Activation of $G_{\alpha q}$ Signaling Enhances Memory Consolidation and Slows Cognitive Decline

Highlights
- Increased $G_{\alpha q}$ signaling enhances memory and slows age-related cognitive decline
- CREB activity underlies enhanced consolidation and memory maintenance with age
- The AWC neuron is the site where $G_{\alpha q}$ signaling regulates memory
- $G_{\alpha q}$ activation in the AWC of aged animals rescues cognitive decline

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In Brief
Arey et al. have found that activating the $G_{\alpha q}$ signaling pathway in sensory neurons enhances memory in young animals, utilizing the canonical CREB pathway. This pathway also slows age-related memory loss and reverses age-related memory impairment.
Activation of Gαq Signaling Enhances Memory Consolidation and Slows Cognitive Decline

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SUMMARY

Perhaps the most devastating decline with age is the loss of memory. Therefore, identifying mechanisms to restore memory function with age is critical. Using C. elegans associative learning and memory assays, we identified a gain-of-function Gαq signaling pathway mutant that forms a long-term (cAMP response element binding protein [CREB]-dependent) memory following one conditioned stimulus-unconditioned stimulus (CS-US) pairing, which usually requires seven CS-US pairings. Increased CREB activity in AIM interneurons reduces the threshold for memory consolidation through transcription of a set of previously identified “long-term memory” genes. Enhanced Gαq signaling in the AWC sensory neuron is both necessary and sufficient for improved memory and increased AIM CREB activity, and activation of Gαq specifically in aged animals rescues the ability to form memory. Activation of Gαq in AWC sensory neurons non-cell autonomously induces consolidation after one CS-US pairing, enabling both cognitive function maintenance with age and restoration of memory function in animals with impaired memory performance without decreased longevity.

INTRODUCTION

Although life expectancy is increasing (Lenart and Vaupel, 2017), therapies to treat age-related memory decline are lacking, creating a growing public health threat. Cognitive function decreases in mid-life and worsens with age (Park et al., 2002). These deficits are caused by subtle changes in the plasticity of circuits that regulate learning and memory (Arey and Murphy, 2017; Burke and Barnes, 2006), which may provide insight into critical mechanisms involved in memory formation and targets for the prevention of age-related cognitive decline. Recent studies have identified interventions that have memory-promoting effects in aged rodents. For example, young blood parabiosis improves hippocampus-dependent learning and memory in aged mice (Villeda et al., 2014). Aged mice receiving chronic, systemic administration of low-dose tetrahydrocannabinol (THC) also display better spatial learning and long-term memory than vehicle-treated controls (Bilkei-Gorzo et al., 2017). Improved memory performance with both of these treatments is accompanied by molecular and cellular changes, including increased dendritic spine density, elevated expression of plasticity-related genes, and increased cAMP response element binding protein (CREB) transcriptional activity (Bilkei-Gorzo et al., 2017; Villeda et al., 2014). Interestingly, these treatments selectively improve memory in aged mammals and either have no effect (Villeda et al., 2014) or can impair memory (Bilkei-Gorzo et al., 2017) in young animals. Ideally, potential therapeutic agents would have benefits in both younger and aged individuals because cognitive performance in humans begins to decline relatively early in their lifespan (Park et al., 2002).

Like mammals, C. elegans can form associative memories, requiring conserved molecular machinery to learn and remember (Kauffman et al., 2010; Lakhina et al., 2015; Shen et al., 2014; Stein and Murphy, 2014; Vukojevic et al., 2012). Furthermore, associative learning and memory deficits are the earliest features of neuronal aging in C. elegans, preceding age-related changes in neuronal morphology, motility, chemo taxis, and other behaviors (Arey and Murphy, 2017; Kauffman et al., 2010). The processes that regulate memory performance with age in C. elegans appear to also be highly conserved. CREB levels and activity correlate with long-term associative memory (LTAM) performance with age (Kauffman et al., 2010), similar to what has been observed in mammals (Kudo et al., 2005). Maintenance of presynaptic vesicle transport and release is also important for learning and memory in both young and aged animals (Kaletsky et al., 2016; Li et al., 2016). Components of this pathway are necessary for the extended short-term associative memory (STAM) of long-lived C. elegans daf-2 insulin receptor mutants and important for their enhanced learning with age (Kaletsky et al., 2016; Kauffman et al., 2010; Li et al., 2016), and increased synaptic density and plasticity coincide with the beneficial effects of THC and young blood parabiosis (Bilkei-Gorzo et al., 2017; Villeda et al., 2014). Thus, conserved molecules that enhance synaptic function and regulate CREB activity may present new targets for the treatment of cognitive decline.
The G_{aq} signaling pathway is one of the main positive regulators of presynaptic transmission in C. elegans. The C. elegans G_{aq} homolog EGL-30 (Brundage et al., 1996) promotes both neurotransmission (Lackner et al., 1999; Miller et al., 1999) and neuropeptide secretion (Ch'ng et al., 2006) via DAG-Rho signaling (Figure 1A). G_{aq} signaling has been implicated in a number of C. elegans processes, including locomotion, egg laying, adaptation, and learned salt avoidance (Adachi et al., 2010; Brundage et al., 1996; Matsuaki et al., 2006), as well as working memory in mammals (Frederick et al., 2012), but the role of G_{aq} in long-term memory in C. elegans and higher organisms is untested.

Here we report that activation of G_{aq} signaling, which increases presynaptic transmission, lowers the threshold for memory consolidation in young animals so that a long-term memory, which usually requires 7 conditioned stimulus-unconditioned stimulus (CS-US) pairings, is formed after only a single CS-US pairing. A single sensory neuron pair, the AWC, is the site of G_{aq} signaling that is both necessary and sufficient for enhanced consolidation, and AWC-specific activation of G_{aq} signaling slows age-related decline in memory performance. Furthermore, increasing activity of the G_{aq} signaling pathway in the AWC of aged animals that already exhibit cognitive impairment restores the ability to form long-term memories. This improved memory performance is due to non-cell-autonomous G_{aq} signaling regulation of CREB activity in the AIM via neuropeptide signaling, which increases the transcription of a previously unidentified set of CREB/LATAM training-dependent genes (Lakhina et al., 2015). Moreover, activation of G_{aq} signaling specifically in the AWC enhances memory without the lifespan decrease caused by G_{aq} activation in the whole body. Activation of this conserved G_{aq} signaling pathway is a new mechanism to improve memory performance in young and aged animals and may present a target for the development of future therapies that treat age-related cognitive decline.

RESULTS

EGL-30 Gain-of-Function Mutants Exhibit Enhanced Memory Consolidation

We hypothesized that the G_{aq} signaling pathway (Figure 1A), which regulates synaptic transmission and olfactory adaptation, another associative behavioral paradigm (Matsuki et al., 2006), might play a role in associative learning and memory. We subjected G_{aq} signaling component mutants to positive olfactory associative training for both short-term (1 CS-US pairing) and long-term memory (7 CS-US pairings) (Kauffman et al., 2010). Hypomorphic egl-30/G_{aq} mutants display a mild defect in learning (Figure S1A) after 1 CS-US pairing and are unable to form short-term memory (Figures 1A and S1B). Genetic ablation of other components of G_{aq} signaling, including egl-8/PLC\_6 (Figures 1A and S1D), unc-73/Trio guanine nucleotide exchange factor (GEF) (Figures 1A and S1F), and pck-1/protein kinase C \( \varepsilon \) (PKCe) (Figures 1A and S1H), and G_{aq}/goa-1 (Figures 1A and S1J), resulted in defective learning after 1 CS-US pairing. G_{aq} signaling is also necessary for forming associations after long-term memory training because all mutants tested in the pathway showed defective learning after 7 CS-US pairings (Figures 1A, S1C, S1E, S1G, and S1I), obscuring any effects the proteins may have on long-term memory (Lakhina et al., 2015).

Because G_{aq} signaling is essential for normal associative learning and memory, we reasoned that increasing the activity of this pathway may enhance memory performance and improve memory function with age. To test this hypothesis, we subjected animals with a gain-of-function (js126) mutation in egl-30 to positive olfactory associative memory training (Kauffman et al., 2010). egl-30(js126) mutants have a single G-to-A transition that results in methionine rather than valine incorporation at amino acid 180 (V180M) in the GTPase domain (Hawasli et al., 2004), altering EGL-30's endogenous GTPase activity (Hawasli et al., 2004). Remarkably, egl-30(js126) mutants exposed to a single pairing of the neutral odorant butanone (CS) with food (US), which normally results in short-term memory that decays over the course of 2 hr (Figures 1B and S1K; Kauffman et al., 2010), exhibited a more than 10-fold memory extension, lasting more than 24 hr, following a single CS-US pairing (Figures 1B and S1K).

Figure 1. Activation of G_{aq} Enhances Consolidation and Prevents Age-Related Decline in Memory Performance via Increased CREB Activity

(A) Diagram of the G_{aq} pathway. Animals bearing hypomorphic mutations in components of this signaling pathway are defective for normal associative learning and memory (indicated by asterisks); red asterisks, defect in learning after 1 CS-US pairing; blue asterisks, defect in short-term memory; purple asterisks, defect in learning after 7 CS-US pairings. For performance indices, see Figure S1.

(B) Day 1 adult animals with a gain-of-function (js126) mutation in egl-30 exhibit extended memory (~24–30 hr) following 1 CS-US pairing compared with the memory of wild-type animals (~2 hr). Mean ± SEM. n ≥ 9–12 per time point. #p < 0.0001 compared with wild-type animals at same time point (data not shown).

(C) egl-30(js126) animals lacking functional CREB (egl-30(js126);crh-1(tz2)) do not display extended memory, indicating that the extended memory is LTAM (CREB-dependent). Mean ± SEM. n ≥ 24–26 per genotype. ****p < 0.0001.

(D) CREB activity is elevated in AIM/SIA of naive egl-30(js126);pCRE::GFP animals, whereas GFP is undetectable in naive wild-type pCRE::GFP animals. CREB activity is increased immediately following LTAM (1 x) training in wild-type animals, whereas there is no observable increase in CREB activity following training (1 x) in egl-30(js126);pCRE::GFP animals.

(E) Quantification of naive wild-type pCRE::GFP versus naive egl-30(js126);pCRE::GFP. Mean ± SEM. n > 30 animals per genotype. ****p < 0.0001.

(F) egl-30(js126) animals maintain the ability to form a long-term memory after 1 CS-US pairing on day 5 of adulthood. Mean ± SEM. n ≥ 10 per genotype. ****p < 0.0001.

(G) Naive (untrained) egl-30(js126) animals maintain elevated CREB activity in the AIM on day 5 of adulthood, whereas wild-type worms lack AIM GFP.

(H) Quantification of naive wild-type pCRE::GFP versus naive egl-30(js126);pCRE::GFP on day 5. Mean ± SEM. n > 26 animals per genotype. ****p < 0.0001. See also Figures S1 and S2.
Figure 2. Increased CREB Activity in AIM Neurons Decreases the Threshold for Consolidation in Animals with Enhanced G<sub>aq</sub> Signaling by Increasing Transcription of “Memory Genes”

(A and B) Rescue of CREB in the SIA fails to restore memory formation to egl-30(js126);crh-1(tz2) animals (A), whereas CREB rescue in the AIM restores memory performance to a level comparable with egl-30(js126) animals (B). Mean ± SEM. n = 11–12 per genotype. ****p < 0.0001, not significant (n.s.), p > 0.05.

(C) Expression of previously identified “CREB/LTAM-dependent genes” (Lakhina et al., 2015), which are upregulated in wild-type animals following LTAM training in a CREB-dependent manner (center) but remain unchanged following LTAM training in crh-1(tz2) mutants (right), are elevated in naive egl-30(js126) animals (left). Expression of these genes in naive egl-30(js126) animals correlates with their expression LTAM-trained wild-type animals (Pearson correlation = 0.56) and is anti-correlated with crh-1(tz2) mutants after LTAM training (−0.21). Individual columns represent the expression of “CREB/LTAM genes” for a single microarray.

(D) Quantification of egl-30(js126);pCRE::GFP animals up to 6 hr after training shows that CREB activity does not significantly increase after conditioning relative to naive egl-30(js126);pCRE::GFP animals. Mean ± SEM. n = 25–40 per time point. n.s., p > 0.05.

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CS-US pairings (Kauffman et al., 2010) and the activity of the transcription factor CREB/crh-1. CREB is a conserved regulator of LTAM in a number of organisms, including C. elegans (Kauffman et al., 2010; Lakhina et al., 2015; Silva et al., 1998), but is not required for short- or intermediate-term memory (Si/TAM) (Kauffman et al., 2010). egl-30(js126)’s extended one CS-US pairing memory indeed required CREB: egl-30(js126);crh-1(tz2) double mutants no longer maintained an extended positive butanone association (Figure 1C), indicating that CREB-dependent LTAM is formed after one CS-US pairing.

How might consolidation, which usually requires multiple rounds of spaced training, occur after a single CS-US pairing when Gaq activity is elevated? Consolidation can be enhanced by increasing CREB levels or by indirectly stimulating CREB activity in C. elegans, Drosophila, and mammals (Josselyn et al., 2001; Kauffman et al., 2010; Sekeres et al., 2012; Yin et al., 1995). Using a pCRE::GFP transgenic reporter of CREB activity (Suo et al., 2006), we found that naive (un-trained) egl-30(js126);pCRE::GFP animals exhibited GFP fluorescence in AIM and SIA interneurons (Figures 1D and S1E), a pattern resembling wild-type pCRE::GFP worms after 7 CS-US pairings (7× trained, Figure 1D; Lakhina et al., 2015).

Therefore, egl-30(js126) animals display CREB activation under conditions (1 CS-US pairing) that would not normally activate CREB.

**EGL-30 Gain-of-Function Mutants Maintain the Ability to Form Long-Term Memories with Age**

By days 4–5 of adulthood, wild-type C. elegans lose their LTAM ability, and learning and Si/TAM decline quickly thereafter (Kauffman et al., 2010). CREB levels and activity correlate with LTAM ability with age (Kauffman et al., 2010). We examined whether high CREB activity in egl-30(js126) mutants conferred LTAM ability with age. On day 5 of adulthood, egl-30(js126) mutants still exhibited LTAM after one CS-US pairing (Figure 1F), whereas wild-type animals exhibit no LTAM after 7 CS-US pairings (Kauffman et al., 2010). To confirm that this was indeed LTAM formation, rather than the development of a butanone preference with age, day 5 wild-type animals were tested after one CS-US pairing; they exhibited no detectable change in butanone preference at the LTAM time point (16 hr after training; Figure 1F), indicating that egl-30(js126) animals indeed maintain the ability to form associative memories.

We found that LTAM ability correlated with maintenance of CREB activity in AIM interneurons of aged (day 5) egl-30(js126);pCRE::GFP animals (Figures 1G and 1H). Although CREB activity is elevated in both young and aged egl-30(js126) mutants, their crh-1 mRNA levels are similar to wild-type animals (Figures S2A and S2B), suggesting that EGL-30 activity increases CREB’s activity rather than its transcription.

**AIM CREB Activity Is Required for Enhanced Consolidation in egl-30(js126) Mutants**

We next determined where CREB activity is required for egl-30(js126)’s enhanced memory. AIM interneurons and CREB activation in AIM interneurons (but not SIA neurons) are required for long-term memory formation after 7 CS-US pairings (Lakhina et al., 2015). We found that, although CREB activity is elevated in the SIA after training (Figure 1D), SIA-specific CREB rescue in egl-30(js126);crh-1(tz2) animals failed to restore memory (Figure 2A), whereas rescue of CREB activity in the AIM restored LTAM ability to egl-30(js126);crh-1(tz2) mutants (Figure 2B). The requirement for CREB in AIM interneurons is in agreement with the previous observation that rescue of crh-1/CREB in AIM interneurons, but not SIA neurons, rescues the ability to form long-term memories in crh-1(tz2) mutants (Lakhina et al., 2015).

**Increased AIM CREB Activity Leads to Elevated Transcription of CREB/LTAM-Dependent Genes in Naive Animals**

Following LTAM training in wild-type animals, increased CREB activity in the AIM induces the expression of a set of 757 CREB-dependent/LTAM training-dependent genes (Lakhina et al., 2015). The induction of this CREB/LTAM gene set is not merely indicative of memory training but is also required for normal memory formation; reducing the activity of many of these genes, either through loss-of-function mutation or RNAi treatment, causes long-term memory impairment (Lakhina et al., 2015).

Because CREB activity in the nervous system of naive egl-30(js126) animals resembled that of wild-type animals following LTAM training, we asked whether elevated CREB activity in the AIM interneurons induces the expression of CREB/LTAM-dependent genes (Lakhina et al., 2015) in egl-30(js126) animals prior to conditioning. Microarray analysis of untrained egl-30(js126) worms and wild-type worms revealed that, in the naive state, egl-30(js126) animals have a CREB/LTAM-dependent gene expression pattern strikingly similar to LTAM-trained wild-type animals (Pearson correlation = 0.56; Figure 2C), suggesting that elevated CREB activity and downstream target gene expression prior to training could permit LTAM formation after a single training session. Training did not further increase GFP fluorescence in egl-30(js126);pCRE::GFP animals for up to 6 hr after conditioning (Figures 1D, 2D, and S2C), suggesting that CREB activity is
not further elevated following training. Therefore, naive CREB activation and subsequent upregulation of CREB/LTAM genes may be sufficient for long-term memory formation in egl-30(js126) animals.

**Elevated CREB-Mediated Transcription in Naive EGL-30 Gain-of-Function Mutants Is Sufficient for Long-Term Memory Formation**

LTAM requires new transcription and is blocked by actinomycin D treatment (Kauffman et al., 2010; Vukojevic et al., 2012). To test whether the pre-existing elevated transcription of CREB/LTAM-dependent genes observed in egl-30(js126) animals was sufficient to induce LTAM, we treated egl-30(js126) worms with actinomycin D during the training paradigm. Blocking new transcription (Figure S2D) does not affect learning or LTAM in egl-30(js126) worms (Figures 2E and 3E), indicating that they are already “primed” for LTAM without the need for new transcription. By contrast, cycloheximide treatment during training of egl-30(js126) worms prevents LTAM, suggesting that translation of the naïve transcriptional state is necessary for memory consolidation in egl-30(js126) worms (Figures 2F and S2F).

**Increased G\(_{aq}\) Signaling in the AWC Sensory Neuron Is Necessary and Sufficient for Enhanced Memory Consolidation**

gegl-30(js126) mutants express constitutively active EGL-30 throughout the animal; therefore, we next sought to identify the site of EGL-30 activity that results in CREB activation and enhanced consolidation. egl-30(js126)’s LTAM only required training (1 CS-US) that usually forms S/ITAM, so we hypothesized that EGL-30 acts in neurons required for both LTAM and S/ITAM forms of memory. CREB activity is necessary in the AIM interneurons for LTAM (Lakhina et al., 2015) but not necessary for S/ITAM (Lakhina et al., 2015; Figure S3D). Butanone is sensed by the AWC sensory neuron (Bargmann et al., 1999), which is required for both LTAM and S/ITAM. The AIA interneuron is the sole interneuron directly connected to the AWC that synapses onto the AIM (Chen et al., 2006). Although the AIA/AIY is required upstream of CREB activity in the AIM for LTAM formation in wild-type animals (Figures S3A–S3C), like AIM, it is not required for S/ITAM (Figure S3E). We therefore examined whether activation of G\(_{aq}\) signaling solely in the AWC would affect memory consolidation.

We tested two constitutively active forms of EGL-30 (Q205L and V180M) under an AWC-specific promoter (Podr-1) and found that activation of G\(_{aq}\) in this neuron was sufficient to extend memory following one CS-US pairing to a degree that was indistinguishable in duration from egl-30(js126) animals (Figures 3A, S3F, and S3G). To verify that the extended memory we observed in animals expressing an AWC-specific gain of function EGL-30 was due to elevated G\(_{aq}\) signaling, we examined the memory ability of egl-8(n488);Podr-1::egl-30(Q205L) mutants, which lack PLC\(\beta\) activity downstream of EGL-30. egl-8(n488) suppresses Podr-1::egl-30(Q205L)’s extended memory (Figure 3B), indicating that the memory phenotype of Podr-1::egl-30(Q205L) was due to increased G\(_{aq}\) signaling in the AWC sensory neuron.

Although increased G\(_{aq}\) signaling in the AWC sensory neuron should increase synaptic transmission, it does not alter butanone sensation in the animal; Podr-1::egl-30(Q205L), egl-30(js126), and wild-type animals display normal naive butanone chemotaxis (Figures S4A and S4B), indicating that increased butanone preference only occurs in the context of behavioral conditioning. Furthermore, training increases egl-30(js126)’s preference for butanone, but not benzaldehyde, another AWC-sensed odorant, at the memory time point, indicating that memory is specific to the CS (butanone) and not due to a general increase in AWC activity (Figure S4C).

**Podr-1::egl-30(Q205L) worms’ memory was also CREB-dependent (Figure 3C) and required LTAM-specific neurons, the AIM and AIA/AIY (Figures 3D and 3E). Therefore, AWC-specific activation of G\(_{aq}\) signaling is sufficient to induce LTAM following a single CS-US pairing. Moreover, repressing G\(_{aq}\) signaling in the AWC of egl-30(js126) animals via targeted expression of a constitutively active negative regulator of G\(_{aq}\), goa-1, abolished LTAM (Figure 3F), despite the fact that G\(_{aq}\) signaling was enhanced elsewhere in the nervous system. Furthermore, learning and STM remained intact (Figures 3D and 4E) in egl-30(js126);Podr-1::goa-1 (Q205L) animals, indicating that the effects of inhibiting the enhanced G\(_{aq}\) signaling were specific to LTAM. G\(_{aq}\) signaling in the AWC is not only necessary but sufficient for enhanced consolidation.

**Increased G\(_{aq}\) Signaling in the AWC Sensory Neuron Results in Non-cell-autonomous Regulation of CREB Activity in the AIM**

The enhanced memory consolidation observed in egl-30(js126) animals is due to elevated CREB activity specifically in the AIM interneurons (Figures 1D, 1E, and 2B). Because increased G\(_{aq}\) signaling specifically in the AWC also resulted in the formation of a long-term memory after one CS-US pairing, we hypothesized that AIM CREB activity may be regulated non-cell-autonomously by G\(_{aq}\) signaling in the AWC. To test this, we examined pCRE::GFP reporter activity in animals with an AWC-specific gain-of-function EGL-30 (Podr-1::egl-30(V180M);pCRE::GFP) and found that increased G\(_{aq}\) signaling solely in the AWC was sufficient to increase CREB activity in the AIM in naive (untrained) animals, similar to that observed in egl-30(js126) mutants (Figures 4A and 4B).

**Increased Neuropeptide Secretion from the AWC Is Necessary and Sufficient for Enhanced Memory**

We next investigated how G\(_{aq}\) signaling in the AWC might non-cell autonomously regulate CREB activity. G\(_{aq}\) signaling positively regulates both neurotransmission and neuropeptide secretion (Ch’ng et al., 2008; Lackner et al., 1999; Miller et al., 1999). To determine which signal from the AWC is necessary for enhanced consolidation, we expressed RNAi targeting either the vesicular glutamate transporter eat-4, which is required for neurotransmission, or unc-31, which is required for neuropeptide secretion (Leinwand and Chalasani, 2013; Sieburth et al., 2007), in the AWC of egl-30(js126) mutants. Reduction of glutamatergic (neurotransmission) signaling from the AWC did not affect egl-30(js126) memory (Figure S4F),...
but neuropeptide signaling was required because unc-31 reduction impairs LTAM (Figure 4C). We next examined whether the requirement for neuropeptidergic signaling from the AWC was specific to enhanced memory or also necessary for normal long-term memory. We found that AWC-specific knockdown of unc-31 in wild-type animals resulted in a long-term memory deficit without any effect on learning (Figures 4Da and S4G). This result further supports the hypothesis that elevated \(G_{\alpha_q}\) signaling enhances memory consolidation through increased activity of pathways necessary for normal long-term memory formation.

We next examined whether increasing neuropeptide release from the AWC was sufficient to enhance memory. To test this hypothesis, we expressed a gain-of-function PKC-1(A160E), which selectively regulates the release of neuropeptide-containing dense-core vesicles, specifically in the AWC of wild-type animals (Dekker et al., 1993; Sieburth et al., 2007; Tsunozaki et al., 2008). We found that animals expressing this AWC-specific gain-of-function PKC-1 exhibited an extended (24-hr) memory after one CS-US (Figure 4E) pairing, indicating that increased neuropeptide secretion from the AWC is sufficient for enhanced memory.
Increasing \( G_{aq} \) Signaling in the AWC of Aged Animals Rescues Impaired Memory Function

We next determined whether enhanced \( G_{aq} \) signaling in the AWC alone was sufficient for memory maintenance with age. Animals expressing two different constitutively active forms (Q205L or V180M) of EGL-30 in the AWC exhibited LTAM following one CS-US pairing on day 5 of adulthood (Figures 5A and S5A). This memory maintenance also correlated with maintenance of elevated CREB activity in the AIM interneurons of aged (day 5) Podr-1::egl-30(V180M); pCRE::GFP animals (Figures 5B and 5C). Although whole-life activation of \( G_{aq} \) signaling in the AWC slows age-related cognitive decline, we wondered whether there were any beneficial effects of increasing \( G_{aq} \) signaling in the AWC specifically in aged animals because wild-type worms can no longer form LTAM by day 4 (Kauffman et al., 2010). We utilized a FLP recombinase driven by a heat shock protein (hsp-16.48) promoter (Davis et al., 2008) to permanently activate an FLP-inducible form of a GFP-tagged, constitutively active EGL-30 (HS Inducible Podr-1::egl-30(Q205L)). This inducible EGL-30 (Figure 5D) was functional; early larval L1 animals subjected to heat shock exhibited LTAM after one CS-US pairing, similar to worms expressing EGL-30(Q205L) in the AWC throughout their whole lifetime (Figures 5E and S5B).
Figure 5. Activation of $G_{aq}$ Signaling Solely in the AWC Slows Cognitive Decline and Restores Cognitive Function to Aged Animals

(A) Podr-1::egl-30(Q205L) animals maintain the ability to form long-term memory after one CS-US pairing on day 5 of adulthood. Mean ± SEM. n ≥ 13–14 per genotype. ****p < 0.0001.

(B) Naive (untrained) Podr-1::egl-30(V180M);pCRE::GFP animals maintain elevated CREB activity in the AIM on day 5 of adulthood.

(C) Quantification of day 5 naive Podr-1::egl-30(V180M);pCRE::GFP versus naive wild-type pCRE::GFP. Mean ± SEM. n > 40 animals per genotype. ****p < 0.0001.

(D) 1 hr of heat shock (HS) at 34°C induces expression of a GFP-tagged gain-of-function EGL-30 in HS Inducible Podr-1::egl-30(Q205L) animals.

(E) Heat shock of HS Inducible Podr-1::egl-30(Q205L) animals at the L1 larval stage enables them to form long-term memories after one CS-US pairing on day 1 of adulthood, whereas non-transgenic siblings are unaffected. Mean ± SEM. n ≥ 3 per genotype. ****p < 0.0001.
examine the effects of activation of G_{aq} signaling in the AWC specifically in aged animals, we induced EGL-30(Q205L) expression in HS Inducible Podr-1::egl-30(Q205L) animals on day 4 of adulthood and compared their behavior following one CS-US pairing on day 5 of adulthood to both wild-type siblings or HS Inducible Podr-1::egl-30(Q205L) animals without transgene induction (see Figure 5F for a schematic). Although both wild-type siblings and HS Inducible Podr-1::egl-30(Q205L) animals without transgene induction were unable to form memory (Figures 5G and 5SC), the induction of AWC-specific activation of G_{aq} signaling on day 4 of adulthood rescued the ability to form LTAM (Figures 5H and 5SD). Thus, increased G_{aq} signaling in the AWC rescues LTAM in aged animals with impaired memory.

AWC-Specific G_{aq} Signaling Improves Memory without Decreasing Lifespan

Although increased G_{aq} signaling allows maintenance of cognitive performance with age, it has also been associated with a decrease in lifespan because egl-30(js126) mutants exhibit a decreased lifespan (Ch’ng et al., 2008). Because increased G_{aq} signaling in the AWC is sufficient to improve memory and slow cognitive decline, we examined its effects on longevity. As reported previously (Ch’ng et al., 2008), egl-30(js126) mutants are short-lived (Figure 5I); however, animals expressing either Q205L or V180M gain-of-function alleles of egl-30 specifically in the AWC have a normal lifespan (Figures 5I and 5SE). Therefore, identification of the neural site of G_{aq} signaling-mediated memory consolidation enables memory improvement without compromising longevity.

DISCUSSION

Here we identified G_{aq} signaling as a novel positive regulator of memory consolidation. This function is unlikely to be limited to C. elegans because EGL-30 shares 82% identity with the mammalian GNAQ (Brundage et al., 1996). Although G_{aq} signaling has been implicated in C. elegans associative behaviors (Matsuki et al., 2006) and in mammalian working memory (Frederick et al., 2012), it has not been specifically identified as a long-term memory regulator in higher organisms. We provide the first direct evidence that G_{aq} regulates associative memory. The downstream targets of G_{aq} have also been implicated in cognitive function in higher organisms. Loss of PLC/EGF-8 and TRIO/UNC-73 have been linked to deficits in working memory and learning in mice, respectively (Kim et al., 2015; Zong et al., 2015). PKC isoforms play an essential role in mammalian learning and memory (Abeliovich et al., 1993; Sacktor and Hell, 2017; Sun and Alkon, 2014; Sutton et al., 2004; Weeber et al., 2000); however, the mammalian ortholog of pck-1, PKC1Δε, has not been as well characterized in memory formation. Furthermore, little is known about G_{aq} and its downstream targets in the context of normal aging and cognitive decline.

We found that enhanced G_{aq} signaling enables memory formation in aged animals when LTAM normally no longer functions. The memory-enhancing effect of G_{aq} signaling in both young and old animals is due to elevated CREB activity in AWM interneurons, and the subsequent up-regulation of a previously identified memory gene set (Lakhina et al., 2015). The up-regulation of these CREB-dependent/training-dependent genes primes the animals for memory formation even in the presence of transcriptional blockage.

The correlation between memory function with age and CREB activity appears to be a highly conserved mechanism. Recently, virus-mediated overexpression of CREB in the CA1 was found to improve memory in aged but not young rats (Yu et al., 2017). Increased CREB activity is also involved in the memory-promoting effects of caloric restriction (Fusco et al., 2012), young blood parabiosis (Villeda et al., 2014), and low-dose Δ9-THC treatment of aged mice (Bilkei-Gorzo et al., 2017). However, here we have identified a pathway that enhances memory performance in young animals, slows cognitive decline, and rescues age-related memory impairment.

G_{aq} signaling influences CREB and the LTAM network non-cell autonomously (Figure 6); activating G_{aq} in the AWC is both necessary and sufficient for LTAM following one CS-US pairing. Moreover, G_{aq} signaling in the AWC slows cognitive decline and restores LTAM ability at an age when associative memory ability has been abrogated. Non-cell-autonomous regulation of memory formation is not well understood; because long-term memory components and processes are highly conserved between C. elegans and higher organisms (Kauffman et al., 2010; Lakhina et al., 2015; Stein and Murphy, 2014), the molecules downstream of G_{aq} signaling may present new targets for the study of learning, memory, and aging in mammals. This non-cell-autonomous regulation occurs via neuropeptide signaling (Figure 6), which has previously been implicated in C. elegans LTAM (Lakhina et al., 2015). Neuropeptides from the AWC likely activate signaling pathways upstream of CREB, such as Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) and c-Jun N-terminal kinase (JNK) signaling, which potentially regulate CREB activity in a positive feedback loop during memory training (Lakhina et al., 2015), although a number of other signaling pathways could be involved.

We also found that increasing G_{aq} signaling in the AWC alone dissociates the positive influence of this pathway on memory formation from its previously reported deleterious effects on

(F) Schematic of induction of AWC-specific gain-of-function EGL-30 in aged animals. 1 hr of heat shock at 34°C on day 4 of adulthood is sufficient to induce transgene expression that is detectable in day 5 animals (1 hr HS).

(G) Animals without transgene induction (no HS) fail to form a long-term memory on day 5 of adulthood. Mean ± SEM. n ≥ 4 per genotype. n.s., p > 0.05.

(H) Induction of AWC-specific gain-of-function EGL-30 in aged (day 4) animals enables long-term memory formation after one CS-US pairing on day 5 of adulthood. Mean ± SEM. n ≥ 13 per genotype. ***p < 0.0001.

(†) egl-30(js126) mutants have a significantly shortened lifespan (****p < 0.0001), as reported previously (Ch’ng et al., 2008), whereas the lifespan of transgenic Podr-1::egl-30(Q205L) animals does not significantly differ from wild-type siblings (p = 0.47). n ≥ 100 per genotype.

See also Figure 5S.
lifespan (Ch’ng et al., 2008). Our elucidation of the neural site where G\textsubscript{aq} signaling regulates memory may enable identification of molecules that promote memory maintenance without shortening lifespan. These could represent targets for novel strategies to manipulate memory consolidation and may potentially provide new therapeutic agents that treat cognitive decline or prevent deleterious consolidation.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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  ○ RNA Isolation, CDNA Synthesis, and qRT-PCR
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● QUANTITATIVE AND STATISTICAL ANALYSIS

● DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and can be found with this article online at https://doi.org/10.1016/j.neuron.2018.03.039.

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We thank J. Ashraf for assistance with the generation of transgenic strains. M. Wright and R. Jin for assistance with behavioral assays, the CGC for strains, and the Murphy lab for valuable discussions. FLP cloning plasmids were a kind gift from C. Bargmann. C.T.M. is the Director of the Paul F. Glenn Center for Biology of Aging Research at Princeton and an HHMI-Simons Faculty Scholar. This work was supported by the NIH (R01AG034446 to C.T.M. and F32AG048108 to R.N.A.).

AUTHOR CONTRIBUTIONS


DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES


## STAR Methods

### Key Resources Table

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**Oligonucleotides**

| pmp-3 qPCR Forward primer AGTTCCGGTTGAGTTGTCC | This paper | N/A |
| pmp-3 qPCR Reverse primer CAGACACGATAAGGCAGAT | This paper | N/A |
| hsp-70 qPCR Forward primer CCGGTGAAAAGGCACCTCG | This paper | N/A |
| hsp-70 qPCR Reverse primer GAGGGTTAGGTCTTCCC | This paper | N/A |
| crh-1 qPCR Forward primer CCAACAACAAACGGGACTCGC | This paper | N/A |
| crh-1 qPCR Reverse primer ATGAGCCCCAGATGTGCCTTC | This paper | N/A |

**Recombinant DNA**

| Plasmid: pl4440 RNAi control | Ahringer RNAi library | N/A |
| Plasmid: pl4440-ttx-3 RNAi | Ahringer RNAi library | N/A |

**Software and Algorithms**

| PUMAdb | Princeton University MicroArray database | http://puma.princeton.edu |
| Cluster 3.0 | Eisen et al., 1998 | http://bonsai.hgc.jp/~mddehoon/software/cluster/software.htm |

(Continued on next page)
All strains were maintained at 20°C. *C. elegans*

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

**3::eat-4 sense, odr-3::eat-4 antisense, eat-2::GFP**]) was generated by crossing NM1380 (sl2mCherry, odr-3::unc-31antisense::sl2mCherry, unc-122::RFP]) was generated by crossing NM1380 (sl2mCherry, odr-3::unc-31antisense::sl2mCherry, unc-122::RFP]) was generated by crossing JN897 (ueEx4[odr-3::unc-31sense::sl2mCherry, odr-3::unc-31antisense::sl2mCherry, unc-122::RFP]) and IV18 (ueEx4[odr-3::eat-4 sense, odr-3::eat-4 antisense, eat-2::GFP]) were gifts from S. Chalasani (The Salk Institute for Biological Studies, La Jolla, CA), and CX9735 (gcy-28(tm2411);kyEX2139[odr-3::pck1(A160E)];sl2GFP, elt-2::GFP) was a generous gift from C. Bargmann (The Rockefeller University, New York, NY).

### Strains

Wild-type: (N2 Bristol); Mutant strains: NM1380 (egl-30(js126)), OH161 (txx-3([t22]), RB816 (sra-11(ex630)), UTK2 (mbr-1(qa5901)), MT1434 (egl-30(n686)), MT1083 (egl-8(n488)), KG1278 (unc-63(ce362)), YT17 (chr-1([t22]), DG1856 (goa-1(sa374)), and KP2342 (pck-1(n488)) were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN). The AIM CREB Rescue CQ159 (chr-1([t22]); wqEx29 [pmyo-2::mCherry]; puroR; pmbr-1::chr-1::β-FLAG) and SIA CREB Rescue CQ160 (chr-1([t22]); wqEx29 [pmyo-2::mCherry]; puroR; pceh-24::chr-1::β-FLAG), and CQ161 (pCRE::GFP) lines were described previously (Lakhina et al., 2015; Suo et al., 2006). Strains JN897 (Podr-1::egl-30(Q205L)) and JN819 (Podr-1::goa-1(Q205L)) were gifts from Y. Iino (University of Tokyo, Tokyo, Japan), IV183 (ueEx104[odr-3::unc-31sense::sl2mCherry, odr-3::unc-31antisense::sl2mCherry, unc-122::RFP]) and IV8 (ueEx4[odr-3::eat-4 sense, odr-3::eat-4 antisense, eat-2::GFP]) were gifts from S. Chalasani (The Salk Institute for Biological Studies, La Jolla, CA), and CX9735 (gcy-28(tm2411);kyEX2139[odr-3::pck1(A160E)];sl2GFP, elt-2::GFP) was a generous gift from C. Bargmann (The Rockefeller University, New York, NY).

The following strains were generated by crosses: CQ210 (egl-30(js126); chr-1([t22])) was generated by crossing NM1380 (egl-30(js126)) with YT17 (chr-1([t22]), CQ243 (egl-30(js126); chr-1([t22]); wqEx29 [pmyo-2::mCherry]; PuroR; pceh-24::chr-1::β-FLAG)] was generated by crossing CQ210 (egl-30(js126); chr-1([t22])) with SIA CREB Rescue CQ160 (chr-1([t22]); wqEx29 [pmyo-2::mCherry]; PuroR; pceh-24::chr-1::β-FLAG), CQ245 (egl-30(js126); chr-1([t22]); wqEx29 [pmyo-2::mCherry]; PuroR; pmbr-1::chr-1::β-FLAG]) was generated by crossing CQ210 ([egl-30(js126); chr-1([t22])] with AIM CREB Rescue CQ159 ([chr-1([t22]); wqEx29 [pmyo-2::mCherry]; PuroR; pmbr-1::chr-1::β-FLAG], CQ252 ([egl-30(js126]; wqEx28[pCRE::GFP]) was generated by crossing NM1380 (egl-30(js126)) with CQ161 (pCRE::GFP), CQ253 ([egl-30(js126); Podr-1::goa-1(Q205L)]) was generated by crossing NM1380 (egl-30(js126)) with JN819 (Podr-1::goa-1(Q205L)), CQ294 (egl-8(n488); Podr-1::egl-30(Q205L)) was generated by crossing JN897 (Podr-1::egl-30(Q205L)) with MT1083 (egl-8(n488)), CQ406 (mbr-1(qa5901); Podr-1::egl-30(Q205L)) was generated by crossing JN897 (Podr-1::egl-30(Q205L)) with UTK2 (mbr-1(qa5901)), CQ407 (egl-30(js126); ueEx104[odr-3::unc-31sense::sl2mCherry, odr-3::unc-31antisense::sl2mCherry, unc-122::RFP]) was generated by crossing CQ159 (egl-30(js126)) with IV183 (ueEx104[odr-3::unc-31sense::sl2mCherry, odr-3::unc-31antisense::sl2mCherry, unc-122::RFP]), CQ412 ([egl-30(js126]; ueEx4[odr-3::eat-4 sense, odr-3::eat-4 antisense, eat-2::GFP]) was generated by crossing CQ159 ([egl-30(js126]) with IV8 (ueEx4[odr-3::eat-4 sense, odr-3::eat-4 antisense, eat-2::GFP]), CQ415 (chr-1([t22]); Podr-1::egl-30(Q205L)) was generated by crossing JN897

### CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Coleen Murphy (ctmurphy@princeton.edu).

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

**C. elegans** Genetics

All strains were maintained at 20°C on plates made from standard nematode growth medium (NGM: 3 g/L NaCl, 2.5 g/L Bactopeptone, 17 g/L Bacto-agar in distilled water, with 1 mL/L cholesterol (5 mg/mL in ethanol), 1 mL/L 1M CaCl2, 1 mL/L 1M MgSO4, and 25 mL/L 1M potassium phosphate buffer (pH 6.0) added to molten agar after autoclaving; (Brenner, 1974) or high growth medium (HGM: NGM recipe modified as follows: 20 g/L Bacto-peptone, 30 g/L Bacto-agar, and 4 mL/L cholesterol (5 mg/mL in ethanol); all other components same as NGM), with OP50 E. coli as the food source. Experiments that did not involve RNAi treatments were performed using NGM plates seeded with OP50 E. coli for *ad libitum* feeding (Brenner, 1974); for RNAi experiments, the standard NGM molten agar was supplemented with 1 mL/L 1M IPTG (isopropyl β-d-thiogalactopyranoside) and 1 mL/L 100 mg/mL carbenicillin, and plates were seeded with HT115 E. coli or OP50(xu3363) E. coli for *ad libitum* feeding. Hypochlorite-synchronization to developmentally synchronize experimental animals was performed by collecting eggs from gravid hermaphrodites via exposure to an alkaline-bleach solution (e.g., 5.5 mL water, 1.5 mL 5N KOH, 3.0 mL sodium hypochlorite), followed by repeated washing of collected eggs in M9 buffer (6 g/L Na2HPO4, 3 g/L KH2PO4, 5 g/L NaCl and 1 mL/L 1M MgSO4 in distilled water; Brenner, 1974). For aging experiments, animals were transferred at the L4 larval stage onto NGM plates supplemented with 500 eggs in M9 buffer (6 g/L Na2HPO4, 3 g/L KH2PO4, 5 g/L NaCl and 1 mL/L 1M MgSO4 in distilled water; Brenner, 1974). For aging experiments, animals were transferred at the L4 larval stage onto NGM plates supplemented with 500 μL 0.1M FUdR (5-Fluoro-2’-deoxyuridine) for a final concentration of 0.05M FUdR.
2400bp upstream of the Actinomycin D treatment blocked transcription, a separate cohort of animals were exposed to heat shock at 33°C while in M9 buffer 

\[ \text{(Podr-1::egl-30(Q205L))} \text{ with YT17 (chr-1(2z2))}, \text{CQ465 (ttx-3(ot22);Podr-1::egl-30(Q205L)) was generated by crossing JN897 (Podr-1::egl-30(Q205L)) with OH161 (ttx-3(ot22)), CQ547 (Podr-1::egl-30(V180M);pCRE::GFP)) was generated by crossing CQ477 (Podr-1::egl-30(V180M)) with CQ161 (pCRE::GFP), CQ563 (Podr-3::pkc1(A160E)::siz2GFP, elt-2::GFP) was generated by crossing CX9735 (gcy-28(tm2411);kyEX2139[Podr-3::pkc1(A160E)::siz2GFP, elt-2::GFP]) with N2 (Bristol) and selecting heterozygotes for self-fertilization, followed by genotyping of F1 progeny to isolate N2 animals that expressed kyEX2139[odr-3::pkc1(A160E)::siz2GFP, elt-2::GFP].

Construction of Transgenic Lines

Extrachromosomal transgenic arrays were generated as previously described (Mello et al., 1991). For CQ477 (Podr-1::egl-30(V180M)), the egl-30 gene (bearing the V180M mutation) was PCR amplified from egl-30/js128 mutants. The egl-30(V180M) was PCR ligated to 2400bp upstream of the odr-1 start site and the unc-54 3' UTR. Podr-1::egl-30(V180M) was injected into animals at 15ng/ul with 1 ng/ul Pmyo-2::mCherry. The HS Inducible Podr-1::egl-30(Q205L) lines CQ429 (pHSP16-48::FLPase; Podr-1::FRT::egl-30(Q205L); Pmyo-2::mCherry) and CQ430 (pHSP16-48::FLPase; Podr-1::FRT::egl-30(Q205L); Pmyo-2::mCherry) #2 were constructed as described previously (Davis et al., 2008). FLP cloning plasmids were a gift of E. Jorgensen. Briefly, the upstream 2.4kb promoter of odr-1 was used to drive expression of a FRT-flanked mCherry expression cassette, followed by GFP N-terminally fused to egl-30(Q205L) and the unc-54 3’ UTR. N2 worms were injected with 25 ng/ul of the inducible odr-1p::FRT-mCherry-FRT::GFP-egl-30(Q205L) plasmid, 45 ng/ul of hsp16.48p::FLPase, and 1 ng/ul Pmyo-2::mCherry.

METHOD DETAILS

Behavioral Assays

Olfactory Associative Paradigms

Wild-type, mutant, and transgenic animals were trained and tested for either long-term or short/intermediate term memory as previously described (Kauffman et al., 2010). Briefly, synchronized day 1 adult hermaphrodites were washed from HGM or NGM plates with M9 buffer, allowed to settle by gravity, and washed again with M9 buffer. After washing, the animals are starved for 1 hr in M9 buffer. For 1 CS-US pairing, worms were then transferred to 10 cm NGM conditioning plates (seeded with OP50 E. coli bacteria and with 6 l 10% 2-butanone (Acros Organics) in ethanol on the lid) for 1 hr. For 7 CS-US pairings, worms alternate between 30 min conditioning cycles and 30 min starvation cycles (on NGM plates with no food). After conditioning, the trained population of worms were tested for chemotaxis to 10% butanone vs. an ethanol control either immediately (0 hr) or after being transferred to 10 cm NGM plates with fresh OP50 for specified intervals before testing (30 min-48 hr), using standard, previously described chemotaxis assay conditions (Bargmann et al., 1993).

Chemotaxis indices were calculated as follows: (#wormsButanone - #wormsEthanol)/(Total #worms). Performance index is the change in chemotaxis index following training relative to the naive chemotaxis index. The calculation for Performance Index is: Chemotaxis Index_{trained} - Chemotaxis Index_{naive}. Performance indices for extrachromosomal transgenic strains (including strains that expressed AWC-specific RNAi) were analyzed by hand counting GFP or mCherry positive and negative worms at different locations on the chemotaxis plates. Wild-type controls for these experiments were the transgenic worms’ GFP or mCherry negative siblings.

Assays Using Drug Treatments

100 μg/mL Actinomycin D ≥ 95% (Sigma Aldrich, Saint Louis, Missouri) was added to M9 buffer for the 1 hr pre-conditioning starvation and added to S-saline (5.8 g/L NaCl, 50 mL/L 1M potassium phosphate buffer (pH 6.0), and 5 mL/L cholesterol (5 mg/mL in ethanol)) during conditioning along with 1:1000 Butanone and OP50 E. coli bacteria that had been grown overnight. After treatment, worms were transferred to NGM plates seeded with OP50 E. coli until memory was assessed 24 hr post-conditioning. To verify that Actinomycin D treatment blocked transcription, a separate cohort of animals were exposed to heat shock at 33°C while in M9 buffer or 100 μg/mL Actinomycin D in M9 followed by RNA isolation and qPCR to measure hsp-70 gene expression. Cycloheximide ≥ 94% (Sigma Aldrich, Saint Louis, Missouri) was added to NGM at 0.8 mg/mL. Plates were poured and solidified overnight at 4°C, then seeded with OP50 E. coli. Animals were exposed to cycloheximide during conditioning and for 1 hr post-conditioning, after which they were transferred to NGM plates seeded with OP50 E. coli until long-term memory was assessed 24 hr post-conditioning.

RNA Collection and Microarray Hybridization

Worms of a particular genotype were crushed in liquid nitrogen and added to Trizol (Thermo Fisher Scientific). RNA was extracted and purified using an RNAeasy Mini Kit (Qiagen, Germantown, MD, USA). cRNA was linearly amplified and Cy3/Cy5 labeled (Agilent, Santa Clara, CA, USA), and hybridized to Agilent 44k C. elegans microarrays, at 60°C overnight, as previously described (Shaw et al., 2007). Three biological replicates of egl-30/js126 versus wild-type N2 arrays were hybridized to determine expression profiles of CREB-dependent, training dependent genes that had been previously identified (Lakhina et al., 2015).

Microarray Analysis

Data from scanned microarrays were loaded onto the Princeton University MicroArray database (PUMAdb) (http://puma.princeton.edu), and analyzed as previously described (Shaw et al., 2007). Genes were filtered for presence in at least 60% of arrays (uncentered...
correlation, average linking). Log2 expression ratios of “CREB/LTAM” genes were hierarchically clustered after filtering for genes that were present in at least 60% of the arrays (uncentered correlation, average linking) in Cluster and displayed in TreeView (Eisen et al., 1998).

**RNA Isolation, CDNA Synthesis, and qRT-PCR**

Worms of a particular genotype were crushed in liquid nitrogen and added to Trizol reagent (Thermo Fisher Scientific). RNA was isolated per manufacturer’s instructions, followed by DNase treatment (Qiagen). cDNA was synthesized with an oligo dT primer and Superscript III reverse transcriptase enzyme (Thermo Fisher Scientific). cDNA was mixed with buffers, primers, SYBR green, and hot start Taq polymerase in a master mix prepared by a manufacturer (Thermo Fisher Scientific). Using a Real-Time PCR machine (7500 Real-Time PCR machine, Applied Biosystems) PCR reactions were run followed by a dissociation reaction to determine specificity of the amplified product. The amount of gene expression was quantified using the ΔΔCt method using *pmp-3* as a reference gene. Primer sets are listed in the key reagents and resources table.

**Microscopy**

Z-stack multi-channel (DIC, GFP) of *pCRE::GFP* animals were imaged every 1 mm at 60X magnification; Maximum Intensity Projections and 3D reconstructions of head neurons were built with Nikon *NIS-Elements*. To quantify *pCRE::GFP* levels worms were synchronized by bleaching, and eggs were plated on OP50-seeded NGM plates until Day 1 of adulthood. For imaging at Day 5 of adulthood, animals were transferred to OP50-seeded NGM plates containing 0.05 mM FUdR to prevent progeny contamination. GFP was imaged at 60X magnification and quantified using *NIS-Elements* software. Average pixel intensity was measured over a standard region of interest (the AIM) in every animal.

**Survival Analysis**

Lifespans were performed on *Podr-1::egl-30(Q205L)* and *Podr-1::egl-30(V180M)* transgenic animals and their wild-type siblings bleached on to OP50 seeded NGM plates. At the L4 Larval stage, transgenic worms (expressing GFP or mCherry) or non-fluorescent wild-type siblings, were picked for survival analysis. Worms were transferred every other day to freshly seeded plates. Day 1 of adulthood was defined as t = 0, and the log-rank (Mantel-Cox) method was used to test the null hypothesis in Kaplan-Meier survival analysis, and evaluated using OASIS survival analysis software (Yang et al., 2011). All experiments were carried out at 20°C; n = 108 per strain/trial. Two biological replicates were performed.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Lifespan assays were assessed using Kaplan-Meier log rank tests. For the comparison of performance indices between two genotypes (i.e., *egl-30(js126)* vs wild-type), two-tailed unpaired Student’s t tests with Welch’s corrections were used. For the comparison of relative fluorescence between two genotypes (i.e., *egl-30(js126)* vs wild-type), two-tailed unpaired Student’s t tests with Welch’s corrections were used. When relative fluorescence was measured across timepoints (0 hr, 1 hr, 2 hr, 4 hr, 6 hr), one-way analysis of variances followed by Bonferroni post hoc tests for multiple comparisons were performed. 2-way ANOVAs were used for evaluating effects between genotype ( *egl-30(js126)* and wild-type) and timepoint (0 hr, 4 hr, 24 hr) on chemotaxis and performance indices with a significant interaction between factors (p < 0.0001) leading to the performance of Bonferroni post-hoc comparisons to determine differences between individual groups. Experiments were repeated on separate days with separate populations, to confirm that results were reproducible. Prism 7 software was used for all statistical analyses; software and further statistical details used for microarray analyses are described in the Method Details section of the STAR Methods. Additional statistical details of experiments, including sample size (with n representing the number of chemotaxis assays performed for behavior, RNA collections for qPCR and arrays, and number of worms for microscopy), can be found in the figure legends.

**DATA AND SOFTWARE AVAILABILITY**

Raw microarray datasets are publically available through PUMAdb (http://puma.princeton.edu).
Supplemental Information

Activation of G\textsubscript{aq} Signaling
Enhances Memory Consolidation
and Slows Cognitive Decline

Rachel N. Arey, Geneva M. Stein, Rachel Kaletsky, Amanda Kauffman, and Coleen T. Murphy
Inventory of Supplemental Information.

Figures S1-S5. Contains supplemental data that supports the data found in the main figures.

**Figure S1** relates to **Figure 1** and includes behavioral characterization of mutants in the G\textsubscript{aq} signaling pathway and a chemotaxis timecourse for *egl-30(js126)* mutants.

**Figure S2** relates to **Figures 1&2**. It contains *crh-1* mRNA quantification as well as further *pCRE::GFP* images and quantification, as well as controls pertaining to chemical treatments performed for experiments in **Figure 2**.

**Figure S3** relates to **Figure 3**. It includes further behavioral testing of mutants used in **Figure 3**, and behavioral testing of EGL-30 gain-of-function alleles

**Figure S4** relates to **Figures 3&4**. It includes naïve chemotaxis data for mutant animals used in Figure 3, further behavioral testing for animals used in **Figures 3&4**, and behavioral testing of animals with reduced AWC glutamatergic transmission.

**Figure S5** relates to **Figure 5**. It includes behavioral testing and lifespan analysis of additional EGL-30 gain-of-function alleles and transgenic lines used in **Figure 5**.

Supplementary Information

**Figure S1 (Related to Figure 1).** A-C) Animals with hypomorphic mutations in *egl-30* have a near significant defect in learning after 1CS-US pairing (A), and have defective short-term memory (B) and learning after 7 CS-US pairings (C). D-I) Animals lacking functional *egl-8/PLCβ* (D,E), *unc-73/Trio GEF* (F,G), and *pkc-1/PKCε* (H,I) display defective learning after 1 CS-US pairing or 7 CS-US pairings. Mean ± SEM. n ≥ 8-15 per genotype. *p<0.05, **p<0.001, ****p<0.0001. J) *goa-1/G\textsubscript{i0}* mutants display defective learning after 1 CS-US pairing. n ≥ 15 per
genotype. ****p<0.0001. K) Chemotaxis indices of Day 1 adult egl-30(js126) animals following 1 CS-US pairing when compared to wild-type animals from Figure 1B. Mean ± SEM. n ≥ 9-12 per timepoint. #p<0.0001 when compared to wild-type animals at same timepoint (data not shown).

Figure S2 (Related to Figures 1 & 2). A,B) crh-1 mRNA levels measured by qPCR are not significantly different in egl-30(js126) animals when compared to wild-type animals at Day 1(A) or Day 5 (B) of adulthood. Mean ± SEM. n = 4-5 per genotype. n.s. = p>0.05. C) Representative images of egl-30(js126);pCRE::GFP animals post-conditioning displaying no detectable change in GFP fluorescence. D) 2-hours of Actinomycin D treatment blocks induction of the hsp-70 gene as measured by qPCR following heat shock at 33⁰C. Mean ± SEM. n ≥ 4-5 per treatment. *p<0.05. E,F) Actinomycin D (E) and cycloheximide (F) treatment have no detectable effect on learning in egl-30(js126) animals. Mean ± SEM. n ≥ 10-15 per treatment. n.s. = p>0.05

Figure S3 (Related to Figure 3). A) Mutants with disrupted development and function of the AIA/AIY (ttx-3(ot22) and sra-11(ok630)) fail to form long-term memory after 7CS-US pairings. Mean ± SEM. n ≥ 6 per genotype. ****p<0.0001. B,C) The AIA/AIY is required for activation of CREB in the AIM following LTAM training (7CS-US pairings). Disrupting AIA/AIY development and function in pCRE::GFP animals by ttx-3 RNAi treatment significantly attenuates CREB activity as measured by pCRE::GFP fluorescence following LTAM training when compared to vector control. Mean ± SEM. n ≥ 35-45 per RNAi. ****p<0.0001. D,E) Mutations that affect development of the AIM (D, mbr-1(qa5901)) or AIA/AIY (E, ttx-3(ot22)) have no effect on S/ITAM. Mean ± SEM. n ≥ 5-9 per genotype. n.s. = p>0.05. F) After 1 CS-US pairing, no detectable difference was observed in memory performance or duration of Podr-1::egl-30(Q205L)) and egl-30(js126) animals. Mean ± SEM. n ≥ 7-8 per genotype, per timepoint. n.s. =
p>0.05 G) After 1 CS-US pairing, no detectable difference was observed in the duration of extended memory of Podr-1::egl-30(Q205L) and Podr-1::egl-30(V180M) animals, which express the gain-of-function allele of EGL-30 present in egl-30(js126) animals specifically in the AWC. Mean ± SEM. n ≥ 3-6 per genotype, per timepoint. n.s. = p>0.05

**Figure S4 (Related to Figures 3 & 4).** A,B) Naïve chemotaxis to butanone in both egl-30(js126) mutants (A) and Podr-1::egl-30(Q205L) (B) animals does not significantly differ from wild-type animals. Mean ± SEM. n ≥ 5-7 per genotype. n.s. = p>0.05. C) egl-30(js126)’s memory is specific to the CS used in the training paradigm. A training-dependent increase is observed in chemotaxis to 10% butanone (CS), but not other neutral AWC-sensed odorants (0.1% Benzaldehyde), is observed 24 hours post-conditioning. Mean ± SEM. n ≥ 8 per group. ****p<0.0001. D,E) Repressing EGL-30 activity in the AWC of egl-30(js126) animals by AWC-specific expression of a gain-of-function inhibitor of Gαq signaling (egl-30(js126);Podr-1::goa-1(Q205L)) has no detectable effect on learning (D) or STAM (E). Mean ± SEM. n ≥ 9 per genotype. n.s. = p>0.05 F) Knockdown of glutamatergic transmission in the AWC (egl-30(js126);Podr-3::eat-4 RNAi)) has no detectable effect on long-term memory in egl-30(js126) animals. Mean ± SEM. n ≥ 12 per genotype. n.s = p>0.05. G) Knockdown of neuropeptidergic transmission in the AWC (Podr-3::unc-31 RNAi) has no detectable effect on learning after 7 CS-US pairings. Mean ± SEM. n ≥ 9 per genotype. n.s = p>0.05.

**Figure S5 (Related to Figure 5).** A) Podr-1::egl-30(V180M) animals maintain the ability to form long-term memory at Day 5 of adulthood after 1 CS-US pairing. Mean ± SEM. n ≥ 13 per genotype. ****p<0.0001 B) Confirmation of ability of Day 1 HS Inducible Podr-1::egl-30(Q205L) animals to form extended memory after heat shock at L1 larval stage in a second transgenic line. Mean ± SEM. n ≥ 3 per genotype. ****p<0.0001 C,D) Heat shock-induced activation of Gαq
signaling in the AWC at Day 4 of adulthood also restores memory formation at Day 5 of adulthood in a second transgenic line of *HS Inducible Podr-1::egl-30(Q205L)* (D), while worms without transgene induction (C) have no memory. Mean ± SEM. n ≥ 10 per genotype. ****p<0.0001. E) Lifespan of transgenic *Podr-1::egl-30(V180M)* animals does not significantly differ from wild-type siblings (p = 0.09). n ≥ 100 per genotype.
**Fig. S1**

A. Learning (1 CS-US pairing)  
Wild-type  
egl-30(js126)  
P = 0.056

B. Short-Term Memory  
Wild-type  
egl-30(js126)  
*

C. Learning (7 CS-US Pairings)  
Wild-type  
egl-30(js126)  
****

D. Learning (1 CS-US pairing)  
Wild-type  
egl-9(js69)  
****

E. Learning (7 CS-US Pairings)  
Wild-type  
egl-9(js69)  
****

F. Learning (1 CS-US pairing)  
Wild-type  
ung-73(co362)  
****

G. Learning (7 CS-US Pairings)  
Wild-type  
ung-73(co362)  
***

H. Learning (1 CS-US pairing)  
Wild-type  
plc-1(nu488)  
****

I. Learning (7 CS-US Pairings)  
Wild-type  
plc-1(nu488)  
****

J. Learning (1 CS-US pairing)  
Wild-type  
gac-1(nu39)  
****

K. Chemotaxis Index  
Wild-type  
egl-30(js126)  

Time (hours)
Fig. S2
A. Naive chemotaxis to 10% butanone

B. Naive chemotaxis to 10% butanone

C. egl-30(js126) memory for AWC-sensed odorants (CS-10% Butanone)

D. Learning (0hr)

E. Short-Term Memory

F. Glutamatergic Transmission Knockdown

G. Learning (7 CS-US Pairings)

Fig. S4
Wild-type Podr-1::egl-30 (V180M) -0.2 -0.1 0.0 0.1 0.2 0.3
Day 5 (Aged) Long-term Memory

****

Wild-type Podr-1::egl-30 (Q205L) #2 -0.4 -0.2 0.0 0.2 0.4 0.6
Day 1 (HS at L1) Adult LTAM after 1 CS-US pairing

Performance Index

-0.2 0.0 0.2
Day 5 Adult (No HS) Long-Term Memory

n.s.

Wild-type Podr-1::egl-30 (Q205L) #2 -0.2 0.0 0.2
Day 5 Adult (HS at D4) Long-term Memory

****

Wild-type siblings

Podr-1::egl-30(V180M)

Percent survival

0 10 20 30 40
Days

Fig. S5