Supporting Information

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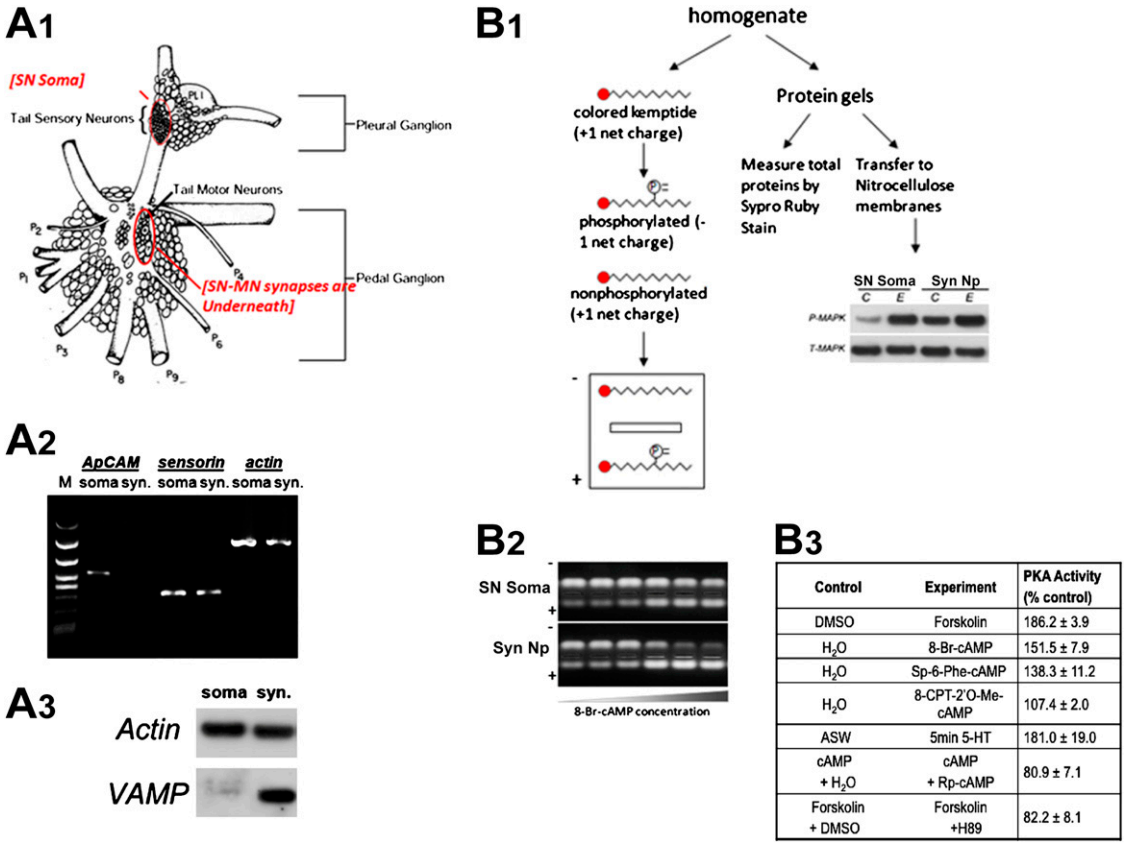
SI Materials and Methods

To analyze biochemical changes induced by tail nerve shock (TNS), tissues enriched in sensory neuronal somata and sensory-to-motor neuron (SN-MN) synapses were excised in ice-cold 1:1 $MgCl_2$:ASW either 1 min (immediately) or 1 h after the last TNS. We waited for 1 min after the last TNS for the "immediate" harvesting time point because it took about 40 s for serotonin (5-HT) release triggered by TNS to return to baseline (1). For harvesting "SN soma," pleural sensory cluster was excised and neuropil under the SN somata was removed carefully. For harvesting "synaptic neuropil," pedal MN cluster with underneath neuropil was excised and MN somata were then carefully shaved off. Previous analyses showed that SN and MN form synapses in this part of neuropil; in addition, they receive 5-HT triggered by TNS (1). To verify these tissue samples, we performed RT-PCR analysis of the mRNA level of sensorin, an SN-specific neuropeptide, as well as Western blot analysis of synaptic proteins (Fig. S1*A*). We acknowledge that these samples are not pure SN somata and SN synaptic terminals, and that they likely are contaminated with serotonergic, interneuronal, and glial processes. However, we would like to take this first step to examine compartmental differences in signaling cascades underlying synaptic facilitation, and use data from the current analyses to guide hypotheses for future investigation using more advanced techniques.

These tissue samples were homogenized quickly in 20 μL lysis buffer (25 mM Tris, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 1% Nonidet P-40, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM 2-mercaptoethanol, 1 × sigma protease inhibitor mixture) and immediately frozen on dry ice/ethanol mixture. The harvesting step typically was finished within 5 min. Subsequently, protein kinase A (PKA) activity in each sample was measured using the PepTag PKA assay (Promega) following the manufacturer's protocol with a few modifications. Briefly, 7 μL SN soma or 5 μL synaptic neuropil homogenate was incubated in a 20-μL reaction mixture with a fluorescent-tagged A1 peptide (PKA substrate, 1.5 μL), 1 × reactivation buffer, and protease inhibitor for 30 min. The reactivation was terminated by boiling the mixture for 10 min. The nonphosphorylated A1 peptide is +1 charged. Upon phosphorylation by active PKA in the samples, it becomes −1 charged and thus can be separated by electrophoresis on a horizontal 0.8% agarose gel. To visualize the migration pattern of A1 peptide in the gel, the fluorescent tag of A1 was excited by a UV lamp and the emission was captured by a CCD camera (Kodak). Densitometric analysis of phosphorylated and nonphosphorylated A1 peptide was performed using Image J [National Institutes of Health (NIH)]. Fifteen microliters of the reaction mixture was used for electrophoresis analysis of A1 peptide phosphorylation, whereas 4 μL of the mixture was resolved on 4–12% Bis-Tris gel (Invitrogen) and stained by Sypro Ruby (Bio-Rad) to estimate the amount of total protein in the homogenate. The relative PKA activity in each sample was calculated as the amount of phosphorylated A1 peptide divided by the total A1 peptide, and normalized to the amount of total protein in the homogenate.

Remaining homogenate was resolved on 4–12% Bis-Tris gel (Invitrogen) for Western blot analysis of MAPK activation, as previously described (2, 3). The relative MAPK activity in each sample was calculated as the amount of phosphorylated MAPK normalized to the amount of total MAPK in the homogenate. Densitometric analysis was performed using Image J (NIH).

1. Sharma SK, et al. (2003) Differential role of mitogen-activated protein kinase in three distinct phases of memory for sensitization in Aplysia. *J Neurosci* 23(9): 3899–3907.
2. Ghirardi M, et al. (1992) Roles of PKA and PKC in facilitation of evoked and spontaneous transmitter release at depressed and nondepressed synapses in Aplysia sensory neurons. *Neuron* 9(3):479–489.
3. Sharma SK, et al. (2003) Differential role of mitogen-activated protein kinase in three distinct phases of memory for sensitization in Aplysia. *J Neurosci* 23(9):3899–3907.



Control	Experiment	PKA Activity (% control)
DMSO	Forskolin	186.2 ± 3.9
H_2O	8-Br-cAMP	151.5 ± 7.9
H_2O	Sp-6-Phe-cAMP	138.3 ± 11.2
H_2O	8-CPT-2'O-Me-cAMP	107.4 ± 2.0
ASW	5min 5-HT	181.0 ± 19.0
cAMP + H_2O	cAMP + Rp-cAMP	80.9 ± 7.1
Forskolin + DMSO	Forskolin +H89	82.2 ± 8.1

Fig. S1. Parallel analysis of MAPK and PKA activation in SN soma and synaptic neuropil (Syn Np). (*A1*) Diagram of *Aplysia* pleural–pedal ganglia highlighting pleural cluster of SN somata and the region below pedal MN cluster that is relatively enriched in SN-MN synapses and harvested as synaptic neuropil. (*A2*) RT-PCR shows that both SN soma and synaptic neuropil are enriched in sensorin mRNA, a marker for *Aplysia* SNs. In contrast, ApCAM mRNA, which is known to be expressed only in neuronal somata, is absent in synaptic neuropil. Actin mRNA is shown in parallel as a loading control. (*A3*) Western blotting shows that synaptic neuropil is enriched in vesicle-associated membrane protein (VAMP), a synaptic protein marker. (*B1*) A flowchart demonstrates parallel analysis of MAPK and PKA activation from a common sample (*SI Materials and Methods*). (*B2*) Representative PKA gel shows increased phosphorylation of PKA substrate peptide with increasing concentration (in nanomolar from left to right: 1, 10, 10^2, 10^3, 10^4, 10^5) of a PKA activator, 8-Br-cAMP in the assay. (*B3*) A table summarizes characterization of the PKA assay using *Aplysia* extract treated with a panel of drugs known to activate PKA (forskolin, 8-Br-cAMP, PKA-specific cAMP analog Sp-6-Phe-cAMP, 5-HT), inhibit PKA (Rp-cAMP, H89), or not change PKA activity (Epac-specific cAMP analog 8-CPT-2'O-Me-cAMP). n = 3–5 per group. Data are presented as mean percent ± SEM of control.

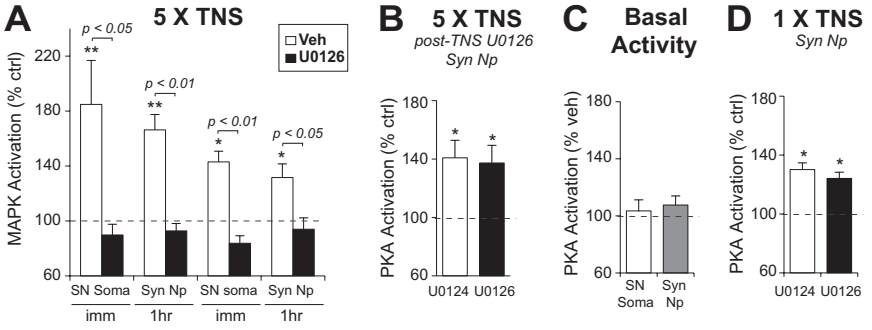


Fig. S2. Control experiments testing specificity of U0126 on blocking PKA activation in synaptic neuropil after repeated TNS. (*A*) In the same examples in which U0126 during repeated TNS blocks PKA activation in synaptic neuropil, but not in SN soma after TNS, MAPK activation by TNS is uniformly blocked by U0126 at all time points in both compartments. (*B*) Post-TNS application of U0126 does not affect PKA activation in synaptic neuropil. (*C*) U0126 does not affect basal PKA activity. (*D*) U0126 does not block the transient PKA activation in synaptic neuropil induced by a single TNS. Data in *A*, *B*, and *D* are presented as mean percent ± SEM of mock-treated controls that did not receive TNS. Data in *C* are presented as mean percent ± SEM of vehicle-treated controls.