Transcriptional Changes Before and After Forgetting
of a Long-Term Sensitization Memory in *Aplysia californica*

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Abstract

Most long-term memories are forgotten, becoming progressively less likely to be recalled. Still, some memory fragments may persist beyond forgetting, as savings memory (easier relearning) can persist long after recall has become impossible. What happens to a memory trace during forgetting that makes it inaccessible for recall and yet still effective to spark easier re-learning? We are addressing this question by tracking the transcriptional changes that accompany learning and then forgetting of a long-term sensitization memory in the tail-elicited siphon withdrawal reflex of *Aplysia californica*. First, we tracked savings memory. We found that even though recall of sensitization fades completely within 1 week of training, savings memory is still robustly expressed at 2 weeks post training. Next, we tracked the time-course of regulation of 11 transcripts we previously identified as potentially being regulated beyond the decay of recall. Remarkably, 3 transcripts still show strong regulation of expression 2 weeks after training and an additional 4 are regulated for at least 1 week. These long-lasting changes in gene expression always began early in the memory process, within 1 day of training. We present a synthesis of our results tracking gene expression changes accompanying sensitization and provide a testable model of how sensitization memory is forgotten.

Highlights:

- Recall of long-term sensitization memory in *Aplysia* decays within 1 week of training, but savings memory (easier relearning) persists for 2 weeks.
- A small set of transcriptional changes also persist for 1-2 weeks after sensitization training.
- We propose that learning induces synaptic outgrowth and increased synaptic efficacy, but that recall is progressively eroded by an active forgetting process reversing gains in synaptic efficacy.

Keywords

active forgetting; latent memory; long-term memory; savings memory

1. Introduction

Long-term memories are distinguished from short-term memories not only by duration but also by sensitivity to transcriptional inhibitors during learning (Goelet, Castellucci, Schacher, & Kandel, 1986). These features are related: long-term memories can endure because their encoding is accompanied by transcriptional changes which alter neuronal function, producing lasting changes in neuronal excitability, synaptic efficacy, and/or patterns of connectivity.

Although long-term memories *can* persist for very long durations, many are forgotten, becoming progressively less likely to be recalled. Forgetting, however, is complex. First, there is increasing evidence that forgetting is an active process that can be manipulated, not just a passive decay of maintenance mechanisms (reviewed in Davis & Zhong, 2017). For example, in fruit flies, forgetting of an intermediate-term olfactory memory can be accelerated or forestalled by manipulations that activate or impede functioning of a dopamine-sensitive G-protein-coupled receptor (Berry, Cervantes-Sandoval, Nicholas, & Davis, 2012; Himmelreich et al., 2017). In mice, a similar bidirectional modification of forgetting for long-term object recognition memory can be produced by enhancing or inhibiting the function of Rac1, a signaling G-protein (Liu et al., 2016). Thus, neuromodulation via G-protein signaling may play a critical role in producing forgetting.

Although forgetting may be active, it may not be complete. Even when recall has completely decayed, traces of a memory may be detectable under the right conditions. One classic example is the phenomenon of savings (Ebbinghaus, 1885). Savings is when a memory that seems completely forgotten (via recall tests) can nevertheless be re-acquired more rapidly or easily, indicating that some aspect of the original memory persisted. Savings has been documented across a wide range of learning tasks (Nelson, 1985) and species (Antzoulatos, Wainwright, Cleary, & Byrne, 2006; Menges, Riepe, & Philips, 2015; Philips, Tzvetkova, Marinesco, & Carew, 2006). Similarly, reminders and contextual cues

can rescue recall not only from time-dependent forgetting but also from amnestic interventions such as electroconvulsive shock and the disruption of protein synthesis (reviewed in Riccio & Richardson, 1984).

If forgetting belies the persistence of subtle memory traces, what exactly persists? Moreover, what changes in the memory system to impair recall but preserve savings? We know surprisingly little about these issues (Davis & Zhong, 2017; Hardt, Nader, & Nadel, 2013). One intriguing finding comes from the study of classical conditioning in rats, which has shown that phosphorylation of mitogen-activated protein kinase can persist in the amygdala after a conditioned fear memory is forgotten (Kim, Li, Hamlin, McNally, & Richardson, 2012). Similarly, structural plasticity induced by visual perturbation can persist after normal vision is restored (Hofer, Mrsic-Flogel, Bonhoeffer, & Hübener, 2009; Linkenhoker, von der Ohe, & Knudsen, 2005). Thus, changes in both protein function and connectivity can outlast the typical behavioral expression of a long-term memory, perhaps providing a substrate for savings memory.

We have recently extended these findings by showing that transcriptional changes can also be detected after the decay of recall for a long-term memory (Perez, Patel, Rivota, Calin-jageman, & Calin-Jageman, 2018). Specifically, we used microarray and qPCR to identify changes in gene expression that accompany forgetting of a long-term sensitization memory in *Aplysia californica*. We found that the decay of recall is accompanied by the dissolution of most of the transcriptional changes initially evoked by learning. Despite this widespread collapse of the transcriptional response, a small set of transcripts remains regulated beyond the decay of recall (Table 1).

The significance of transcriptional regulation beyond recall is unclear. Some of the remaining transcriptional changes could be part of a fragmentary memory trace that helps support savings. Another possibility is that some residual transcriptional changes are part of an active forgetting process, expressing proteins that help suppress the recall of sensitization memory. These possibilities are not

Table 1: Putative 'beyond recall' transcripts		7-day regulation (Perez et al., 2018)	
Transcript	Genome Match	M_{LFC}	95% MoE
EB243511	not discernable	(0.56)	0.30
EB342172	none	(0.31)	0.31
EB255259 (ApSpectrin)	PREDICTED: Aplysia californica spectrin beta chain-like (LOC101859991)	(0.31)	0.19
EB241258	none	0.24	0.21
EB240636	not discernable	0.27	0.24
EB254334	none	0.32	0.24
EB239594	none	0.41	0.35
EB257711	PREDICTED: Aplysia californica uncharacterized LOC101857556 (LOC101857556)	0.56	0.28
EB312493	none	0.57	0.27
M11283 (FMRFa)	Aplysia californica FMRF5 mRNA mRNA, FMRFamide precursor	0.68	0.32
FF066943	PREDICTED: Aplysia californica uncharacterized LOC106013098 (LOC106013098)	0.89	0.41

Table Note: Not discernable is above 70% identity to multiple mRNAs with query coverage of greater than 45%. The FMRFa accession number M11283 has now been deprecated for AH005259. Regulation at 7-days indicated the log-fold change on the trained side relative to the untrained side (both normalized to H4 expression) along with the 95% margin of error.

mutually exclusive. To help shed light on these issues we undertook a time-course analysis. First, we examined if savings is present long after recall fades, testing at 14 days after sensitization training. Next, we used qPCR to track the expression of the 11 putative “beyond recall” transcripts from memory encoding (1 hour after training) to long after forgetting (14 days). We find that both savings and transcriptional regulation can persist long after the end of the normal behavioral expression of sensitization (> 14 days). We present a synthesis of our results tracking gene expression changes accompanying sensitization and provide a testable model of how sensitization memory is forgotten.

1.1. Sensitization of the Tail-Elicited Siphon Withdrawal Reflex

The learning paradigm used in these studies is sensitization, an increase in reflex responsiveness due to a painful stimulus (Figure 1A). This form of memory is ubiquitous in the animal kingdom (Abramson, 1994) and may reflect basal plasticity mechanisms from which other memory systems have evolved (Hawkins & Kandel, 1984).

In *Aplysia*, sensitization seems to represent a general behavioral state induced by pain, producing changes not only in reflexes but also across a spectrum of behaviors (e.g. Leod, Seas, Wainwright, & Mozzachiodi, 2018). We track sensitization memory by monitoring the tail-elicited

siphon-withdrawal reflex (T-SWR), a defensive reflex of the siphon, a respiratory organ. The T-SWR can be evoked by weak electrical stimulation to the left or right side of the tail; it is measured as the duration of time the siphon contracts after stimulation.

Long-term sensitization of the T-SWR can be evoked by repeatedly applying a strong noxious shock to one side of the body. This produces a dramatic increase in the duration of the T-SWR. Conveniently, sensitization of the T-SWR is unilateral; reflexes on the untrained side of the body remain unperturbed (Scholz & Byrne, 1987). Thus, both behavioral and mechanistic studies can proceed using powerful within-subjects designs comparing measures from the trained and untrained sides of the same animal.

The duration of T-SWR sensitization depends on the training protocol. With repeated noxious shock, sensitization memory can last more than 24 hours and requires changes in transcription (Sutton, Bagnall, Sharma, Shobe, & Carew, 2004). In the protocol we use, sensitization is forgotten within ~7 days, indicated by a progressive decline in T-SWR duration back to baseline levels (Conte et al., 2017). This apparent forgetting, however, belies a persistent savings memory, as a brief re-training can produce a long-lasting re-expression of the sensitization memory (Perez et al., 2018).

Study of the neural circuitry mediating the T-SWR has shown that sensitization of this reflex is encoded across the T-SWR circuit (Leonard J Cleary, Lee, & Byrne, 1998). One prominent site of storage, though, is the ventro-caudal (VC) cluster of the pleural ganglia. The neurons in the VC cluster are nociceptors; each VC cluster forms a somatotopic map of one side of the *Aplysia* body surface (Walters et al., 2004). Nearly all VC nociceptors on the side of training are strongly activated by the sensitizing stimulus (Walters, 1987b, 1987a). This barrage of activity coupled with shock-induced serotonin release (Marinesco & Carew, 2002) can produce long-lasting physiological changes in nearly all VCs, including increased excitability (Scholz & Byrne, 1987), spike narrowing (Antzoulatos & Byrne, 2007), and long-

Figure 1: Sensitization and savings in the tail-elicited siphon withdrawal reflex (T-SWR). a) Overview of the behavioral paradigm. Sensitizing stimuli (lightning bolts) were applied to one side of the body. Sensitization was monitored as the duration of the T-SWR, a defensive withdrawal of the siphon evoked by applying a weak electrical shock to the left or right side of the tail (test site). To document savings, a brief re-training protocol was administered to the midline of the tail (re-training site, wide arrow). In naïve animals the re-training protocol is not sufficient for evoking a long-term sensitization memory. b) Experimental protocol. T-SWR responses were evoked on the left and right tail (Pre-test, hash lines, four on each side). Long-term sensitization training was then administered (4 strong shocks 30 minutes apart, lightning bolts). Sensitization was monitored by measuring T-SWR on each side at 1, 7, and 14 days after training. After the 14-day post-tests, the brief re-training protocol was administered (wide arrow). Savings was probed by measuring the T-SWR on both sides 20-minutes and 1 day after the re-training. c) Behavioral results ($n = 21$). The graph shows changes in T-SWR duration as % change from baseline by side of training. The dashed line at 0 represents no change. The thick lines represent mean responses $\pm$ the 95% margin of error. The faded lines represent each individual animal in the study. Where the 95% margin of error is smaller than the change in behavior the result is statistically significant at $\alpha = .05$.

and others). These physiological changes can be accompanied by outgrowth of VC neurites (Wainwright, Zhang, Byrne, & Cleary, 2002).

In this study we track transcriptional changes associated with sensitization using qPCR conducted on RNA extracted from the pleural ganglia. These ganglia contain the VC clusters, T-SWR interneurons (Buonomano, Cleary, & Byrne, 1992; L J Cleary & Byrne, 1993; Mackey et al., 1987), and other neurons unrelated to defensive withdrawal. In general, gene expression measured in whole pleural ganglia correlates strongly with expression measured from isolated VC clusters (Herdegen, Conte, Kamal, Calin-Jageman, & Calin-Jageman, 2014b), but transcripts expressed in other cell types can also be detected. For example, analysis of pleural ganglia shows that long-term sensitization produces a strong up-regulation of a transcript encoding the peptide neurotransmitter Phe-Met-Arg-Phe NH₂ (FMRFa, GenBank: M11283; Schaefer et al., 1985). This transmitter is not expressed in the VC cluster but is expressed in a number inhibitory interneurons in the T-SWR circuit (Mackey et al., 1987; Small, Cohen, Kandel, & Hawkins, 1992; Xu, Cleary, & Byrne, 1994).

2. Materials and Methods

We report how we determined our sample size, all data exclusions (if any), all manipulations, and all measures in these studies (Simmons, Nelson, & Simonsohn, 2012). All data for this project is posted to the Open Science Framework (<https://osf.io/deq5g/>).

2.1. Animals

Animals (75-125g) were obtained from the RSMAS National Resource for *Aplysia* (Miami, FL) and maintained at 16° C in one of two 90-gallon aquariums with continuously circulating artificial sea water (Instant Ocean, Aquarium Systems Inc.).

2.2. Long-term sensitization training

To induce long-term sensitization we used a one-day training protocol adapted from Wainwright et al. (2002) as previously described (Bonnick et al., 2012). Training consisted of 4 rounds of noxious shock applied at 30-minute intervals to one side of the body with a hand-held electrode (Figure 1A and B). Each round of shock consisted of 10 pulses (60Hz biphasic) of 500ms duration at a rate of 1hz and an amplitude of 90mA (DC). As each shock was applied, the stimulating electrode was moved slowly from anterior (just behind neck) to posterior (just in front of tail) and back, ensuring stimulation of nearly the entire surface of that side of the body. Animals were matched in pairs and each member of the pair was randomly assigned to be trained on the left or right side of the body.

2.3. Behavioral measurement

To observe sensitization we monitored the duration of the tail-elicited siphon-withdrawal reflex (T-SWR) (see Walters & Erickson, 1986). T-SWRs were evoked by applying a weak shock to one side of the tail using a hand-held stimulator (60Hz biphasic DC pulse for 500ms at 2ma of constant current). Each T-SWR was quantified as the duration of withdrawal from the moment of stimulation to the first sign of siphon relaxation. T-SWR responses were collected before training (baseline) and then again at different time points after training (1, 5, 7, and/or 14 days). For each time point, T-SWR responsiveness was characterized by a series of 8 responses evoked on alternating sides of the body at a 10-min ISI. Scores were split by side of stimulation (trained vs. untrained) and averaged (4 responses/side for each time point characterized). Behavioral measures were conducted blind to the side of training.

2.4. Reminders to elicit savings

To elicit savings, animals received a weak re-training protocol adapted from Philips et al. (2006). This consisted of two moderate shocks (60Hz biphasic DC pulse for 2s at 20ma of constant current) applied to the midline of the tail with a 15-minute rest between the shocks. This protocol is strong enough to produce short-term sensitization in naïve animals, but not sufficient to produce long-term sensitization (Perez et al., 2018; Philips et al., 2006).

2.5. Isolation and processing of pleural ganglia RNA

We compared gene expression from pleural ganglia on the trained vs. untrained side of the animal. To control for lateralized gene expression, samples from two animals trained on opposite sides were pooled. Isolation and homogenization was exactly as described in Herdegen et al. (2014b).

2.6. Reverse-transcription quantitative PCR (qPCR)

Reverse transcription was performed using Maxima cDNA kit with dsDNase (Thermo Scientific, Carlsbad CA). Quantitative PCR was conducted using Maxima SYBR Green/Fluorescein qPCR Master Mix (Thermo Scientific, Carlsbad CA) and the MyIQ real time PCR system (Bio-Rad, Los Angeles CA). Primers were validated for correct PCR efficiency and are listed in Supplemental Table 1. qPCR samples were analyzed in duplicate and the relative amounts of each transcript were determined using the ddCT method and the Bio-rad IQ5 gene expression analysis (Carlsbad CA). All qPCR expression levels were normalized to levels of histone H4, a transcript which is stable during LTS training (Bonnick et al., 2012).

2.7. Sample size determination

For the savings experiment, we set a goal of collecting 21 animals, as this had been sufficient for our previous study of savings (Perez et al., 2018). For qPCR, we have previously found that 8-10 samples is sufficient to regularly detect the large changes in gene expression that can accompany long-term sensitization training (Conte et al., 2017). We set a goal of 8-16 samples, with the higher end targeted for the 5-day and 7-day time points when there is more variation in the response to training.

2.8. Quality controls and exclusions

To ensure suitable samples, we established the following behavioral screens prior to data collection: 1) the protocol had to be administered properly (no inadvertent shocks to the wrong side), 2) animals had to show strong sensitization of the T-SWR on the trained side (< 30% increase in T-SWR duration from baseline to 1-day post-test), and 3) animals had to show stable responding on the untrained side

(no more than a 30% change in T-SWR duration from baseline to 1-day posttest). In addition, we established that qPCR samples would be discarded if RNA concentrations were too low ($< 7 \text{ ng}/\mu\text{l}$) or had genomic contamination.

For the savings experiment all 21 animals met the behavioral screens and were included in analysis.

For the time-course experiment 138 animals were trained. Of these, 10 failed the behavioral screen (2 due to protocol errors, 6 for insufficient sensitization on the trained side, 2 for unstable responses on the untrained side). We did not utilize or further analyze these 10 animals nor the 10 animals they had been paired with. This left 118 animals, yielding 59 pairs that were dissected for RNA isolation. Of these, 3 pairs yielded poor RNA concentrations and 5 had genomic contamination one at least one side. Thus, we obtained 51 valid samples: 11 harvested 1 hour after training, 16 harvested 5 days after training, 15 harvested 7 days after training, and 9 harvested 14 days after training. For the 1-day timepoint we drew on a library of 11 samples collected for a previous study (Conte et al., 2017); all the qPCR measures for this time-point are unique to this manuscript.

2.9. Statistical analyses

2.9.1. Behavioral data

Behavioral responses were averaged by time point (baseline and post-tests) and side of testing (trained or untrained). Changes scores were then calculated by normalizing each post-test to baseline: $\text{Trained_Change} = (\text{AvgPostTest}_{\text{TrainedSide}} / \text{AvgBaseline}_{\text{TrainedSide}}) - 1$. Thus, 0 represents no change from baseline.

Consistent with modern reporting guidelines and statistical practice we focus on reporting and interpreting effect sizes and uncertainty (Cumming & Calin-Jageman, 2017; e.g. Levitt et al., 2018). At each time point paired comparisons were made between the average change on the trained side and the average change on the untrained side: $M_{\text{diff}} = (M_{\text{trained_change}}) - (M_{\text{untrained_change}})$. The 95% margin of

error (MoE) for this contrast was then calculated. Along with raw-score effect sizes we report standardized effect size estimates (Cohen's d). These are corrected for bias (Hedges, 1981) and calculated so that positive values represents a stronger increase in response on the trained side (sensitization).

We do not report p values or null-hypothesis tests, but readers can translate our results into this format: any effect size reported that is larger in absolute value than its accompanying margin of error is statistically significant at $\alpha = .05$. For example, an increase of response by 30% over baseline is statistically significant if the 95% MoE is even fractionally less than 30%; otherwise $p > .05$. The contrast we present (M_{diff}) is equivalent to the interaction term in a 2 (side: trained vs. untrained) x 2 (time: baseline, post-test) within-subjects ANOVA.

2.9.2. qPCR Data

For analysis of quantitative qPCR data, we analyzed multiple transcripts at 5 different time points. To control for multiple comparisons we used the same analysis pipeline we normally use for microarray data (Herdegen, Conte, et al., 2014b; Herdegen, Holmes, Cyriac, Calin-Jageman, & Calin-Jageman, 2014). First, a fold-change score was calculated for each animal as the ratio of trained to untrained expression. Fold change scores were then log transformed (base 2). This ensures equal weight to both up- and down-regulated measures. With this calculation, a score of 0 represents no change in expression. Log-fold-change scores were assessed for regulation using an empirical Bayes-moderated t -test (Smyth, 2004) against a null hypothesis of 0. As an effect size we report the mean log-fold change when comparing expression on the trained vs. untrained side (M_{LFC}). Each effect size is reported with a 95% margin of error (MoE) expanded using Benjamini-Hochberg correction for multiple comparisons and a false-discovery rate of 5% (Benjamini & Hochberg, 1995; Jung, Friede, & Beissbarth, 2011). Corrections for multiple comparisons was applied over all contrasts conducted (13 transcripts x 5 timepoints = 60 contrasts). Analysis was conducted using limma (Smyth, 2005) from the Bioconductor

suite of tools (Gentleman et al., 2004) for R (Ihaka & Gentleman, 1996). Again, statistical significance can be determined by comparing each M_{LFC} to its 95% margin of error.

2.10. Transcript names

Transcripts are identified in this paper by the accession number for their corresponding EST or mRNA. Due to the incomplete nature of the current draft of the *Aplysia* genome, most of these transcripts do not have clear matches to genomic gene models and are not currently annotated.

3. Results

3.1. Savings Memory Persists for 2 Weeks After Sensitization Training

We have previously shown that savings memory is apparent 7 days after sensitization training, a time point at which recall has decayed for most animals (Perez et al., 2018). Here we sought to determine if savings memory persists far after the decay of recall, 14 days after training (protocol in Figure 1B). Before collecting data for this experiment, we pre-registered our sample-size plan and our analysis plan on the Open Science Framework: <https://osf.io/vhgqf/>. All (and only) the pre-registered analyses are reported below.

Sensitization training was successful (Figure 1C). All 21 animals showed a sharp increase in T-SWR durations: $M_{\text{trained_change}} = 61\%$, $\text{MoE} = 9\%$ (average duration was 61% higher than baseline). On the untrained side responses were slightly habituated, indicated by a modest decline relative to baseline: $M_{\text{untrained_change}} = -12\%$, $\text{MoE} = 5\%$. Thus, there was a large difference in how the trained and untrained sides responded to training: $M_{\text{difference}} = 73\%$, $\text{MoE} = 9\%$, $d = 4.5$ [3.1, 6.2], $r = .08$. The slight habituation on the trained side was likely due to repeated measurement of the T-SWR for this protocol.

At 7 days after training, recall of sensitization had decayed substantially. On the trained side, responses were only slightly elevated above baseline: $M_{\text{trained_change}} = 6\%$, $\text{MoE} = 9\%$. There was still weak habituation of responses on the untrained side: $M_{\text{untrained_change}} = -16\%$, $\text{MoE} = 4\%$. Thus, there was still a

small difference in how responses had changed between the trained and untrained sides: $M_{\text{difference}} = 22\%$, $\text{MoE} = 10\%$, $d = 1.4 [0.7, 2.2]$, $r = -.04$. This seemed driven by individual variation; 2 animals had above-baseline responses on the trained side at this time-point.

At 14 days after training, recall was fully decayed in all animals. On the trained side, responses were slightly habituated relative to baseline ($M_{\text{trained_change}} = -13\%$, $\text{MoE} = 5\%$) and no animal had more than a 10% increase in response relative to baseline. Nearly the same pattern was observed on the untrained side: $M_{\text{untrained_change}} = -14\%$, $\text{MoE} = 5\%$. Thus, the difference in response to training was very small: $M_{\text{difference}} = 1\%$, $\text{MoE} = 6\%$, $d = 0.1 [-0.4, 0.6]$, $r = .20$.

With sensitization fully decayed we tested for savings by applying a weak sensitization protocol to the middle of the tail, influencing both the trained and untrained sides. This second round of training was much less extensive than the original training protocol, differing in shock number (2 vs. 4), intensity (20ma vs. 90ma), and duration (2s vs. 10s). In naïve animals this protocol produces some short-term sensitization but is not sufficient to induce a long-term sensitization memory (Perez et al., 2018; Philips et al., 2006).

Responses to the weak sensitization protocol depended on previous training history, indicating savings memory. At 20 minutes after the reminder, there was short-term sensitization on both the previously trained ($M_{\text{trained_change}} = 24\%$, $\text{MoE} = 13\%$) and previously untrained sides ($M_{\text{untrained_change}} = 10\%$, $\text{MoE} = 10\%$). Notably, however, this change was stronger on the previously trained side: $M_{\text{difference}} = 14\%$, $\text{MoE} = 8\%$, $d = 0.7 [0.2, 0.9]$, $r = .72$.

One day after the reminder, responses on the previously-trained side remained somewhat elevated over baseline: $M_{\text{trained_change}} = 8\%$, $\text{MoE} = 11\%$. In contrast, responses on the previously-untrained side were again slightly habituated: $M_{\text{untrained_change}} = -9\%$, $\text{MoE} = 6\%$. Thus, there was a moderate difference in the long-term response to the weak training protocol: $M_{\text{difference}} = 17\%$, $\text{MoE} = 9\%$, $d = 0.8 [0.3, 1.4]$, $r =$

.43. This differential response represents a long-term savings memory, though this was substantially weaker than what we have observed 7 days after training (Perez et al., 2018).

Normalizing to the 14-day measures rather than the original baseline did not alter these results. Moreover, animals given sham training followed by a reminder did not show any long-term sensitization (data not shown). Overall, these results indicate that savings memory persists long after the normal expression of a long-term sensitization memory.

3.2. Time course of persistently-regulated transcripts

We previously identified a set of 11 transcripts which seem to be regulated 7 days after sensitization training, a time when recall has decayed for most animals. We next sought to examine the time course of regulation for these transcripts, examining gene expression 1 hour, 1 day, 7 days, and 14 days after long-term sensitization training (protocol in Figure 2A).

3.2.1. Behavioral Results

Training was successful (Figure 2B). As expected, training produced robust but unilateral long-term sensitization of the T-SWR. On the trained side, responses 1 day after training were substantially stronger than at baseline: $M_{\text{trained_change}} = 79\%$, $\text{MoE} = 5\%$, $N = 102$. On the untrained side, responses were essentially unchanged relative to baseline: $M_{\text{untrained_change}} = 0\%$, $\text{MoE} = 3\%$. Thus, training produced a much larger change on the trained side than on the untrained side, $M_{\text{difference}} = 79\%$, $\text{MoE} = 5\%$, $d = 4.2$ [3.6, 4.9], $r = .02$.

At 5 days after training, there was some forgetting of sensitization training, but responses remained elevated on the trained side ($M_{\text{trained_change}} = 43\%$, $\text{MoE} = 11\%$, $N = 32$). Responses on the untrained side remained unchanged relative to baseline ($M_{\text{untrained_change}} = 3\%$, $\text{MoE} = 4\%$, $N = 102$), so there was now a moderate difference in how each side changed after training: $M_{\text{difference}} = 40\%$, $\text{MoE} = 13\%$, $d = 1.6$ [1.0, 2.3], $r = .22$. Note that there was considerable individual variation at this time point: some animals

Figure 2. Time course study. a) Experimental protocol. Long-term sensitization training consisted of 4 rounds of noxious shocks (lightning bolts). T-SWR responses were evoked on both the trained and untrained side before and after training. Pleural ganglia RNA was harvested for qPCR analysis (curved arrows to test tubes) at 1 hour ($N = 11$), 1 day ($N = 11$), 5 days ($N = 16$), 7 days ($N = 15$), or 14 days ($N = 9$) after training. No behavioral measures were obtained for 1 hour animals. All others were tested before training, at 1-day after training, and just before dissection. b) Behavioral results combined over all groups. The graph shows changes in T-SWR duration as % change from baseline by side of training. The dashed line at 0 represents no change. The thick lines represent mean responses $\pm$ the 95% margin of error. The faded lines represent each individual animal in the study. Where the 95% margin of error is smaller than the change in behavior the result is statistically significant at $\alpha = .05$.

maintained relatively strong sensitization while others had already relapsed back to baseline responding.

At 7 days after training forgetting was complete for most animals. Responses on the trained side were now within 1% of baseline: $M_{\text{trained_change}} = 1\%$, $\text{MoE} = 5\%$, $N = 48$. On the untrained side, responses

were also close to baseline: $M_{\text{untrained_change}} = -3\%$, $\text{MoE} = 4\%$. Thus, there was no longer a large difference in how each side changed after training: $M_{\text{difference}} = 4\%$, $\text{MoE} = 4\%$, $d = 0.3$ [0.0, 0.6], $r = .45$.

At 14 days there were no behavioral manifestation of sensitization. On the trained side there was slight habituation relative to baseline: $M_{\text{trained_change}} = -6\%$, $\text{MoE} = 9\%$, $N = 18$. On the untrained side, responses were essentially unchanged relative to baseline: $M_{\text{untrained_change}} = 0\%$, $\text{MoE} = 8\%$. Thus, are no more than negligible traces of sensitization at 14 days after training: $M_{\text{difference}} = -6\%$, $\text{MoE} = 7\%$, $d = -0.3$ [-0.7, 0.1], $r = .63$.

3.2.2. Transcriptional Changes

We used qPCR to track the transcriptional changes occurring 1 hour ($n = 11$), 1 day ($n = 11$), 5 days ($n = 16$), 7 days ($n = 15$), and 14 days ($n = 9$) after long-term sensitization training. We measured each of the 11 transcripts we had previously identified as regulated at 7 days after training. For comparison, we also examined expression of transcripts encoding ApBiP (GenBank: NM_001204652; Kuhl, Kennedy, Barzilai, & Kandel, 1992) and ApEgr (GenBank: KC608221; Cyriac et al., 2013). Both of these transcripts are very strongly regulated 1-day after training (Conte et al., 2017) but were not flagged as regulated in our initial microarray screen for transcripts regulated 7 days after training (Perez et al., 2018).

Remarkably, we found that some transcriptional changes persist for at least 14 days after training (Figure 3). For EB342172 there was no evidence of regulation at 1 hour ($M_{\text{LFC}} = 0.07$, $\text{MoE} = 0.37$), but then down-regulation at 1 day ($M_{\text{LFC}} = -0.36$, $\text{MoE} = 0.33$) that seemed to persist relatively unchanged at 5-days ($M_{\text{LFC}} = -0.28$, $\text{MoE} = 0.34$), 7-days ($M_{\text{LFC}} = -0.62$, $\text{MoE} = 0.35$), and 14-days ($M_{\text{LFC}} = -0.48$, $\text{MoE} = 0.39$) after training. Similarly, EB243511 showed very rapid down-regulation evident at 1 hour ($M_{\text{LFC}} = -0.51$, $\text{MoE} = 0.53$) and even stronger down-regulation 1-day after training ($M_{\text{LFC}} = -1.08$, $\text{MoE} = 0.47$). Although evidence was not as clear-cut at the 5-day ($M_{\text{LFC}} = -0.47$, $\text{MoE} = 0.48$) and 7-day measures ($M_{\text{LFC}} = -0.23$, $\text{MoE} = 0.50$), the overall trend for persistent down-regulation was clear 14 days after

Figure 3. Very long-lasting changes in gene expression after sensitization training. This figure shows changes in gene expression 1 hour (N = 11), 1 day (N = 11), 5 days (N = 16), 7 days (N = 15), or 14 days (N = 9) after training after long-term sensitization training for 3 transcripts which are regulated at the 14-day timepoint. The y axis gives the log (base 2) change in expression on the trained side relative to the control side. The dashed line at 0 represents no change in expression. In each panel the empty circles represent individual measurements, the dark circles represent mean differences, and the gray band represents the 95% margin of error, expanded to correct for multiple comparisons across all qPCR data in this experiment.

training ($M_{LFC} = -0.72$, $MoE = 0.55$). For FF066943 there was not strong regulation at 1 hour ($M_{LFC} = 0.24$, $MoE = 0.47$), but then a strong up-regulation at 1 day ($M_{LFC} = 1.25$, $MoE = 0.41$) that decayed somewhat in measures taken 5-days ($M_{LFC} = 0.36$, $MoE = 0.42$) and 7-days after training ($M_{LFC} = 0.38$, $MoE = 0.44$) but which was still detectable at 14-days after training ($M_{LFC} = 0.74$, $MoE = 0.48$).

For other transcripts, there was clear evidence of regulation only through the 7-day measures (Figure 4). For the transcript encoding the FMRFa there was not strong regulation at 1-hour ($M_{LFC} = 0.05$, $MoE = 0.54$), but then robust up-regulation at 1-day ($M_{LFC} = 1.44$, $MoE = 0.47$). This then seemed to

decay, with progressively weaker regulation detected at the 5-day ($M_{LFC} = 0.77$, $MoE = 0.48$), 7-day ($M_{LFC} = 0.53$, $MoE = 0.51$), and 14-day timepoints ($M_{LFC} = 0.43$, $MoE = 0.56$). There was a very similar pattern of regulation for EB254334, with little regulation at 1 hour ($M_{LFC} = 0.32$, $MoE = 0.54$) followed by a very strong up-regulation at 1 day ($M_{LFC} = 2.41$, $MoE = 0.47$) that decayed progressively (5-day: $M_{LFC} = 0.74$, $MoE = 0.48$; 7-day: $M_{LFC} = 0.51$, $MoE = 0.50$; 14-day: $M_{LFC} = -0.1$, $MoE = 0.55$). For EB257711 there was delayed up-regulation that decayed just barely below the level of expected sampling error by 14 days (1-hour: $M_{LFC} = 0.34$, $MoE = 0.49$; 1-day: $M_{LFC} = 1.17$, $MoE = 0.43$; 5-day: $M_{LFC} = 0.19$, $MoE = 0.44$; 7-day: $M_{LFC} = 0.52$, $MoE = 0.46$; 14-day: $M_{LFC} = 0.47$, $MoE = 0.50$). For ApBiP, we had not previously detected long-lasting regulation via microarray (Perez et al., 2018), but our qPCR results suggest that it also up-regulated for at least 7 days after training (1-hour: $M_{LFC} = 0.11$, $MoE = 0.49$; 1-day: $M_{LFC} = 1.06$, $MoE = 0.43$; 5-day: $M_{LFC} = 0.51$, $MoE = 0.44$; 7-day: $M_{LFC} = 0.63$, $MoE = 0.46$; 14-day: $M_{LFC} = 0.15$, $MoE = 0.51$).

We did not observe clear-cut evidence for long-lasting regulation in all transcripts (Figure 5). This includes ApEgr, which our previous microarray study suggested would not be regulated persistently (1-hour: $M_{LFC} = 1.86$, $MoE = 0.58$; 1-day: $M_{LFC} = 1.74$, $MoE = 0.52$; 5-day: $M_{LFC} = -0.07$, $MoE = 0.53$; 7-day: $M_{LFC} = -0.43$, $MoE = 0.55$; 14-day: $M_{LFC} = 0.29$, $MoE = 0.61$). But this also included 5 transcripts which previous results had led us to expect to be persistently regulated (Perez et al., 2018). For EB255259 (ApSpectrin), this seemed primarily due to the subtlety of the regulation, as there was a consistent trend for long-lasting down-regulation beginning 1 day after training, but this rarely exceeded the expected level of sampling error (1-hour: $M_{LFC} = 0.16$, $MoE = 0.43$; 1-day: $M_{LFC} = -0.21$, $MoE = 0.38$; 5-day: $M_{LFC} = -0.43$, $MoE = 0.38$; 7-day: $M_{LFC} = -0.30$, $MoE = 0.40$; 14-day: $M_{LFC} = -0.28$, $MoE = 0.44$). For the other 4 transcripts (EB312493, EB240636, EB239594, EB241258), however, there was no clear evidence of regulation beyond 1 day after training.

4. Discussion

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Figure 4. Very long-lasting changes in gene expression after sensitization training. This figure shows changes in gene expression 1 hour (N = 11), 1 day (N = 11), 5 days (N = 16), 7 days (N = 15), or 14 days (N = 9) after training after long-term sensitization training for 3 transcripts which are regulated at the 14-day timepoint. The y axis gives the log (base 2) change in expression on the trained side relative to the control side. The dashed line at 0 represents no change in expression. In each panel the empty circles represent individual measurements, the dark circles represent mean differences, and the gray band represents the 95% margin of error, expanded to correct for multiple comparisons across all qPCR data in this experiment. Note the different scale for panel 3 (EB254334).

Figure 5. Transient changes in gene expression after sensitization training. This figure shows changes in gene expression 1 hour ($N = 11$), 1 day ($N = 11$), 5 days ($N = 16$), 7 days ($N = 15$), or 14 days ($N = 9$) after training after long-term sensitization training for 6 transcripts which do not show clear evidence of regulation beyond the decay or recall. The y axis gives the log (base 2) change in expression on the trained side relative to the control side. The dashed line at 0 represents no change in expression. In each panel the empty circles represent individual measurements, the dark circles represent mean differences, and the gray band represents the 95% margin of error, expanded to correct for multiple comparisons across all qPCR data in this experiment.

weak but discernable savings memory and transcriptional regulation 14 days after training. The very-persistent changes in transcription are always initiated early, within 1 day after training. In some cases, it seems that regulation after recall is due to very strong early regulation that has not fully decayed by the time recall has lapsed (e.g. FMRFa, EB254334, and ApBiP). In other cases, it seems that initial regulation may be relatively stable across long durations (e.g. EB342172, EB243511 and possibly

EB255259 (ApSpectrin)). Unfortunately, the majority of these transcripts are currently uncharacterized; future work will need to determine the proteins they encode.

Although we were able to confirm ‘beyond recall’ transcriptional changes, 5 of the 11 transcripts we tested were not clearly regulated beyond 1 day, contrary to our previous results (Perez et al., 2018). To some extent, this should be expected due to basic sampling error. Our initial estimates of regulation were somewhat imprecise (margins of error were close to the observed effect size, see Table 1). Moreover, transcriptional signals fade after training while individual differences increase, presenting the challenge of discerning trends in transcription as signal to noise deteriorates. Finally, even though we obtained reasonable sample sizes and used a powerful within-subjects design, we also used aggressive corrections for multiple comparisons. For example, the consistent but weak down-regulation of EB255259 (ApSpectrin) may actually be reliable but obscured by our correction for multiple comparisons. Even when calibrating expectations to the vagaries of sampling error, it is disappointing that some putative ‘beyond recall’ transcripts did not hold up well to additional scrutiny (EB312493, EB240636, EB239594, and EB231258). On the other hand, the long-lasting transcriptional changes we did find have now been confirmed in 3 independent samples, providing confidence that these changes are reliable and robust enough for additional study. Furthermore, it may be possible to improve the precision of future work by harvesting and isolating specific cell-types within the pleural ganglia, as these tend to show larger and more consistent changes than when analyzed at the level of whole ganglia (Herdegen, Conte, Kamal, Calin-Jageman, & Calin-Jageman, 2014a).

With these experiments, we have completed a comprehensive analysis of the transcriptional changes that occur as a long-term sensitization memory is encoded, maintained, and subsequently forgotten. Figure 6A provides an overview of our findings from microarray analysis, where 4 distinct patterns of regulation can be observed: encoding only, encoding and maintenance, maintenance only, and beyond recall. About 60 transcripts show the encoding-only pattern of regulation (light blue); all are

Figure 6. Microarray Expression overview and model. a) Time course of transcriptional changes 1h, 1 day, and 7 days after long-term sensitization memory. This figure summarizes microarray data from three prior experiments. Significantly regulated transcripts were grouped by time-course of regulation: encoding only (light blue), maintenance only (gray), encoding and maintenance (dark blue) and beyond recall (red). Each line represents the average fold change for all up- or down-regulated transcripts in each category. Line thickness represents the relative number of transcripts in each category. b) Model of encoding, forgetting, and savings of a long-term sensitization memory. We propose training produces two parallel changes in VC sensory neurons (and potentially other T-SWR circuit elements): synaptic outgrowth (shown as increased branching) and increased synaptic efficacy (black circles). Forgetting is postulated to represent an active reversal of increased synaptic efficacy via G-protein mediated signaling from FMRFa inhibitory interneurons (black bar). Savings persists due to the insensitivity of structural plasticity to active forgetting, enabling the memory to be re-expressed when the circuit is potentiated.

2014). Transcription factors are heavily represented in this set, including C/EBP and CREB1. Many transcripts show the maintenance-only pattern (gray), where regulation develops more slowly and then fades within 1 week (Conte et al., 2017). About 750 transcripts are up-regulated in the maintenance-only pattern; many are related to protein production, cytoskeletal functioning, synaptic functioning, and neuronal signaling. Another 450 show maintenance-only down-regulation, especially transcripts related to catabolism and plasticity. A small set of transcripts (~25) exhibit regulation at both encoding and maintenance (dark blue) but still fade within a week. All of these are up-regulated and regulation is typically stronger at 1 day than at 1 hour. This includes memory-related transcripts such as the *ApEgr* (Cyriac et al., 2013) and a Tob-1 like protein (transducer of ErbB-1 Jin et al., 2005). Finally, our results confirm that a very limited set of transcripts (perhaps as few as 7) are regulated from early in the memory process to beyond recall (red). These numbers probably under-estimate the scope of regulation, as the microarray we used is estimated to represent only 50-60% of all neuronally expressed transcripts (Moroz et al., 2006). Still, these results give a broad sense of the different waves of transcriptional regulation evoked by the acquisition of a long-term memory. Each of these distinct patterns of regulation may relate to different functions in mediating sensitization memory. Moreover, these time-course groupings may relate to distinctive response elements in promoter regions for these transcripts. We are currently exploring this possibility.

How do these underlying molecular dynamics relate to memory recall, forgetting, and savings? Figure 6B presents a tentative model. It is adapted from similar frameworks proposed by others (e.g. Hofer, 2010; Tonegawa, Pignatelli, Roy, & Ryan, 2015) but incorporates hypotheses developed specifically from our study of sensitization. We propose that initial training evokes two parallel mechanisms: one which promotes synaptic outgrowth and one which enhances synaptic efficacy. These mechanisms would operate in the VC nociceptors to increase responses to subsequent stimuli; similar changes may also occur in other elements of the T-SWR circuit. The initial net effect of these two

mechanisms would be strong recall of sensitization. What happens, then, during forgetting to disrupt recall but preserve savings? We propose that changes in synaptic efficacy fade, but that structural changes persist. The decay of synaptic strength could occur through entropy, but we speculate that it also reflects an active process, specifically G-protein mediated neuromodulation from FMRFa released by inhibitory interneurons in the T-SWR circuit. Forgetting would thus represent an active erosion of synaptic plasticity, but persistent structural changes would prime the T-SWR circuit for savings any time circuit activity is enhanced (e.g. through a re-training protocol).

Reviewing all of the evidence underlying this tentative model is beyond the scope of this paper, but we note the following. First, there is growing evidence that successful recall requires both structural and synaptic plasticity, and that recall failure can be due to a greater sensitivity of synaptic plasticity to disruption (Roy, Muralidhar, Smith, & Tonegawa, 2017; e.g. Ryan, Roy, Pignatelli, Arons, & Tonegawa, 2015). There is some evidence for the same trend in *Aplysia*. Bailly and Chen used electron microscopy to track structural and synaptic properties of nociceptive neurons following sensitization of the siphon-elicited siphon-withdrawal reflex (1989). They found that sensitization training increased both outgrowth (more varicosities and active zones) and synaptic properties (increased active zone size and vesicle complement). Tracking these changes showed that outgrowth persisted for 1-3 weeks whereas synaptic properties relapsed to baseline within a few days. One complication for interpreting these results, however, is that the training protocol was so strong that relatively little forgetting occurred over the 3 weeks of the experiment.

Others have proposed that differential time-courses of structural and synaptic plasticity could underlie forgetting and savings (e.g. Hofer, 2010). One extension to this model we are proposing is that the shorter time course of synaptic plasticity is due to an active forgetting process that does not alter structural plasticity. In both mice and flies, there is evidence that G-protein-mediated neuromodulation can facilitate forgetting (Berry & Davis, 2014; Liu et al., 2016). We have identified a suggestive parallel in

Aplysia: long-term sensitization is associated with a very persistent up-regulation of a FMRFa transcript (this paper) as well as maintenance-only up-regulation of a transcript encoding a putative FMRFa receptor (EB234154; Conte et al., 2017). This is intriguing because FMRFa has been characterized as a “memory suppressor” (Fioravante, Smolen, & Byrne, 2006 p. 239) due to antagonistic effects on the expression of LTS memory (e.g. Abrams, Castellucci, Camardo, Kandel, & Lloyd, 1984).

The model we are proposing is speculative but provides a number of testable predictions. First, if FMRFa mediates an active forgetting process it should be possible to accelerate or forestall forgetting of a long-term sensitization memory by manipulating FMRFa signaling. Second, if structural plasticity is not subject to active forgetting it should persist beyond the decay of recall and be insensitive to manipulations that accelerate forgetting. Finally, if savings represents a re-activation of persistent structural plasticity, then the expression of a savings memory through re-training should be accompanied by only a limited transcriptional response and therefore be relatively insensitive to manipulations that disrupt changes in transcription. We are now working to test these predictions using an *in vivo* model of long-term sensitization (e.g. Noel, Scholz, Eskin, & Byrne, 1991).

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6. Supplemental Materials

Supplemental Table 1: qPCR Primers

EST	Forward qPCR Primer	Reverse qPCR Primer
FF066943.1	TACGTTGTCGTATGCTGGGG	TTGGCAGCTTCTGTCGTCTT
EB312493.1	CGCTTTGGTGAGTCATTACGG	ACACCAATCCAGGCAACCAA
EB241258.1	TAGGGTTGAAACAGCCCCAC	CGTTCAGTGTCACTCACTCCA
EB257711.1	GACGAGTTCCGGGCGATATT	TGACTTCATTTCAAAGCAACCA
EB342172	GACCCGGTCAGTTGAGTGAG	AAGGTCTGACCCTCTTGTC
EB240636.1	CCTTCCTACACAGGGAAGCA	ATGCCCCAAAAGTACAAGC
EB254334.1	GTCTCGTCCTGCGAGTTTCA	AGCTGGACGATTTGAGGCAA
EB243511.1	CGGCATTTTGCCTCATT	GCAAGAAAATTGCCGCCCTA
EB239594.1	GCTTGGATTGTGGGGAGTCT	AGACGGCGATGTGTGAATGA
mRNA	Forward qPCR Primer	Reverse qPCR Primer
FMRFa	GATGTGCAGGATCTGACGGA	CTTATCCGGTTCCCGTCTTC
ApSpectrin	CAGCGTTCAGTCTCTGCTCA	CGATCGATGGAGGTCAGACG
ApEGR	ATACCCATGTCCCGTTGAAA	AATGGAAGGGCTTCTGTCCT
ApBiP	ACGGCTGCTGACAACCAGCC	ACACCACGAGGAGCTGGGGG

7. Figure captions

Figure 1: Sensitization and savings in the tail-elicited siphon withdrawal reflex (T-SWR). a) Overview of the behavioral paradigm. Sensitization training consisted of 4 rounds of strong noxious shock to one side of the body (training site: lightning bolt). The effect of training was monitored by measuring the duration of the T-SWR, a defensive withdrawal of the siphon evoked by applying a weak electrical shock to the left or right side of the tail (test site). To document savings, a brief re-training protocol was administered to the midline of the tail (re-training site, wide arrow). In naïve animals this brief re-training protocol is not sufficient for evoking a long-term sensitization memory. b) Experimental protocol. T-SWR responses were evoked on the left and right tail (Pre-test, hash lines, four on each side). Long-term sensitization training was then administered (4 strong shocks 30 minutes apart, lightning bolts). Sensitization was then monitored by measuring T-SWR on each side at 1, 7, and 14 days after training. After the 14-day post-tests, the brief re-training protocol was administered (wide arrow). Savings was then probed by measuring the T-SWR on both sides 20-minutes and 1 day after the re-training. c) Behavioral results ($n = 21$). The graph shows changes in T-SWR duration as % change from baseline by side of training. The dashed line at 0 represents no change. The thick lines represent mean responses $\pm$ the 95% margin of error. The faded lines represent each individual animal in the study. Where the 95% margin of error is smaller than the change in behavior the result is statistically significant at $\alpha = .05$.

Figure 2. Time course study. a) Experimental protocol. Long-term sensitization training consisted of 4 rounds of noxious shocks (lightning bolts). T-SWR responses were evoked on both the trained and untrained side before and after training. Pleural ganglia RNA was harvested (curved arrows to test tubes) for qPCR analysis 1 hour ($N = 11$), 1 day ($N = 11$), 5 days ($N = 16$), 7 days ($N = 15$), or 14 days ($N = 9$) after training. No behavioral measures were obtained for 1 hour animals. All others were tested before training, at 1-day after training, and just before dissection. b) Behavioral results combined

over all groups. The graph shows changes in T-SWR duration as % change from baseline by side of training. The dashed line at 0 represents no change. The thick lines represent mean responses +/- the 95% margin of error. The faded lines represent each individual animal in the study. Where the 95% margin of error is smaller than the change in behavior the result is statistically significant at $\alpha = .05$.

Figure 3. Very long-lasting changes in gene expression after sensitization training. This figure shows changes in gene expression 1 hour (N = 11), 1 day (N = 11), 5 days (N = 16), 7 days (N = 15), or 14 days (N = 9) after training after long-term sensitization training for 3 transcripts which are regulated at the 14-day timepoint. The y axis gives the log (base 2) change in expression on the trained side relative to the control side. The dashed line at 0 represents no change in expression. In each panel the empty circles represent individual measurements, the dark circles represent mean differences, and the gray band represents the 95% margin of error, expanded to correct for multiple comparisons across all qPCR data in this experiment.

Figure 4. Somewhat long-lasting changes in gene expression after sensitization training. This figure shows changes in gene expression 1 hour (N = 11), 1 day (N = 11), 5 days (N = 16), 7 days (N = 15), or 14 days (N = 9) after training after long-term sensitization training for 4 transcripts which are regulated up to the 7-day timepoint. The y axis gives the log (base 2) change in expression on the trained side relative to the control side. The dashed line at 0 represents no change in expression. In each panel the empty circles represent individual measurements, the dark circles represent mean differences, and the gray band represents the 95% margin of error, expanded to correct for multiple comparisons across all qPCR data in this experiment. Note the different scale for panel 3 (EB254334).

Figure 5. Transient changes in gene expression after sensitization training. This figure shows changes in gene expression 1 hour (N = 11), 1 day (N = 11), 5 days (N = 16), 7 days (N = 15), or 14 days (N = 9) after training after long-term sensitization training for 6 transcripts which do not show clear

evidence of regulation beyond the decay or recall. The y axis gives the log (base 2) change in expression on the trained side relative to the control side. The dashed line at 0 represents no change in expression. In each panel the empty circles represent individual measurements, the dark circles represent mean differences, and the gray band represents the 95% margin of error, expanded to correct for multiple comparisons across all qPCR data in this experiment.

Figure 6. Microarray Expression overview and model. a) Time course of transcriptional changes 1h, 1 day, and 7 days after long-term sensitization memory. This figure summarizes microarray data from three prior experiments. Significantly regulated transcripts were grouped by time-course of regulation: encoding only (light blue), maintenance only (gray), encoding and maintenance (dark blue) and beyond recall (red). Each line represents the average fold change for all up- or down-regulated transcripts in each category. Line thickness represents the relative number of transcripts in each category. b) Model of encoding, forgetting, and savings of a long-term sensitization memory. We propose training produces two parallel changes in VC sensory neurons (and potentially other T-SWR circuit elements): synaptic outgrowth (shown as increased branching) and increased synaptic efficacy (black circles). Forgetting is postulated to represent an active reversal of increased synaptic efficacy via G-protein mediated signaling from FMRFa inhibitory interneurons (black bar). Savings persists due to the insensitivity of

structural plasticity to active forgetting, enabling the memory to be re-expressed when the circuit is potentiated.

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