Central dogma in modern neuroscience: Learning is mediated by changes in the strength of synaptic connections
Central dogma in modern neuroscience: Long-term learning is mediated by changes in synaptic connectivity within the brain.

Santiago Ramon y Cajal (Nobel Prize 1906)
Central dogma in modern neuroscience: Long-term learning is mediated, in part, by changes in synaptic connectivity within the brain.
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Basic Problem:
The human brain is composed of ~ 100 billion nerve cells (neurons), and each neuron connects to $10^3$-$10^5$ other neurons.
Reductionist approach to memory

Eric Kandel
Aplysia californica
Gill- and siphon-withdrawal reflex

A

Mantle Shelf

Gill

Siphon

B

Parapodium

Mantle shelf

Siphon

Gill
Gill- and siphon-withdrawal reflex
Nervous system of *Aplysia*

**FIGURE 2.** The nervous system of *Aplysia, in situ*, showing the four paired head ganglia and the single unpaired abdominal (or pleurovisceral) ganglion and their relation to other internal organs. (Modified from Eales, 1921.)
Abdominal ganglion (dorsal surface)
Forms of learning exhibited by *Aplysia*

• Habituation
  – short-term (minutes)
  – long-term (hours-to-weeks)
Habituation of the defensive withdrawal reflex
Forms of learning exhibited by *Aplysia*

- **Habituation**
  - short-term (minutes)
  - long-term (hours-to-weeks)
- **Sensitization**
  - short-term (minutes)
  - long-term (hours-to-days)
Sensitization of the withdrawal reflex: a simple form of learning

Kandel (1978)
All forms of learning in *Aplysia* are due, in part, to changes in the “monosynaptic” sensorimotor connections

*Transmitter at this synapse is glutamate.*
The presynaptic model for sensitization in *Aplysia*
Model for short-term presynaptic facilitation

Two cellular processes:
1) Closure of K+ channels—produces presynaptic spike broadening
2) Enhanced mobilization of presynaptic vesicles
Long-Term Memory in *Aplysia*: Mechanisms of Induction and Maintenance
Phases of memory in *Aplysia*

Sutton and Carew (2000)
Phases of memory/facilitation in *Aplysia*

- Short-term: posttranslational modifications (protein phosphorylation/dephosphorylation)
- Intermediate-term: translation, but not transcription
- Long-term: translation and transcription
Long-term memory in *Aplysia*: long-term sensitization

From Kandel (2001)
Long-term sensitization is accompanied by long-term facilitation (LTF) of sensorimotor connections in the abdominal ganglion.

From Frost et al. (1985.)
Aplysia sensorimotor synapses in dissociated cell culture
LTF can be induced in synapses in cell culture by repeated, spaced application of 5-HT. LTF depends on both translation and transcription.

From Montarolo et al. (1985)
LTF requires protein synthesis as well as gene transcription. The protein synthesis and gene transcription must occur during a specific critical period.

LTF depends upon translation within a specific time period. A. Cultures were exposed to anisomycin (A) for 3 hr at four different times: starting 12 hr before the first recording session (bar at -12 to -9 hr); 1 hr before 5-HT application until 0.5 hr after the last 5-HT application (bar at -1 to 2 hr); starting either 0.5 hour or 4 hr after the last 5-HT application (bars at 2 to 5 hr and 5.5 to 8.5 hr, respectively). The height of each bar is the mean ± SEM of the % change in amplitude of the EPSP recorded at 0 hr compared to the EPSP of the same connection retested at 24 hr. Notice that when anisomycin was present from 1 hr before 5-HT application until 0.5 hr after the last 5-HT application LTF was inhibited. B1. Inhibition of macromolecular synthesis for the entire period following 5-HT application does not block LTF. Anisomycin (A in B1) or α-amanitin (α-A in B2) was added to cultures 0.5 hr after the last 5-HT application. (Control cultures received the same test regimen and drug treatment, but no 5-HT applications.) (From Montarolo et al. 1986.)
Short-term facilitation does not require either translation or transcription

Results from experiments in which sensorimotor synapses in cell culture were tested at 1/20 sec. After five tests 5-HT was applied to the synapses a single time for 5 min. A. Short-term, 5-HT-induced facilitation in a Control culture. The tests were performed at 0 hr (filled symbols) and 24-hr later (open symbols). B. 5-HT-induced facilitation of synapses in the presence (solid symbols) and after washout (open symbols) of the protein synthesis inhibitor anisomycin. C. 5-HT-induced facilitation of synapses in the presence (solid symbols) and after washout (open symbols) of the RNA synthesis inhibitor α-amanitin. (From Montarolo et al. 1986.)
LTF is partly mediated by morphological growth in sensory neurons.

Green fluorescence = SN; red fluorescence = MN. Blue arrows indicate some of the presynaptic varicosities.
LTF is partly mediated by morphological growth in sensory neurons.
LTF of sensorimotor synapses depends on CREB activity

A. **Design of experiment used to test the role of cyclic AMP response element binding protein (CREB) in LTF of *Aplysia* sensorimotor synapses in culture.** The experimenters attempted to block LTF by injecting oligonucleotides specifically encoding CRE into the sensory neuron before applying the 5 X 5-HT treatment to the cultures. (Note that the CRE sequence of the gene for rat somatostatin was used in these experiments.) A. CREB is phosphorylated by PKA, which translocates to the nucleus with its prolonged activation by cAMP. Binding of phosphorylated CREB to the CRE of cAMP-inducible genes in the nucleus of the sensory neuron initiates transcription of these genes. B. Injecting excess CRE oligonucleotides into the sensory neurons would be expected to interfere with transcription of cAMP-inducible genes (due to 5 X 5-HT treatment), because the injected oligonucleotides will compete with the normal binding of CREB to these genes. (From Dash *et al*. 1990.)
Injection of CRE oligonucleotides into the presynaptic sensory neuron blocks LTF

A. Sensorimotor EPSPs at 0 hr (before 5-HT treatment) and at 24 hr after 5-HT treatment. Injection of the CRE oligonucleotide blocks LTF, whereas injection of a control oligonucleotide (NFκB) does not. Short-term facilitation (tested at the 24-hr time point) due to a single, 5-min application of 5-HT is not disrupted in cultures that received the CRE oligo injection. B. Summary of LTF data for the various experimental groups. (The HSE and mutant oligo data are from additional control injection groups.) C. Summary of short-term facilitation data for various experimental groups. (From Dash et al. 1990.)

No effect of CRE oligonucleotide injection on short-term facilitation.
Induction of LTF depends on the coordinated regulation of CREB-1 and CREB-2

- CREB-2 is an inhibitory transcriptional regulator expressed in *Aplysia* sensory neurons. (Homologs of CREB-2 are expressed in other nervous systems, including the mammalian CNS.)
- CREB-2 represses CREB-1 mediated gene transcription.
- CREB-2 is a substrate for protein kinases, particularly MAP kinase.
- Activation of MAP kinase, as well as, possibly, other kinases removes the inhibition of CREB-1 by CREB-2.
- The removal of CREB-2-mediated repression of CREB-1 may be the source of so-called “flashbulb memories.”
Injection of anti-bodies to CREB-2 into sensory neurons facilitates the induction of LTF

A. Time course of changes in EPSP amplitude in various groups of sensorimotor cultures. Some of the cultures received only a single, 5-min application of 5-HT, while others received the 5 X 5-HT treatment. Still other cultures did not receive any treatment with 5-HT. In some cultures the sensory neuron received an injection of antibodies to CREB-2 before the start of the experiment (CREB-2 Ab). Finally, some cultures were treated with the translational inhibitor anisomycin (Aniso,) or the transcriptional inhibitor actinomycin-D (Actino), during training with 5-HT. B. Comparison of the time course of the changes in EPSP amplitude during the first 2 hr after application of a single 5-min pulse of 5-HT with or without prior injection of the CREB-2 antibodies. Note the dip in EPSP amplitude in the 1 X 5-HT + CREB-2 Ab group (indicated by the red arrow) at 20-40 min. This dip may reflect an intermediate memory period that depends upon translation, but not upon transcription. C. Sample EPSPs recorded from the experiments in A. D. Sample EPSPs from cultures that received the single 5-HT application after CREB-2 antiserum injection. (From Bartsch et al. 1995.)
Presynaptic model of LTF of sensorimotor synapses during long-term sensitization of the withdrawal reflex of Aplysia
Role of Postsynaptic Mechanisms in Long-Term Sensitization in Aplysia
Does long-term facilitation (LTF) depend on postsynaptic changes?
LTF requires elevated postsynaptic intracellular Ca$^{2+}$

LTF requires **postsynaptic** protein synthesis

Note: Gelonin is a cell membrane impermeant protein synthesis inhibitor.

Modulation of AMPA receptor trafficking during long-term potentiation (LTP)

Malenka & Nicoll (1999) Science
LTF, but not STF, is accompanied by modulation of postsynaptic AMPAR trafficking.

**Fig. 1 LTF but Not STF Is Associated with the Remodeling of Glutamate Receptors**

ApGluR1/EGFP was expressed in L7 MN cocultured with SN after 1 day in vitro. Cells were stimulated with 5-HT on day 5. Induction of STF does not lead to any significant redistribution of ApGluR1 in L7 MN cocultured with SN. By contrast, induction of LTF is accompanied by increases in the expression of ApGluR1 (number of ApGluR1 “puncta”) in L7 MN cocultured with presynaptic SN.

Li et al. (2009) *Neuron* 61:527-40
Long-term facilitation depends on:

- Postsynaptic Ca$^{2+}$ (5-HT causes Ca$^{2+}$ release from intracellular stores)
- Postsynaptic protein synthesis
- Exocytotic insertion of AMPARs into MN cell membrane
Old presynaptic model of synaptic facilitation in *Aplysia*
New cellular model for synaptic enhancement in *Aplysia*

Problem: If LTF depends on protein synthesis and gene transcription, which are supposed to occur in the nucleus and cell body of neurons, then how do you get synapse specificity of facilitation?

John Lisman: “What does the nucleus know about memories?”
*Method for inducing LTF: five bouts of 5-HT; each bout consisted of five 5-s pulses @ 10-s intervals were given @ 10-min intervals. Total number of pulses = 25, the total time of perfusion = 2 min 5 s, and the total time of treatment (including 5-HT application and inter-5-HT-intervals = 45 min 25 s.

Kandel (2001) *Science*
Photomicrograph of a single, bifurcated sensory neuron making synaptic contact with two spatially separated motor neurons. A perfusion pipette is used to deliver puffs of serotonin (5-hydroxytryptamine or 5-HT) locally to the connection made onto one of the motor neurons.

b | Five puffs, but not a single puff, of serotonin produce a long-lasting (24 hour) increase in the amplitude of the excitatory postsynaptic synaptic potential (EPSP), providing a case of synapse-specific long-term facilitation (LTF). This branch-specific facilitation is blocked by the bath application of actinomycin D and by the microinjection of anti-CREB (cyclic-AMP-responsive element (CRE)-binding protein) antibodies into the sensory neuron, indicating that LTF requires CREB-mediated transcription in the sensory neuron.

c | The LTF produced by five puffs of serotonin can be 'captured' by the opposite branch if a single pulse of serotonin is given within a discrete time window with respect to the five puffs. Applying one puff of serotonin to the other branch, either simultaneously (left) or within 1 hour of the five puffs (middle), results in LTF in both branches. This is not the case if the single pulse is given after 4 hours (right).

d | Schematic of these phenomena. Five puffs of serotonin activate a retrograde signal that induces CREB-mediated transcription in the nucleus (blue arrow). The products of gene expression are delivered throughout the cell (red arrows), but only function to increase synaptic strength at sites that have been 'tagged' by synaptic activity. Five puffs of serotonin produce a synaptic tag and also turn on gene expression; a single puff of serotonin produces a synaptic tag and captures the products of gene expression that are induced by the five puffs to the other branch. From Martin & Kosik (2002) Nat. Rev. Neurosci.