The Sparse Engram of the Lateral Amygdala

by

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I, AUTHORNAME confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Memories are stored in the brain by discrete physiological changes, collectively referred to as an engram, that allow patterns of activity present during learning to be retrieved in the future. The work in this thesis tests the prediction from computational studies and experimental data that, within a given brain region such as the lateral amygdala, only a small proportion of neurons encodes any one memory and that this proportion remains relatively stable across different memories. First, it demonstrates that the size of the LA component of an engram remains constant between memories of different strengths. Then, it implicates parvalbumin-positive interneurons in the mechanisms that constraing the engram to a sparse proportion of neurons. Together, these results thesis provide additional support for the notion that the LA employs a sparse distributed coding scheme to store memories.

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Chapter 1

Introduction

1.1 The engram

1.1.1 A history of ideas

If we accept the philosophical (and now scientifically incontrovertible) position that all activity of the mind is mediated by activity of the brain, then it follows that whenever an event is stored in mind such that it can be recalled at a later time (ie. a memory), there is some concurrent change in the biological substrate of the brain. To understand how such changes or 'engrams' occur, and how they give rise to the gift of memory that shapes our thoughts, beliefs, and behaviours is one of the great goals of neuroscience. Many scientists have devoted their careers to studying this question. Among them, the Canadian neurophysiologist Donald Hebb stands out for his compelling theory of how information could be encoded into cell assemblies (Hebb, 1949). Hebb was not the first to propose the concept of the engram, the German biologist Richard

Semon had done so years earlier (Semon, 1921), but he advanced the field greatly by presenting a theory of memory that drew upon all the neurophysoiological data that had been collected at that time. His thesis, remembered today in the simplified adage "neurons that fire together, wire together", was built on the concept that changes in the strength of connections between neurons lead to the formation of cell assemblies, groups of cells whose coincident activation give rise to certain behaviours or perceptions. Such cell assemblies, formed during the encoding of a memory, increase the chance that the same pattern of activity could be recreated at a later time and trigger memory recall.

Hebb's postulate arrived at a time when many neuroscientists believed that the brain carried out many of its functions holistically, with no particular area of the brain or group of cells being more important than any other. Indeed, Hebb's own doctoral advisor Karl Lashley had come to this conclusion after his systematic attempt to locate the engram in 1917 had failed. Lashley had examined the effects of lesions in various cortical areas on the retention of a maze and inclined plane learning task in rodents (Bruce, 2001). He failed to find any specific region of the cortex that, when lesioned, would erase memory. Instead, he only observed memory impairments after large areas of the cortex were damaged, regardless of location. This lead him to propose that memory was broadly distributed throughout 'equipotential' neural circuits and that finding the engram was impossible. This belief would provide a source for debate between the two thinkers and within the scientific community until Lashley's death.

It was not until technologies for probing and manipulating the activity of the brain became sufficiently precise that Hebb's ideas could be validated. First, more detailed more detailed lesion studies in mammals proved that subcortical

areas of the brain were necessary for memory storage (Mishkin, 1978, Morris, Garrud, Rawlins, & O'Keefe (1982), Weiskrantz (1956)). Additionally, the first evidence for a discrete molecular change underlying a simple form of learning was discovered in the flatworm Aplysia (Kandel, 1978). Most importantly, the discovery of long-term potentiation (LTP) (Bliss & Lomo, 1973) provided the strongest vindication for Hebb's theory that memories are stored by durable changes in neural connectivity. LTP refers to a phenomenon in which high frequency activity across a synapse increases the strength of that synaptic connection (R. C. Malenka & Bear, 2004). The scientists who discovered LTP wisely left open the question of whether the phenomenon, which was identified in cultured hippocampal cells, could be used by living organisms for memory storage. However, LTP, especially when considered alongside the phenomenon of spike-timing-dependent plasticity, wherein coordinated firing between pre- and post-synaptic neurons drives the induction of plasticity (Markram, Lübke, Frotscher, & Sakmann, 1997), corresponds strikingly well with the mechanisms described by Hebb.

1.1.2 Observing the engram

More recently, tools that allow neurons active during the time of memory formation to be identified and tagged for future manipulation have been able to answer questions about memory information beyond the level of the synapse. By analyzing the activity of groups of neurons, it has been possible to demonstrate how neurons activated during memory encoding form neuronal ensembles that underlie memory retrieval. Furthermore, these techniques has important questions at the heart of Lashley and Hebb's debates to be answered: to what

degree are engrams distributed throughout the brain?

Strategies for identifying the engram have often relied on immediate early genes (IEGs) such as Fos, Zif268, or Arc. These genes are reliably induced by neural activity (John F. Guzowski et al., 2005) and return to baseline low levels of expression within minutes. Pairing IEGs with expression of molecular tags such as green-flourescent protein (GFP) or LacZ allows researchers to permanently label populations of cells active during learning. By combining these permanent tagging approaches with methods to detect IEG expression induced by memory retrieval, researchers have been able to test one key hypothesis made by engram theorists: neurons active during the experience of an event are reactivated during the memory of that event. Several studies have now shown that there is a high degree of overlap between the populations of neurons active during memory encoding and recall (Denny et al., 2014, Reijmers, Perkins, Matsuo, & Mayford (2007), Tayler, Tanaka, Reijmers, & Wiltgen (2013)). Although this pattern of reactivation could be traced to specific neurons, those neurons were distributed broadly throughout both cortical and subcortical areas of the brain (Denny et al., 2014, Tayler et al. (2013)). Therefore, the engram of a memory appears to involve a widely distributed collection of neurons distinguished on the basis of their activity during the events that lead to the formation of that memory.

1.1.3 Research showing necessity

Studies that merely observe the engram are unable to demonstrate more than a correlational relationship between brain changes and memory. Thus, many studies have made us of techniques to ablate or silence the neuronal ensembles

tagged during memory formation to demonstrate the necessity of their activation for memory retrieval. In addition to proving that the engram resides in specific cells, these studies have been able to demonstrate the importance of brain regions such as the amygdala and hippocampus in storing and retrieving memories.

By selectively introducing inhibitory or toxic receptors into neurons that undergo engram-related changes, many studies have demonstrated that preventing the reactivation of engram cells in specific networks of the brain during re-exposure to memory cues prevents memory recall. Either through selective genetic ablation (Han et al., 2009), inhibitory optogenetics (Tanaka et al., 2014), or DREADDS (Hsiang et al., 2014), when those neurons that were most active during learning are prevented from firing, memory retrieval is either impaired or eliminated. In comparison to Lashley's lesion experiments, these studies succeeded by specifically targeting subcortical brain regions known to be involved in each particular form of learning under investigation. Furthermore, they were able to target those specific neurons that had presumably undergone changes in synaptic connectivity during encoding. The ability of localized inhibition to interfere with the reactivation of a widely distributed engram demonstrates the importance of certain regions in initiating memory retrieval. For example, the silencing of only a small subset of cells in the CA1 region of the hippocampus (Tanaka et al., 2014) was sufficient to wipe out a memory that involved distributed representations throughout the brain. This results suggest that distinct areas of the brain, especially those involved in processing contextual or sensory input such as the CA1 or the lateral nucleus of the amygdala, may contain critically important neuronal ensembles that 'trigger' a broad pattern of reactivation across the brain when activated by appropriate retrieval cures. This

idea has been supported by the finding that directly reactivating cortical engram neurons is able to bypass the effects of subcortical inactivation (Cowansage et al., 2014).

1.1.4 Research showing sufficiency

In order to demonstrate the sufficiency of engram reactivation for memory retrieval, many researchers have focused their efforts on artificially activation neuronal ensembles formed during learning. In one study, artificially inducing the firing of hippocampal neurons tagged during the formation of a fearful memory was shown to increase the amount of time mice displayed fearful behaviour when placed into a completely novel context (Liu et al. (2012)). Many studies have since demonstrated that artificial activation of engram neurons in both the hippocampus and amygdala can trigger of memory recall in the absence of appropriate retrieval cues (Garner et al., 2012, J. Kim, Kwon, Kim, & Han (2013), Yiu et al. (2014)). Such artificial memory activation has also been shown to trigger reactivation of widely distributed populations of neurons outside the area of stimulation, such as would be observed during natural memory retrieval (Cowansage et al., 2014).

Studies involving artificial reactivation of memories have been able to go further than simply demonstrating the necessity of engram reactivation for memory retrieval. For example, artificially reactivated memories have been shown to have effects on long-term emotional behaviour (Ramirez et al., 2015). Furthermore, intense artificial activation of engram neurons in the amygdala or hippocampus during exposure to new contexts is capable of modifying engrams and producing false memories (Ohkawa et al., 2015, Redondo et al. (2014)).

This is a compelling area of current research that provides the strong evidence that memories are mediated by the activity of discrete ensembles of cells and may one day permit a greater understanding of how the contents of memory are encoded.

1.1.5 Memory allocation

Understanding how neuronal ensembles are formed during memory formation should provide insight into how information is stored in an engram. Memory allocation refers to the processes by which specific cells and synapses are selected to undergo changes that will become part of the engram. The neural mechanisms by which this occurs have not been specifically defined, but both theoretical and experimental work has proposed that memory allocation is driven by a process of sparse coding that provides efficient information storage.

A critical component of the engram supporting an auditory fear memory can be localized in the lateral nucleus of the amygdala (LA). In the LA, many different sensory input streams converge, allowing associations to be formed between stimuli and the expectation of threat or reward (J. E. LeDoux, 2000). Several experiments exploring auditory fear conditioning in the LA have demonstrated that neurons expressing high levels of the transcription factor CREB are more likely to be allocated to memory ensembles (Hsiang et al., 2014, Han et al. (2009), Han et al. (2007)). CREB is a necessary component of the synaptic plasticity pathways that underlie long-term potentiation (A. J. Silva, Kogan, Frankland, & Kida, 1998), but also increases intrinsic neuronal excitability (Y. Zhou et al. (2009)). Thus, excitability has been proposed to play an important role in memory allocation, with more excitable cells more likely to be allocated

to an engram in the LA. Recent studies have confirmed this hypothesis, showing that manipulating cellular excitability directly without altering CREB expression also increases the likelihood that neurons will become part of an engram (Yiu et al. (2014)).

However, artificially increasing the excitability of LA neurons in these studies did not increase the overall size of the of the LA component of the engram (in terms of number of neurons). This suggests that memory allocation is a competitive process determined by relative rather than absolute excitability (Han et al., 2007; Yiu et al., 2014). In addition, a wide range of studies involving different analytical techniques has repeatedly shown that only a small, sparse proportion of principal neurons in the LA become part of any one fear memory trace (An, Hong, & Choi (2012), Ghosh & Chattarji (2015), Gouty-Colomer et al. (2015), Herry et al. (2008), Quirk, Repa, & LeDoux (1995), Reijmers et al. (2007), Rumpel, LeDoux, Zador, & Malinow (2005)] despite the fact that more than 70% of neurons in the LA receive the appropriate sensory innervation (Repa et al. (2001), Romanski, Clugnet, Bordi, & LeDoux (1993)]. Together, these results suggest that the number of neurons involved in representing sensory events remains constrained to a sparse proportion of neurons despite variations in excitability and sensory input. The concept of small, consistently-sized representations corresponds with theories of sparse coding and data collected from other brain regions (Hromádka, DeWeese, & Zador, 2008, Sanes & Donoghue (2000), Weliky, Fiser, Hunt, & Wagner (2003), Wixted et al. (2014)). Sparse distributed coding, in which discrete units of information are encoded across small subsets of neurons in a large network, is thought to provide a structure for high-capacity memory storage that is robust to noise and capable of being implemented in a rapidly-changing biological substrate (Ahmad & Hawkins, 2015,

Druckmann & Chklovskii (2012), Krieg & Triesch (2014)). Memory allocation in the LA may proceed in such a way as to promote sparse coding.

1.2 The amygdala

1.2.1 The Epicenter of conditioned emotional behaviour

Research in psychology and neuroscience has made great progress towards understanding how the brain learns and remembers by focusing its efforts on simple forms of learning such as sensitization and Pavlovian conditioning. These simple models of memory provide the dual advantage of allowing research to be performed in animal models and proceeding through relatively simple neural mechanisms that permit underlying features of neural learning to be identified more rapidly.

Famously, Ivan Pavlov identified a form of simple learning that could be described by a formalized system of stimulus association and conditioning. While studying gastric physiology in dogs, he discovered that they would often began to salivate as soon as he entered the room, even if he had no food to feed them with. This inspired him to perform his classic experiment, in which he paired feeding with the ringing of a bell and recorded the amount of saliva the dogs eventually produced after hearing the bell alone. He observed that repeated pairing of the bell with food delivery created an association between the two stimuli, such that the presentation of the bell was sufficient to produce the behavioural response that would normally only accompany presentation of