CellPress

Neuron
Article

Neurons Are Recruited to a Memory Trace Based on Relative Neuronal Excitability Immediately before Training

Adelaide P. Yiu,[1,2,3,4,8] Valentina Mercaldo,[1,2,3,4,8] Chen Yan,[1,2,3,4] Blake Richards,[1,2,3,4,5] Asim J. Rashid,[1,2,3,4] Hwa-Lin Liz Hsiang,[1,2,3,4] Jessica Pressey,[6] Vivek Mahadevan,[6] Matthew M. Tran,[1] Steven A. Kushner,[1,7] Melanie A. Woodin,[6] Paul W. Frankland,[1,2,3,4] and Sheena A. Josselyn[1,2,3,4,*]

[1]Program in Neurosciences & Mental Health, Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G 1X8, Canada
[2]Department of Psychology
[3]Institute of Medical Sciences
[4]Department of Physiology
University of Toronto, Toronto, ON M5G 1X8, Canada
[5]Department of Biological Sciences, University of Toronto Scarborough, Toronto, ON M1C 1A4, Canada
[6]Department of Cell and Systems Biology, University of Toronto, Toronto, ON M5G 1X8, Canada
[7]Department of Psychiatry, Erasmus Medical Center, 3000 CA Rotterdam, the Netherlands
[8]Co-first author
*Correspondence: sheena.josselyn@sickkids.ca
http://dx.doi.org/10.1016/j.neuron.2014.07.017

SUMMARY

Memories are thought to be sparsely encoded in neuronal networks, but little is known about why a given neuron is recruited or allocated to a particular memory trace. Previous research shows that in the lateral amygdala (LA), neurons with increased CREB are selectively recruited to a fear memory trace. CREB is a ubiquitous transcription factor implicated in many cellular processes. Which process mediates neuronal memory allocation? One hypothesis is that CREB increases neuronal excitability to bias neuronal recruitment, although this has not been shown experimentally. Here we use several methods to increase neuronal excitability and show this both biases recruitment into the memory trace and enhances memory formation. Moreover, artificial activation of these neurons alone is a sufficient retrieval cue for fear memory expression, showing that these neurons are critical components of the memory trace. These results indicate that neuronal memory allocation is based on relative neuronal excitability immediately before training.

INTRODUCTION

Although different brain regions may specialize in storing different types of memories, computational and observational findings suggest that only a small portion of neurons within a given region is necessary to encode any particular memory (Guzowski et al., 1999; Kanerva, 1988; Reijmers et al., 2007; Rolls and Treves, 1990; Wilson and McNaughton, 1993). For instance, it is generally agreed that the amygdala, in particular the lateral nucleus of the amygdala (LA), is important for auditory fear (Davis, 1992; Duvarci and Pare, 2014; Fanselow and Gale, 2003; Maren, 2003) or threat (LeDoux, 2014) conditioning, in which a tone is paired with an aversive shock. However, while over 70% of pyramidal/principal neurons in the rodent LA respond to both tone and shock presentation (suggesting these neurons are "correctly wired" and therefore eligible to become part of the memory trace), only a small portion (~10%–30%) of these eligible neurons seem to be recruited into any one fear memory trace (Han et al., 2007; Reijmers et al., 2007; Repa et al., 2001; Rumpel et al., 2005). Similarly, there is evidence for a stable sparse fear memory trace in the human amygdala (Bach et al., 2011). Here we examine the mechanisms that help determine which particular neurons are selected or allocated (Zhou et al., 2009) to a sparsely encoded fear memory trace in the mouse LA.

Previously, we and others showed that LA neurons compete against one another for allocation to a fear memory trace, and furthermore that it was possible to bias the outcome of this competition by manipulating CREB (cAMP/Ca^{2+} *r*esponsive *e*lement *b*inding protein) function in individual LA neurons. Neurons with relatively higher CREB function were more likely to be included, whereas neurons with relatively lower CREB function were more likely to be excluded from the memory trace (Han et al., 2007, 2009; Zhou et al., 2009). Importantly, the overall size of the LA memory trace remained stable despite these various CREB manipulations, and indeed did not vary with the strength of the fear memory. This suggests that there is a limit or constraint on the overall size of the LA fear memory trace. Interestingly, decreasing CREB function in a small population of random LA neurons did not disrupt memory formation. This result is likely because the small population of neurons with decreased CREB function was excluded from the memory trace (which was composed of nonmanipulated neurons). In contrast, increasing CREB function in a similar small portion of LA neurons was sufficient to enhance memory formation (Han et al., 2007). Together, these results suggest that neuronal competition is important for neuronal allocation and memory formation, and that neurons with relatively higher CREB are

competitively advantaged (and therefore, more likely to "win" this competition).

CREB is a ubiquitous transcription factor implicated in many diverse cellular processes, including proliferation, survival, apoptosis, differentiation, metabolism, glucose homeostasis, and neuronal excitability (Lonze and Ginty, 2002). For instance, CREB bidirectionally modulates neuronal excitability (increasing CREB function increases the propensity of neurons to fire action potentials, while decreasing CREB function decreases neuronal excitability) (Dong et al., 2006; Viosca et al., 2009; Zhou et al., 2009). Which of these CREB-mediated processes is important for neuronal allocation during memory formation? One plausible mechanism is that neurons with high levels of CREB are preferentially recruited to a memory trace because these neurons are more excitable than their neighbors (a postsynaptic neuron that is more excitable than its neighbor would be "primed" for allocation into a given memory trace). Although an increase in excitability has been proposed as a mechanism mediating neuronal allocation during memory formation (Kim et al., 2013; Zhou et al., 2009), this idea has not been directly tested experimentally. Here we used three different methods to determine whether relatively higher excitability before training confers a competitive advantage for neuronal allocation to a fear memory.

RESULTS

HSV Microinjected into the LA Preferentially Infects Excitatory Pyramidal/Principal Neurons

To manipulate excitability in a small, arbitrarily chosen subpopulation of LA neurons, we used replication-defective herpes simplex viral (HSV) vectors. To phenotype the type of LA cells infected by HSV, we microinjected mice with HSV expressing GFP (to allow for easy visualization of infected cells) into the LA and performed immunohistochemistry for different cell markers (NeuN, neuronal nuclei, as a marker of neurons; αCaMKII, alpha Ca^{2+}/calmodulin-dependent protein kinase II, as a marker of excitatory pyramidal/principal neurons; GFAP, glial fibrillary acidic protein, as a marker of astrocytes; and GAD67, glutamate decarboxylase 67, as a marker of inhibitory neurons). We observed complete overlap between LA cells infected by HSV (GFP^+) and cells expressing a neuronal marker ($NeuN^+$), but no overlap between infected cells and cells expressing GFAP, confirming that HSV is neurotropic (Cole et al., 2012; Fink et al., 1996). Moreover, HSV predominantly (~98%) infected pyramidal/principal excitatory ($\alpha CaMKII^+$) neurons in the LA, with only a very small number (<2%) of infected neurons costaining with the inhibitory neuronal marker GAD67 (Figures 1A and 1B). Therefore, consistent with previous reports, we found that following microinjection into the LA, HSV preferentially infects pyramidal/principal neurons (Cole et al., 2012). Because of this tropism, we used HSV to manipulate excitability in a portion of pyramidal/principal neurons in the LA.

Manipulating Neuronal Excitability Using Voltage-Dependent K^+ Channels

Neuronal excitability is determined by the composition, distribution, and properties of ion channels (e.g., Na^+, K^+, and Ca^{2+}) in the plasma membrane. Increasing CREB function increases neuronal excitability, in part, by decreasing voltage-gated K^+ currents (Dong et al., 2006; Lopez de Armentia et al., 2006; Viosca et al., 2009; Zhou et al., 2009). This, in turn, inhibits the postburst afterhyperpolarization (AHP, a hyperpolarizing current which mediates the "undershoot phase" or refractory period following an action potential). Two members of the voltage-dependent K^+ family of channels, KCNQ2 and related KCNQ3, help mediate the AHP and function as molecular brakes on neuron firing (Delmas and Brown, 2005; Gu et al., 2005). Expression of a dominant-negative KCNQ2 mutant (hQ2-G279S; dnKCNQ2) coassembles with native KCNQ2/3 subunits, disrupts their function, and thereby increases neuronal excitability (Peters et al., 2005; Schroeder et al., 1998; Wuttke et al., 2007). We observed that LA neurons in adult mice endogenously express KCNQ2-containing channels (Figure 1C), suggesting that expression of the dnKCNQ2 construct could be a viable method for increasing excitability in LA neurons. To test this, we first transfected primary hippocampal neurons with the dnKCNQ2 construct. The dnKCNQ2 construct was also observed near the axon initial segment (Figure 1D), consistent with the notion that dnKCNQ2 coassembles with, and blocks the function of, endogenous KCNQ2/3 channels. Therefore, as our first method to increase excitability without directly manipulating CREB function, we used HSV vectors to express dnKCNQ2. To decrease excitability without directly decreasing CREB function, we used HSV to express Kir2.1, an inwardly rectifying K^+ channel, which reduces neuronal input resistance and decreases evoked action potential firing (Dong et al., 2006).

To verify that expression of our excitability constructs indeed manipulated neuronal excitability, we assessed excitability (as measured by firing rate) of cultured hippocampal neurons transfected with GFP, CREB, dnKCNQ2, Kir2.1, and CREB+Kir2.1 constructs. Consistent with previous reports, we found that expression of dnKCNQ2 (Peters et al., 2005) or CREB (Dong et al., 2006; Han et al., 2006; Lopez de Armentia et al., 2006; Viosca et al., 2009; Zhou et al., 2009) increased excitability, while Kir2.1 decreased (Dong et al., 2006) excitability. Moreover, the CREB-induced increase in excitability was blocked by coexpression with Kir2.1 in the same neurons (Figure 2A; one-way ANOVA on firing rate of transfected/not-transfected neurons with between-group factor Transgene [GFP, CREB, dnKCNQ2, Kir2.1, and CREB+Kir2.1], $F_{4,28} = 7.18$, $p < 0.001$, posthoc Newman-Keuls tests showed that dnKCNQ2 and CREB increased excitability over GFP, whereas CREB+Kir2.1 condition was not different from GFP). To confirm that HSV-CREB and HSV-dnKCNQ2 increased excitability in infected LA neurons, we also assessed excitability in ex vivo LA slices following microinjection of HSV expressing GFP, CREB, or dnKCNQ2 into mice. LA neurons overexpressing dnKCNQ2 or CREB showed higher firing rates than LA neurons expressing GFP (Figure 2B; one-way ANOVA conducted on firing rate of infected/not-infected neurons with between-group factor Vector (GFP, CREB, and dnKCNQ2), $F_{2,21} = 4.15$, $p < 0.05$, LSD posthoc tests showed that both neurons with dnKCNQ2 or CREB fired more action potentials than neurons expressing GFP and did not differ from each other). Therefore, although we did not directly determine excitability following our manipulations in vivo, results from cell culture and ex vivo LA neuron slices confirm previous reports that dnKCNQ2 and CREB enhance excitability.