach to ϕ_∞ is monotonic if the derivative is less than 1, oscillatory if greater than 1.

The interval τ is related to the stable latency by

$$\tau = \sigma + f(\phi_\infty), \qquad (3)$$

and hence the stable latency is given by

$$\phi_\infty = f^{-1}(\tau - \sigma). \qquad (4)$$

The domain of stability for τ (or for frequency $\nu = 1/\tau$) is that set of values for which the delay function $f(\tau - \sigma)$ has an inverse, and has a slope between 0 and 2. Similar criteria can be derived for intervals in which more than one postsynaptic potential arrives between successive firings. The delay function satisfies these criteria for a single arriving IPSP when the latency lies between ρ (the length of the absolute refractory period, before which time an IPSP does not delay firing) and σ, the natural interspike interval, at which time firing will occur spontaneously. The domain of stability is then given by

$$\sigma + f(\rho) \leqq \tau < \sigma + f(\sigma). \qquad (5)$$

At all intervals between arrivals within this zone, the IPSP latency will become stable, and the cell's spikes will "lock in" with the IPSP's in a one-to-one manner, not in synchrony but delayed by the stabilized latency, as shown in Fig. 1*c*. If the IPSP frequency is increased, the activity enters an "un-

stable" zone, with shifting interval lengths and latencies. With further increases in IPSP frequency, there is a second period of stability, in which

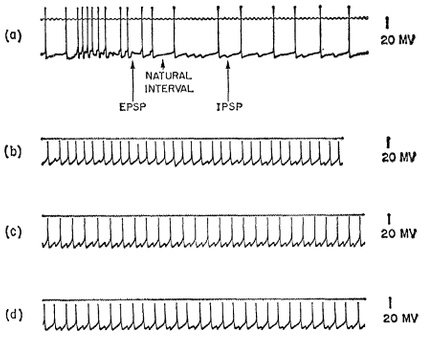

Fig. 1. Intracellular recordings from pacemaker neurons in the abdominal ganglion of *Aplysia californica*. (*a*) Cell with EPSP input shortening the firing interval and IPSP input lengthening the interval. (*b*) Approach to a stable pattern, with IPSP after every third spike. (*c*) Primary stable IPSP firing mode; one IPSP per spike. (*d*) Stable pattern with IPSP after every other spike. The time base is 20 cy/sec.

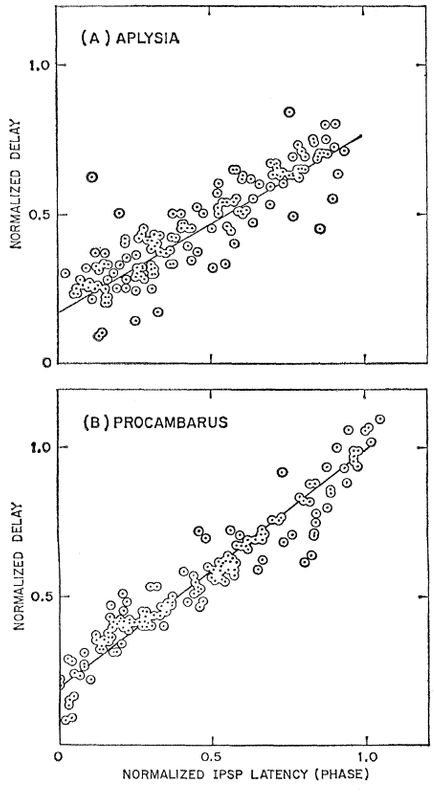

Fig. 2. Delay function for a pacemaker cell subject to single IPSP's. The lengthening of the interspike interval is plotted as a function of the arrival phase of the IPSP. Each value is expressed as a fraction of a "natural" interspike interval. (*a*) *Aplysia californica*, abdominal ganglion. (*b*) *Procambarus clarkii*, tonic thoracic stretch receptor.

two IPSP's arrive, at stabilized latencies, between each successive pair of spikes; the activity then enters another unstable zone. The sequence of stability zones continues in this fashion until the IPSP frequency is sufficiently great to cause total inhibition of the cell. At lower frequencies, similar zones are found in which an IPSP occurs after every other spike (Fig. 1*d*), every third, fourth, and so on. The stability of each pattern will vanish if either (i) the mean IPSP frequency is changed to one outside the critical ranges or (ii) sufficiently large fluctuations are imposed upon the intervals between IPSP's. Analogous zones exist for EPSP's, with the difference that at frequencies in the stable ranges, EPSP's regularly cause immediate firing of the cell.

The digital-computer program that simulates nerve-cell functioning is written in Fortran, and is run on the IBM 7090 and 7094 machines. The program uses a continuous time parameter (*3*) and it embodies a moderately complex, flexible mathematical model of a functioning neuron. For the illustrative calculations summarized in Fig. 3, values of cell parameters were selected from those observed in pacemakers in the abdominal ganglion of *Aplysia*. Some of the parameters are chosen at random from normal distributions after each spike, with means and standard deviations as stated. After each firing, there is an absolute refractory period of 10 ± 1 msec, during which postsynaptic potentials have no effect. Then the transmembrane potential is restored at -100 ± 5 mv, from which it exponentially approaches a "resting level" of -40 mv with a time constant of 6.93 sec^{-1}. The threshold for firing a spike is restored at the end of the absolute refractory period to a value of -20 mv, from which it undergoes an exponential approach to its asymptotic value of -50 mv with a time constant of 3.47 sec^{-1}. Since the asymptotic value of the potential (-40 mv) is less negative than that of the threshold (-50 mv), the cell will fire spontaneously (in the absence of synaptic input), with a "natural" interspike interval of 436 ± 5 msec. Synaptic input is simulated here by an instantaneous shift of 25 mv in the membrane potential, positive-going (EPSP's) or negative-going (IPSP's).

Figure 3 summarizes the principal computer results. The mean spike-firing (output) rate is plotted against mean

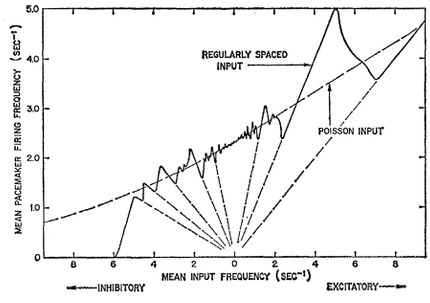

Fig. 3. Mean firing frequency of computer-simulated neuron, as a function of mean input frequency. Inhibitory frequencies are plotted to the left of the origin, excitatory frequencies to the right.

EPSP or IPSP (input) rate, with IPSP's to the left of the origin and EPSP's to the right. Uniformly spaced arrivals (solid line) produced markedly different responses from those of a Poisson process or "random" arrivals with the same mean rate (broken line). The stability zones correspond to the straight-line segments of the solid curve which coincide with lines radiating from the origin. These zones are characterized not only by a linear frequency-response curve, but also by the constancy of pattern at any frequency within a stability zone after a brief initial transition period. This was shown, for example, by the standard deviations of interspike intervals, which are small inside each stability zone as compared with those at frequencies between these zones. The principal zones, whether excitatory or inhibitory, have slopes of ± 1, with output frequencies equal to input frequencies. Zones of

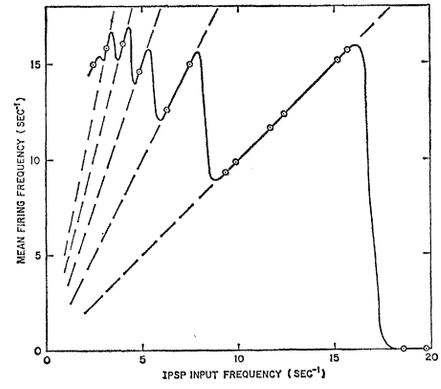

Fig. 4. Mean firing frequency of monosynaptically inhibited sensory neuron in thoracic stretch receptor of *Procambarus clarkii*. Input pulses were regularly spaced. Left-hand portion of curve joining experimental points was drawn in accordance with theory.

higher integral and fractional order may also be distinguished.

The overall trend of the curve is positive, corresponding to the fact that, in general, an increased excitation frequency, or a decreased inhibition frequency, will result in an increased firing rate of the cell. With regularly spaced input (solid curve in Fig. 1), however, some portions of the curve have a slope opposed to the overall trend. These portions give rise to the "paradoxical" effect: namely, that an increased frequency of inhibition, or a decreased frequency of excitation, will cause an increase in the firing rate of the "inhibited" or "less excited" cell.

The "paradoxical" regions for inhibited cells correspond to the stability zones; within each such region, the firing rate is proportional to the inhibition rate, with a different constant of proportionality for each zone. The corresponding "paradoxical" effect for excitation takes place just outside the stability zones, where a small increase in EPSP's frequency can cause a drastic decrease in firing rate. The change is not proportional, however, and the pattern of EPSP's and spikes is constantly shifting, as contrasted with the stable temporal patterns produced by "paradoxical" frequencies of IPSP's.

With "random" Poisson arrivals, a relatively smooth response curve is observed. Additional curves were computed in which the intervals between the postsynaptic potentials were normally distributed; with increasing variance in the arrival interval, the stability zones become narrower and less clearly delineated (*2*).

In pacemaker cells in the isolated abdominal ganglion of *Aplysia,* the observed input often consists of regularly spaced IPSP's, evoked (presumably) by another pacemaker cell. A constant latency between spike and IPSP is frequently observed; if disrupted, for example by stimulation of a nerve, the constant latency is eventually reestablished (Fig. 1*b*). Various stable sequences of interspike intervals are found, corresponding to various stability zones (Fig. 1, *b–d*); unstable "interzonal" sequences are also found. Where the delay function was known, the order of the zone and the input and output frequencies conformed to the theoretical predictions.

The stretch receptor of the crayfish (*Procambarus clarkii*) operates as a pacemaker when submitted to moderate stretch (*4*). A single inhibitory fiber makes a direct monosynaptic contact with the neuron; this fiber was subjected to electrical stimulation at regular intervals. The curve of the mean output frequency plotted against the mean input frequency (Fig. 4) is essentially similar to that of the simulated pacemaker (Fig. 3). Some of the low-frequency zones are visible, but the higher-order ones are absent, because the IPSP was of sufficient magnitude to cause total inhibition at frequencies beyond the primary stability zone. The "paradoxical" effect of regularly spaced inhibition is apparent.

The mechanism described determines stable patterns in which, over a clearly defined frequency range, the output discharge is locked in phase and frequency with the input; these phase relationships are restored spontaneously after interruption of the input. This is accomplished without feedback, that is, without closed-loop or reciprocal interaction (*5*). A mathematical term related to the slope of the delay function plays a role analogous to that of a feedback signal and accounts for the self-stabilizing properties. Many physiological possibilities suggest themselves; we may mention the following: (i) Such systems are capable of maintaining stable phase relationships among discharging units, such as would be required of antagonistic and synergistic motor units responsible for coordinated movements. (ii) Since small changes in rate of synaptic input or in input variance can markedly alter the output pattern, highly selective filtering or switching becomes possible in these networks, particularly if coupled with cells that are pattern sensitive, as have been previously described (*6*).

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5-Methoxytryptophol: Effect on Estrus and Ovarian Weight

Abstract. *Daily injection of 5-methoxytryptophol in microgram quantities in rats decreased the incidence of estrus and reduced ovarian weight in maturing animals.*

The administration of extracts from the pineal gland of rats caused a decrease in ovarian weight and provided histologic evidence of retarded ovarian maturation (*1, 2*). The reduction in incidence of spontaneous estrus and antagonism of light-induced estrus in rats has been reported (*3*). Wurtman, Axelrod, and Chu (*4*) have shown that some of the effects of the pineal gland on gonad function might be mediated by melatonin. Melatonin (5-methoxy-*N*-acetyltryptamine) is localized in the pineal gland (*5*) and is the product of hydroxyindole-O-methyl transferase (*6*) acting on the substrate *N*-acetylserotonin, a metabolite of serotonin (*7*). There is evidence that the pineal gland also contains other indolic compounds including 5-hydroxytryptophol and 5-methoxytryptophol (*8*). Hydroxyindole-O-methyl transferase, present only in pineal tissue (*6*), converts 5-hydroxytryptophol to 5-methoxytryptophol (*8*).

We have studied compounds known or thought to be present in the pineal gland in relation to the incidence of estrus and ovarian weight in rats. Since *N*-formyl-5-methoxytryptamine is indistinguishable by chromatographic and spectroscopic examinations from melatonin, it was also tested.

In the first experiment, 25 immature 27-day-old female Sprague-Dawley rats (average weight 60 grams) were divided at random into five groups of five each. The compounds tested, *N*-acetyl-5-methoxytryptamine, *N*-formyl-5-methoxytryptamine, 5-hydroxytryptophol, and 5-methoxytryptophol, were