Aprendizaje, memoria y plasticidad neuronal en Aplysia
10.7 The neural circuit for siphon withdrawal

(A) The major ganglia that constitute the central nervous system of *Aplysia* are superimposed here (in color) on a top view of the animal. (B) A highly simplified schematic wiring diagram of the neural circuit for the gill withdrawal reflex. Only eight sensory neurons from the siphon are illustrated, although there are many more. They are shown connecting directly to motor neurons that cause contraction of the gill. The sensory neurons also connect to excitatory (EXC) and inhibitory (INH) interneurons (the terminals of which are depicted as white and shaded triangles, respectively). A after Kandel 1976; B after Squire and Kandel 1999.
Habituation and dishabituation in *Aplysia*

*Aplysia* exhibit habituation and dishabituation. (A) This top view of *Aplysia* shows the gill in a relaxed state (left) and contracted (right) following a tactile stimulus to the siphon (dotted lines show relaxed position of fill for comparison). (B) Photocell records of gill contractions show that the response habituates after repeated tactile stimuli. Each stimulus event is indicated at the bottom of each trace; the number below each trace indicates the stimulus number. The interstimulus interval was 1.5 minutes. Between the thirteenth and fourteenth stimuli a shock was delivered to the tail, producing dishabituation. After Kandel and Schwartz 1982.
The cellular mechanisms of habituation have been investigated in the gill-withdrawal reflex of the marine snail *Aplysia*.

A. A dorsal view of *Aplysia* illustrates the respiratory organ (gill), which is normally covered by the mantle shelf. The mantle shelf ends in the siphon, a fleshy spout used to expel seawater and waste. A tactile stimulus to the siphon elicits the gill-withdrawal reflex. Repeated stimuli lead to habituation.

B. This simplified circuit shows key elements involved in the gill-withdrawal reflex as well as sites involved in habituation. In this circuit about 24 mechanoreceptors in the abdominal ganglion innervate the siphon skin. These glutaminergic sensory cells form synapses with a cluster of six motor neurons that innervate the gill and with several groups of excitatory and inhibitory interneurons that synapse on the motor neurons. (For simplicity, only one of each type of neuron is illustrated here.) Repeated stimulation of the siphon leads to a depression of synaptic transmission between the sensory and motor neurons as well as between certain interneurons and the motor cells.
HABITUACIÓN EN APLYSIA

A

Control  Habituated (1 week)

Motor neuron

Sensory neuron

5 mV

10 mV

50 ms

B Inactivation of synaptic connections by long term habituation

Mean percent of connections

Control 1 day 1 week 3 weeks

Habituated
10.6 Long-term memory in *Aplysia*

(B) The left-hand graph shows the pretraining siphon withdrawal responses, and then the retention of sensitization in tests at 1 day (R1), 1 week (R2), and 3 weeks (R3). The right-hand graph shows the cumulative sensitization exhibited in each retention test. After Kandel 1976; data from Carew, Pinsker, and Kandel 1972 (A) and Pinsker et al. 1973 (B).
SENSIBILIZACIÓN EN APLY西亚

Estímulo sensibilizador

Estímulo test

A

1 Control Sensitized

Sensory neuron

Motor neuron

Sensitizing stimulus

Facilitating interneuron

Sensory neuron

Interneuron

Motor neuron

Gill

Tail

Tactile stimulus

Siphon

Respiratory organ (gill)

Mantle shelf

Snack

2 Synaptic potentials

3 Behavior

Median PSP amplitude (mV)

Median duration of siphon withdrawal (s)

Control Sensitized

Control Sensitized

Pre Post Pre Post
SENSIBILIZACIÓN EN APLYsIA

(A) Siphon withdrawal duration (median pretraining-posttraining) (s)
- 4 days, 4 trains (4 shocks)
- 4 trains (4 shocks)
- 4 single shocks
- Control

Time after training (days)

(B) Control Sensitized
MN 12 mV
SN 20 mV 100 ms

Control Sensitized
One day after training

Median EPSP amplitude (mV)
Experimental preparations for cellular analysis

The cellular basis of gill and siphon withdrawal can be studied at several levels of analysis. (A) In the most intact preparation the abdominal ganglion is externalized, and recordings from neural elements are made during reflex actions. (B) In what is known as the semi-intact preparation, the entire central nervous system (CNS) is removed. In some cases peripheral organs (such as the gill, siphon, and tail) are left attached to the CNS by their peripheral nerves. (C) In a third preparation, single ganglia (or pairs of ganglia) are removed. Recordings are made from identified neurons in the neural circuit for siphon and gill withdrawal. (D) Finally, in the most fundamentally reduced preparation, isolated sensory and motor neurons are placed in cell culture, where functional synapses form.
Sensitization increases the synaptic response between sensory and motor neurons

The connection between the sensory neuron and motor neuron is facilitated during sensitization. In each case illustrated here, intracellular recordings from a motor neuron and a sensory neuron are shown. A spike is elicited in the sensory neuron (by the injection of depolarizing current into the cell), which produces an EPSP in the motor neuron. (The preparation used in these experiments is shown in Figure 10.8C.) (A) Electrical stimulation of the connectives, which carry input from the tail, induces synaptic facilitation. (B) Application of serotonin, which is released in the CNS in response to tail shock, induces synaptic facilitation. (C) Injection of cyclic AMP into the sensory neuron induces synaptic facilitation. B after Kandel 1983; data from Brunelli, Castellucci, and Kandel 1976.
A Calcium control of vesicle fusion and pore opening

Figure: The mobilization, docking, and function of synaptic vesicles are controlled by low-molecular-weight GTP-binding proteins and Ca$^{2+}$.

A. Synaptic vesicles in nerve terminals are present in a sequestered compartment (sometimes called the storage compartment) where they are tethered to the cytoskeleton as well as in a compartment (sometimes called the releasable compartment) where vesicles are docked to the fusion pore complex. Entry of Ca$^{2+}$ into the nerve terminal leads to the opening of the fusion pore complex and neurotransmitter release. Calcium entry also mobilizes vesicles from the storage compartment, thus increasing their availability for docking into the presynaptic plasma membrane.
Figure 12 The cAMP pathway is typical of neuronal second-messenger pathways. Adenyl cyclase converts ATP into cAMP. Four cAMP molecules bind to the two regulatory subunits of the cAMP-dependent protein kinase, liberating the two catalytic subunits, which are then free to phosphorylate specific substrate proteins that regulate a cellular response. Two kinds of enzymes regulate this pathway. Phosphodiesterases convert cAMP to AMP (which is inactive), and protein phosphatases remove phosphate groups from the regulator (substrate) proteins.

(A) Inject current | Record voltage
(B) Membrane depolarization | Action potential

-40 mV
0 mV
Rising phase
Falling phase
-65 mV
(Restoring potential)

The action potential
(A) When a small pulse of positive current is injected into a neuron, small depolarizations are produced. Once threshold is achieved, an action potential is generated. (B) The different phases of the action potential (top) and the ionic events underlying it (bottom).
Memoria / comportamiento
Aumento de la respuesta:
Sensibilización de
Corto término (STS)

Neuronal / sináptico.
Aumento del potencial post-sináptico excitatorio:
Facilitación de corto término
(STF)
FIGURE 6  The Schwartz and Kandel model of short-term and long-term regulation of PKA in *Aplysia* sensory neurons. This is a mechanism for short- and intermediate-term facilitation of neurotransmitter release. See references 8 and 9 and explanation of pathway in text. PKA shown as tetramer of two regulatory (Reg.) and two catalytic (Cat.) subunits. The catalytic site is shown in yellow. PDE = Phosphodiesterase.
Facilitación de término intermedio (ITF)

Depende se síntesis de proteínas pero no de transcripción
Depende de actividad persistente de PKA
Facilitación de largo término (LTF)

Depende se síntesis de proteínas
Depende de transcripción vía cAMP (CREB)
CREB se une a la secuencia consenso CRE

Foreign CRE sequences block long-term synaptic facilitation
Injection of foreign oligonucleotides (oligos) containing foreign CRE sequences blocks the induction of long-term synaptic facilitation. (A) EPSPs at 0 h and 24 h after multiple exposures to serotonin. In the control case (no oligonucleotides, top traces) and in cases in which inactive oligonucleotides were injected (middle traces), long-term facilitation was induced. But the CRE-containing oligonucleotide blocks long-term facilitation (bottom traces). (B) Summary of the data. In the control case and in several cases in which inactive oligonucleotides were injected, long-term facilitation was induced. However, injection of the CRE-containing oligonucleotide (black bar at far right) blocks long-term facilitation. After Dash, Hochner, and Kandel 1990.
Facilitación de largo término (LTF)

Depende de síntesis de proteínas
Depende de transcripción vía cAMP (CREB)
CREB se une a la secuencia consenso CRE
Gen temprano:
CAATT enhancer binding protein (C/EBP)

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Figure 19: Induction of ApC/EBP mRNA
(A) ApC/EBP mRNA expression in CNS of untreated Aplysia, of Aplysia treated in vivo with the indicated drugs for 2 hr at 18°C, or dissected without treatment and kept at 18°C in culture medium. Shown here are four independent experiments, in which 10 µg of total RNA extracted from the CNSs of untreated (minus sign) or treated Aplysia, as indicated, were electrophoresed, blotted, and hybridized with 32P-labeled ApC/EBP (top) or S4 (bottom) probes. The latter encodes the Aplysia homolog of S4 ribosomal protein (Thomas et al., 1987), which is constitutively expressed and used as a loading control. Zero indicates RNA extracted immediately after dissection of Aplysia CNS. The 2 hr dissection represents RNA extracted from Aplysia CNS, dissected, and incubated in culture medium for 2 hr at 18°C. IBMX, isobutyl methylxanthine.

(B) Time course of ApC/EBP mRNA induction following 5-HT treatment. Times of treatment, in minutes, are indicated. Five micrograms of the total RNA extracted from the total CNS of in vivo treated Aplysia were analyzed as described in (A).
C/EBP se une a la secuencia ERE
CREB se une a la secuencia CRE
Ventana temporal del efecto de la inyección del oligonucleótido ERE
Facilitación de largo término (LTF)

Depende se síntesis de proteinas
Depende de transcripción vía cAMP (CREB)
Depende de transcripción vía C/EBP
Intermediate-term memory in *Aplysia*

*Aplysia* exhibit a unique form of intermediate-term memory. (A) Five pulses of serotonin give rise to three distinct phases of synaptic facilitation: short-term, lasting 15 minutes (produced by a single pulse); intermediate-term, lasting 1 to 2 h; and long-term, lasting at least 24 h. Intermediate-term facilitation decays to baseline several hours before the expression of long-term facilitation. (B) PKA activation in the sensory neurons induced by five pulses of serotonin shows the same three phases of facilitation that synaptic facilitation shows. (C) One tail shock induces short-term memory; five induce intermediate- and long-term memory for sensitization. Note that the temporal profiles for all three measures are quite similar. In addition, all three measures of memory processing have the same mechanistic requirements. A after Mauelshagen, Parker, and Carew 1996; B after Müller and Carew 1998; C after Michael A. Sutton and Thomas J. Carew, unpublished observations.
Facilitación de término intermedio (ITF)

Depende se síntesis de proteínas pero no de transcripción
Depende de actividad persistente de PKA
MAPK Establishes a Molecular Context That Defines Effective Training Patterns for Long-Term Memory Formation

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**Figure 1.** Long-term memory for sensitization of the tail-elicited tail withdrawal reflex has a narrow temporal two-trial training window. *a,* Schematic of the reduced behaving preparation used to study the tail-elicited tail withdrawal reflex (Inset: representative tail withdrawal [TW] response to test stimulation [TS] as measured by a strain gauge). *b,* Summary of average 18–22 h (LTM) scores in preparations trained with two shocks spaced by 15 min (2×15), 45 min (2×45), 60 min (2×60), or untrained (NS). Data are presented as mean ± SEM. Asterisk indicates enhanced responding at 18–22 h in 2×45 trained animals over untrained controls (unpaired t-test).
ERK se activa a los 45 min

**Figure 2.** Serotonin can serve as a proxy for training shock in the restricted temporal activation of protein synthesis-dependent MAPK. *a*, A 5 min 5HT exposure to the isolated pleural-pedal ganglia induced significant MAPK activation in tail SNs at 45 min compared with paired ASW-treated control ganglia. No activation was observed at 15 min or at 1 h following the same treatment. *b*, Exposure to the protein synthesis inhibitor emetine (eme.) for 30 min before 5HT treatment prevented the 5HT-induced MAPK activation at 45 min.
La activación de ERK es necesaria para que el 2do ensayo sea efectivo.
Contexto molecular para el espaciamiento óptimo

Training Shock 1 (TS1) ↓
5HT

immediate

15 min

+ TS2 → No LTM

45 min

MAPK-dependent
“molecular context”

+ TS2 → LTM

1 hr

+ TS2 → No LTM

No LTM

Figure 6. A model depicting how MAPK establishes a molecular context that defines effective training intervals for LTM. A single training shock (TS) releases 5HT onto tail SNs and sensorimotor synapses (Marinesco and Carew, 2002; Philips et al., 2011). 5HT acts at SN membranes through a receptor-coupled adenylyl cyclase to increase levels of cAMP and activate PKA in sensory neuron somata and synaptic terminals (Bacsikai et al., 1993; Muller and Carew, 1998). cAMP/PKA activation is transient and is inactivated within 15 min following TS. MAPK activation by the same TS is delayed, and appears in the cell body and nucleus at 45 min, but not at 15 min or 1 h. Trial 1 MAPK activation requires protein synthesis which may account, at least in part, for its delayed activation. Trial 1 MAPK activity establishes a unique molecular context within tail SNs by 45 min that is required for two-trial LTM formation. By 45 min, MAPK has activated the CREB pathway as observed in the increased phosphorylation of the CREB kinase p90rsk and increased ApC/EBP expression. By 1 h, the MAPK-dependent molecular context has ended. Although required for LTM, this MAPK-dependent molecular context is not sufficient for LTM and must interact with additional signaling from a second TS at 45 min to induce LTM (two-trial LTM induction). The necessary signaling provided by the second TS during two-trial LTM induction may be the recruitment of additional 5HT release, PKA activity and the PKA-dependent activation of the ApC/EBP/APAF complex which is sufficient to drive long-term synaptic facilitation at SN-MN synapses (Lee et al., 2006). The delayed onset of the MAPK-dependent molecular context may be sufficient to explain the failure of the short (15 min) training interval to support LTM formation. Longer (1 h) training intervals may similarly fail to support LTM induction because the required MAPK signaling and MAPK-dependent gene expression has ended by 1 h.
10.3 Classical conditioning in *Aplysia*

*Aplysia* exhibit classical conditioning. (A) This diagram shows the site of delivery of the CS and US. (B) The experimental paradigm. (C) The results show that *Aplysia* retain classical conditioning for several days. In tests following training, siphon withdrawal is greatest for the group receiving paired CS–US training. The US-alone group exhibits sensitization that is significantly weaker than the responses of the paired group. The unpaired group shows the weakest responding. After Kandel 1984; data from Carew, Walters, and Kandel 1981.
10.4 Differential conditioning in *Aplysia*

*Aplysia* exhibit differential classical conditioning. (A) This diagram illustrates the locations of the two CSs and the US. (B) The experimental paradigm. (C) Results of the training show that when the siphon was the site of the CS+, in subsequent tests responding was greater to a siphon stimulus than to a mantle stimulus. The opposite pattern was observed when the mantle served as the site of the CS+ and the siphon as the site of the CS−. (D) The data from both groups in part B are pooled, showing that CS+ training produces significantly greater responding in the test phase than does CS− training. After Carew, Hawkins, and Kandel 1983.
Condicionamiento en Aplysia. CS estímulo táctil. US shock

Interstimulus interval function for classical conditioning of siphon withdrawal
The bar indicates the temporal “position” of the US. Data points to the left of 0 s represent backward conditioning; data points to the right represent forward conditioning (see inset above). Learning is best when the CS–US interval is about 0.5 s. After Hawkins, Carew, and Kandel 1986.
14. Cellular analog of differential conditioning

(A and B) The experimental paradigm. MN = motor neuron; SN = sensory neuron.
(C) The top graphs show the overlap of EPSPs from a paired and an unpaired preparation (note that the paired EPSP is larger). The results shown in the bottom graphs are the same as those in the top graphs, except that a paired and a US-alone preparation are compared (again note that the paired EPSP is larger). After Hawkins et al. 1983.
Figure 15 A molecular model of the synaptic action underlying classical conditioning. The model is based on the hypothesis that activity in the sensory neurons mediating the conditioned stimulus prior to the presentation of the unconditioned stimulus permits an influx of Ca\(^{2+}\) that enhances the activity of calcium-dependent adenylyl cyclase.

A. In the unpaired pathway (CS\(^{-}\)) the sensory neuron is not active prior to presentation of the CS, so its Ca\(^{2+}\) channels are closed when the unconditioned stimulus (US) is presented. (5-HT, serotonin.)

B. In the paired pathway (CS\(^{+}\)) the sensory neuron is active prior to the CS and thus its Ca\(^{2+}\) channels are open when the US is presented. The intracellular Ca\(^{2+}\) binds to calmodulin and in turn interacts with adenylyl cyclase. As a result, the adenylyl cyclase undergoes a conformational change that enhances its ability to synthesize cAMP in response to serotonin released in the US pathway. The greater amount of cAMP activates more cAMP-dependent protein kinase and leads to a substantially greater amount of transmitter release than would occur without paired activity.
Reforzamiento sináptico tipo LTP en Aplysia
Homosynaptic plasticity

Depression

Facilitation

Heterosynaptic plasticity

Inhibition

Facilitation

28 Synaptic plasticity

Homosynaptic plasticity is produced by activity within the presynaptic cell (cell A; the postsynaptic neuron is cell B). Heterosynaptic plasticity is produced by the activity of another (modulatory) neuron (cell C).