A computational approach enhances learning in *Aplysia*

L F Abbott & Eric R Kandel

A mathematical model based on the dynamics of molecular signaling pathways predicts an optimal training regimen that enhances learning and memory.

Like forgetting, learning is a dynamic process involving a multitude of timescales. If we understood these timescales and the processes that produce them, could we enhance learning by devising training schedules that are matched to the underlying dynamics of learning? A paper by Zhang *et al.* in this issue of *Nature Neuroscience*[1] shows that, for the sea slug *Aplysia californica* at least, the answer to this question seems to be yes.

Aplysia is an excellent system for studying the dynamics of learning because of a long history of results that relate changes in its behavior to modifications in synaptic strength between its sensory and motor neurons[2]. In addition, a great deal is known about the molecular processes responsible for these synaptic modifications[3]. *Aplysia* retract their siphon and gill in response to a weak stimulus applied to the siphon. This reflex is greatly enhanced by sensitization, a form of learned fear, produced by applying a noxious stimulus, such as an electric shock, to the tail of the animal. The duration of this enhanced withdrawal is increased for days following exposure to a series of repeated shocks[4].

The characteristics of *in vivo* long-term sensitization can be reiterated *in vitro* by exposing synaptically connected cultured *Aplysia* sensory and motor neurons to repeated pulses of serotonin[5], the modulatory transmitter released by tail shocks. Five pulses of serotonin spaced at 20-minute intervals produce a long-lasting enhancement of synaptic strength termed long-term facilitation. It has been established previously that a series of pulses spaced in this manner is more effective at inducing long-term facilitation than the rapid delivery of the same number of pulses[6], an effect that has also been observed in other systems[7,8]. Interestingly, spaced protocols are also more effective than massed training in human memory tasks[9,10], as typified by the common advice to students not to cram for exams.

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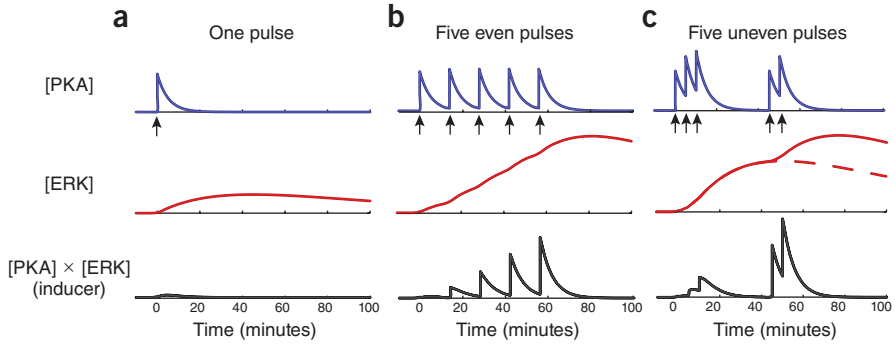

Figure 1 A simple, descriptive model of concentration time courses. Graphs show concentrations of active PKA (top) and active ERK (middle, solid red curves), and the product of their concentrations (bottom). Times of serotonin pulses are denoted by arrows. (**a**) The effect of a single serotonin pulse. [PKA] rises rapidly and decays exponentially, becoming negligible in 15 minutes. [ERK] rises and falls more slowly, reaching its maximum after 45 minutes. A single pulse produces only a small transient increase in the product of these concentrations. (**b**) The arrangement of five serotonin pulses with even separations that produces the highest peak value in the inducer, [PKA] × [ERK]. (**c**) The optimal arrangement of five serotonin pulses with separations of 5, 5, 35 and 5 minutes (5 minutes is the minimum allowed spacing) produces a higher peak level of inducer. Dashed red curve, expected time course of [ERK] due to the first three pulses if the final two pulses had not occurred; solid red, result of all five pulses. This pulse spacing produces the highest peak value of the inducer because the final two pulses begin at the time when the rise in [ERK] produced by the first three pulses reaches its peak.

Critical for the work of Zhang *et al.*[1] is the dependence of long-term facilitation on an increase in the enzymatic activities of two kinases, protein kinase A (PKA) and extracellular signal-related kinase (ERK), which in turn increase the phosphorylation of cyclic AMP response element-binding factor 1 (CREB1; ref. 3), a key transcription factor in the establishment of long-term memories. Because of the dual involvement of PKA and ERK, the authors postulated that the magnitude of CREB1 phosphorylation and long-term facilitation, as well as the degree of behavioral sensitization, should depend cooperatively on the activation of both proteins. In particular, they assumed that the magnitude of the synaptic changes and the strength of learning are determined by an 'inducer' that has an efficacy proportional to the peak value of the product of the concentrations of active PKA and ERK, [PKA] × [ERK].

The authors used a mathematical model that describes the time evolution of [PKA] and [ERK] to determine the temporal sequence of five serotonin pulses that produces the largest possible peak value of the inducer. The optimal intervals between these pulses turned out not only to be different from the standard 20 minutes, but also unequal to one another. Therefore, the optimal exposure is not a set of five evenly spaced serotonin pulses but rather an irregular sequence. Most studies of learning have considered varying the intervals between training sessions but have restricted consideration to cases in which all the intervals are identical. The modeling work by Zhang *et al.*[1] suggests that a non-uniform training schedule may be optimal.

The authors tested this prediction using three measures of learning: the magnitude of synaptic long-term facilitation, the amount of CREB1 phosphorylation and, finally and most impressively, the degree and persistence of behavioral long-term sensitization. For all three measures, they compared the effects of the unevenly spaced protocol suggested by their model to those produced by serotonin pulses delivered at uniform 20-minute intervals. In all three cases, unevenly spaced pulses outperformed even spacing, producing larger and longer-lasting effects.

A highly simplified model based on the work of Zhang *et al.*[1] (**Fig. 1**) illustrates why

uneven training intervals outperform even intervals. In the simplified description, [PKA] rises rapidly (approximated as instantaneous) following a serotonin pulse and then decays exponentially with a time constant of 5 minutes (top, **Fig. 1a**). [ERK] rises and decays more slowly, described by an alpha function ($te^{-\alpha t}$) that peaks 45 minutes after a serotonin pulse ($1/\alpha = 45$ minutes; middle, **Fig. 1a**). A sequence of five evenly spaced serotonin pulses gives a peak value of the inducer (that is, the product [PKA] × [ERK]) that is greater than that of single pulses (**Fig. 1b**). However, the sequence of five pulses that generates the greatest possible peak value of the inducer has uneven interpulse spacings (**Fig. 1c**). The optimal arrangement involves three pulses, spaced as closely together as possible, to initiate an increase in ERK activation, followed by a pause to allow the rise in ERK activity to reach its maximal value. Finally, the remaining pulses are used to increase the activity of PKA near the time of this maximum. The optimal sequence thus puts 60% of its resources into an initial burst to lift ERK activation and saves 40% to produce a final increase in PKA activity (**Fig. 1c**). This produces a higher peak value of the inducer than any sequence with equally spaced intervals (**Fig. 1b**). This simpler (and less accurate) model does not reproduce the exact interval pattern that was predicted to be optimal by Zhang *et al.*[1], but it matches the result that uneven intervals give the largest effect.

Learning is not simply a matter of flipping biochemical switches inside neural circuits. Training sets off a cascade of genetic, biochemical and structural changes that are established over and last for a wide range of different times. The multi-timescale nature of these events accounts for the complex dynamics of forgetting. For example, curves measuring the retention of memory over time do not fall exponentially, but rather they are proportional to time raised to a negative power, so-called power-law forgetting[11,12]. The similarly extended timescales involved in generating synaptic modifications make the dynamics of learning equally rich. The work of Zhang *et al.*[1] opens up the possibility that, as we understand more about the mechanisms of neuronal circuit modification that lead to learning and begin to construct predictive models, we may be able to develop more effective, rationally designed training schedules that enhance learning.

Because elementary forms of learning, such as habituation, sensitization and classical conditioning, are extremely general, not only behaviorally but also at the molecular level, it is likely that features of stimulus sequences and reinforcement schedules are also general. The approach taken by Zhang *et al.* may be productive in the context of various implicit forms of learning in mammals, and this could, ultimately, lead to more effective learning protocols with clinical applications, for example to rehabilitation medicine.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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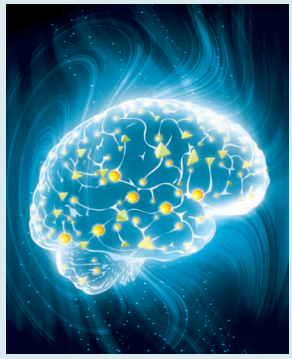
Zooming in on seizures



Epilepsy affects 1–2% of the general population and treatment options for this debilitating disorder are limited. Although the mechanisms that underlie seizure initiation and generation are still not fully understood, an imbalance between excitation and inhibition in the cortex is thought to be a critical factor. Fast-spiking interneurons that express the calcium-binding protein parvalbumin (parvalbumin-positive interneurons) make up the dominant inhibitory system of the neocortex and critically regulate the output of the pyramidal neurons. Two studies in this issue of *Nature Neuroscience* examine the mechanistic link between parvalbumin-positive interneurons and seizures and causally implicate parvalbumin-positive interneuron excitability to seizure generation in rodent models.

Neuregulin 1 (NRG1) is a neurotrophic factor that activates the kinase activity of ErbB4 receptors. Previous work had shown that ErbB4 expression is high in parvalbumin-positive interneurons and that NRG1 can regulate GABAergic transmission.

Li *et al.* on page 267 use transgenic mice, biochemistry, pharmacology and electrophysiology to manipulate NRG1-ErbB4 signaling and examine its effects on parvalbumin-positive interneuron excitability. They find that NRG1 and ErbB4 normally enhance parvalbumin-positive interneuron excitability by suppressing the voltage-activated potassium channel Kv1.1, altering the membrane threshold for the generation of action potentials. Although the detailed mechanism by which NRG1 affects Kv1.1 activity is not known, the authors find that NRG1 regulates the level of tyrosine phosphorylation of the Kv1.1 channel. Crucially, they find that mice lacking ErbB4 specifically in parvalbumin-positive interneurons have more severe seizures in response to convulsants. Li *et al.* also find that ErbB4, but not ErbB2, expression is reduced in cortical tissue excised from humans suffering from temporal lobe epilepsy.

In an independent report, Tan *et al.* on page 258 corroborate the critical involvement of NRG1-ErbB4 signaling in parvalbumin-positive interneurons to epilepsy. They find that, in a kindling model of epilepsy, seizure activity increases NRG1 levels. In the same time frame, the phosphorylated form of the ErbB4 receptor is also increased. Tan *et al.* also find that intracerebral infusion of NRG1 delays the onset of kindling, whereas specific deletion of ErbB4 in parvalbumin-positive interneurons promotes kindling progression. Notably, they generate mice in which ErbB4 is specifically ablated in CaMKIIα-positive neurons (presumed to be pyramidal cells) but observed no effect on seizures. Moreover, the authors report that NRG1-ErbB4 signaling affects the sprouting of hippocampal mossy fibers into the dentate inner layer, a pathological feature of limbic epilepsy.

Together, both studies provide powerful evidence to causally implicate the NR1-ErbB4 pathway in the regulation of parvalbumin-positive interneuron excitability and seizure generation, and also point to new targets for future anticonvulsive therapy.

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