reflect a physiological contribution of such a subset to the collective behavior of the network. Therefore, for each neuron, the activity of its "distant" neighbors has been compared with the mean value of all such subsets in the network.

Results of simulations. Simulations have begun with the excitation of a localized subset of neurons in the described network. The following results appear most promising and bear close attention:

(a) In contrast with the above model, which has a metastable mode of excitatory behavior, the lifetime of the mode is increased by at least two orders of magnitude, and the initial localized excitation never diffuses over the network. The power spectrum of the temporal dynamics demonstrates a power law dependence, $f^{-\alpha}$, between the amplitude of the fluctuations in the level of excitation (taken as the ratio of excited neurons to the total number of neurons in the network) and the frequency of the fluctuations. In our case the analysis indicates that large fluctuations appear with a lower probability than those of low amplitude.

(b) The average topological distance (ATD) between excited elements (a measure of the minimum number of synaptic junctions that information must traverse in going from one randomly chosen element to another) is within the topological distance interval (2.2–2.3), until a steady state is approached. According to experimental data (3), the ATD in the cortex is close to this interval: about 2. This result indicates that the metastable mode is inherent only to a subset of excited elements which has an ATD reasonably close to what is found in a real biological object.

(c) The average number of excited "distant" neighbors for most neurons at the border of the group of excited neurons is larger than for most neurons inside the group. Thus, there is dynamic selectivity which operates without the synaptic modulation that usually underlies selectivity.

Analysis and discussion: First, what kind of approximation can be applied to the part of the input signal to each neuron that comes from "distant" neighbors? It may be regarded as Gaussian noise with amplitude $\sigma^2 \simeq S*(1 - S)$, where S is the fraction of excited neurons. Next we can pass to a diffusion approximation (4) which leads to an interesting explanation of the persistence of the metastable mode in the system: the system moves over the stochastic potential surface, but is never able to reach a stable state, because the latter is changing its position as the system changes its level of excitation and, therefore, the amplitude of noise. Remember that the amplitude of the noise determines the location of stable states on the stochastic potential surface.

These results lead us to the following conclusion: the noise component in neural network dynamics may play, not only a destructive, but also a stabilizing role in neural activity. Indeed noise can originate naturally, not from the "imperfectness" of biological systems, but rather from the interactions between different structural levels of a deterministic system.

Literature Cited

1. **Kirillov, A. B., and V. I. Makarenko. 1991.** In *Neurocomputers and Attention II*, A. V. Holden and V. I. Kryukov, ed. Manchester University Press.
2. **Little, W. A. 1974.** *Math. Biosci.* **19**: 101–120.
3. **Clark, J. W., G. C. Littlewort, and J. Rafelski. 1986.** 'Topology, structure and distance in quasirandom neural networks', preprint, University of Cape Town.
4. **W. Horsthemke and R. Lefever. 1984.** *Noise-Induced Transitions,* Springer Series in Synergetics, Vol. 15, Hermann Haken, ed. Springer-Verlag.

Ca²⁺ Fluxes Around Pollen Grains and Pollen Tubes of Lily; Normal Development and Effects of Thermal Shock, BAPTA-Type Buffer Microinjection and Depletion of Boric Acid From the Medium

E. S. Pierson (Biologia Ambientale, University of Siena, I-53100 Siena, Italy), P. J. S. Smith, A. M. Shipley, L. F. Jaffe, M. Cresti, and P. K. Hepler

Under favorable conditions, pollen grains germinate to form a long tubular extension, the pollen tube, through which the plant male gametes reach the egg apparatus. Cytosolic free Ca^{2+} ions, which are distributed in a steep gradient along the growing pollen tube tip (5), are assumed to play a role in regulating the growth of the tip, the organelle movement, and the maintenance of the cytoplasmic zonation (5,6). From $^{45}Ca^{2+}$ labeling (1) we know that the high Ca^{2+} domain in the cell apex is at least partly created by a transient uptake of the ion. With the Ca^{2+}-selective vibrating probe technique (2,9), minute Ca^{2+} fluxes can be detected around single cells (2). Here, we have used this technique

to survey the net Ca^{2+} flux pattern around *in vitro* germinating pollen grains and growing pollen tubes of the lily (*Lilium longiflorum* 'White Nellie'). We have also explored the effect on the net Ca^{2+} flux of three growth-disturbing treatments: thermal shock, microinjection of 4,4' difluoro 1,2-bis (o-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid [2F-BAPTA] buffer, and depletion of boric acid from the culture medium.

Pollen tubes were grown at room temperature in a film of medium composed of 10% sucrose, 100 μM $CaCl_2$, 160 μM H_3BO_3, 15 mM 2 n-morpholino ethane sulfonic acid buffer adjusted to pH 5.5 with KOH, and about 1% low temperature

gelling agarose. Ca^{2+} fluxes were measured according to standard procedures (2, 9), except that the tip of the electrode was vibrated inside the agarose layer, as close as possible to the cell.

Normally developing pollen grains and tubes typically showed a marked pattern of Ca^{2+} influx at the cell tip, but with great variability among cells (estimated range: 15–180 μV after subtraction of the background value $\approx$ 0 μV, at a vibration amplitude of 10 μm and a background [Ca^{2+}] of about 100 μM). Ca^{2+} diffusion is obviously an important component of the total ionic current that traverses pollen tubes (10,11). No significant Ca^{2+} flux signal could be detected in the other regions of the grain. The influx magnitude appeared to be positively correlated with the growth rate (1–14 μm/min) of the cell. The voltage differences ($\approx Ca^{2+}$ flux) were higher for *Lilium* pollen tubes than for *Arabidopsis* root hairs (7).

Exposure of short (10 μm) and long (400 μm) pollen tubes to a thermal shock (45°C for 10 s) immediately halted growth and concomitantly arrested Ca^{2+} influx. Moreover, large organelles, which normally were absent from the tip zone, penetrated into the apical dome. Resumption of growth, characterized by the bulging of the tip and the restoration of the organelle zonation, was in all cases accompanied by recovery of the Ca^{2+} influx.

BAPTA-type buffers possibly act as calcium shuttle buffers when they are introduced inside cells (8), dissipating or diminishing the natural calcium gradients (see reference 5 for pollen tubes). In previous work (E. S. Pierson *et al.*, in prep.) BAPTA-type buffers with a K_d between 0.2 and 4.6 μM were found to inhibit pollen tube growth in a reversible way. In the present study we injected a low dose of 2F-BAPTA in normally growing pollen tubes to block cell growth without seriously affecting the cytoplasmic streaming. As a consequence, the Ca^{2+} flux at the cell tip fell rapidly to about zero. Spontaneous resumption of growth, probably due to the sequestration of buffer into the vacuole, proceeded together with recovery of the Ca^{2+} ionic flux.

This observation suggests that a certain level of intracellular Ca^{2+} is required for transcellular Ca^{2+} entry (positive feedback), and that this entry pathway is not directly linked to passive diffusion of the ion.

Boric acid has been reported to enhance the chemotropic response of pollen tubes to calcium (4). In preliminary experiments we progressively washed boric acid out of the culture and found that most pollen tubes burst at their tip. The cells had a normal morphology, growth behavior, and Ca^{2+} influx pattern at the moment of disruption. Explanations for the above findings are that boric acid may affect cell wall integrity or plasma membrane water permeability, rather than the Ca^{2+} flux mechanism.

Literature Cited

1. Jaffe, L. A., M. H. Weisenseel, and L. F. Jaffe. 1975. *J. Cell Biol.* 67: 488–492.
2. Kühtreiber, W. M., and L. F. Jaffe. 1990. *J. Cell Biol.* 110: 1565–1573.
4. Mascarenhas, J. P., and L. Machlis. 1964. *Plant Physiol.* 39: 70–77.
5. Miller, D. D., D. A. Callaham, D. J. Gross, and P. K. Hepler. 1992. *J. Cell Sci.* 101: 7–12.
6. Pierson, E. S., and M. Cresti. 1992. *Int. Rev. Cytol.* 140: 73–125.
7. Schiefelbein, J. W., A. Shipley, and P. Rowse. 1992. *Planta* 187: 455–459.
8. Speksnijder, J. E., D. W. Corson, C. Sardet, and L. F. Jaffe. 1989. *Dev. Biol.* 135: 182–190.
9. Smith, P. J. S., R. H. Sanger, and L. F. Jaffe. 1994. In *Methods in Cell Biology* 40, R. Nuccitelli, ed. Academic Press (in press).
10. Weisenseel, M. H., R. Nuccitelli, and L. F. Jaffe. 1975. *J. Cell Biol.* 66: 556–567.
11. Weisenseel, M. H., and L. F. Jaffe. 1976. *Planta* 133: 1–7.

Endogenous Currents During Wound Healing and Regeneration in Sabellid Fanworms

Susan Douglas Hill (Michigan State University), Alan M. Shipley, and Peter J. S. Smith

The concept that endogenous currents play a significant role in wound healing and regeneration has gained considerable support over the past 20 years (1, 2). It is well-documented that in urodele amphibians, an outward ionic current leaves the wound surface shortly after limb amputation (2, 3, 4) and persists at low levels until the time of blastema formation. Much of the impetus for the present interest in endogenous currents has resulted from the development of the vibrating probe by Jaffe and Nuccitelli (5), subsequently modified (6, 7), which allows precise current measurements to be made noninvasively at specific sites. We have used the vibrating probe to detect the weak voltages associated with the steady state ionic currents generated during anterior regeneration in sabellid fanworms following segment amputation.

Sabellids have remarkable powers of regeneration. Following transection at almost any level of the body, anterior-facing cuts result in the regeneration of a new head complete with branchial crown, collar, mouth, and anterior neural ganglia and connectives. Posterior amputations result in the replacement of the pygidium and the addition of new posterior segments. Immediately after amputation, muscle contractions constrict the wound surface. Within 24 h, the wound surface is usually thinly covered with epidermis. This initial phase of wound healing probably results primarily from cell migration, since few cell divisions (as detected by H^3 thymidine incorporation) are observed (8). Following the initial healing, cells accumulate to form the regeneration blastema that gives rise to the new outgrowth. New branchial filaments appear as finger-like projections, and