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Testing the Network Reset Hypothesis: Noradrenergic Modulation of Hippocampal Representations

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Noradrenergic Modulation of Hippocampal Representations

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DISSERTATION

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Abstract

The locus coeruleus (LC) responds to salience cues, including novelty, and sends a major noradrenergic projection to the hippocampal formation (HF). Novelty-associated LC activation may help to sculpt contextual representations in the HF, but modulatory influence of norepinephrine (NE) over HF representations remains poorly understood. One possible mechanism is that NE provides a "*reset*" signal causing the HF to recruit distinct neural populations, thereby providing a molecular switch to dictate if hippocampal circuits should generate new representations or update existing ones to incorporate novel information. This hypothesis suggests that NE release should cause the HF to recruit a unique population even in the presence of the same stimuli an animal has just experienced, a phenomenon referred to as "*global remapping*". The compartmental expression of immediate early genes (i.e. *arc* & *zif268)* allowed us to test this by mapping the activity history of individual neurons as animals engaged in spatial processing following LC-NE manipulation.

Recruitment of new neurons is part of the memory encoding process involved in separating memories. Tasks involving memory retrieval require reactivation of representations formed during encoding. If those representations "*remapped*" (i.e. a new cellular ensemble was recruited, rather than reactivation of the cells comprising the previously formed representation), this should theoretically result in a retrieval error. Therefore, switching the system back to a state of encoding would prove maladaptive in situations where retrieval is necessary to perform a task, unless new information was at hand. We hypothesize that NE resets the system causing the HF to move from a state of retrieval back to encoding when it is necessary, when novel information needs to be incorporated. This hypothesis suggests the effect of modulating NE on memory critically depends on the stage of training. To further understand how NE modulation of hippocampal circuits affects spatial memory, we tested whether infusions of the β-adrenergic agonist isoproterenol would impair working and reference memory retrieval (i.e., switching the

system back to encoding when it is maladaptive) and in contrast, promote cognitive flexibility thus improving reversal learning (i.e., switching the system back to encoding when it is adaptive).

Dedication

To Alex and Rina

(The Time Travelers)

With Love

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I would like to express my sincerest gratitude to my supervisor, Dr. Diano F. Marrone, for taking a chance on me, believing in me, and for giving me every opportunity to succeed. Without your guidance and mentorship, I would not have had the confidence and motivation to navigate this odyssey. I would also like to thank my committee, Dr. Bruce E. McKay, Dr. Noam Y. Miller, and Dr. Scott Ramsay for endless support and feedback throughout the years of my graduate work. I was privileged to conduct this work under the tutelage of Dr. Carolyn W. Harley, and to her I am eternally grateful. I would also like to thank all the students that helped with this project. To my parents, thank you for constantly encouraging me to pursue my dreams, giving me the space I needed to do that, and knowing that I could, even when I didn't. You are the stars I look up to, for always. I am also grateful to my grandparents, paragons of kindness who gave me a nurturing environment to call home and unwavering love. To Genevieve and Anthony, thank you for keeping me grounded and connected to all that matters. And to Joe, thank you for walking every step of this unpaved road with me.

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AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Arc = activity regulated cytoskeleton-associated protein
BAR = β-adrenergic receptor
BLA = basolateral amygdala
CA (1-4) = Cornu Ammonis
CAMK-IV = calcium and calmodulin dependent kinase IV
catFISH = cellular compartmental analysis of temporal *in situ* hybridization
CFC = contextual fear conditioning
CREB = cAMP response element binding protein
DAPI = 4',6-Diamidino-2-Phenylindole, Dihydrochloride
DBM = dopamine β monooxygenase
DG = dentate gyrus, fascia dentate
EC = entorhinal cortex
GABA = gamma-aminobutyric acid
GPCR = G-protein coupled receptor
HD = head direction
HF = hippocampal formation
HPC = hippocampus
IEG = immediate early gene
LC = locus coeruleus
lEC = lateral entorhinal cortex
LS = lateral septum
LTD = long-term depression
LTP = long-term potentiation
OFC = orbitofrontal cortex
PaS = parasubiculum
PFC = prefrontal cortex
PKA = protein kinase A
PND = post-natal day
PrS = presubiculum
PP = perforant path
PTSD = post-traumatic stress disorder
MAPK = mitogen activated protein kinase
mEC = medial entorhinal cortex
MF = mossy fibers
mRNA = messenger ribonucleic acid
MS = medial septum
NE = norepinephrine
NMDA = N-methyl-D-aspartate
RSC = retrosplenial cortex
RTF = regulatory transcription factor
SC = Schaffer collaterals
SPS = single prolonged stress
SRF = serum response factor
Sub = subiculum
TH = thalamus
VD = ventricular dialator

“The true art of memory is the art of attention”

- Samuel Johnson (1709-1784)

1.0 General Introduction

For centuries, in attempts to gain insight to the processes of higher cognition, we have been trying to understand how the brain makes sense of the world around us, specifically, how we are able to extract meaning from a seemingly chaotic environment. In psychology, Gestalt theory, derived primarily from observations regarding the interaction between an organism and its environment, approaches "*perception*" from a perspective where we create global or holistic representations of the environment, and holds that these unified representations are distinct from the individual sensory inputs from which they are created. The specific way in which the brain unifies these sensory inputs remains an open question. One example is how we view and mentally represent the space that is all around us to form a coherent layout - one that we are a part of. What features of the environment does the human brain attend to (e.g. angles, distance, and position)? How is this integrated with movement? We often take for granted the neural sophistication necessary simply to avoid collisions during navigation. And yet as sophisticated as animal movement is, we sometimes still bump into things. How do these errors arise? The negotiation of space, in other words, movement, is often goal-directed; therefore, memory for places associated with salient information is adaptive (resulting in place learning). The link between spatial learning (and memory) and animal movement has been well established (Collins et al., 2006; Mueller and Fagan, 2008; Nathan et al., 2008; Paul et al., 2009; Fagan et al., 2013; Gautestad et al., 2013). Given the relatively recent technological advancements in navigational tools (e.g., animal tracking), the field of movement ecology has received much attention in recent years.

1.1 The Negotiation of Space: Movement Ecology & Cognitive Maps

Borrowed from mathematics, the term isomorphism is used to describe relatedness or formal correspondence between systems or entities (Gallistel, 1989). Gallistel (1989) described aspects of the environment (e.g. an object in your path), and the neural processes that function to direct an organism's behaviour in such a way that allows the individual to cope with the

environment in an adaptive way (e.g. maintaining or changing a course or trajectory to avoid the object), as functioning isomorphisms. He made the important assertion that creating representations alone is not interesting, what is interesting is how the brain operates in a manner of combinatorial processing to give rise to these spatial representations while also integrating motivational and attentional states, internal and external sensory information, along with movement, to promote adaptive behaviour (Gallistel, 1989; Mizumori et al., 2000).

How is the space around us represented in the brain? Are the mechanisms that support spatial memory innate or does experience play a role? Questions such as these gave rise to the "*geometric module*" (Cheng and Newcombe, 2005) and "*cognitive map*" theory (Tolman, 1948), a notion originating from the ideology that our brains are preconfigured, or at least semi-preconfigured, with navigational hardware (Ainge and Langston, 2012). The idea of an *a priori* representation of space dates back to Immanuel Kant's *Critique of Pure Reason* first published in 1781 (Kant, 1922; Burnham, 2008; Janiak, 2009), where he theorized upon the metaphysical nature of the universe asking himself questions concerning the "*problem of space*" (Burnham, 2008). In his writings, Kant rejected philosopher David Hume's empirical description of space, which he first published in *A Treatise of Human Nature* in 1738 (Hume, 1738). This was partially inspired by a feud regarding frames of reference and velocity between "*absolutist*" Isaac Newton, and **"***relationalist*" Gottfried Wilhelm von Leibniz. Newton's understanding of motion was based on the idea that space is" *absolute*" and that the entities (objects, people etc.) occupying and moving through that space are separate, existing only in relation to absolute space. If these entities suddenly ceased to exist, space would still be space. Contrary to this conceptualization of space, von Leibniz believed that the relationship between entities in fact, defined space (Janiak, 2009). Kant suggested that space was not subject to empirical testing, and surmised that since we cannot perceive the "*absence of space*" we must therefore, have innate "*knowledge of space*" (Burnham, 2008). In other words, space was not a thing to be perceived but instead a way of perceiving, and therefore, did not exist without someone to perceive it (O'Keefe & Nadel, 1978).

Whether or not we possess the faculties to process our surroundings innately as the nativist Kantian perspective would suggest, or whether it is a result of acquired experience as empiricist Jean Piaget would argue (Piaget & Inhelder, 1967), we do seem to be able to form coherent neural representations of the space we traverse. In fact, there is even evidence to suggest that we can form representations of the space we have not yet traversed, but plan to (Maurer & McNaughton, 2007; Dragoi & Tonegawa, 2011, 2013; Azizi et al., 2013; Cona & Ursino, 2015; Ólafsdóttir et al., 2015). Some have referred to these representations as our "*cognitive map*" or contextual code (Nadel et al., 1985; Teyler & DiScenna, 1986; McNaughton et al., 1996). Understanding this code, and what is specifically being encoded within these contextual representations (e.g., space and time) has been the goal of many scientists in the last century. And while significant progress has been made, there remains much to decipher.

Based on early work with laboratory rats (*Rattus norvegicus*) in mazes, it became clear that rats could complete certain spatial tasks, not by remembering a series of turns or responses, but by somehow orienting themselves with respect to landmarks and cues. This led behaviourist Edward Tolman to the conceptualization of the "*cognitive map*", a term he coined in his 1948 publication "*Cognitive Maps in Rats and Men*" (Tolman, 1948). Tolman viewed the cognitive map as a mental representation of the environment that functioned somewhat like a paper map indicating which routes or paths to take (Bennett, 1996). This map was primarily based on a Euclidean metric and the concept of novel shortcutting - flexible rather than inflexible lines of movement to goal locations (O'Keefe & Nadel, 1978; Gould, 1986). Since this initial description by Tolman, John O'Keefe in the early 1970's provided a clear neural substrate for this theory with the discovery of place cells in the hippocampal formation (HF). Place cells are highly specialized, spatially tuned neurons that encode an animal's location in space (O'Keefe, 1976). Shortly after this breakthrough discovery, John O'Keefe & Lynn Nadel published a comprehensive book titled "*The Hippocampus as a Cognitive Map*" (1978) which they dedicated (in part) to Tolman for "*first dreaming of cognitive maps in rats and men*" (O'Keefe & Nadel, 1978). Fittingly, they

referred to the concept of a cognitive map as "*terra incognita*", a phrase used by cartographers to mean uncharted territory or "*un-mapped land*". In this work, they described a neo-Kantian view of place representation and presented specific anatomical data to support the theory that the HF is critical to spatial cognition and contextual processing.

Tolman saw the cognitive map as a global representation of the environment. Notably, his definition included an element of flexibility in the animal's ability to navigate from unfamiliar locations (novel shortcutting). From this perspective, one can consider the cognitive map a *relational representation*. Animals that have sustained damage to the HF are impaired in this type of flexible and adaptive navigation, which is consistent with the notion that the cognitive map involves creating global representations of the environment, and with the theory that the HF is the neural substrate for this type of relational representation. However, other theories also emerged. Gallistel (Gallistel, 1989, 1993) defined the cognitive map simply as a record of geometric relations, in other words, a utility-based, vector archive or repository of routes; a definition that had been heavily criticized for not delineating which specific geometric relations would be encoded. Cartwright and Collett's "*snapshot model*" of the cognitive map described an image-matching process (Gould, 1986; Cartwright & Collett, 1987) whereby an animal takes a mental snapshot (or several) of its panoramic view and stores it in memory. Later, the animal compares its current view with the stored view to help orient. Image matching does not require a relational representation, as relational representations are defined by their flexibility (Eichenbaum et al., 1990). Therefore, this type of strategy can be successfully completed without an intact HF. It is unlikely that the sophisticated spatial abilities observed in so many animals are subserved by such a simple model.

Unfortunately, the term cognitive map has been used to describe many different concepts over the years (Bennett, 1996) and according to O'Keefe & Nadel (1978), is too vague a term to be translated into a neural model. Nonetheless, there is evidence that animals possess some type of mapping system to account for their complex behaviour in space. And although the

organization of this system is still unclear, it is widely accepted that a neural mapping system exists, at least in the mammalian brain, and possibly in other species of animals. Despite the advances that have been made in this field in the last century or so, we are still wondering if the integration of spatial information used when navigating is supported by a neural architecture that is modularly connected within a system that is distributed (Gallistel, 1989; Bennett, 1996; Bingman & Cheng, 2005; Cruse & Wehner, 2011) or whether spatial information is sent to a global "*coordinator*" (Moser et al., 2008). Undoubtedly, spatial orientation in mammals relies on many cortical structures that interact with each other in an integrated framework. For instance, the retrosplenial cortex (RSC) encodes sensory input from allocentric frames of reference (Andersen, 1997; Colby & Goldberg, 1999; Parron & Save, 2004), the perirhinal cortex (PC) is involved in cue and object recognition (Mumby and Pinel, 1994), and the thalamus (TH) codes for visual and vestibular cues (Shine et al., 2016). However, current models support the theory that the brain is not organized in a completely modular fashion as early phrenology charts would suggest, but rather, memory involves distributed, but synchronous activation of different regions working together, a level of analysis referred to as *systems neuroscience*. It is these dynamic interactions which support a flexible and adaptive navigational system, although, there is much we still do not understand in terms of the organizational principles of functional and structural connectivity across brain structures involved in mnemonic processing. Scientists use animal models to elucidate the patterns of neural processing and circuitry involved in spatial memory in order to develop sophisticated computational models and algorithms characterizing these cognitive processes.

Although synchronous communication across brain regions is necessary, converging evidence from animals and humans has demonstrated that the HF is centrally involved in the formation of episodic, spatiotemporal, context-rich memory (Holland and Bouton, 1999; Burgess et al., 2002). Episodic memory refers to our ability to recollect the specific events in our lives in a spatiotemporal context (Eichenbaum et al., 2012). It is the difference between *remembering* and

simply *knowing* (Tulving, 1972). This type of memory has also been referred to as *autobiographical* memory. Not only is the HF critical to the formation of episodic memories but it is hypothesized to act as a *detector* of prediction errors. That is, the HF stores contextual representations, and defines the expectations for these contexts. For example, you have a representation stored of the coffee shop down the street. You go to get coffee one morning, and you find the door is locked. The coffee shop is usually open at this time of day. When the current experience doesn't match the expected, this results in a context prediction error (Mizumori, 2013). An example of this in a research setting involves fear conditioning. Animals that have been fear-conditioned in a particular context form a representation of that context. Upon re-exposure to the context the following day, these animals "predict" that they will again receive a shock in that environment, but during the extinction session this does not occur. In the first context presentation, given the association between the shock and the context, they learn to fear the context. During the second context presentation, despite that the context itself is the same, the absence of the shock suggests that the context is indeed safe. Identification of mismatches provides a signal that a new representation is needed, or that the old representation needs updating. This process allows for the distinction of memories into separate, meaningful epochs (Mizumori, 2013) and most importantly, allows for learning to occur. And indeed, representations do change as a result of different stages of learning (Wang et al., 2012b).

The complexity of this system is extended when you take into consideration that contextual representations may be externally or internally driven (Pastalkova et al., 2008). Furthermore, the HF contains not only contextually-mediated but also temporally-mediated cells (MacDonald et al., 2011) and the fields of these cells may be biased by sensory information (Ranck, 1973; O'Keefe., 1976; O'Keefe & Conway, 1978; Olton et al., 1978; Muller & Kubie, 1987; Gothard et al., 1996; O'Keefe & Burgess, 1996; Wiener, 1996; McEchron & Disterhoft, 1999; Save et al., 2000) task demands (Markus et al., 1995; Wood et al., 2000; Smith & Mizumori, 2006; Satvat et al., 2011), or motivational states (Breese et al., 1989; Kobayashi et al.,

1997; Fyhn et al., 2002; Hölscher et al., 2003; Tabuchi et al., 2003; Kennedy & Shapiro, 2009). In other words, representations can and do change. This process, commonly referred to as remapping, is likely involved in the updating of memories, and in new learning, however, the mechanism by which remapping occurs is not currently known.

The following chapters outline a novel hypothesis involving the noradrenergic pathway originating in a brain stem structure (locus coeruleus) projecting to the HF, and how this system may be able to sculpt contextual HF representations possibly providing insight to mechanisms of remapping. The subsequent chapters provide a brief description of the material basis of contextual representations as well as the functional properties of the cells and subfields within the HF. This is followed by an exploration of how the mutability of memory is conducive to learning and evolutionarily advantageous, but more specifically, how neuromodulators such as norepinephrine (NE) play a role in altering mnemonic processes at a cellular and behavioural level.

1.2 Contextual Encoding: Recruitment of Neuronal Ensembles as Representations

The last 50 years have provided us with compelling evidence that the HF is essential in the processing of spatial and contextual information (Hirsh, 1974; O'Keefe, 1976; Phillips & LeDoux, 1992; Kim & Fanselow, 1992; Holland & Bouton, 1999; Fanselow, 2000; Burgess et al., 2001, 2002; Guzowski & Worley, 2001; Schmolck et al., 2002; Anderson et al., 2003; Rudy et al., 2004; Vazdarjanova & Guzowski, 2004; Smith & Mizumori, 2006; Acheson et al., 2012; Maren et al., 2013; Nees & Pohlack, 2014; Sadeh et al., 2014; Smith & Bulkin, 2014). Lesions to the HF produce severe spatial memory impairments in rats (Morris et al., 1982; Sutherland et al., 1982; Kesner et al., 1989), birds (Colombo et al., 1997; Fremouw et al., 1997), and primates (Murray et al., 1998) including humans (Bohbot et al., 1998). These impairments observed after damage to the HF are not limited to the spatial domain, as they also produce deficits in episodic memory (Sun et al., 2005; Didic et al., 2011). This is obvious in patients with Alzheimer's disease which is characterized by progressive degeneration of the HF. In fact, Dr. Alois Alzheimer in his initial description of the long-term study of his patient Auguste D and her "*peculiar disease*"

(Hippius and Neundörfer, 2003) mentioned the existence of abnormalities in the cerebral cortex were revealed in the autopsy.

More conclusive evidence linking the HF to spatial, contextual, and episodic memory came from studies involving a patient who, in 1953, suffered from severe and intractable epileptic seizures. Most first-year psychology textbooks refer to him as patient H.M., but after his recent passing in 2008, we now know him as Henry Gustav Molaison. To treat his condition, neurosurgeon Dr. William Beecher Scoville performed a bilateral temporal lobotomy on Mr. Molaison. Following the removal of his HF and adjacent structures, H.M. suffered severe anterograde amnesia; essentially the procedure rendered him unable to acquire new memories (Scoville & Milner, 1957). This demonstrated quite convincingly, the involvement of the HF in forming episodic memories (procedural memories for things like how to write or how to walk remained intact) and spatial memories as he was impaired on many spatial tasks (Corkin, 2002). The duality of function with respect to this brain area in processing both spatial and episodic memories, is not surprising since episodic memory is spatiotemporal by definition. Since this discovery, Brenda Milner a Canadian professor of neurology and neurosurgery at McGill University, and Suzanne Corkin, a former student of hers, who conducted most of her research at MIT, continued to study H.M. for decades.

Contextual memory is less easily defined than spatial or episodic memory. Contextual memory includes, but is not limited to the inclusion of spatial information and also relies heavily on the HF (Holland and Bouton, 1999). It refers to the abstract components of experience providing meaning, placing events in time, encompassing perceptions, emotions, socially relevant information, and learned contingencies (Maren, Stephen, Phan, & Liberzon, 2013). Therefore, contextual information extends beyond spatial information to include other dimensions such as the physiological, motivational, social, and cognitive states of the organism. In the learning literature, contexts are distinguished from cues and typically refer to the set of circumstances surrounding an event or the physical location that an event takes place in. This suggests that

contexts can be considered separate from the elements they encompass (Maren et al., 2013) and are yet still connected to them. Nadel and Wilner (1980) describe context as being paradoxical for this reason (Anderson et al., 2003). No wonder Kant described it as the "*problem of space*".

For contextual learning to occur, a representation of the context must be formed in the HF. A widespread hypothesis central to all neurobiological investigations of memory is the hypothesis that memory formation should result in a structural, observable memory trace (Hebb, 1949). Although this idea is often credited to Donald Hebb's postulate of memory residing in specific "*cell assemblies*", this notion was inspired by Rafael Lorente de Nó's (Lorente de Nó, 1933) reverberating "*neural loops*", and is consistent with Richard Wolfgang Semon's idea of the engram as the "*enduring though primarily latent modification*" of the brain by experience (Semon, 1921; Schacter et al., 1982). Semon (1921) coined the term "*engram*" (p. 12) to refer to these putative "*memory traces*", or neuronal ensembles (contextual representations), that we believe embody our experiences quite literally. These ensembles can be tracked (Guzowski et al., 1999), tagged, and even reactivated them using optogenetics (Liu et al., 2013; Denny et al., 2014) in attempts to recapitulate experiences. Scientists have looked at the manipulation of two separate engrams simultaneously (Yokose et al., 2017) and have even been able to investigate how two distinct engrams formed at different times interact with each other (Won & Silva, 2008; Rogerson et al., 2014; Rashid et al., 2016). Today, we think of memory traces or engrams as the biochemical changes occurring following experience, set in a sparse population of neurons. These changes, which persist, involve the transcription of genes and the formation of proteins. Moreover, these populations of neurons are reactivated when the memory is recalled (Guzowski et al., 1999; Reijmers et al., 2007; Han et al., 2009; Silva et al., 2009; Garner et al., 2012; Deng et al., 2013; Liu et al., 2013; Ramirez et al., 2013; Taylor et al., 2013; Rogerson et al., 2014). For several decades scientists have been trying to study these traces debating about where they may be stored (Mayes and Roberts, 2001). The most quintessential example of this being the experiments Karl Lashley conducted where he progressively removed more and more of the

cortex in famously failed attempts to locate the engram. From this, Lashley argued against the functional specialization and modularity of the brain, and concluded that memory could not be localized to one structure, but rather it must be distributed throughout the cortex (Lashley, 1950). Lashley was correct to conclude that memory is widely distributed, but this does not mean that copies of our memories are redundantly stored all over the brain. Lashley tried to locate a complex memory (maze running) that likely had different cues which could lead to the memory trace. If he eliminated the area involved in vision, the animal could use kinesthetic cues; if he removed these cues, the animal could rely on olfaction etc. Lashley wrote:

> *"I sometimes feel, in reviewing the evidence on localization of the memory trace, that the necessary conclusion is that learning just is not possible. It is difficult to conceive of a mechanism which can satisfy the conditions set for it. Nevertheless, in spite of such evidence against it, learning does sometimes occur".*
>
> (Lashley, 1950, pp.477-478)

What Lashley could not envision was that the brain engages in a high degree of region specificity, yet there are multiple pathways to get to the same place. And despite his failed attempts, we now know that the HF is a core brain structure supporting memory (Eichenbaum et al., 2012). But prior to any hard evidence that the HF contained memory traces, David Marr (1971) proposed in his basic model of simple memory, that pyramidal cells within the HF could be regarded as populations of cells in which simple representations of various input events are formed. He postulated that the HF acts a temporary storage space for sensory experiences which are encoded by specific patterns and that these patterns are retrieved when confronted with a cue. He also believed that this information would eventually be transferred to the neocortex (Marr, 1971). Given the lack of evidence at the time, and the astounding accuracy of his predictions, his theory was extremely prescient. Marr was also very much interested in computational modelling; thus, it is befitting that computational neuroscience models developed some 20 years later, would also predict that representations of the surrounding environment were formed in the HF (Gluck & Myers, 1993; Treves & Rolls, 1994). We are now aware that the HF does indeed form contextual representations of the surrounding environment (Hirsh, 1974; Fanselow, 2000; Guzowski et al.,

2001; Rudy et al., 2004; Vazdarjanova and Guzowski, 2004). In fact, contexts can be robustly encoded very rapidly (<30s) (Fanselow, 1986; Wiltgen et al., 2001). Moreover, lesions of the HF impair contextual learning (Sutherland et al., 1982; Winocur & Gilbert, 1984; Selden et al., 1991; Kim & Fanselow, 1992; Phillips & LeDoux, 1992; Young et al., 1994; Chen et al., 1996; Gerlai, 1998).

The concept of a memory trace can seem very abstract. Our brief acquaintance with the material basis of memory (Tonegawa et al., 2015b) as engram cells has not included a specification of what information is encoded. For instance, an animal is placed in an environment and undergoes a specific experience (e.g. another mouse is placed in the box) and as a result, a distinct set of neurons is recruited in the DG, the activity in which is considered to be *a component* of the distributed memory trace for that experience, encoding what we believe, is at least the contextual component of that episodic memory (Gerrard et al., 2001; Fyhn et al., 2007; Nalloor et al., 2012; McKenzie et al., 2013; Orsini et al., 2013; Takahashi, 2013; Cai et al., 2016; Kelemen & Fenton, 2016). But what does that mean, the contextual component? Different aspects of a memory may be encoded in different sub-regions. In the dorsal HF, this includes spatial information given that neurons which make up these ensembles are spatially responsive (discussed in more detail below) e.g. place cells, and traversing the environment, sampling its spatial properties, activates these cells (Chawla et al., 2005; Ramirez-Amaya et al., 2005; Vazdarjanova et al., 2006). Information related to the valence of a memory (positive or negative) may be encoded in the basolateral amygdala (BLA), social elements may be encoded in the CA2, and emotional aspects of a memory in the ventral HF. Therefore, the full context of an experience may only be recaptured when all of these populations are reactivated in concert. This can potentially increase neural coding space significantly (Holtmaat and Caroni, 2016). It is also believed that memories become less dependent on the HF as they become more remote and more reliant on engram populations in the prefrontal cortex (PFC). Engram research is in its infancy and therefore the answers to these questions are still being teased out and investigated.

Contextual information present at the time of memory encoding may be different than contextual information present at the time of retrieval. Endel Tulving (1972) described remembering as "*the joint product of information stored in the past, and information present in the immediate cognitive environment of the rememberer*" (p. 352). This view emphasizes how memory retrieval can be affected by factors present at the time of retrieval e.g. mood, hormones, vigilance, stress etc., and that retrieval efficacy depends not only on the integrity of the memory trace alone, but also on these relevant contextual circumstances (Sara, 1985; Rimmele et al., 2016). Contextual cues play an important role in triggering or facilitating memory retrieval processes (Sara and Devauges, 1989). This is what Lashley failed to fully appreciate. In addition to the term engram, Richard Semon (1921) also coined the term "*ecphory*" to describe the automatic process that occurs during memory retrieval between contextual elements and the memory traces they reactivate *". . . the influences which awaken the mnemic trace or engram out of its latent state into one of manifested activity...*" (p.12). Presentation of contextual cues before a test of memory retention can help to mitigate experimentally induced amnesia (Sara, 1974), natural time-dependent forgetting (Sara et al., 1980; Sara & Deweer, 1982) and memory deficits in rats with lesions to the HF (Winocur & Kinsbourne, 1978). Contextual cues can also elicit changes in an individual through an influence on neurophysiological and attentional states and can even be conditioned to elicit biological changes that match the internal state of the individual during memory acquisition. The arousal experienced at the time of learning is essentially recapitulated in the brain during retrieval (Sterpenich et al., 2006) and can influence the retrieval process (Rimmele et al., 2016). One of Ivan Pavlov's students, Pyotyr S. Kupalov first noticed this while studying conditioned behaviour in dogs. He noticed that the strength of the dog's conditioned response was greater when the dog was tested under the same conditions of illumination and noise that occurred during training (Giurgea, 1989) and posited that the context was able to affect his cortical tone such that this tone facilitated memory retrieval. He called this the *truncated conditioned reflex* (Sara, 1985). Other studies have replicated this finding in various

circumstances to demonstrate that contextual information not only exerts a powerful influence over memory encoding and retrieval, but also over physiological responses that directly influence memory.

1.3 Understanding the Spatial Code: The Hippocampal Formation & Behavioural Correlates of Individual Neurons

In 1894, Santiago Ramón y Cajal (Jones, 1994) first described the relay of unidirectional synaptic transmission in the HF commonly referred to as the trisynaptic loop (Andersen, 1975). The entorhinal cortex (EC) is considered the main gateway or interface between the HF and the neocortex. It is typically subdivided into medial and lateral domains, and is organized into distinct layers. The EC is the major source of cortical input to this circuit sending efferent signals to the dentate gyrus (fascia dentata, DG) via granule cell fibers known collectively as the perforant path (PP). The hippocampus proper, known as the Cornu Ammonis (CA), is divided into different subregions (CA1-4). The DG synapses on CA3 (regio inferior) pyramidal cells via mossy cell fibers (MF) and pyramidal cells in the CA3 project to the CA1 (regio superior) via Schaffer collaterals (SC). Finally, CA1 pyramidal cells send information to the subliculum, which then loops back to the EC (Marr, 1971; Andersen, 1975).

The mammalian HF is characterized by the presence of spatially responsive neurons, *specifically head direction cells, place cells, grid cells,* and *boundary vector cells* (Barry et al., 2007; Moser et al., 2008). These cells are highly specialized; for instance, place cells fire when an animal visits a particular location in an environment (O'Keefe and Dostrovsky, 1971). The activity of these cells encodes the animal's location in space, each cell with a different place field, with activity in local cell populations covering the rat's entire environment (O'Keefe, 1976). These cells are organized in a manner where adjacent place cells do not necessarily give rise to adjacent place fields. In fact, neurons adjacent to a place cell are more likely to be silent within a given environment (Thompson and Best, 1989). This lack of topographical organization

demonstrates that 1) not all HF cells are place cells, 2) inputs are not topographically organized and 3) that the lack of firing may be just as important as the presence of firing. This orchestration of activity and no-activity has been hypothesized to contribute to the way in which hippocampal circuits synchronize frequencies (oscillations such as gamma and theta) (Thompson & Best, 1989; Mizumori, 2013) and may be related to plasticity within the HF. Interestingly, place fields exhibit plasticity in that they can change when alterations in the environment occur. For instance, in one environment, a single place cell may become active when the animal visits the left corner of the room; in a different environment that same cell does not respond at all when the animal visits the left corner, and in a third environment, that cell becomes active in the right corner. This phenomenon in which place cells can alter their firing patterns in response to environmental changes was discovered by Muller and Kubie (1987) and is referred to as "*remapping*". It is specifically this property that emphasizes the multi-representational nature of the HF (Colgin et al., 2008). Place cells have been shown in numerous studies to remap in response to novel environmental stimuli, and although place cells can possess more than one place field within the same environment (Maurer et al., 2006), in some instances, they can also remap in the same environment as a result of experience (Navratilova et al., 2012). Most of the electrophysiology studies conducted to date have identified place cells in the HF but there is some evidence for place cells in other brain regions such as the medial entorhinal cortex (mEC) (Quirk et al., 1992; Hargreaves et al., 2005; Savelli et al., 2008), the lateral septum (LS) (Nishijo et al., 1997; Zhou et al., 1999; Leutgeb & Mizumori, 2002), the TH (Jankowski et al., 2015), the RSC (Tayler and Wiltgen, 2013; Cowansage et al., 2014) and the PFC (Zelikowsky et al., 2014). Cells in these regions have been identified as having "*place-like"* properties but typically have a much lower spatial resolution (Grieves & Jeffery, 2017). Several studies have also suggested that other types of neurons such as granule cells in the DG, which also encode spatial information, may function similarly to place cells (Tonegawa et al., 2015a) (figure 1). The formation of contextual representations is hypothetically driven by place cell activation, at least in rodents (Chawla et al.,

2005; Ramirez-Amaya et al., 2005; Vazdarjanova et al., 2006; Rowland et al., 2011), which is coupled to the initiation of second messenger systems and gene transcription leading to protein synthesis (Miyashita et al., 2008).

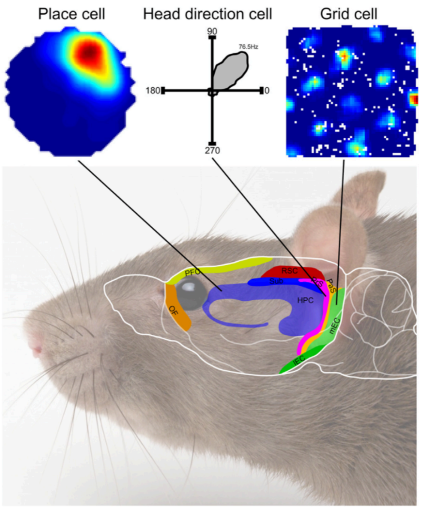


Figure 1. Example of spatially responsive neurons and representation of their anatomical distribution in the rat brain. Top left: firing rate heat map of a place cell, which was recorded as a rat explored a circular arena. Top middle: hypothetical head direction cell firing rate polar plot in which the cell fires at a high rate when facing north east. Top right: example firing rate map of a grid cell where multiple firing fields can be seen that form a tessellating hexagonal grid covering the environment. Bottom: anatomical distribution these cell types, black lines highlight the region where each cell was discovered (however, they are found in other areas as well). Brain region abbreviations: HPC = hippocampus; Sub = subiculum, RSC = retrosplenial cortex; PrS = presubiculum; PaS = parasubiculum; mEC = medial entorhinal cortex; lEC = lateral entorhinal cortex; PFC = prefrontal cortex; OFC = orbitofrontal cortex. Reprinted from Behavioural Processes 135, Grieves RM & Jeffery KJ, The representation of space in the brain 113-131., Copyright (2017), reprinted with permission from Elsevier.

Shortly after the discovery of place cells, James B. Ranck, Jr. determined that within the presubiculum there were cells that fired in response to the specific direction an animal's head was pointed; cells had different preferences for different orientations (Taube et al., 1990) and he called these head-direction (HD) cells. HD cells have since been localized to other regions of the brain where the presubiculum projects to (e.g. TH, RSC) (Mizumori & Williams, 1993; Chen et al., 1994; Taube, 1995; Sherrill et al., 2013; Shine et al., 2016). The firing rates of both place, and HD cells are often coupled, and to some degree controlled by an interaction between external landmarks and idiothetic cues (Yoganarasimha & Knierim, 2005), although further research is needed to characterize this interaction.

In layers II and III of the mEC there are cells that have multiple firing fields arranged in a tessellated, grid-like array that covers the surface of the environment, with firing occurring maximally for a cell when the animal is at any vertex of a grid of equilateral triangles (Hafting et al., 2005; Fyhn et al., 2007; Moser et al., 2008), hence, Edvard & May-Britt Moser named these cells grid cells when they discovered them in 2005. The grid fields tend to increase in size from the dorsal to ventral regions of the mEC (Hafting et al., 2005). In darkness, and when landmarks are removed, the cells maintain their fields suggesting that they may play a prominent role in path integration, especially given that their fields are arranged in a way that could theoretically allow for vector algebra (Hafting et al., 2005; but also see Barry et al., 2007). Path integration is a method of navigating, also called dead reckoning, which involves summing vectors of distance and direction as one travels (McNaughton et al., 2006).

Several studies have shown that place cells can *predict* an animal's trajectory or goal location, demonstrating anticipatory properties of firing (Johnson & Redish, 2007; Schmidt & Redish, 2013), therefore, it is biologically plausible that grid fields contribute to the generation of place fields (Moser et al., 2008). Several theoretical models have emerged recently inspired at least in part by the recent characterization of the bat HF, hypothesizing the existence of a grid-like representation of space that includes the vertical as well as the horizontal plane since organisms travel in a three-dimensional environment (Jeffery et al., 2013). Three-dimensional representation of space by cells in the HF has been examined in flying bats (Yartsev & Ulanovsky, 2013; Sarel et al., 2017). The place fields of place cells in the HF of free-flying Egyptian fruit bats are spherical volumes (Yartsev & Ulanovsky, 2013), however, others theorize that these planes are processed separately i.e. bi-coded system (Phillips & Ogeil, 2013) while some predict that it depends on the direction the animal is moving (Jeffery et al., 2013). In deeper layers of the EC, conjunctive place and grid cells have also been found (Moser et al., 2008). In addition, a fourth type of neuron exhibiting a spatial profile has been discovered in the subiculum - boundary-vector cells, also called border cells (Hartley et al., 2000; Lever et al., 2009). These

cells are sensitive to geometric properties of the environment, specifically boundaries, and have also been proposed as inputs to place cells (Lever et al., 2009).

Learning more about the way in which these neurons communicate with each other would give us a better understanding of how mammals represent space. It is believed that the collective firing of such cells, specifically place cells, within a given environment comprises the spatial or contextual code for that environment (Pevzner et al., 2012). And we have come to call neuronal ensembles comprised of spatially-, temporally-, and contextually-mediated cells – engram cells. In fact, current models provide evidence for the possibility of an associated temporal code (Moser et al., 2008). Certain cells in the CA1 and CA3 have been shown to fire at specific "*times*" during a task and have been dubbed time-cells (MacDonald et al., 2011; Eichenbaum, 2014, 2017a, 2017b; Salz et al., 2016). Cells in the HF exhibit a strong background low frequency (4-8Hz) theta oscillation typically when an animal is engaged in active locomotion (e.g. exploration) or during REM sleep (Maurer & McNaughton, 2007), a rhythm that is entrained by the medial septum (MS) (Mizumori et al., 1989) and can be measured in the extra-cellular space (local field potential). To relate the timing of spikes to the theta frequency, each spike is assigned a phase (0-360 degrees, based on when it fires relative to the theta oscillation; 0 degrees corresponds to the trough of the oscillation). For a place cell that is anchored to a landmark, the phase can theoretically tell how far the landmark is, and whether the animal is entering or exiting that place field (Maurer et al., 2006; Moser et al., 2008). As the rat moves through a place field, towards or away from the landmark, the phase at which it fires changes from theta cycle to theta cycle and therefore spatial information is encoded in the timing of spikes with respect to the theta rhythm (negative correlation of spike phase to animal position), a phenomenon called phase precession (Mehta et al., 2002). Neural activity across brain regions is thought to be synchronized by gamma (~ 40Hz) oscillations (Colgin & Moser, 2010), which occur in a phasic manner (grouped bursts) while theta oscillations occur in a tonic (ungrouped, stochastic) pattern (Bragin et al., 1995). HD cells may exert control over grid cells to aid in path integration. There is

preliminary evidence to show that gamma oscillations can synchronize activity in different cell populations (Colgin & Moser, 2010) and may be linking HD to place or grid cell activity in an associative "*Hebbian*" manner (Hebb, 1949). In summary, contextual information encoded as representations in the dorsal HF, is multi-sensory, externally- and internally-driven, spatiotemporal information (Burgess et al., 2002).

- 1893,1911, Santiago Ramon y Cajal – anatomical description of HF circuitry, focus on synaptic flow of information
- 1921, Richard Wolfgang Semon – coined the word *engram*: neuronal HF ensembles considered to contain contextual representations of experience
- 1934, Lorente de Nó - HF subdivisions / nomenclature, concept of *reverberating neural loops* contributes to understanding of auto-associative CA3 network
- 1948, Edward C. Tolman – *cognitive map theory*: idea that we form mental representations of space, based on Euclidean metric, involves "novel-shortcutting"
- 1949, Donald O. Hebb – concept of *neuronal assemblies*: strengthening of synaptic connections, plasticity and consolidation
- 1957, Wlliam B. Scoville & Brenda Milner – *epileptic patient H.M.'s* medial temporal lobe removed: resulted in global amnesia, HF is critical to episodic memory encoding
- 1971, John O'Keefe & Jonathan Dostrovsky – *place cells* discovered in HF
- 1971, David C. Marr – *computational model*: HF acts as a temporary storage space for experiences encoded via specific patterns also important for retrieval
- 1972, Endel Tulving – coined the term *episodic memory*: to distinguish between knowing and remembering
- 1973, Timothy V.P. Bliss & Terje Lømo – discovery of *long-term potentiation* in the HF: evidence for *Hebbian* plasticity
- 1982, Richard G.M. Morris - invention of *Morris water maze task*: showed spatial memory impairments in rodents following HF lesions
- 1984, 1990, James B. Ranck Jr. – discovery of *head direction cells* in the subiculum
- 1986, Richard G.M. Morris – *blocked LTP* using NMDA antagonist AP5
- 1987, Larry R. Squire – characterization different types of memory (e.g. declarative, implicit)
- 1987, Robert U. Muller & John L. Kubie – concept of *remapping:* HF stores multiple representations / place fields can be altered in the presence of new stimuli
- 1998, Jian-Zhong Xiang & Malcolm W. Brown – characterization of neuronal responses (anterior temporal lobe) to novel vs. familiar stimuli in monkeys
- 1998, Peter S. Eriksson – discovery of *neurogenesis* in the HF
- 1999, John F. Guzowski, Bruce L. McNaughton, Carol A. Barnes, & Paul F. Worley – *environment-specific* immediate early gene expression in HF neuronal ensembles
- 2000, Emma R. Wood, Paul A. Dudchenko, R. Jonathan Robitsek, & Howard Eichenbaum – showed that HF cells encode more than just spatial information (e.g. task demands)
- 2001, Richard G.M. Morris - Puts the concept of episodic memory inside the realm of experimental study in *non-human species*
- 2004, 2005, Marianne Fyhn & Torkel Hafting (Edward I Moser & May-Britt Moser Lab) – discovery of *grid cells*
- 2004, Joseph R. Manns, Marc W. Howard, & Howard Eichenbaum – showed that gradual changes in the pattern of HF activity served as a *temporal context*
- 2006, George Dragoi, György Buzsaki – concept that *brain rhythms e.g. theta* → temporally bind activity between anatomically distributed groups of *Hebb's cell assemblies*
- 2007, Jill K. Leutgeb, Stefan Leutgeb, Edward I Moser & May-Britt Moser – *pattern separation* in the dentate gyrus with minimal changes in the shape of the environment
- 2008, Eva Pastalkova (György Buzáki Lab) – cell assemblies can be *internally-generated* as well as externally-driven, and can be used to predict *future* behavioural responses
- 2009, Colin Lever (Neil Burgess Lab) – discovery of *boundary vector cells* in the subiculum (presence of these cells still being debated)
- 2011, Christopher J. MacDonald, Kyle Q. LePage, Uri T. Eden, & Howard Eichenbaum – discovery of *time cells:* HF neuronal ensembles are *temporally-mediated* as they are spatially / contextually mediated
- 2012, Xu Liu, Steve Ramirez & Susumu Tonegawa – identification and manipulation of memory engrams in the HF
- 2017, Takashi Kitamura (Susumu Tonegawa Lab) – evidence supporting *systems consolidation theory:* episodic memories are gradually consolidated in neocortical networks for permanent storage relying less on the HF with the passage of time

Figure 2. Some of the significant milestones in the last 125 years that have contributed to a greater understanding of how the HF process contextual information.

1.4 How Hippocampal Subfields Contribute to the Maintenance of Multiple Representations

Pattern separation was first described as a computational process associated with the DG, where the output firing patterns are dissimilar, despite that input patterns (e.g. sensory input) may be highly similar (Deng et al., 2010). In other words, the DG is thought to act as a mediator of stimulus representations, which can perform stimulus discrimination while reducing interference from redundant stimuli by enhancing dissimilarity between representations (Leutgeb

et al., 2007; Bakker et al., 2008; Berron et al., 2016; Kesner et al., 2016). This is achieved using a sparse coding scheme. The DG receives incoming spatial information via excitatory inputs from the EC and must process this information before sending excitatory outputs to the CA3 but this structure is also under a high level of inhibitory control due to the presence of inhibitory (GABA-ergic) interneurons (e.g. basket cells) via feedback and feed-forward inhibition (Ribak, 1992; Jonas & Lisman, 2014). Moreover, the DG contains only a small fraction of neurons displaying activity at any given time. Therefore, low levels of basal activity combined with a vast number of granule cells (~1 million neurons) contribute to a relatively orthogonal coding scheme that can support different traces assigned to different memories promoting a reduction in interference from similar stimuli. For instance, if you were to vacation at the same resort every year, and some years you went with family, and some years you went with friends, it may be difficult to remember which years you went with who. This interference can hypothetically be overcome by employing a neural system that can maintain different representations for each experience, despite the similarity of these experiences (Colgin et al., 2008). The process of orthogonalizing output despite similarity in input has been termed pattern separation and the DG is thought to be critical to this function (Gilbert et al., 2001; Chawla et al., 2005; Leutgeb et al., 2007) (Figure 3).

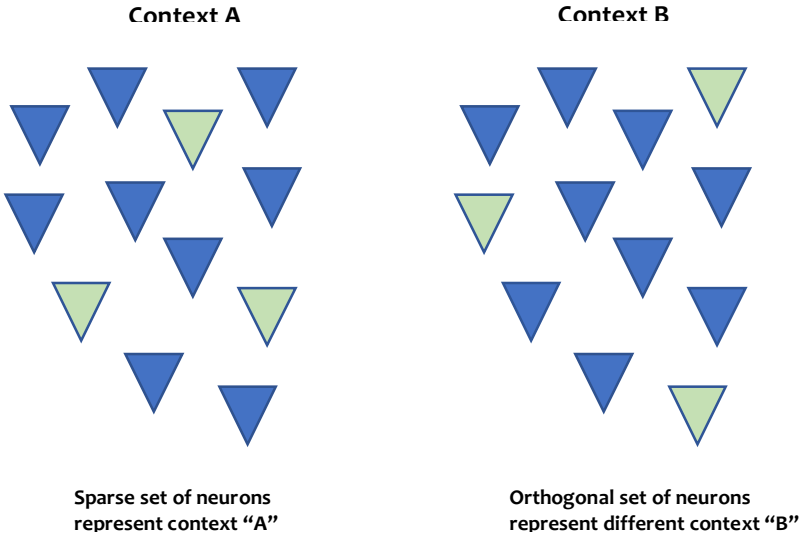


Figure 3. Schematic depicting two distinct memory traces after exposure to different contexts. DG granule cells represent each context by using non-overlapping orthogonalized sets of neurons.

Consistent with Marr's (1971) description of the CA3 as part of an auto-associative network that can give rise to recall of complete memories with only partial cues due to a relatively high level of interconnectivity there (recurrent collaterals) (Colgin et al., 2008), and the notion of Lorente de Nó's reverberating loops, it has been proposed that the CA3 engages in an opposing process called *pattern completion* (Gluck & Myers, 1993; Treves & Rolls, 1994). It may seem intuitive that the ability to reconstruct complete memories from only partial cues would promote interference but it has been shown that the CA3 indeed also utilizes a sparse coding scheme to form contextual representations and this is supported by lower levels of neuronal activity observed in the CA3 compared to the CA1, but a higher degree of overlap when comparing ensembles after visiting the same context twice (Vazdarjanova & Guzowski, 2004). This is further supported by the fact that the CA3 seems to play an important role in one-trial learning; this region is extremely sensitive to environmental changes and can encode contexts very rapidly (Cravens et al., 2006; Miyashita et al., 2009).

Both the DG and the CA3 can engage in pronounced remapping (Leutgeb et al., 2004) and the sparse coding scheme allows for similar events to be encoded by distinct populations of cells specifically to reduce interference. The generation of multiple contextual representations (global remapping) permits the distinction between similar experiences in different environments. It is important to note though, that cells can also engage in rate remapping where maintenance of the same representation is preserved but the firing rate of the cells within that representation changes, theoretically to allow for differentiation of two different experiences in the same spatial context (Leutgeb et al., 2005). Neural mechanisms such as remapping are considered to be adaptive in an ever-changing environment, where animals must be able to update contextual representations to incorporate new information. For example, if an animal learns that a food reward is present in a certain location within an environment, this information would theoretically be encoded within a specific neuronal ensemble. If the next day, the reward is no longer present in that location, but has moved to a different location, then the animal would need to update the

representation associated with this experience and this could be achieved via a remapping effect. While significant advances have been made in the last 50 years towards a better understanding of how representations of the surrounding environment are created and stored, the way in which these representations change and are modulated is not well understood.

To appreciate how contextual representations, change or remap globally, we must consider that sensory input is relayed to the DG / CA3 via the EC and that the mEC is also where grid cells reside. Therefore, it is plausible that grid cells contribute to global remapping. One piece of evidence supporting this hypothesis is the finding that changes in the environment that induce rate remapping in the CA3 do not shift grid cell representations whereas changes that induce global remapping in the CA3 not only cause grid cells to shift, but do so in a temporally synchronous manner (Fyhn et al., 2007; Colgin et al., 2008). Moreover, partial inactivation of the mEC causes remapping in the CA3 (Miao et al., 2015). Another possibility is that changes in contextual representations are initiated through perturbations in HF theta rhythm, which depend on projections from the MS. It is possible that the MS induces synaptic changes in the mEC, which are then relayed to place cells in the DG/CA3 resulting in remapping. This may be achieved via cholinergic inputs from the MS to the DG (Bergado et al., 2007). Finally, a third possibility involves a direct projection from the EC to the CA1 that acts in parallel to the traditional monosynaptic pathway (EC →DG→CA3→CA1) which posits the CA1 as a novelty / prediction error detector (Basu & Siegelbaum, 2015) that compares stored representations in the DG/CA3 with ongoing, direct sensory inputs from the EC. The CA1 sends excitatory projections to layer V of the EC, which in turn, loops back to layers II and III (Naber et al., 2001). This hypothesis suggests that through this connection the CA1 translates the detected prediction error into a signal, which recruits new cells in the DG/CA3 to become active (Lee et al., 2005; Chen et al., 2011; Duncan et al., 2012). Interestingly, acute inactivation of the mEC induced remapping in the HF (Miao et al., 2015) while bilateral excitotoxic lesions of the mEC had no effect, or could

not abolish remapping (Schlesiger et al., 2015) therefore, it is unclear what role the mEC or grid cells play in global remapping.

The mechanisms by which remapping occurs are not fully elucidated. We believe that remapping may involve activation of a brain stem structure long implicated in novelty detection and the regulation of behavioural flexibility and attention (Aston-Jones et al., 1999), the locus coeruleus (LC). The LC sends a major noradrenergic projection to the DG (Jones et al., 1977; Loughlin et al., 1986a; Harley, 1987, 2007a; Lee et al., 1993) as well as the MS (Bergado et al., 2007) and activation of the LC can initiate theta rhythm in the HF (Berridge & Foote, 1991), therefore the LC is well positioned to play this role from a theoretical standpoint. Enhanced theta following LC activation fits well with the hypothesis that the LC causes a disengagement from established representations and an enhancement of processes that promote the incorporation of new information (Bouret & Sara, 2005; Harley, 2007). It is also plausible that the LC noradrenergic system could theoretically be involved in sculpting HF contextual representations since there is a vast literature describing the nature of neuromodulator-induced alterations in memory.

1.5 Mnemonic Processes and The Malleability of Memory

By virtue of Hebbian plasticity, mnemonic processes are malleable, a property that allows for neuromodulators such as catecholamines to play a pivotal role in shaping our memories. To understand how catecholamines such as norepinephrine (NE) modulate memory, we must first evaluate the basis of mnemonic construction, deconstruction, and reconstruction. A commonly purported conjecture regarding memory formation is that it occurs in stages assuming a linear direction (e.g. encoding, storage, retrieval), and that with the passage of time, memories become more stable [short-term memory, long-term memory (Miyashita et al., 2009) through a process of *consolidation* (Müller & Pilzecker, 1900; Lechner et al., 1999). Consolidation is thought to depend on *de novo* protein synthesis as protein synthesis inhibitors disrupt late-phase long-term potentiation (LTP) thus interfering with the expression of long-term memory (Huang et al., 1996;

Flexner et al., 1963; Agranoff et al., 1965; Davis & Squire, 1984; Krug et al., 1984; Goelet et al., 1986; Frey et al., 1988). The molecular mechanisms by which memories become crystalized, involving protein synthesis, describe *synaptic consolidation* (Dudai, 2004; Frankland & Bontempi, 2005), a process that allows synapses to retain their strength over time (Clopath, 2012) through the growth of new connections, the restructuring of existing ones, the recruitment of second messenger systems, and the activation of transcription factors (Frankland & Bontempi, 2005). This is different than the concept of *systems consolidation* involving a more prolonged process regarding the interaction and reorganization of several brain circuits simultaneously to support memory (Frankland & Bontempi, 2005; Nadel et al., 2007). Some of the evidence for this theory of memory stabilization and transition from short-term memory to long-term memory (Dudai, 2004) came from patients with brain damage such as H.M., who were able to form short-term memories but could not convert these experiences into long-term memory. We describe memory in this organized way because it is easier to comprehend, but in reality, memory is a complex construct that is neither unitary nor linear, and is highly malleable.

Encoding is the first step in memory formation. It is the process that takes place which recruits engram cells at the time of learning. Following episodes of learning, different contextual elements pertaining to those episodes / experiences are encoded, elements which can later facilitate memory retrieval processes. Delineating which specific contextual elements are encoded has been the subject of research for many decades. As memories are acquired, they can either last a short time where the memory trace is temporary or transient in nature (i.e. short-term or working memory), or they can gain permanence through consolidation (Frey et al., 1988; Meiri & Rosenblum, 1998; Schafe et al., 1999; Miyashita et al., 2008). After a discovery made by Yarmolinksy and de la Haba (1959) that the antibiotic puromycin produced profound inhibition of protein synthesis, Flexner, Flexner, & Steller (1963) reported that hippocampal injections of puromycin in mice resulted in memory impairments. These initial findings led researchers to establish a clear connection between cellular modifications such as protein synthesis as well as

morphological changes at the synapse, and the formation of long-term memories (Bailey & Kandel, 1993; Bailey et al., 2008; Mayford et al., 2012). Interestingly, a recent study has challenged the canonical nature of this notion. In this study, Ryan et al., (2015) showed that memory consolidation was associated with increased synaptic strength and dendritic spine density. When they gave mice a protein synthesis inhibitor, which induced amnesia as expected, these plasticity-associated changes were not observed. However, using direct optogenetic activation of the memory engram cells activated during memory encoding, they could restore memory retrieval bypassing protein synthesis and the associated plasticity. It would be incorrect to assume that natural memory recall post-consolidation occurs in the absence of protein-synthesis associated plasticity but studies such as this demonstrate the potential ways in which memory systems can be manipulated to function in ways that may be different than the manner in which they operate under natural conditions.

Early research on memory consolidation suggested that memories were time-dependently stored in the HF and then later transferred to neocortical sites such as the PFC (Nadel et al., 2007), an idea first proposed by Theodule A. Ribot (Ribot, 1882) and later by David C. Marr (Marr, 1971). Despite the widely-accepted view of systems consolidation, there have been few studies conducted to directly test this theory empirically. In support, a recent study using time-lapse two-photon microendoscopy showed that dendritic spines in the CA1 turnover after approximately one month (the hypothesized length of time memory is limited to the HF) (Attardo et al., 2015). Additional evidence comes from another recent study that showed that HF (EC) engram cells involved in contextual fear conditioning gradually became silent with time, whereas BLA engram cells were maintained. They also showed that "*immature engram*" cells in the PFC became gradually active with time consistent with the hypothesis that remote memory is formed in the cortex by a slow transfer of HF memory (Kitamura et al., 2017). Therefore, current models describing systems consolidation (Squire & Alvarez, 1995) suggest the HF not only plays an integral role in encoding memories, but also in binding associated extra-hippocampal

(neocortical) structures in a long-term fashion to promote memory retrieval (Nadel et al., 2007). Memories become independent of the HF and rely more so on cortical connections (Yassa & Reagh, 2013). This is in contrast to *multiple trace theory* (Nadel & Moscovitch, 1997), which proposes memory retrieval processes always involve the HF despite how recent or remote the memory is and that different attributes of a memory (e.g. space, time, valence, salience) may be processed and stored in different cortical structures (Yassa & Reagh, 2013). In the first model, damage to the HF would only affect recent memories but in the second model more remote memories would also be affected. Similarly, to patients like H.M. who have sustained insult to the HF, in addition to *anterograde amnesia,* this type of damage is typically associated with greater deficits on more recently acquired memories or *temporally-graded retrograde amnesia*. This suggests a time-dependent process is involved (Frankland & Bontempi, 2005). However, both theories are supported in the literature. Although memories do seem to reorganize in the brain becoming more dependent on cortical structures and less dependent on the HF with time, it appears the HF is always required for rich contextual or spatial detail when retrieving a memory (Nadel & Moscovitch, 1997). Many of the memories we recall from the distant past are more semantic (and less episodic) than we perceive them to be (Lambon & Patterson, 2008; Yokoyama & Matsuo, 2016). In a study by Goshen et al. (2011), where they leveraged the precise temporal resolution of optogenetic methods, they were able to interrogate this problem further to try and reconcile these two theories of consolidation. Surprisingly, they showed that inhibition of the HF (CA1) in a precise manner (for 5 min) during acquisition or recall of a fear conditioned memory at a remote time point did result in impairments in freezing. However, when they inhibited the CA1 for a prolonged period of time (30 min prior to + acquisition or recall), no impairments were observed suggesting that in that 30-minute window, compensatory mechanisms were recruited and a reorganization of information took place in the brain (Goshen et al., 2011). They followed this up by showing that indeed, neuronal markers of activity were present in the neocortex (c-Fos expression in the anterior cingulate cortex). Together these findings suggest that the HF is a

"default activator" even for remote memories, but as time goes on dependence on the HF is lessened lending credence to both systems consolidation and multiple trace theory. In fact, recent findings in the literature call for a reconciliation of these theories and the emergence of a new theory. A few years ago, Yassa and Reagh (2013) proposed an integrated theory called the *competitive trace theory*, which suggests there are overlapping traces that compete over time - the HF lies at one end of the continuum encoding highly-contextual information and the neocortex at the other end as a final storage site of memories that have been decontextualized. Reactivation of a memory at any time point will result in a new trace that overlaps with other traces to the left and right as these traces compete.

Memory storage refers to the "*maintenance and preservation*" of memories (Squire, 1987; p33) whereby flexible synaptic connections reshaped by learning serve as essential components in this process (Ryan et al, 2015). The idea that memories may be stored as synaptic "weights" has a long history – for instance, Ramon y Cajal (1894) hypothesized that enhancement in synaptic efficacy could be a mechanism of memory storage. However, it is important to note that simple enhancement in synaptic efficacy is not sufficient to store a complex memory but that these changes must occur in the context of an ensemble of neurons (Mayford et al., 2012). Donald Hebb theorized that cells, which were repeatedly active at the same time, would become associated with each other calling these *neuronal assemblies* (Hebb, 1949). We refer to the reactivation of a memory trace that has been previously encoded and consolidated, as memory retrieval, however, if a memory deficit is observed, how can you deduce whether the deficit is one of retrieval or storage? It is especially difficult to differentiate between the true underlying neurobiology of memory storage versus memory retrieval since typical conceptualizations of memory processes in the literature conflate the two (Tonegawa et al., 2015). In the experiment where Ryan et al., (2015) rescued protein synthesis inhibitor-induced retrograde amnesia, demonstrating retrieval of an ostensibly lost contextual memory using optogenetic activation of

engram cells in the DG, they found that cells in the BLA were also active. They measured the "*connectivity*" between these two neuronal populations following contextual fear conditioning (CFC). Despite administration of a protein synthesis inhibitor given after CFC, the downstream activity in BLA cells was surprisingly still maintained and so they concluded that the connectivity between these populations was resistant to protein synthesis inhibition. This high "*preferential engram-to-engram cellular connectivity*" exists between the DG and the CA3 as well as between the DG and the BLA (Ryan et al., 2015; Redondo et al., 2015; Ohkawa et al., 2015) and likely between the HF (EC) and the PFC (Kitamura et al., 2017). Optical activation of cells in the DG induced plasticity (increased cFos expression) in the downstream BLA engram population associated with the CFC memory. This suggests that: a) memory storage is a function of how well these connections are established in the hours following an experience and how well they are preserved, b) these connections are protein synthesis independent, and c) memory deficits that have been deemed a result of a consolidation failure or retrieval failure may in fact be the result of a storage failure. It will be interesting to see how future studies investigate the dynamics of engram connectivity and plasticity.

For a long time, memories were considered "*static*" or "*inflexible*" and once laid down in the neural net of consciousness, there they resided perfect snapshots of the past, filed away for later use. But we now know that memories are quite dynamic, malleable in nature, and can be modified (Otis, Werner, & Mueller, 2015). For instance, when memories are retrieved, they are also reconsolidated. *Reconsolidation* is the process by which a reactivated memory trace becomes transiently susceptible to disruption (Nader, 2015). Theoretically, this change in the mnemonic reconstruction process (Reisberg et al., 1988; Rimmele et al., 2016) can result in strengthening, weakening, or simply altering the memory trace such that new contextual elements are incorporated in a way that may "*overwrite*" the original memory. This is due to factors present at the time of retrieval and their effect on the processes governing the reconsolidation of that memory trace. These factors may include internal motivational states, hormonal profile,

emotional condition, level of attention etc.

Why aren't memories fixed so that you always remember events as perfectly as they occurred in real-time? The functional significance of a system where memories can be strengthened, weakened, or altered is highly debated and may even seem maladaptive in some cases (Rodriguez-Ortiz & Bermúdez-Rattoni, 2007). Despite our proclivity to view memory as an accurate depiction of past events (Lee, Nader, Schiller, 2017), mnemonic processes do not operate like a recorder that can be played back later for review. For instance, encoding can be distorted in such a way that elaborates certain semantic details of an event to achieve a sense of coherence (Fairfield, Altamura, Padalino, Balzotti, Di Domenico, & Mammarella, 2016). For this reason, eyewitness testimonies can be unreliable (Bartlett, 1932); people fill in gaps with imagined elements to create a complete picture in their mind. In these instances, especially if an individual is experiencing a highly emotional state, focus tends to be on the emotional content rather than neutral contextual details (Fairfield et al., 2016). However, memory doesn't always need to be accurate to be adaptive. Although the flexibility of memories may not bode well in a court room, this sort of memory modulation can potentially have survival value in many other contexts. For example, the generalization of fear memories has clear potential to be adaptive: you don't need to know which rattlesnake tried to bite you, but the sound is enough to serve as a warning signal and keep you away. Conversely, when fearful memories such as those acquired after experiencing a traumatic event become strengthened in a maladaptive way, this can lead to disorders such as post-traumatic stress disorder (PTSD). However, from a functional perspective this type of system allows for new information to be incorporated into a memory trace since memories can be "*updated*". Imagine writing a letter. After saving the first draft you later open the file and notice several spelling errors, so you edit the document. Subsequently, when you save the document, you can either choose to overwrite the old draft with the new edited content or save two separate drafts, the old one and the new one. Overwriting allows you to have the most updated version stored but you lose the original copy. Saving two drafts allows you to keep the

original, but as you continue to edit, and save, and edit, and save, eventually you may have so many drafts it will be difficult to figure out which one is the most recent unless you have another system in place that can keep track of that. In terms of memory, editing and saving is like learning and (re) consolidation, and in some cases, having all these traces would create too much "*interference*" and not being able to update memories could lead to a loss of discrimination and / or impairments in learning (e.g., reversal learning). This would be maladaptive. For example, if an animal learned to associate the colour red with an aversive stimulus (e.g., a shock) and then this contingency was changed such that the colour green now predicted the shock and red was deemed safe, a loss of discrimination or an increase in interference could lead to generalized behaviour where the animal demonstrated avoidance or fear to both colours. This idea is thought to underlie generalized anxiety disorder (Kheirbek, Klemenhagen, Sahay, & Hen, 2012). Therefore, when memories become labile upon reactivation, this gives rise to a reduction in interference but the consequence is that the original learning trace may be inaccessible over time. For this reason, each time you remember an experience, the process of recapitulating that experience alters the memory trace so that the memory is in fact a "*newly reconstructed*" memory each time and less of a true representation of the actual events that took place originally. This reflects the dynamic nature of the reconsolidation process whereby new information is incorporated into existing traces (Nader, Schafe, & LeDoux, 2000). The fact that memories are not immutable and that contextual details may be lost or possibly generalized over time, could in fact underlie the formation semantic memories or schemas (Lambon, Ralph, & Patterson, 2008; Yokoyama & Matsuo, 2016) and govern the transition of memories from the HF to the neocortex over time.

1.6 The Temporal Dynamics of Immediate Early Gene Transcription

One of the first steps in long-term plasticity is the transcription of immediate early genes (IEGs) such as *arc* (Activity Regulated Cytoskeletal-Associated Protein) also known as *arg3.1* (Link et al., 1995), and *zif268* also known as *egr1* (Guzowski, 2002). Unlike most genes, these

genes do not require *de novo* protein synthesis to be transcribed as constitutive regulatory transcription factors (RTFs) such as cAMP response element binding protein (CREB) and serum-response factor (SRF) are available in the nucleus and capable of recruiting transcriptional machinery (Finkbeiner & Greenberg, 1998; Ginty, 1997). RTFs are activated by second messengers such as protein kinase A (PKA), calcium and calmodulin dependent kinase IV (CaMK-IV), and mitogen activated protein kinase (MAPK) following NMDA receptor mediated synaptic stimulation.

IEG transcription occurs at low levels under basal conditions (Rao et al., 2006; Miyashita et al., 2008) and is highly dependent on synaptic input (Lyford et al., 1995) although once initiated, can occur extremely rapidly (Cole, Saffen, Baraban, & Worley, 1989; Guzowski, McNaughton, Barnes & Worley, 1999; Vazdarjanova, McNaughton, Barnes, Worley, & Guzowski, 2002). Some IEGs regulate the transcription of other genes (RTFs) (e.g. *zif268*) and may play a role in metaplasticity[1] (Guzowski, 2002), and other non-RTF IEGs (called effector IEGs) such as *arc,* are involved in a wide range of cellular functions (Miyashita et al., 2008). Suggestive of a highly specific function (Miyashita et al., 2008) *arc* is only found in vertebrates (Lyford et al., 1995; Link et al., 1995; Mattaliano, Montana, Parisky, Littleton, & Griffith, 2007) and is thought to promote plasticity via synaptic modifications (Rial-Verde, Lee-Osbourne, Worley, Manilow, & Cline, 2006) such as the scaling / trafficking of AMPA receptors which mediate neuronal transmission (Chowdhury, Shepherd, Okuno, Lyford, Petralia, Plath, Kuhl, Huganir, & Worley, 2006; Xiao et al., 2000) and initiating changes in the actin cytoskeleton of the cell required for changes in dendritic spine structure and density (Dillon & Goda, 2005). Following transcription, *arc* mRNA is rapidly transported outside of the nucleus to the dendrites for local storage, translation, and decay (Steward, Wallace, Lyford, & Worley, 1998). *Arc* is one

[1] **Metaplasticity**: a term originally coined by W.C. Abraham and M.F. Bear - refers to the idea that a synapse's previous history of activity determines its current plasticity (Abraham & Bear, 1996).

of the most tightly regulated proteins (Bramham, Alme, Bittins, Kuipers, Nair, et al., 2010) with a half-life of only 47 minutes (Rao et al., 2006).

Consequently, IEGs have been widely used as neuronal markers of activity and due to the kinetics of IEG mRNA following transcription they can be used to map the activity history of individual neurons (Guzowski et al., 1999). A sensitive molecular protocol referred to as cellular compartmental analysis of temporal fluorescent *in situ* hybridization (catFISH) allows for the tracking of neuronal populations at two distinct time points by exploiting the distribution dynamics of IEG transcription. Following neuronal stimulation, the induction of *arc* mRNA occurs in the nucleus; these transcripts then translocate to the cytoplasm after approximately 15 minutes targeting the dendrites and return to basal levels after approximately 60 minutes (Guzowski et al., 1999).

In experiments utilizing this protocol, animals are typically placed in an environment that they are permitted to explore thus activating place cells, which drives *arc* transcription. After 5 minutes of context exploration, animals are placed back in their home cage, where any further transcription can be attributed to, and is associated with the context that was just explored (Marrone, Schaner, McNaughton, Worley, & Barnes, 2008). Twenty-five minutes later animals are given another context exposure for 5 minutes. Given the distribution dynamics of *arc* transcription, cells active during the second exploration will still contain *arc* mRNA in the nucleus but those cells, which were active during the first context exploration, will contain *arc* mRNA in the cytoplasm, and cells that were active in both behavioural epochs, will contain *arc* in both locations. Therefore, the sub-cellular localization of *arc* visualized via fluorescent confocal microscopy allows for the neuronal populations activated by two distinct experiences to be discriminated and quantified (Guzowski et al., 1999).

The catFISH protocol, developed by John Guzowki (1999), allows us to look at large numbers of cells, within many different brain regions simultaneously. Furthermore, it has demonstrated that *arc* expression is induced in the CA1 in a context-dependent manner. When

animals visit the same context twice, as opposed to two different contexts, this results in a higher degree of overlap in the cells being activated across time points. This effect does not disappear or habituate following repeated context presentations across days and only after four exposures to the same context each separated by 25 minutes does *arc* induction begin to diminish (Guzowski et al., 2006). However, when the animal is presented with a new environment, even after nine exposures to the same context, this attenuation in *arc* transcription is rescued. The fact that *arc* induction is not easily disengaged when an animal is presented with familiar stimuli suggests that it does not distinguish between new learning and memory retrieval (Guzowski et al., 2006; Miyashita et al., 2008). This effect is also consistent with electrophysiology studies involving place cell remapping. Remapping occurs when an animal visits two different contexts in the same way different neuronal ensembles are recruited to activate *arc* in different contexts using the catFISH protocol (figures 4-7). The tracking of IEGs in a temporal fashion has also been useful in determining the differential contributions of distinct subfields within the HF to contextual coding. For instance, novel contexts appear to be encoded more rapidly in the CA3 compared to the CA1 (Pevzner et al., 2012) and spatially selective IEG expression has been demonstrated in the CA1 (Guzowski et al., 2006), the CA3 (Vazdarjanova & Guzowski, 2004) and the DG (Marrone et al., 2011; Schmidt et al., 2012).

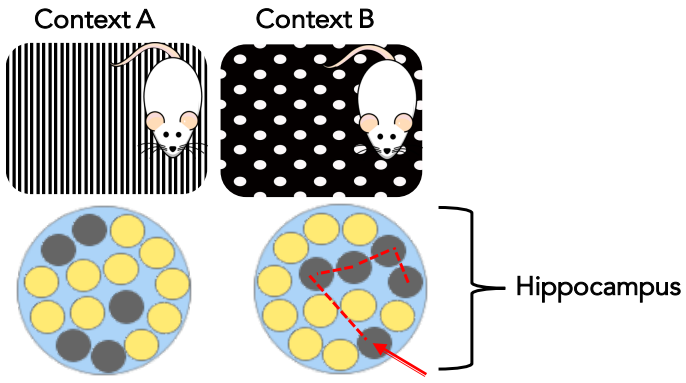


Figure 4. Schematic depicting a hypothetical memory trace in the HF.

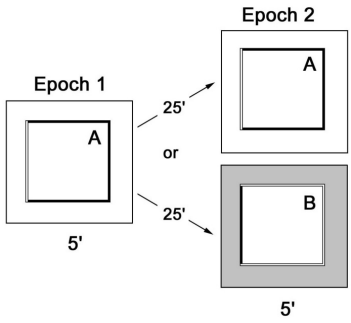


Figure 5. Animals are given two context exposures (5 min) separated by a 25-minute delay. Animals are placed in context A twice (A/A) or placed in context A and then context B (A/B).

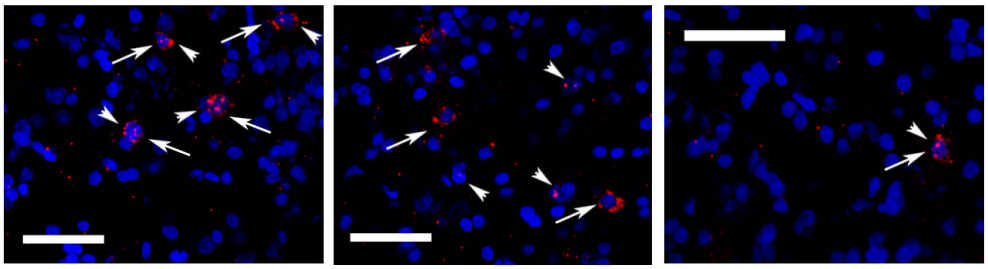


Figure 6. IEG expression is context-dependent. Sample confocal images (scale bar = 50µm) showing intra-nuclear foci signal (second context exposure, short arrows) (pink dots), and cytoplasmic signal (first context exposure, long arrows) (pink ring). Nuclei are counterstained with DAPI. (Grella et al., 2016)

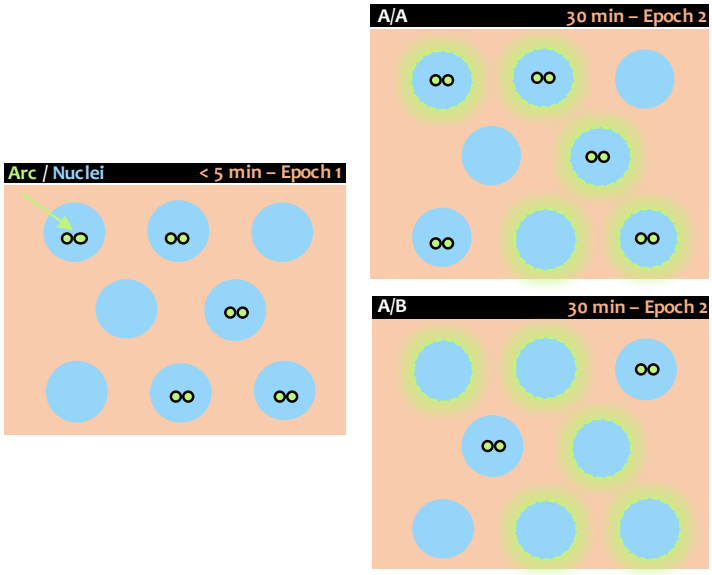


Figure 7. Cell nuclei = blue circles. Red dots inside the nuclei represents cellular activity within a 5-minute time window (intranuclear foci signal <5min after activation); green ring around the nuclei represent those same cells following the translocation of IEG mRNA transcripts to the cytoplasm 30-35 minutes later (cytoplasmic signal ~ 35 min after activation). Animals that visit the same context twice (AA) will have more cells labeled with both intranuclear + cytoplasmic signal while animals that visit two different context (AB) will show these signals separately.

1.7 The Locus Coeruleus Norepinephrine System

The release of the neurotransmitter NE throughout the mammalian brain is important for modulating attention, arousal, and cognition (Aston-Jones et al., 1991; Sara & Segal, 1991; Schwarz & Luo, 2015). Classified as a catecholamine structurally, this chemical is also an important mediator of the mammalian stress response. It is generated by the amino acid tyrosine, and exerts its effects by binding to adrenoreceptors. Pharmacodynamically, noradrenergic receptors are G-protein coupled receptors (GPCRs) and include α and β-subtypes. Found post-synaptically, α1 receptors are coupled to the guanine nucleotide-binding regulatory protein Gq, and when activated, this causes an increase in intracellular levels of Ca^{2+}, and subsequently the release of NE. Conversely, α2 receptors are presynaptic, coupled to Gi; activation of these receptors inhibits NE release acting as a negative feedback mechanism. β-receptors (1-3) are post-synaptic and positively coupled to Gs resulting in an increase in NE release when activated. Generally, NE release is associated with increased heart rate, and blood glucose levels. In preparation for fleeing or fighting, activation of the sympathetic nervous system and the release of catecholamines such as NE, occurs in mammals when faced with a threatening situation (Jansen et al., 1995). Walter Cannon (1915) coined the term "*fight or flight*" to describe this hyper arousal reaction.

In 1970, Seymour Kety introduced the idea that biogenic amines such as NE, not only have an effect on arousal, but also emotion and learning (Kety, 1970) acting as "*neuromodulators*". Neuromodulation is often contrasted with fast synaptic transmission where transmission is slow-acting on GPCRs rather than fast-acting on ligand-gated ion channels. Rather than initiating spiking, effects can be to modulate ongoing spiking activity, are typically long lasting and groups of cells are affected as opposed to one or two cells. Evolutionarily, neuromodulators emerged quite early and have been highly conserved. For instance, both dopamine and acetylcholine are present in invertebrate species of animals, however, NE is unique in that it is only present in vertebrates. Since Vittorio Erspamer (Erspamer & Boretti, 1951)

discovered the biogenic amine octopamine in the salivary gland of an octopus and characterized it as having NE-like properties affecting physiology and behaviour, it is thought that this chemical is the precursor to NE. The similarity between NE and octopamine demonstrate the conservation and need for such a molecule. Kety's hypothesis regarding NE was quite specific; he believed that forebrain NE acted to selectively enhance cell firing in neurons receiving inputs during affectively important events and that this served to promote memory (Kety, 1967, 1970).

"The state of arousal by means of adrenergic input to each (cerebral, hippocampal, and cerebellar cortices) may serve to concurrently reinforce and to consolidate the significant sensory patterns, the affective associations and the motor programs necessary in the learning of a new adaptive response"

Seymour Kety (1970)

Kety's ideas were quite novel given the limited evidence at the time demonstrating that neuromodulators could affect more than simply neuronal responses, but could also improve cognitive performance (Sara and Segal, 1991). In the brain, NE is produced in a small pontine cluster called the locus coeruleus (LC). This bilateral structure contains approximately 1600 densely packed neurons per nuclei in the rodent brain (Robertson et al., 2013) all which produce NE, all which provide a neuromodulatory influence in the brain. Segal and Bloom (1976) showed in rats, that electrical stimulation of the LC selectively enhanced hippocampal responses to stimuli conditioned with an appetitive reward. Following this study, Sara et al., (1980) showed that LC stimulation improved memory retrieval for a spatial task and (Dekeyne et al., (1987) replicated this effect. Devauges and Sara, (1991) later discovered that this effect was dependent on β-adrenergic receptors (BARs) in the HF. Furthermore, LC activation facilitated memory encoding for spatial information and this was also dependent on BARs (Lemon et al., 2009).

The survival of an organism depends highly on their ability to remember certain information; therefore, it is adaptive for that organism to possess a mechanism by which it can detect what is important, highlight that information, and filter out what is irrelevant (Berridge, 2008). Kety believed that the neurotransmission of NE played this role. The ability to demonstrate an insensitivity to the environment when rest is required, have a broad "*diversive*"

focus in a manner of reconnaissance exploration when searching for resources, and maintain an "*inspective*" vigilant hold on an identified predator, food source, or potential mate when necessary, is key (Berlyne, 1966; Flicker & Geyer, 1982). This great task of processing such a wide variety of stimuli in a constantly changing environment is achieved by one of the smallest nuclei in the brain (Schwarz & Luo, 2015), the LC.

1.8 Tonic and Phasic Modes of Discharge: Salience, Novelty, & Reversal Learning

As the major source of NE in the brain (Tanaka et al., 1976; Foote et al., 1983; Sara et al., 1994; Kitchigina et al., 1997) and the only source of NE in the HF (Blackstad et al., 1967; Fuxe et al., 1968; Ungerstedt, 1971; Lindvall & Björklund, 1974; Pickel et al., 1974; Ross & Reis, 1974; Morrison et al., 1978) the LC exhibits distinct modes of output. Neurons in the LC typically discharge with *phasic* burst activity patterns essentially superimposed onto steady, yet stochastic *tonic* discharge rates (Devilbiss & Waterhouse, 2011) that serve as background activity. Tonic LC activation can occur in the absence of presynaptic activity to maintain NE levels in the brain where inhibition or increases in presynaptic input can be transmitted. In contrast, phasic LC activation is driven by presynaptic activity. Importantly, it is thought that these two modes differentially modulate information processing in LC targets such as the HF (Devilbiss & Waterhouse, 2011).

At rest, LC neurons exhibit a tonic basal activation pattern characterized by relatively slow frequencies (1-5 Hz), roughly correlated with the sleep/wake cycle (e.g. higher levels observed when the animal is awake compared to asleep) (Aston-Jones & Bloom, 1981; Harley, 1987; Aston-Jones et al., 1991; Berridge & Waterhouse, 2003). Typically, LC neurons are quiescent and tonus is low when animals are asleep, but also when they are awake but not actively engaged in their surrounding environment such as when they are grooming or eating (Aston-Jones & Bloom, 1981; Grant et al., 1988; Aston-Jones et al., 1991; Rajkowski et al., 1994). Michel Jouvet (1969) first observed this tonic variation with behavioural state in his

characterization of sleep states. These findings paralleled those seen in cats (Hobson et al., 1975; Rasmussen et al., 1986) and monkeys (Foote et al., 1980; Rajkowski et al., 1998).

In contrast, phasic discharge (5-20 Hz) (Hobson et al., 1975; Foote et al., 1980; Aston-Jones & Bloom, 1981) consists of short bursts of activity (e.g. typically restricted to 2-3 action potentials) followed by a brief pause in firing lasting approximately 200-500 milliseconds (Aston-Jones & Bloom, 1981b; Harley & Sara, 1992; Klukowski & Harley, 1994; Kitchigina et al., 1997) and is typically triggered by surprising, or novel stimuli (bottom up) (Aston-Jones & Bloom, 1981; Vankov et al., 1995; Berridge & Waterhouse, 2003; Harley, 2007b; Aston-Jones & Waterhouse, 2016) that are either appetitive or aversive (Rasmussen et al., 1986; Sara & Segal, 1991). When stimuli are associated with task-related decision processes (top down), phasic LC activation can be driven by outcomes and tied to behavioural responses in a way that helps to optimize task performance (Aston-Jones & Cohen, 2005). The computational neuromodulatory *adaptive gain* model of LC-NE activity describes how these different modes can "*adaptively adjust the gain of these cortical circuits*" proposing that phasic LC activation facilitates task performance where the organism is namely "*exploitive*" in its behaviour while tonic LC activation promotes the disengagement of task-related behaviour allowing the organism to be more "*explorative*" (Aston-Jones & Cohen, 2005).

Some evidence for this comes from a study where cynomolgus monkeys (*Macaca fascicularis*) were trained to perform an oddball visual discrimination task while recordings were obtained from the LC (Aston-Jones et al., 1991). Monkeys performed for a juice reward where they were required first to depress a lever to initiate the task, then to fixate on a spot in the middle of a screen. Following this, a target cue was presented on the screen (20% of the time) and the monkeys were to release the lever in the presence of the cue, and suppress this response when the cue was not present in order to obtain the reward. The researchers found that LC neurons selectively responded to the target with relatively short latencies. From this experiment, it was not possible to tell if LC responses facilitated performance but it was suggested by the authors that

this might be the case; the LC may have been directing behaviour. These findings demonstrated that LC neurons are involved in processing salient stimuli. Commensurate with this view, Hervé-Minvielle and Sara (1995) used the hole board test, which essentially tests exploratory behaviour by measuring head dipping, rearing, and locomotor behaviour in rodents as they search and discover novel objects within different holes, and found that when the animals encountered a novel object, LC neurons responded with a phasic burst. The results of this study were later replicated (Kitchigina et al., 1997). When the rats were placed in the novel hole board environment, there was a tonic increase in population spike amplitude consistent with an exploratory role for this firing pattern. This effect habituated, and was blocked partially when the animals were administered the BAR antagonist propranolol. When the animals encountered a novel stimulus in one of the holes, there was a strong phasic response in LC neurons lasting approximately 50-75 seconds; this effect was completely absent in rats pretreated with propranolol.

Around the same time, (Rajkowski et al., 1994) showed that differences in performance on the visual discrimination task were closely associated with rates of tonic discharge. During times of behavioural agitation, distractibility, and poor task performance, LC tonus was high whereas moderate levels of tonic LC discharge were correlated with optimal task performance. NE exerts feedback inhibition of its own release through an interaction with presynaptic α-2 adrenergic inhibitory autoreceptors in the LC (Washburn & Moises, 1989). Clonidine, which is an α-2 adrenoceptor agonist, serves to inhibit LC neurotransmission (Aghajanian, 1978; Aghajanian & VanderMaelen, 1982). Administration of clonidine was able to help the animals with high tonic LC activity regain focus, direct attention and enhance performance (Ivanova et al., 1997). A moderate level of activity in the tonic mode of sustained environmental surveillance allows an organism to appropriately and readily responds to salient stimuli (Rajkowski et al., 1994). These data suggest that optimal task performance involves phasic LC responding to salient stimuli superimposed on a background of moderate, or optimal tonic LC activity to promote focused

attention (Aston-Jones & Cohen, 2005). One interesting discrepancy in the literature concerns the rate at which these responses habituate. In the studies done in monkeys, LC responses did not habituate even after 100 presentations (Aston-Jones et al., 1991; Rajkowski et al., 1994) and were greatest when stimuli elicited an orienting response (Aston-Jones & Cohen, 2005). In contrast, in the studies conducted in rats, LC neurons did habituate, with different populations of LC neurons habituating at different rates (Sara & Segal, 1991), and in some cases, this occurred very rapidly (Sara et al., 1994; Hervé-Minvielle & Sara, 1995). Habituation of LC neurons has also been shown to occur in mice exposed to a novel environment (Takeuchi et al., 2016). This discrepancy has never been resolved in the literature.

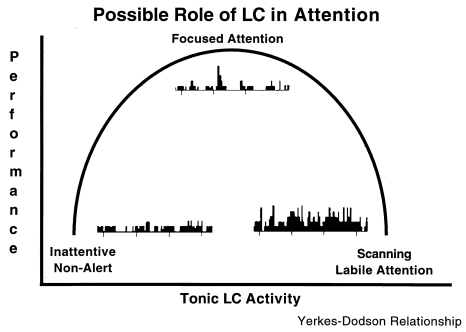


Figure 8. Reprinted from: Biological Psychiatry, 46(9), Aston-Jones, Rajkowski, & Cohen, Role of locus coeruleus in attention and behavioral flexibility, 1309-1320, Copyright (1999) with permission from Elsevier.

Notably, in the oddball task performed by the monkeys, the LC did not respond to distractors but only to the target (Aston-Jones et al., 1994). Furthermore, during reversal training when the distractor became the target and vice versa, LC responding switched to the new cue (Aston-Jones et al., 1994, 1997; Rajkowski et al., 1994; Aston-Jones & Cohen, 2005). This demonstrated importantly that the LC was not responding simply to a set of attributes related to the specific cue used but instead to the fact that the cue was meaningful in that it signalled reward (Aston-Jones et al., 1999). According to the adaptive gain model, which suggests that tonic LC activity facilitates behavioural response disengagement; the switch in phasic LC responding to the appropriate target cue must depend on tonic activity in some way. However, this interaction has not been elucidated.

In the rat, Sara and Segal (1991) also showed that LC neurons exhibit remarkable plasticity as a function of environmental contingencies. In a classical conditioning experiment, LC neurons responded with a phasic burst when a stimulus was novel, and when it acquired salience during conditioning, but also during reversal training and during extinction. The authors noted that the most striking and consistent observation in the study was that LC neurons responded immediately to any change in stimulus-reinforcement contingency with respect to both appetitive and aversive stimuli, and that these responses were even stronger than the initial responses to novel stimuli. A separate study was conducted where rats performed a spatial task in a radial arm maze using extra-maze cues.

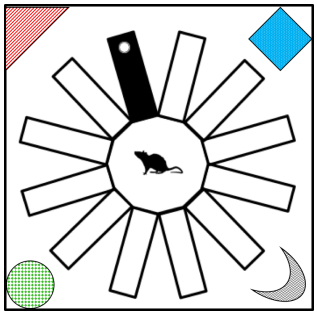


Figure 9. Schematic depicting a 12-arm radial maze with extra-maze cues on the wall which the rat uses to navigate the spatial task.

When the cues were removed, or changed, performance dropped significantly. When the rats were given a drug that elevated NE levels, their performance recovered more quickly, as they were better able to shift their attention to the newly relevant cues which were necessary to perform the task well (Sara et al., 1994). Therefore, activation of LC neurons, specifically phasic activation, has been demonstrated to occur in association with cognitive shifts in attention (Hagena et al., 2016) and likely plays a integral role in reversal learning.

1.9 Locus Coeruleus Induced Plasticity in the Hippocampus

Cells in the LC are exquisitely sensitive to changes in stimulus contingencies and phasic and tonic modes of LC output have different antecedents but both appear to function in such a way that increases the signal to noise ratio (Woodward et al., 1979; Sara, 1985; Servan-Schreiber

et al., 1990; Aston-Jones et al., 1991, 1999; Hagena et al., 2016) and both phasic and tonic LC activity are equally capable of inducing downstream learning-facilitated plasticity effects such as LTP (Bliss et al., 1983; Neuman & Harley, 1983; Gray & Johnston, 1987; Hopkins & Johnston, 1988; Harley, 1991; Walling et al., 2011, 2004; Almaguer-Melian et al., 2005; Lashgari et al., 2008; Lim et al., 2010; Hagena et al., 2016) long-term depression (LTD) (Lemon et al., 2009; Lemon & Manahan-Vaughan, 2012; Hansen & Manahan-Vaughan, 2015a) and NE release in the DG (Dahl & Winson, 1985; Harley & Milway, 1986; Harley et al., 1989; Babstock & Harley, 1992; Frizzell & Harley, 1994; Klukowski & Harley, 1994; Walling et al., 2004; Lemon et al., 2009) and CA1 (Lemon et al., 2009). For instance, activation of the LC with glutamate, which mimics phasic signalling, results in a ~200% increase in NE in the HF within 20 minutes measured by microdialysis (Walling et al., 2004) and electrical simulation also results in stable NE signals measured by voltammetry (Yavich et al., 2005). Phasic LC activation has also been shown to potentiate LTP at CA3-CA1 synapses (Takeuchi et al., 2014) and elevated levels of NE can increase somatic and dendritic excitability in the DG (Lacaille & Harley, 1985; Stanton & Sarvey, 1985; Harley, 1991; Hagena et al., 2016) as well as in CA1 and CA3 (Mueller et al., 1981; Heginbotham & Dunwiddie, 1991; Dunwiddie et al., 1992; Jurgens et al., 2005a, 2005b), effects which are mediated by BARs (Kitchigina et al., 1997) and which can persist for 24 hours (Walling & Harley, 2004). Therefore, it has been proposed that activation of the LC-NE system can induce changes in network dynamics occurring at critical times when learning is necessary to promote adaptive behaviour (Sara et al., 1994; Bouret & Sara, 2005). These network configurations function to "reset" the system and as such, this hypothesis has been referred to as the *network-reset hypothesis* (Bouret & Sara, 2005).

1.10 The Network Reset Hypothesis

A simple invertebrate model inspired this hypothesis. In lobsters, Hooper and Moulins (1989) demonstrated that a neuron, which was part of a specific network, could switch its functional membership to a different network in certain conditions likely attributed to

neuromodulatory inputs. The crustacean stomatogastric nervous system is composed of four networks containing distinct neuronal populations that control motor patterns. These networks can be simultaneously active. Using *in vitro* preparations of the lobster (*Palinurus vulgaris),* Hooper and Moulins (1989) demonstrated that a ventricular dialator (VD) neuron, which was part of a specific network, could shift, becoming a functional and exclusive member of a different network under certain circumstances. The VD neuron demonstrated two different firing patterns when spontaneous recordings were taken. When the first network was active (pyloric network) the VD neuron exhibited a short, burst firing pattern, but when the second network (caridac sac), which was typically quiescent, became active via experimenter-induced stimulation, the VD neuron assimilated its firing pattern, exhibiting longer bursts like the other cardiac sac neurons, effectively switching networks. What could be the basis for this apparent appropriation of a neuronal constituent from one network to another? They investigated this further and found a relationship with membrane properties that were likely influenced by neuromodulatory input. These findings represent early evidence that neurons could participate in multiple networks suggesting these shifts were under neuromodulatory regulation by catecholamines. This phenomenon observed in crustacean neural networks, where network dynamics could undergo a spontaneous shift, combined with Kety's ideas regarding how biogenic amines can facilitate memory through the enhancement of neuronal activity during affectively important events, led Bouret and Sara (2005) to hypothesize that activation of the LC-NE system may be able to induce a ''reset'' in its target structures, by interrupting existing functional networks and facilitating the emergence of new ones. Moreover, the LC has vast cortical projections and it is this dense innervation of the neuraxis that has fueled the hypotheses presented here (Dahlström & Fuxe, 1964; Jones & Moore, 1977; Jones et al., 1977) encouraging the view that the NE system is implicated in shifting attention to environmental imperatives (Sara, 2009).

Viewing the LC-NE system as a potential mechanism for network resetting does not disentangle phasic from tonic activation, and from the perspective of plasticity promotion, Bouret

and Sara (2005) believed both phasic and tonic LC activation could reset a neural network. In our own experiments, we have interpreted "resetting of a neural network" to include global remapping and hypothesize that both phasic and tonic activation of the LC-NE system may underlie the mechanism by which remapping occurs. The main hypothesis of the current work is that this system acts as a modulatory switch recruiting new neurons to create new contextual representations when necessary and likely updating existing representations in the presence of new information. We also hypothesize that the pathway from the LC to the DG is involved in shifting attention promoting learning that involves contingency changes in the environment (e.g. reversal learning or extinction) and when behavioural changes are necessary.

1.11 Noradrenergic Modulation of Memory

According to the *network reset hypothesis*, activation of the LC-NE system should promote adaptive responses during important events and support learning during changes in contingency, hypothetically, and mechanistically, by means of increased excitability in the HF (Lacaille and Harley, 1985; Stanton and Sarvey, 1985; Harley, 1991) and global remapping of hippocampal contextual representations. The highest concentrations of BARs are found in the DG compared to the CA1 and CA3 (Booze et al., 1993; Milner et al., 2000), and we propose that the LC-NE system via BAR activation in the DG is involved in updating memory traces, specifically to incorporate new information. NE theoretically provides a "reset" signal causing the HF to recruit distinct populations of neurons, thus, providing a molecular switch to dictate if hippocampal circuits should generate new representations or update existing representations to incorporate novel information. In this way, the LC-NE system would be considered a neuromodulator of memory encoding through BARs, where plasticity promotes the long-term storage of salient experiences (Hagena et al., 2016).

There is an abundance of research to show that the noradrenergic system mediates different stages of memory through a neuromodulatory effect (McGaugh et al., 1990; Do Monte et al., 2008) but how does this fit with the network reset hypothesis?

The majority of studies investigating the role of NE on memory have focused on consolidation and reconsolidation, specifically in the context of emotional versus neutral memory (van Stegeren et al., 1998; Przybyslawski et al., 1999; Cahill & Alkire, 2003). Most of this work was carried using human participants administered drugs that act on the peripheral and / or central nervous system (van Stegeren et al., 1998). In humans, activation of BARs results in the augmentation of memory consolidation (specifically emotional memory) and this is thought to be mediated via receptors in the BLA (Cahill et al., 1994; Cahill & McGaugh, 1998; van Stegeren, 2008; Chamberlain & Robbins, 2013; Barsegyan et al., 2014; Kuffel et al., 2014; for reviews see Ferry et al., 1999; McGaugh, 2000; Roozendaal et al., 2009; Roozendaal & McGaugh, 2011), an effect which disappears if participants are pretreated with propranolol (Cahill et al., 1994; van Stegeren et al., 1998; Maheu et al., 2004) or if given propranolol post-learning (Sara et al., 1999; Tronel et al., 2004; Roozendaal et al., 2008; Barsegyan et al., 2014). In rats, when NE was directly infused into the DG, this enhanced memory consolidation while propranolol had the opposite effect (Lee et al., 1993). Moreover, NE is required for the strengthening of synaptic connections e.g. LTP (Stanton & Sarvey, 1985; Thomas et al., 1996; Katsuki et al., 1997; Gelinas & Nguyen, 2005; Tully & Bolshakov, 2010; Qian et al., 2012; Sarabdjitsingh et al., 2012; Hansen & Manahan-Vaughan, 2015b) although propranolol (Hagena & Manahan-Vaughan, 2012) and the BAR antagonist timolol (Dunwiddie et al., 1982) did not block LTP elicited with electrical stimulation.

Like consolidation (Kobayashi & Kobayashi, 2001), NE has also been shown to enhance memories through reconsolidation (Gazarini et al., 2013). When rats were given a single infusion of the non-specific BAR agonist isoproterenol following retrieval of a fear memory, this served to enhance further retrieval and impair extinction of that memory (Green et al., 1992; Dębiec et al., 2011). In contrast, BAR antagonists have been shown to impair or disrupt reconsolidation (Schwabe et al., 2012). Administration of propranolol induced a long-lasting impairment in the subsequent expression of contextual fear memory when administered immediately after retrieval

(Przybyslawski et al., 1999; Schneider et al., 2014; Taherian et al., 2014). Given these findings, and the fact that oral administration of propranolol impaired reconsolidation of drug-related, positive and negative but not neutral, words in abstinent heroin addicts (Zhao et al., 2011), clinical interest in the amnesic effect of propranolol on retrieved fear memories as a possible treatment for anxiety disorders such as PTSD was prompted (Nader et al., 2000; Debiec & Ledoux, 2004; Kindt et al., 2009; Finnie & Nader, 2012; Sevenster et al., 2013; Merlo et al., 2014, 2015). However, data supporting this intervention as a viable treatment option are still lacking (Kroes et al., 2015; Giustino et al., 2016; Steenen et al., 2016; Villain et al., 2016).

BARs also regulate memory retrieval (Brown & Silva, 2004; Thomas, 2015), but the role of the noradrenergic system on memory retrieval is not well understood (Abel & Lattal, 2001; Chamberlain et al., 2006). Experimental manipulations that enhance NE activity have been typically shown to facilitate memory retrieval, and manipulations that inhibit NE release to impair retrieval. These effects were observed when memory retention was tested at least 24 hours after learning and drug manipulations were given prior to testing. Mice deficient for dopamine β-monoxygenase (enzyme which catalyzes the reaction of dopamine to NE) (DBM -/-) showed deficits in contextual and spatial memory; these deficits were recovered when mice were administered a BAR agonist (Murchison et al., 2004). Researchers also found that temporarily depleting NE in a rat's brain by injecting a DBM inhibitor resulted in impaired memory retention in a passive avoidance task when administered 30 minutes prior to test. This occurred after 1, 3, 5, or 7 days following the initial training (Hamburg & Cohen, 1973). In addition, Cohen and Hamburg (1975) were able to replicate their results, again producing amnesia for the passive avoidance task using propranolol. Animals were injected with propranolol 1 or 3 days following training, and memory retention was impaired when tested 2 hours after the injection. In rats, propranolol also led to deficits in spatial reference memory in the water maze (Ji et al., 2003), caused the disruption of retrieval of a cocaine-associated memory (Otis & Mueller, 2011), and abolished the expression of a cocaine conditioned place preference following co-blockade of β1-

and β2-ARs (Fitzgerald et al., 2016). However, in humans, propranolol given prior to a test of memory retrieval had no effect (Rimmele et al., 2016) and in rats, reversibly inactivating the LC with lidocaine had no effect on spatial reference memory retrieval (Khakpour-Taleghani et al., 2009). BARs may also mediate the reinstatement of previously extinguished memories since activation of BARs by NE has been shown to induce reinstatement of fear memories (Morris et al., 2005).

Relatively few studies have been specifically designed to assess the role of BARs in working memory. In rodents, Khakpour-Taleghani et al. (2009) showed that inactivation of the LC had no effect on spatial working memory. Administration of propranolol in rats also had no effect on working memory (Kobayashi et al., 1995; Ohno et al., 1997). In Rhesus monkeys, moderate doses of propranolol (0.01, 0.05 and 0.1 mg/kg) impaired spatial working memory, while a low dose (0.005 mg/kg) and high dose (0.5 mg/kg) had no effect (Wang et al., 2012a). In humans, a low (25 mg) dose of propranolol impaired numerical working memory in subjects with low arousal levels (Müller et al., 2005), and repeated administration of a high (160 mg) dose impaired working memory (Frcka & Lader, 1988). However, several other studies utilizing a moderate dose (40 mg) found no effect at all of propranolol on working memory in humans (Bodner et al., 2012; Becker et al., 2013; Ernst et al., 2016). Therefore, it appears that the role of the noradrenergic system on memory that has not been consolidated is still unclear.

Through the activation of the LC during important events, NE is involved in the acquisition of new information and therefore, plays a crucial role in the encoding of new memories. We hypothesized that the LC-NE system is involved in the recruitment of new neurons during memory encoding whereby post-encoding neuronal activity and plasticity promotes the consolidation of hippocampal dependent memory (Takeuchi et al., 2016). The recruitment of new neurons is part of the memory encoding process and tasks that involve memory retrieval require reactivation of the representations formed during encoding. When new information is encountered or contingencies in the environment are detected, these representations require

updating. We propose that activation of the LC-NE system, involved in the detection of novel and salient environmental imperatives involving spatially relevant information like changes in goal locations or reward contingencies in both the appetitive and aversive domain, can drive global remapping of these contextual representations in the HF.

1.12 Preface

In the first set of experiments we ran we sought to investigate the modulatory role of β-noradrenergic signalling in the HF on contextual memory representations and hippocampal plasticity using a molecular and behavioural approach. We specifically investigated how phasic and tonic LC activation could sculpt neuronal ensembles within the HF, which were tied to a specific episodic-like memory in rats. We hypothesized that NE release would cause the HF to recruit a unique population of neurons even in the presence of the same stimuli an animal had just encountered. This hypothesis was tested by examining global remapping in the HF using the compartmental expression of IEGs such as *arc* and *zif268* and the catFISH procedure. The activity history of individual HF neurons was mapped using this technique as animals engaged in spatial processing following manipulation of the LC-NE system.

In the second set of experiments, we assessed how plasticity associated changes affected behaviour following direct infusion of BAR agonists and antagonists into the HF. More specifically, if those representations involved in the encoding of an experience "remapped", meaning that rather than the cells that comprise the previously formed trace being reactivated, a new cellular ensemble was recruited instead, this should theoretically be detectable at a behavioural level e.g. as a memory retrieval error. Consistent with the idea of the LC-NE system as a network "reset" generator, one might assume that switching the system back to a state of encoding would prove maladaptive in a situation where memory retrieval was necessary to complete a task unless new information was at hand. We hypothesized that NE resets the memory system in such a way that it causes the HF to move from a state of retrieval back to a state of encoding when it is necessary, when novel information needs to be incorporated, an example of

this would be during reversal learning. This hypothesis suggests that the effect of modulating NE on memory will critically depend on the stage of training. To further understand how NE modulation of hippocampal circuits affects spatial working memory, we tested whether infusions of the BAR agonist isoproterenol would impair retrieval (i.e. switching the system back to encoding when it is maladaptive). We also tested how isoproterenol would affect spatial reference memory, hypothesizing that if isoproterenol did impair memory during testing, that the effect of activating BARs would subsequently promote cognitive flexibility thus improving reversal learning (i.e., switching the system back to encoding when it is adaptive).

2.0 Norepinephrine as a Memory Reset Signal: Phasic but Not Tonic Activation of the Locus Coeruleus Drives Remapping in the Hippocampus

2.1 Introduction

The HF is crucial in processing contextual information (Hirsh, 1974; O'Keefe, 1976; Phillips & LeDoux, 1992; Kim & Fanselow, 1992; Holland & Bouton, 1999; Fanselow, 2000; Burgess et al., 2001, 2002; Guzowski et al., 2001; Schmolck et al., 2002; Anderson et al., 2003; Rudy et al., 2004; Vazdarjanova & Guzowski, 2004; Smith & Mizumori, 2006; Acheson et al., 2012; Maren et al., 2013; Nees & Pohlack, 2014; Sadeh et al., 2014; Smith & Bulkin, 2014). Contexts are constantly and very rapidly (<30s) encoded during experience (Fanselow, 1990; Wiltgen et al., 2001; Guzowski et al., 2004; McHugh & Tonegawa, 2009; Pevzner et al., 2012) and these encoded representations are thought to be crucial to accurate memory and recall of information learned within each context. Moreover, although "context" typically refers to the physical location in which an event takes place (Holland & Bouton, 1999; Chawla et al., 2005; Maren et al., 2013), contexts relevant for memory can also include abstract components of experience that can encompass perceptions, emotions, socially relevant information, and learned contingencies related to appetitive or aversive outcomes (Maren et al., 2013).

The formation of contextual representations in the HF is driven by place cell activation, at least in rodents (Chawla et al., 2005; Ramirez-Amaya et al., 2005; Vazdarjanova et al., 2006; Rowland et al., 2011), which is, in turn, coupled to the initiation of second messenger systems and gene transcription (Miyashita et al., 2008). One of the first steps in long-term plasticity is the transcription of immediate early genes (IEGs) such as *arc/arg3.1* (Link et al., 1995), and *zif268/egr1* (Guzowski, 2002). Following transcription, IEG mRNA is rapidly transported outside of the nucleus to the dendrites for local storage, translation, and decay (Steward et al., 1998). Consequently, IEGs have been widely used as neuronal markers of activity. Due to the kinetics of IEG mRNA following transcription, the activity history of individual neurons can be tracked at two distinct time points. This is possible through the use a sensitive molecular protocol referred to as cellular compartmental analysis of temporal fluorescent *in situ* hybridization (catFISH) that exploits the distribution dynamics of IEG transcription (Guzowski et al., 1999; Guzowski & Worley, 2001).

The basis of this technique rests on the fact that following neuronal stimulation, the induction of IEG mRNA occurs in the nucleus; these transcripts then translocate to the cytoplasm after approximately 15 minutes targeting the dendrites and mRNA levels in the peri-nuclear cytoplasm subsequently return to basal levels after approximately 60 minutes (Guzowski et al., 1999, 2001; Guzowski & Worley, 2001). In experiments utilizing this protocol, animals are typically placed in an environment they are permitted to explore to activate place cell firing, which in turn drives IEG transcription. After 5 minutes of context exploration, animals are placed back in their home cage. Twenty-five minutes later, animals are given another context exposure for 5 minutes and then immediately sacrificed. Given the distribution dynamics of IEG transcription, cells active during the second exploration will still contain mRNA (e.g. *arc*) in the nucleus at the time of sacrifice. In contrast, cells that were active during the first context exploration will contain *arc* mRNA in the cytoplasm, while any cells that were active in both behavioural epochs will contain *arc* in both locations. Therefore, the sub-cellular localization of

arc visualized via fluorescent confocal microscopy allows for the neuronal populations activated by two distinct experiences to be discriminated and quantified (Guzowski & Worley, 2001).

This technique has demonstrated that IEG expression is induced in the HF in a context-dependent manner (Guzowski et al., 1999, 2001; Guzowski & Worley, 2001). When animals visit the same context twice, the vast majority of cells that express *arc* during the first behavioural epoch also express *arc* during the second exposure. In contrast, if two different locations are visited, the same cell is significantly less likely to express *arc* during both explorations, an observation that is consistent with unit recordings showing that each location will recruit a unique cell population to express place fields. This effect does not disappear or habituate following repeated context presentations when the experiences are sufficiently spaced over time (Guzowski et al., 2006). That is, *arc* induction begins to diminish only after four exposures to the same context in rapid succession (i.e. each separated by 25 minutes). However, when the animal is presented with a new environment, even after nine exposures to the same context, *arc* transcription is rescued. The fact that *arc* induction is not easily disengaged when an animal is presented with familiar stimuli suggests that it does not distinguish between new learning and memory retrieval (Miyashita et al., 2008). This effect is also consistent with electrophysiology studies involving place cell remapping. Remapping describes a phenomenon where place cells can alter their firing patterns in response to environmental changes (Muller & Kubie, 1987). Global remapping occurs when the positon of place fields shift (e.g. cell fires when animal is in location A in context X, same cell does not fire when animal is in location A in context Y, but now fires when animal is in location B in context Y). It is specifically this property that emphasizes the multi-representational nature of the HF (Colgin et al., 2008). Using the catFISH protocol, remapping is observed when an animal visits two different contexts and quantified by visualizing two different neuronal ensembles recruited to activate *arc* in these contexts. Rate remapping refers to when places don't necessarily alter their place fields but instead change their rate of firing. In this experiment, remapping refers to "global" rather than "rate" remapping.

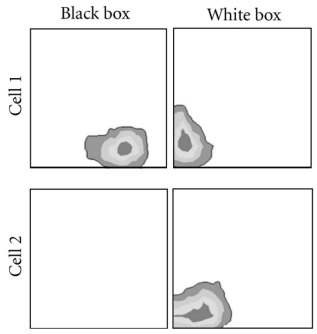


Figure 10. Examples of remapping from two cells in CA1, as the context was changed from black to white, cell 1 remapped by changing the location of its firing field, while cell 2 remapped by switching its field from off to on. Reprinted from Neural Plasticity 2011:182602, Jeffery KJ, Place cells, grid cells, attractors, and remapping, 1-11., Copyright (2011), open access article distributed under the Creative Commons Attribution License.

The way in which contextual representations change or remap globally is not well understood. We hypothesize that remapping may involve activation of the locus coeruleus (LC), a brain stem structure long implicated in novelty detection and the regulation of arousal, behavioural flexibility and attention (Aston-Jones et al., 1999). The LC sends a major norepinephrine (NE) projection to the HF (Jones et al., 1977; Loughlin et al., 1986a; Harley, 1987, 2007a; Lee et al., 1993) and activity in LC neurons is associated with cognitive shifts in attention (Sara, 2009; Hagena et al., 2016). Furthermore, the LC is exquisitely sensitive to changes in both appetitive and aversive stimulus contingencies (Vankov et al., 1995; Berridge & Waterhouse, 2003; Harley, 2007a; Aston-Jones & Waterhouse, 2016), and exhibits remarkable plasticity as a function of these changes (Sara & Segal, 1991). Surprisingly, no studies that we are aware of have examined the properties of place cells in the HF while simultaneously manipulating LC neurons. We hypothesize that activation of the LC causes a disengagement from established representations and the recruitment of new representations towards an enhancement of processes that promote the incorporation of new information (Bouret & Sara, 2005; Harley, 2007a).

This hypothesis was inspired by several observations. In 1970, Seymour Kety proposed that biogenic amines could facilitate memory through the enhancement of neuronal activity during affectively important events (Kety, 1970; Harley, 1987). Twenty years later, in crustacean

neural networks, it was observed that network dynamics could undergo a spontaneous shift hypothesized to be the result of neurmodulatory influence (Hooper & Moulins, 1989). This phenomenon led Bouret & Sara (2005) to hypothesize that activation of the LC-NE system induces a "reset" in its target structures, by interrupting existing functional networks and facilitating the emergence of new ones. According to the network reset hypothesis, activation of the LC-NE system should promote adaptive responses during important events and support learning during changes in contingency (Bouret & Sara, 2005). In our own experiments, we have interpreted "resetting of a neural network" to include remapping of contextual representations in the HF. From a mechanistic standpoint, we hypothesize that this occurs via NE release (Walling & Harley, 2004; Walling et al., 2004) and increased excitability (Lacaille & Harley, 1985; Harley & Milway, 1986; Stanton & Sarvey, 1987; Harley et al., 1989; Klukowski & Harley, 1994; Kitchigina et al., 1997; Brown et al., 2005; Mather et al., 2016) in the HF.

Evidence for this comes from work demonstrating that LC activation induces plasticity in the HF. LC neurons elicit tonic firing patterns (1-5Hz), and also respond with phasic, burst firing patterns (2 or 3 spikes, 10-15Hz, followed by a 200-500ms pause) (Aston-Jones & Bloom, 1981) which are associated with the detection of environmental stimuli. Activation of LC-NE by novel objects or contexts produces brief phasic firing followed by inhibition, which induces transient input along the perforant path (PP) from the entorhinal cortex (EC) to the dentate gyrus (DG) of the HF thereby enhancing DG synaptic transmission via disinhibition (Harley & Sara, 1992; Klukowski & Harley, 1994; Brown et al., 2005). Repeated electrical stimulation of the LC resulted in stable NE signals measured by voltammetry (Yavich et al., 2005) and elevated levels of NE can increase somatic and dendritic excitability in the DG (Lacaille & Harley, 1985; Stanton & Sarvey, 1987; Harley, 1998; Hagena et al., 2016) as well as the CA1 and CA3 regions of the HF (Mueller et al., 1981; Heginbotham & Dunwiddie, 1991; Dunwiddie et al., 1992; Jurgens et al., 2005b) effects which are all mediated by β-noradrenergic receptors BARs (Kitchigina et al., 1997) and which can persist for 24 hours (Walling & Harley, 2004).

Intra-LC glutamate infusions cause phasic activation (Page & Abercrombie, 1999; Palamarchouk et al., 2000, 2002; Dunn & Swiergiel, 2008) producing the same firing pattern (brief burst ~200 msec followed by hyperpolarization lasting 200-500msec). Previous work has shown that these infusions also produce short and long-lasting potentiation of PP-evoked spike amplitude in the DG (Harley & Milway, 1986; Harley & Sara, 1992). The effects in the DG observed after LC glutamate resemble those produced by direct application of NE in vitro (Lacaille & Harley, 1985; Stanton & Sarvey, 1987) or in vivo (Neuman & Harley, 1983; Winson & Dahl, 1985) and depend specifically on BARs. Likewise, glutamatergic activation of the LC also potentiates long-term potentiation (LTP) in the DG (Harley & Sara, 1992; Klukowski & Harley, 1994) and CA3-CA1 synapses (Takeuchi et al., 2016) via activation of BARs (Walling & Harley, 2004). BAR antagonists timolol and propranolol infused locally (Harley and Evans, 1988) or systemically (Harley & Milway, 1986; Harley et al., 1989; Babstock & Harley, 1992; Walling & Harley, 2004; Walling et al., 2004) have been shown to block this potentiation. Both phasic and tonic LC activity are equally capable of inducing downstream learning-facilitated plasticity effects such as LTP (Bliss et al., 1983; Neuman & Harley, 1983; Gray & Johnston, 1987; Hopkins & Johnston, 1988; Walling et al., 2004, 2011; Walling & Harley, 2004; Almaguer-Melian et al., 2005; Lashgari et al., 2008; Lim et al., 2010; Reid & Harley, 2010; Hagena et al., 2016), long-term depression (LTD) (Lemon et al., 2009; Lemon & Manahan-Vaughan, 2012; Hansen & Manahan-Vaughan, 2015a, 2015b) and NE release in the DG (Dahl & Winson, 1985; Harley & Milway, 1986; Harley et al., 1989; Babstock & Harley, 1992; Frizzell & Harley, 1994; Klukowski & Harley, 1994; Walling et al., 2004; Lemon et al., 2009). Furthermore, other pharmacological agents such as orexin A (Walling et al., 2004), the cholinergic agonist carbamyl-β-methyl choline chloride (bethanechol) (Berridge & Foote, 1991; Berridge & Abercrombie, 1999), and corticotropin releasing factor (CRF) (Palamarchouk et al., 2000, 2002; Curtis et al., 1997; Page & Abercrombie, 1999) mimic tonic activation of the LC lasting up to 30 minutes, and contribute to increased levels of NE in the HF (Figures 11-13). For instance, intra-

LC infusions of orexin A resulted in a ~200% increase in NE in the HF (within 20 minutes following LC activation) measured by microdialysis (Walling et al., 2004).

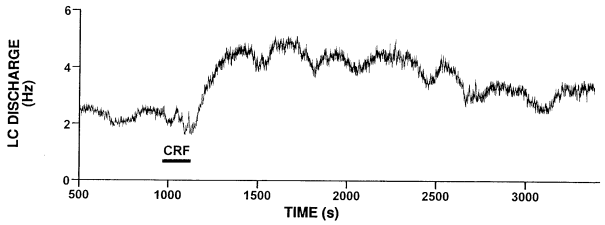


Figure 11. Effects of CRF administered by microinfusion into the LC on LC discharge rate. LC discharge rate began to increase immediately after the termination of the infusion, peaked at 200 sec after infusion, and remained elevated for 30 min. Reprinted from The Journal of Pharmacology and Experimental Therapeutics, 281, Curtis AL, Lechner SM, Pavcovich LA, & Valentino RJ, Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity, 163-172, Copyright (1997), with permission from Aspet.

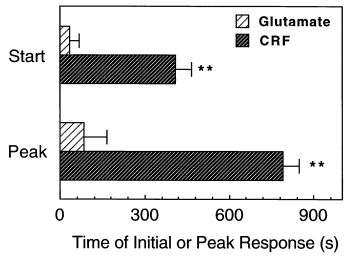


Figure 12. Summary of the time parameters of the hippocampal NE responses to the LC infusion of CRF and glutamate. Reprinted from Brain Research Bulletin 51(4), Palamarchouk VS, Zhang J, Zhou G, Swiergiel AH, & Dunn AJ, Hippocampal norepinephrine-like voltammetretic responses following infusion of corticotropic-releasing-factor into the locus coeruleus, 319-326, Copyright (2000), with permission from Elsevier.

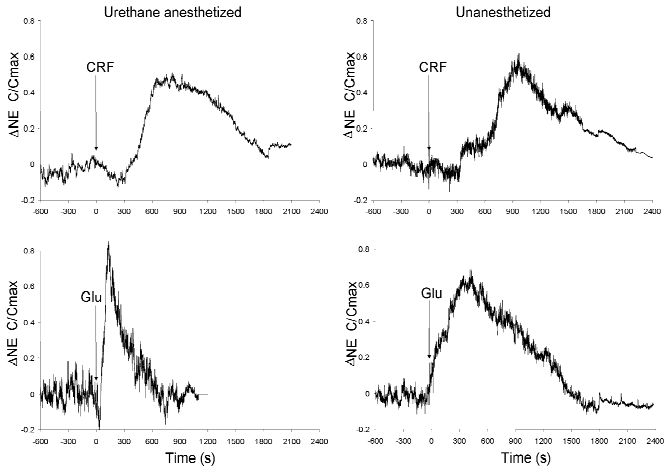


Figure 13. Hippocampal noradrenergic chronoamperometric response following infusions of CRF and glutamate in anesthetized and un-anesthetized rats. Reprinted from Brain Research 950(1-2), Palamarchouk VS, Swiergiel AH, & Dunn AJ, Hippocampal noradrenergic responses to CRF injected into the locus coeruleus of unanesthetized rats, 31-38, Copyright (2002), with permission from Elsevier.

Viewing the LC-NE system as a potential mechanism for resetting cognitive systems does not disentangle phasic from tonic activation, and from the perspective of plasticity promotion, Bouret & Sara (2005) believed both phasic and tonic LC activation could reset a neural network. In each sub-region of the HF, neuronal ensembles are modified by novel input and environmental change. Our central hypothesis is that the both phasic and tonic activation of the LC-NE system may underlie the mechanism by which remapping occurs. We believe that both phasic and tonic activation of the NE pathway from the LC to the DG can act as a modulatory switch recruiting new neurons to create new contextual representations when necessary and likely updating existing representations in the presence of new information (e.g. environmental imperatives, stimulus-contingency changes, reversal learning) promoting learning when behavioural changes are necessary. We propose that the LC-NE system underlies these modifications in such a way that induces plasticity and supports new successful behaviours. The compartmental expression of immediate early genes such as *arc* and *zif268* allowed us to test this hypothesis by mapping the activity history of individual HF neurons as animals engaged in spatial processing following perturbation of the LC-NE system.

2.2 Materials & Method

2.2.1 Subjects & Handling

The current experiments included a total of 108 rats separated into two groups. The first group, used for Experiment 1, consisted of 43 male Sprague Dawley (SD) rats. The second group of rats consisted of 65 male Long-Evans (LE) rats and were used for Experiment 2. Both groups of rats were approximately two months of age at the start of the experiments weighing in the range of 250-300g. SD rats were obtained from the Vivarium Breeding Facility (St. John's, NL) and the LE rats were obtained from Charles River Laboratories (Montreal, QC). All rats were socially housed (2 rats per cage) before surgery and individually housed after surgery. They were kept in a colony room controlled for humidity and temperature, on a reversed 12:12 hour

light/dark cycle with (lights ON 7:00pm). Animals were given *ad libitum* access to food and water and were weighed twice a week. Each rat was handled for 15 minutes a day for 6 days in the colony room under a red light. SD rats were handled for 6 days and then underwent surgery and allowed to recover relatively undisturbed for 4 days. LE rats were handled for 2 days, then underwent surgery, and were handled for an additional 4 days during recovery. All procedures were approved by the Memorial University Institutional Animal Care Committee and carried out in accordance with the guideline provided by the Canadian Council on Animal Care.

2.2.2 Surgical Procedure

Rats underwent implantation of a unilateral (Experiment 1) or bilateral (Experiment 2) 26-gauge guide cannula (Plastics One, Roanoke, VA). For experiment 1, SD rats were anesthetized with a chloral hydrate solution (1ml/100g, PCCA, intraperitoneal). The chloral hydrate solution was prepared at a concentration 40mg/ml in sterile saline (0.9% NaCl). Following administration, rats were undisturbed until unresponsive to a reflexive tail pinch. For experiment 2, LE rats were deeply anesthetized with 5% isoflurane and 70% oxygen (induction) and maintained at a level of 2-3% isoflurane for the duration of the surgery. Once anesthetized, the rat's head was shaved and a sub-cutaneous (s.c.) injection of meloxicam (0.2mg/kg, Sigma-Adrich) was administered at the base of the neck for general analgesia. Meloxicam was prepared at a concentration of 0.25 mg/ml in sterile water. The rat was then anchored in a stereotaxic frame with ear bars to ensure a flat skull surface and prepped for aseptic surgery. Cannulae and obdurators were autoclaved prior to implantation. A midline incision was made on the scalp from anterior to bregma to the base of the skull; the periosteum membrane was cleared and then skull flat position was confirmed. Each rat was implanted with cannulae (7.7mm in length, Plastics One, Roanoke, VA) aimed at the LC (AP -11.8mm, ML +/-1.3mm, DV-5.5 from dura, relative to Bregma) angled 20 degrees from the vertical plane, and anchored by four jeweler's screws and dental acrylic cement mixed with gentamycin (Cellgro, Manassas, VA). The angle was important to avoid a sinus, which when hit can cause excessive bleeding. At the end of the surgery,

obdurators that extended 1mm below the pedestal were screwed into the cannulae to ensure patency. Animals that received unilateral implants were implanted only on the left side. Rats were allowed 4 days for recovery undisturbed except for weighing and to administer 0.1mg/kg of Meloxicam (s.c.) each morning at the base of the neck as an analgesic.

2.2.3 Experimental Procedure

2.2.3.1 Environmental Exploration

After a 4-day post-surgical rest period, each rat was exposed to environment A - a 63cm X 63cm X 35cm box surrounded by black curtains in a dimly lit room (Figure 14). The box featured black walls with a white floor divided into 9 equal sections by small black cross marks. Each rat was placed in the center section of the box for a 5-minute environmental exposure. At 15-second intervals, the rats were picked up and placed randomly in different sections (facing different directions) of the box to ensure that all sections were sampled equally. This was done to maximize the number and consistency of place cells activated driving *arc* expression (Guzowski et al., 1999). Following the first 5-minute exposure, the rats were transported back to the colony room for a 20-minute rest period. The rats were then transported back to either environment A if they were in the A/A (familiar) groups or environment B if they were in the A/B groups (novel). Environment B was a 63cm X 63cm X 35cm box placed within a bright room with no curtains. The box featured black walls with a black floor divided into 9 equal sections by small white cross marks. On each wall of the box was an 8”x10” sheet of vertical black and white bars (Figure 14). The second context exposure also lasted for 5 minutes with random placements every 15 seconds. Immediately after exposure, rats were briefly anesthetized with isoflurane, decapitated, and brains were quickly extracted (< 2min). Brains were blocked prior to histology such that the brainstem was separated from the forebrain. Both sections were flash frozen in isopentane and placed at -80°C to preserve the integrity of IEG mRNA. Forebrain blocks were then packaged in dry ice and

shipped to Wilfrid Laurier University for further processing. Brainstem blocks remained at Memorial University for histological verification of LC drug delivery.

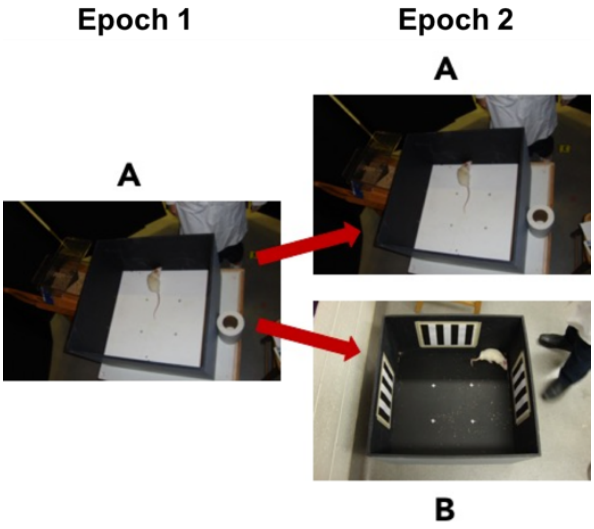


Figure 14. Environmental exploration: Animals were placed in context A for 5 minutes and then back in the home cage for a 20-minute rest period after which they were either placed back in the *familiar* context A (A/A condition), or in the *novel* context B (A/B condition) for 5 minutes. Contexts differed in terms of location and discrete intra- and extra-box cues. IEG expression in the cytoplasm was matched to Epoch 1 and in the nucleus to Epoch 2.

2.2.4 Cage Control and MECS Animals

To serve as a negative control, cage control (CC) SD rats remained in their home cages in the colony room until sacrifice. They received the normal daily handling prior to the sacrifice day. For the LE rats a handling CC condition was employed. On the test day, LE rats were picked up from their home cages under red light in the colony room every 15 seconds a for 5-minute period and this was repeated 20 minutes later to match the experimental open field groups. Immediately after the second 5-minute home cage handling event, decapitation and brain extraction was performed as it was in the other groups. A group of rats in both experiment 1 (n=5) and experiment 2 (n=8) received maximal electroconvulsive shock (Cole et al., 1990); data for these rats is not reported as their purpose was to serve as a positive control for IEG signalling during *in situ* hybridization and confocal imaging.

2.2.5 Infusions

Immediately prior to the second context exposure animals were either unilaterally (Experiment 1) or bilaterally (Experiment 2) infused with a specific drug (see groups below). The infusions took place just outside the testing room where the rat was placed in a small bucket on a transport cart. Obdurators were removed and the infusion cannula(e) (33 gauge, projecting 1mm below the pedestal; Plastics One, Roanoke, VA) connected via PE-50 tubing to two 1-µL gastight Hamilton syringes mounted onto a microfluidic infusion pump (New Era Pump Systems Inc., Farmingdale, NY, Model: NE-1002X) was inserted into the guide cannula(e). The thin tubing was filled with sterile water and a 0.2 µL air bubble was introduced to the tubing and then subsequently the drug solution was drawn up to the maximum capacity of the syringe. The desired volume of drug was infused over a 30 second period. For example, if 0.5µL of a drug was infused, the infusion rate was set to 1µL/min. The infusion cannula was left in place for 1 minute post-infusion to ensure that the liquid had diffused from the injection site. Obdurators were then replaced and animals were placed in the second environment.

2.2.6 Drug Treatments and Groups

Experiment 1. Animals were assigned to either A/A or A/B context exposure conditions or remained in their home cages (CC). Rats in both A/A and A/B groups either received an infusion of L-glutamate (0.2 µL at a concentration of 500 mM) in the LC or they received no infusion. Therefore, there were six groups: GLU-A/A (n=9), NO-GLU-A/A (n=6), GLU-A/B (n=8), NO-GLU-A/B (n=6), and CC (n=9) and MECS (n=5). All drugs were mixed to the desired concentration with 1% methylene blue to allow for localization of the drug delivery event histologically.

Experiment 2. Given the preliminary data that was obtained from experiment 1, we decided to run a second experiment with bilateral infusions to see if our effect would be greater if infusions were performed on both hemispheres. In this experiment, we also decided to include

more control groups and explore the effects of additional pharmacological treatments. There were 10 groups. All groups were assigned to the A/A context exposure condition except one group, which was assigned to the A/B condition. The 1st group GLU-AA (n=6) received bilateral infusions of L-glutamate in the LC. We reduced the dose to 0.2µL of a 200mM solution of glutamate from the first experiment. The 2nd group underwent bilateral implantation of guide cannulae aimed at the LC but did not receive any drug infusions during testing; this was our surgery control group NO-DRUG-A/A (n=6). The 3rd group was a vehicle control group that was given an infusion of artificial cerebral spinal fluid aCSF-AA (n=6) since aCSF served as the solvent for making the other drug solutions. The 4th group was an additional cage-control group that received handling in the home cage similar to the handling that occurred during testing (see above) CCH (n=6). The 5th and 6th groups were also control groups that received infusions of clonidine (0.2µL of a 3.75mM solution). Since NE exerts feedback inhibition of its own release through an interaction with presynaptic α-2 adrenergic inhibitory autoreceptors in the LC (Taylor et al., 1988; Washburn & Moises, 1989) clonidine, which is an α-2 adrenoceptor agonist, serves to inhibit LC neurotransmission. Clonidine infusions were given to animals in both exploration conditions: CLON-AB (n=9) to assess whether we could block remapping and CLON-AA (n=6) as a control. Finally, we decided to include three groups that received infusions targeted at increasing tonic versus phasic LC discharge. While glutamate simulates phasic activation of the LC, orexin A, bethanechol, and CRF increase tonic LC activity (Valentino & Foote, 1988; Berridge & Foote, 1991; Berridge & Abercrombie, 1999; Page & Abercrombie, 1999; Palamarchouk et al., 2000, 2002; Walling et al., 2004; Jedema & Gracce, 2004; Reyes et al., 2008; Snyder et al., 2012). The 7th group (ORX-AA, n=6) received infusions of orexin A (Sigma Alrdich), a highly excitatory neuropeptide given at a dose of 0.2µL of a 100nM solution. The 8th group (BETH-AA, n=6) received infusions of bethanechol (Sigma Aldrich), a drug that selectively stimulates muscarinic receptors at a dose of 0.2µL of a 20mM solution. Finally, the 9th group (CRF-AA, n=6) received infusions of CRF (Sigma Aldrich), a peptide involved in the

mammalian stress response at a dose of 0.3µL of a 100nM. The 10th group consisted of MECS animals (n=8). Artificial cerebrospinal fluid (aCSF) was mixed as a solvent (147mM NaCl, 3mM KCl, 1mM $MgCl_2$, and 1.3mM $CaCl_2$) in sterile water. All other drugs were dissolved in a 1% methylene blue solution made with aCSF to produce a dark blue colour. This was necessary for later verification of cannula placements histologically.

2.2.7 Histological Procedures

Cannula placements and drug diffusion sites were confirmed histologically at the end of the experiment (Figure 16). For the LC, sagittal sections (30µm) were cut on a using a cryostat (Leica, CM3050) and mounted to gelatin-subbed slides. Two sets of slides were taken in alternation, one to localize the position of the dye and visualize the total area where the drug diffused, and the other to Nissl stain using cresyl violet to determine the location of the LC and verify cannula placements. It was necessary for the dye and the LC to overlap for the animal to be included in the study. If the dye and LC were further than 300µm apart from each other it was considered a miss and the animal was removed from the study. For the hippocampus region, using Tissue-Tek OCT compound (Fisher Scientific), blocks were created containing one hemisphere of 8 brains, including negative and positive controls on each block. Coronal sections (20um) were cut using a cryostat (Leica, CM3050), thaw-mounted onto Superfrost-Plus slides (VWR) coated with 3-triethoxysilylpropylamine (TESPA), dried, and stored at -80°C until further processing.

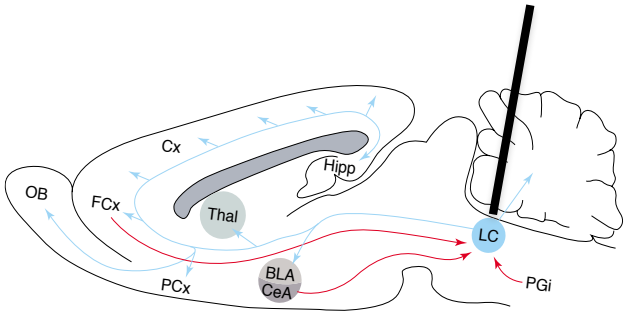


Figure 15. Schematic demonstrating cannula placement in the LC (blue circle) and the noradrenergic projections to downstream regions in the brain including the hippocampus (blue). Reprinted from Trends in Neurosciences 28(11), Bouret S & Sara SJ, Network reset: A simplified overarching theory of locus coeruleus noradrenaline function, 574-582., Copyright (2005), with permission from Elsevier.

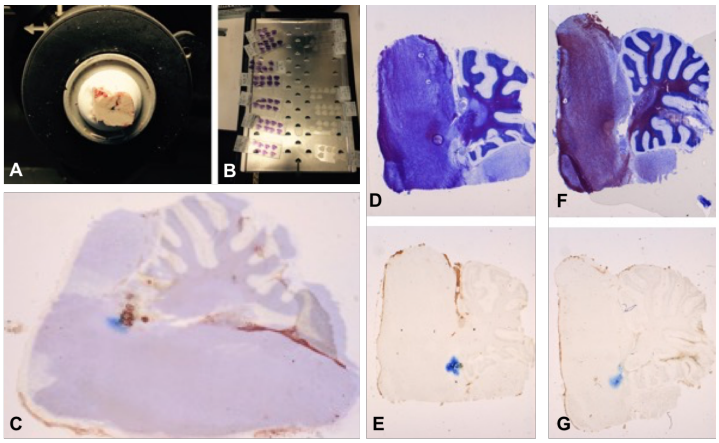


Figure 16. Cannula placements and drug diffusion sites were confirmed histologically A) 1% Methylene blue dye was injected into the LC and visualized following sectioning (sagittal sections). B) Sections were mounted to gelatin-subbed slides. Two sets of slides were taken in alternation, one to localize the position of the dye and visualize the total area (in µm) where the drug diffused, and the other to Nissl stain using cresyl violet to determine the location of the LC and verify cannula placements. C-G) It was necessary for the dye and the LC to overlap for the animal to be included in the study If the dye and LC were further than 300µm apart from each other it was considered a miss and the animal was removed from the study.

2.2.8 Animals Removed

Experiment 1. Four animals were categorized as misses (two from GLU-AA and two from GLU-AB) and removed as a result. One of the GLU-AB animals was also removed due to illness. Experiment 2. Two animals from experiment 2 in the CLON-AB group were also removed as they were infused with the wrong concentration of clonidine. Due to a freezer malfunction the tissue from 1-3 animals was destroyed in each of the following groups BETH-AA, ORX-AA, CRF-AA, CLON-AA, aCSF-AA, NO-DRUG-AA, and CCH. The final N-values for all groups in all brain regions are depicted in Table 1.

Table 1.

Experiment 1 & 2: Number of animals in all groups across hippocampal sub-region

Drug Treatment	Context Exposure	Group	n-value (DG)	n-value (CA1/CA3)	Group Total
Glutamate (Bilateral)	A/A	FPB	6	6	6
Glutamate (Unilateral)	A/A	FPU	7	7	7
aCSF	A/A	F	5	CA1:4; CA3:3	21 (DG) 16 (CA1/CA3)
NO-DRUG (Surgery)	A/A	F	5	4	
Clonidine	A/A	F	5	3	
NO-DRUG (No Surgery)	A/A	F	6	6	
Bethanechol	A/A	FT	5	3	15 (DG) 9 (CA1/CA3)
Orexin A	A/A	FT	5	3	
CRF	A/A	FT	5	3	
Clonidine	A/B	NI	7	7	7
NO-DRUG (No Surgery)	A/B	N	6	6	11
Glutamate (Unilateral)	A/B	N	5	5	
NO DRUG (Cage Control - Handled)	HC	HF	5	5	5
NO DRUG (Cage Control - Undisturbed)	HC	CC	9	9	9

2.2.9 Fluorescence in situ Hybridization

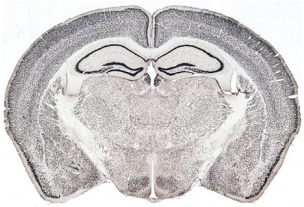
Fluorescence *in situ* hybridization was performed as previously described (Guzowski et al., 1999). Briefly, full length *arc* riboprobes were synthesized using a commercial transcription kit (MaxiScript; Ambion) and RNA labeling mixes (Roche Molecular Diagnostics), verified by electrophoresis. Slides were thawed to -20°C overnight and then to room temperature 1hr before processing. They were fixed in 4% paraformaldehyde (5 min), washed in 2x saline-sodium citrate (SSC) (2 min), and treated with 0.5% acetic anhydride (10 min). Next, they were dipped in deionized water, placed in a methanol / acetone (1:1) solution (5 min), and in 2x SSC (5 min). Slides were then incubated with pre-hybridization buffer (Sigma-Aldrich) for 1hr at room temperature and then overnight (16-18hrs) at 56°C with riboprobe mixed in hybridization buffer (1:50). The following day, the tissue was treated with a series of 2x SSC washes and then in an RNase A / 2x SSC solution (10 mg/ml) at 37°C for 30 min. This was followed by a series of 0.5x SSC washes including 30 min at 55°C. Endogenous peroxidases were then quenched with a 2% H_2O_2 (in 1xSSC) solution. The tissue was blocked with TSA blocking buffer (Perkin Elmer) containing normal sheep serum (0.5%), and incubated with anti-digoxigenin- horseradish peroxidase (HRP) antibody (Roche Molecular Diagnostics) in TSA blocking buffer (1:400) for 2 hours at room temperature. Slides were washed in 0.1M Tris-buffered saline with 0.05% Tween 20 and HRP antibody conjugates were detected using CY3 (TSA kit, Perkin Elmer). The final step involved counterstaining the nuclei with DAPI (Sigma-Adrich), sealing with buffered glycerol (with anti-fade) and cover slipping. Slides were then placed in the fridge for storage.

2.2.10 Image Acquisition and Analysis

Images were collected from coronal sections of the hippocampus (range: AP: -2.5 to -4.2) relative to Bregma, (Paxinos & Watson, 2013), including the suprapyramidal blade of the dentate gyrus (DGS), the CA1 medial (CA1distal) and lateral (CA1proximal) regions, and the CA3a, CA3b, and CA3c sub regions. (Figures 17A-B), using an Olympus FV1000 confocal microscope

at 40x magnification. We decided not to look at the infrapyramidal blade of the DG since several studies (e.g., Chawla et al., 2005; Marrone et al., 2012; Gheidi et al., 2013) have previously demonstrated sparse, but environmentally-specific, IEG expression following behavioural experience is largely restricted to the suprapyramidal blade. For experiment 1, for each animal, two z-stacks (~1.0um optical thickness, step size 0.8um) were collected from 2-3 different slides yielding 4-6 total stacks per region, and images were collected only from the left hemisphere. For experiment 2, one z-stack (~1.0um optical thickness, step size 0.8um) was collected from each hemisphere on 2-3 different slides yielding 4-6 total stacks per region. Data from each hemisphere was then pooled. For each slide, acquisition parameters were kept constant. The median 20% of neurons in each stack was quantified using MetaMorph software (Molecular Devices, Sunnyvale, CA). Neurons and glial cells were differentiated by size and patterns of chromatin staining, and neurons in the CA1 and CA3 were classified as *arc*-negative (neg), *Arc*-positive within the nucleus (foci), *arc*-positive within the cytoplasm (cyto), and *arc*-positive within both the nucleus and the cytoplasm (double) (Figure 18). For the DG, *zif268* was quantified instead of *arc* because *arc* undergoes sustained transcription in the DG following context exposure (Ramirez-Amaya et al., 2013; Maple et al., 2017). Within each animal, an average of 577.44 (SD = 122.72) granule cells were counted in the DG, and 554.19 (SD = 110.46) / 1299.95 (SD = 341.46) pyramidal cells in the CA1/CA3 respectively. Cells found to be *arc*- or *zif268*-positive in the cytoplasm were engaged in transcription 30-45 min prior to decapitation (first behavioural epoch) while cells found to be *arc*- or *zif268*-positive in the nucleus were engaged in *arc* transcription 5 min before (second behavioural epoch).

A



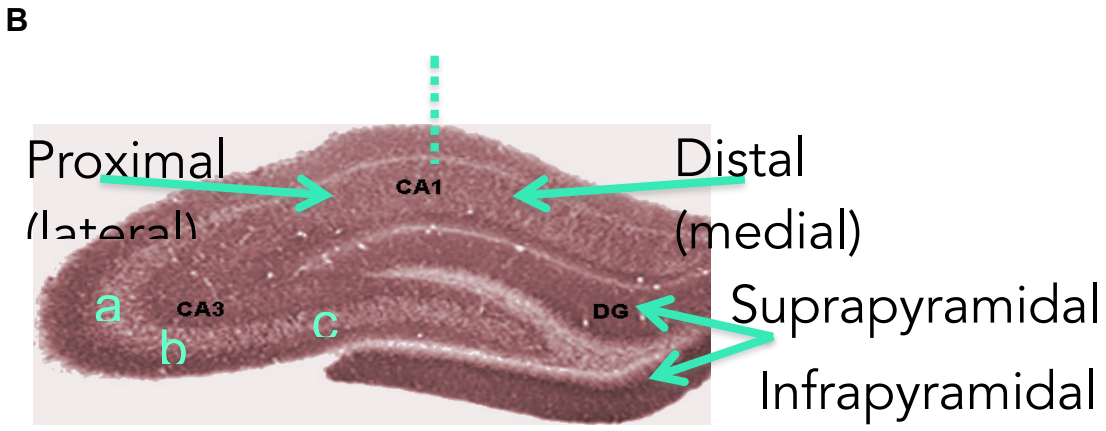


Figure 17. A) Coronal slice of a rat brain showing the location of the hippocampus. B) Close-up of the hippocampus demonstrating anatomically how we labeled and counted cells following imaging.

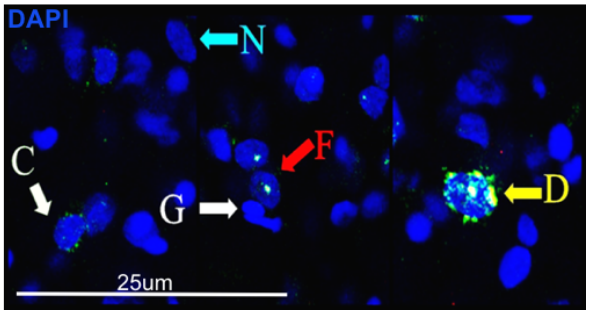


Figure 18. Representative image (scale bar 25 m) of the different IEG localization labels. Cell nuclei are counterstained with DAPI (blue) providing a histological marker of those neurons that were engaged in transcription during Epoch 1 [containing cytoplasmic signal (C, green ring)], Epoch 2 [containing intranuclear foci (F, green dot in the center)], or both Epochs (D, green ring plus dots in the center). Granule cells and glial cells (G, densely-coloured cell) were differentiated and cells with no IEG expression (N) were also counted.

2.2.11 Statistical Analyses

Statistical analyses were conducted using SigmaPlot™ version 11.0 (Systat Software, San Jose, CA). They consisted of two-way repeated measures (RM) analysis of variance (ANOVA), except for total IEG expression which was examined with the use of one-way ANOVAs across groups. For all analyses, alpha was set at 0.05, and post-hoc tests (Tukey's HSD) were conducted when necessary. Each brain region was analyzed separately. Experiment 1&2: Mixed model two-way RM ANOVAs were conducted across groups (between-subject factor: GROUP) on the number of cells that were labeled and counted as IEG-positive solely within (FOCI), or outside (CYTO) the nucleus, as well as within both the nucleus and the

cytoplasm (DOUBLES) – (within-subject factor: IEG-LOCATION) or on total IEG expression within each behavioural epoch (within-subject factor: EPOCH). For experiment 2, several groups were combined following two-way RM ANOVAs to determine that these groups were not different from each other, followed by additional two-way RM ANOVAs on the resulting groups.

2.3 Results

2.3.1 Experiment 1: Unilateral Infusions

2.3.1.1 Total IEG Expression & Context Exploration

Consistent with previous reports (Guzowski et al., 1999; Marrone et al., 2011), we observed significantly higher levels of IEG expression in CA1 (M=53.9%, SD=21.58%) compared to CA3 (M=26.82%, SD=11.73%), and very low expression in DGS (M=5.79%, SD=2.32%; see Figure 19A-F). IEG expression was driven by context exploration (Figure 19A-F) and was not influenced by the presence of a novelty given that comparable numbers of cells expressed IEGs across behavioural epochs in both A/A and A/B groups (Figure 20A-F; Table 1).

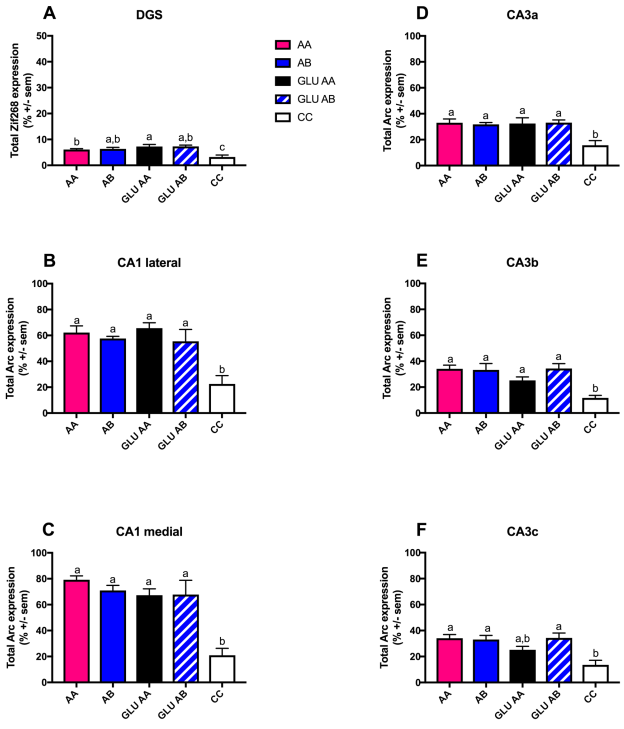


Figure 19. A-F) Total IEG expression across groups for each brain region was driven by context exploration. Letters (a,b,c) denote significant differences ($p < 0.05$) between groups.

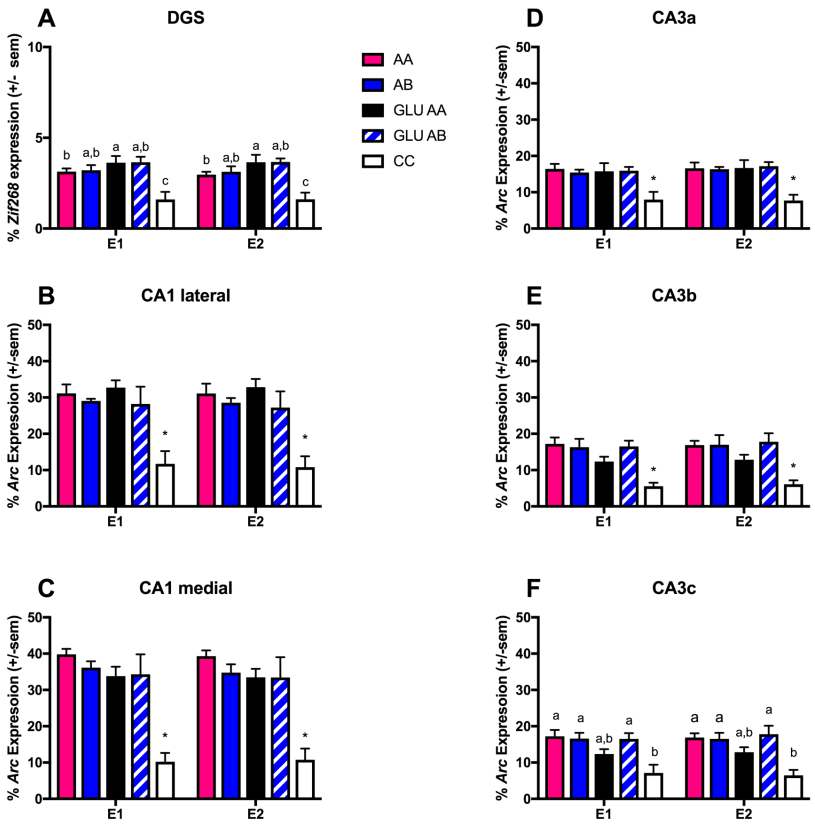


Figure 20. A-F) Total IEG expression was similar across both behavioural epochs (first and second context exposures) in each brain region and higher during context exploration compared to spending the same amount of time undisturbed in the home cage. E1 (Epoch 1): proportion of cells labeled as IEG-positive solely within the cytoplasm (CYTO) plus the cells labeled within both the nucleus and the cytoplasm (DOUBLES); E2 (Epoch 2): proportion of cells labeled as IEG-positive solely within the nucleus (FOCI) plus the cells labeled within both the nucleus and the cytoplasm (DOUBLES). Letters (a,b,c) denote significant differences ($p < 0.05$) between groups. Where there is only one group that is significantly different (B-E) an asterix ($p < 0.05$) is used instead.

2.3.1.2 Environment-Specific IEG Expression and Remapping in DGS

To assess whether perturbation of the LC-NE system could alter contextually-mediated IEG expression in the HF we examined the differential expression of cells labeled FOCI, CYTO and DOUBLES across groups for each HF sub-region. In the DGS, there was a main effect of GROUP ($F_{4,28} = 9.626$, $p < 0.001$), a main effect of IEG_LOCATION ($F_{2,56} = 4.534$, $p = 0.015$), and a significant GROUP x IEG_LOCATION interaction ($F_{8,56} = 5.362$, $p < 0.001$). Post-hoc tests showed that this effect was partially due to differences between the CC group and the GLU-AA ($p < 0.001$) group as well as between the CC group and the NO-GLU-AA group ($p < 0.001$) demonstrating simply more cellular activity in animals that explored an environment compared to

animals that remained in the home cage (Figure 21). This was expected since transcription of *zif268* is observed in very few cells in rats that remain undisturbed in their home cages (baseline cage control). There were also more cells labeled FOCI and CYTO in animals that visited two different contexts compared to those that repeatedly visited the same context. Additionally, a higher proportion of cells were labeled CYTO and FOCI in the GLU AA group compared to the NO GLU AA group suggesting that glutamate is inducing IEG transcription in new cells (FOCI signal) while simultaneously affecting the cells in which transcription has already been engaged (CYTO signal). This is explored further in experiment 2.

Unexpectedly, contexts were not well discriminated in the DGS. While there were fewer cells labeled as DOUBLES in the groups that visited two different contexts compared to the same context twice, there was no statistical difference in the pattern of IEG expression. Because there is such sparse firing in the DGS (~2-5%), we expect to find only small differences in a raw measure like DOUBLES and in turn, statistically analyzing this measure does not adequately convey whether neuronal ensembles activated in epoch 1 are discrete from epoch 2. It is possible that this measure is not sensitive enough. Several other studies using the catFISH protocol have utilized the calculation of single normalized measures (SIMILARITY & OVERLAP) (Vazdarjanova & Guzowski, 2004; Marrone et al., 2011) derived from the raw staining class data (FOCI, CYTO and DOUBLES) (Kubik et al., 2007) to assess context-dependent IEG expression in the HF. Using these measures, we detected a contextually-mediated pattern of *zif268* expression (Figure 22A-B). We ran two separate one-way ANOVAs and found a main effect of GROUP (SIMILARITY: $F_{4,28} = 7.792$, $p < 0.001$; OVERLAP: $F_{4,28} = 7.391$, $p < 0.001$). Post-hoc test showed that this effect was due to a difference between the NO GLU AA group and all other groups in both cases. Therefore, it is better to use one of these normalized measures in DGS. In DGS it does not seem to matter which of the two are used as they produce comparable values (Table 2). However, when the disparity in the raw data (DOUBLES) increases as it does in the

CA1, or CA3, OVERLAP and SIMILARITY yield less comparable values (Table 2) and therefore we decided to continue to use the raw data for these brain regions.

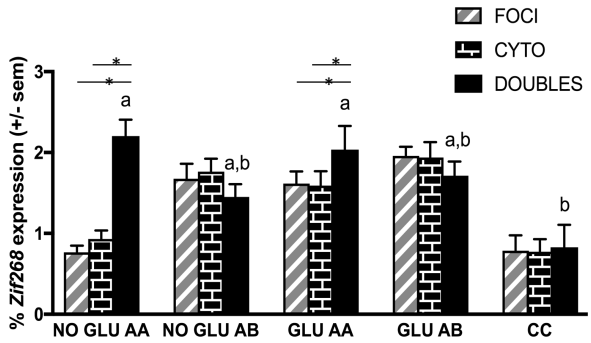


Figure 21. The proportion of granule cells in DGS expressing *zif268* solely within (FOCI) or outside (CYTO) the nucleus, as well as those containing *zif268* mRNA in both cellular compartments (DOUBLES) across groups in the DGS. Following a 5-min exploration session in a novel context "A" and a 20-min home cage period, animals were given unilateral infusions of glutamate (GLU) or no infusions (NO-GLU) and then either placed back in the same context (AA condition) or in a new context "B" (AB condition). Letters (a,b,c) denote significant differences ($p < 0.05$) between groups.

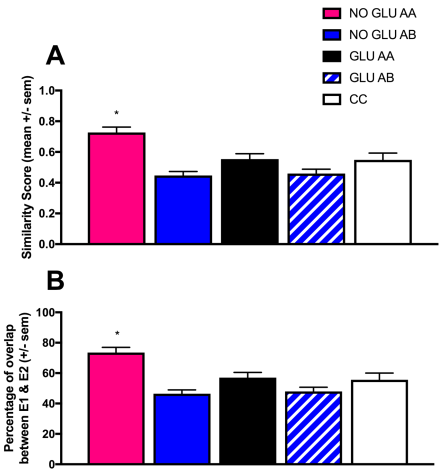


Figure 22. A-B. DGS: SIMILARITY and OVERLAP scores. A) SIMILARITY score, calculated using the formula: [%DOUBLES-(%E1*E2)] / [MIN (E1, E2)] – [E1*E2] of 1 is indicative of a complete overlap in the cellular ensemble activated during the first and second behavioural epochs (E1 & E2). A score of 0 indicates that the degree of overlap is not greater than chance. OVERLAP is a similar measure [%DOUBELS / MIN (E1, E2)] where 100% indicates complete overlap in the E1 and E2 ensembles and 0% represents completely discrete ensembles. * = significant differences ($p < 0.05$) between groups.

Table 2

Sample Raw Staining Class Data: DGS & lateral CA1 (NO-GLU AA & NO-GLU AB groups - experiment 1).

DGS

A/B	**Raw Data**					**Percentages**				**Calculations**				
Animal	NEGATIVE	FOCI	CYTO	DOUBLES	TOTAL	%NEGATIVE	%FOCI	%CYTO	%DOUBLES	%TOTAL IEG	%E1 (C+D)	%E2 (F+D)	SIMILARITY	%OVERLAP
1	165.84	2.83	3.17	2.33	174.17	95.22	1.63	1.82	1.34	6.12	3.16	2.97	0.43	45.16
2	163.25	2.25	2.50	2.00	170.00	96.06	1.32	1.47	1.18	5.15	2.65	2.50	0.46	47.06
3	214.33	4.83	5.00	4.50	228.67	93.73	2.11	2.19	1.97	8.24	4.15	4.08	0.46	48.21
4	216.33	3.00	3.17	4.00	226.50	95.51	1.32	1.40	1.77	6.25	3.16	3.09	0.56	57.14
5	233.17	5.83	5.67	3.83	248.50	93.83	2.35	2.28	1.54	7.71	3.82	3.89	0.38	40.35
6	236.50	3.25	3.50	2.25	245.50	96.33	1.32	1.43	0.92	4.58	2.34	2.24	0.39	40.91
Mean	204.90	3.67	3.83	3.15	215.56	95.11	1.68	1.76	1.45	6.34	3.21	3.13	0.45	46.47
SEM	13.26	0.56	0.50	0.44	14.22	0.45	0.18	0.16	0.16	0.58	0.28	0.30	0.03	2.50
A/A	**Raw Data**					**Percentages**				**Calculations**				
Animal	NEGATIVE	FOCI	CYTO	DOUBLES	TOTAL	%NEGATIVE	%FOCI	%CYTO	%DOUBLES	%TOTAL IEG	%E1 (C+D)	%E2 (F+D)	SIMILARITY	%OVERLAP
1	167.67	1.00	1.00	4.83	174.50	96.08	0.57	0.57	2.77	6.69	3.34	3.34	0.82	82.86
2	181.00	1.50	2.00	3.25	187.75	96.40	0.80	1.07	1.73	5.33	2.80	2.53	0.68	68.42
3	186.75	1.50	1.50	3.25	193.00	96.76	0.78	0.78	1.68	4.92	2.46	2.46	0.68	68..42
4	172.33	2.00	2.33	3.33	180.00	95.74	1.11	1.30	1.85	6.11	3.15	2.96	0.61	62.50
5	228.17	1.33	2.00	6.33	237.83	95.94	0.56	0.84	2.66	6.73	3.50	3.22	0.82	82.61
6	220.40	1.80	2.40	5.80	230.40	95.66	0.78	1.04	2.52	6.86	3.56	3.30	0.75	76.32
Mean	192.72	1.52	1.87	4.47	200.58	96.10	0.77	0.93	2.20	6.11	3.14	2.97	0.73	73.52
SEM	10.39	0.14	0.22	0.57	10.96	0.17	0.08	0.10	0.20	0.33	0.18	0.16	0.04	3.42
Lateral CA1														
A/B	**Raw Data**					**Percentages**				**Calculations**				
Animal	NEGATIVE	FOCI	CYTO	DOUBLES	TOTAL	%NEGATIVE	%FOCI	%CYTO	%DOUBLES	%TOTAL IEG	%E1 (C+D)	%E2 (F+D)	SIMILARITY	%OVERLAP
1	42.00	12.00	9.67	13.67	77.33	54.31	15.52	12.50	17.67	63.36	30.17	33.19	0.38	58.57
2	51.00	12.67	17.00	10.00	90.67	56.25	13.97	18.75	11.03	54.78	29.78	25.00	0.20	44.12
3	55.00	21.00	20.50	11.00	107.50	51.16	19.53	19.07	10.23	59.07	29.30	29.77	0.07	34.92
4	54.00	19.00	21.00	9.50	103.50	52.17	18.36	20.29	9.18	57.00	29.47	27.54	0.05	33.33
5	67.00	17.50	18.50	11.50	114.50	58.52	15.28	16.16	10.04	51.53	26.20	25.33	0.18	39.66
6	47.67	14.00	13.00	12.67	87.33	54.58	16.03	14.89	14.50	59.92	29.39	30.53	0.27	49.35
Mean	52.78	16.03	16.61	11.39	96.81	54.50	16.45	16.94	12.11	57.61	29.05	28.56	0.19	43.32
SEM	3.44	1.50	1.82	0.65	5.71	1.09	0.85	1.21	1.34	1.69	0.58	1.30	0.05	3.89
A/A	**Raw Data**					**Percentages**				**Calculations**				
Animal	NEGATIVE	FOCI	CYTO	DOUBLES	TOTAL	%NEGATIVE	%FOCI	%CYTO	%DOUBLES	%TOTAL IEG	%E1 (C+D)	%E2 (F+D)	SIMILARITY	%OVERLAP
1	57.67	6.33	6.00	19.00	89.00	64.79	7.12	6.74	21.35	56.55	28.09	28.46	0.66	76.00
2	53.25	10.50	9.25	33.00	106.00	50.24	9.91	8.73	31.13	80.90	39.86	41.04	0.63	78.11
3	66.00	3.33	4.67	23.00	97.00	68.04	3.44	4.81	23.71	55.67	28.52	27.15	0.82	87.34
4	60.67	4.00	5.00	14.67	84.33	71.94	4.74	5.93	17.39	45.45	23.32	22.13	0.72	78.57
5	49.00	6.50	7.50	24.50	87.50	56.00	7.43	8.57	28.00	72.00	36.57	35.43	0.67	79.03
6	53.50	8.75	7.00	20.25	89.50	59.78	9.78	7.82	22.63	62.85	30.45	32.40	0.62	74.31
Mean	56.68	6.57	6.57	22.40	92.22	61.80	7.07	7.10	24.03	62.24	31.13	31.10	0.69	78.89
SEM	2.48	1.16	0.70	2.54	3.24	3.28	1.07	0.63	2.00	5.17	2.47	2.72	0l03	1.84

Note: In the CA1, group differences in the raw staining class data (FOCI, CYTO & DOUBLES) translate to a roughly equal magnitude of difference in normalized measures such as OVERLAP and SIMILARITY scores whereas due to the sparse level of firing in DGS, examining a raw measure like DOUBLES is not sensitive enough to capture these group differences. Therefore, in DGS, it is better to use a normalized measure to assess context-dependent IEG expression.

2.3.1.3 Environment-Specific IEG Expression and Remapping in CA3

In CA3a there was a significant main effect of GROUP ($F_{4,28} = 7.224$, $p < 0.001$), and a significant GROUP x IEG_LOCATION interaction ($F_{8,56} = 11.445$, $p < 0.001$. In CA3b, there was a significant main effect of GROUP ($F_{4,28} = 12.315$, $p < 0.001$), main effect of IEG_LOCATION ($F_{2,56} = 3.876$, $p = 0.027$), and a significant GROUP x IEG_LOCATION interaction ($F_{8,56} = 31.571$, $p < 0.001$). In CA3c, there was also a significant main effect of GROUP ($F_{4,28} = 10.168$, $p < 0.001$), and a significant GROUP x IEG_LOCATION interaction

($F_{8,56} = 16.646$, $p < 0.001$). Post hoc tests revealed that in each region, there was higher *arc* expression in groups that explored contexts compared to groups that remained in the home cage (Fig 20A-C). The expression of *arc* was contextually mediated evinced by the finding that the NO-GLU-AA and NO-GLU-AB groups differed significantly in the proportion of DOUBLES in CA3a ($p = 0.02$), CA3b ($p < 0.001$), and the CA3c ($p = 0.004$). Post-hoc tests also revealed a significant difference between the NO-GLU-AA and GLU-AA groups in the CA3b ($p = 0.013$) and the CA3c ($p = 0.036$) but not the CA3a. Since the proportion of DOUBLES for the GLU-AA fell halfway in between the NO-GLU-AA and NO-GLU-AB groups, and was not significantly different than either, we interpret this as a partial resetting of representations. Therefore, while unilateral infusions of glutamate in the LC caused partial remapping in the CA3a, it induced a full resetting of representations in the CA3b and CA3c. Further support for the induction of a full reset in CA3b and CA3c is shown by the lack of difference between the NO-GLU-AB and the GLU-AA groups in CA3b ($p = 0.659$) and the CA3c ($p = 0.807$). Finally, the effects of glutamate were limited to the A/A groups; the NO-GLU-AB and GLU-AB groups did not differ in the CA3a ($p = 1.00$), CA3b ($p = 1.00$), or CA3c ($p = 1.00$) (Figure 23A-C). This observation is consistent with the hypothesis that activation of the LC by glutamate biases the system to remap. That is, under conditions in which you would expect the LC-NE system to be active and for remapping to occur, further perturbation of the system has no observable effect.

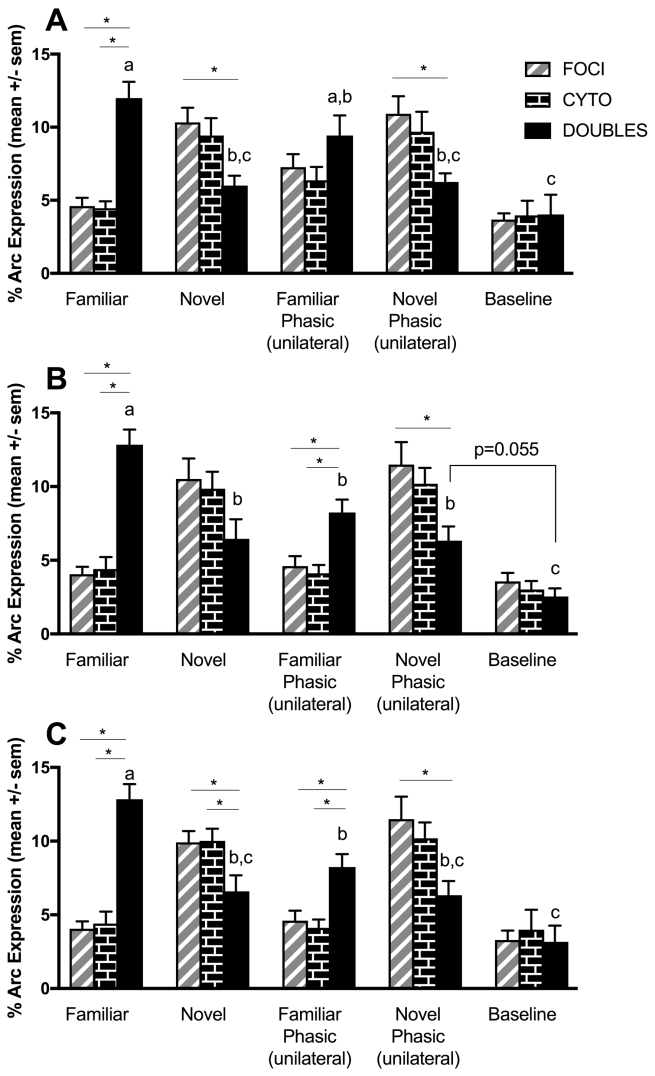


Figure 23. The proportion of pyramidal cells expressing *arc* solely within (FOCI) or outside (CYTO) the nucleus, as well as those containing *arc* mRNA in both cellular compartments (DOUBLES) across groups in the A) CA3a, B) CA3b, and C) CA3c. Following a 5-min exploration session in a novel context "A" and a 20-min home cage period, animals were given unilateral infusions of glutamate (GLU) or no infusions (NO-GLU) and then either placed back in the same context (AA condition) or in a new context "B" (AB condition). Letters (a,b,c) denote significant differences ($p < 0.05$) between groups.

2.3.1.4 Environment-Specific IEG Expression and Remapping in CA1

In lateral CA1 there was a main effect of GROUP ($F_{4,28} = 17.959$, $p < 0.001$), a main effect of IEG_LOCATION ($F_{2,56} = 23.177$, $p < 0.001$), and a significant GROUP x IEG_LOCATION interaction ($F_{8,56} = 18.954$, $p < 0.001$). Post-hoc tests revealed that these effects were attributed in part to differences in the proportion of DOUBLES between the CC group and the GLU-AA ($p < 0.001$) group as well as between the CC group and the NO-GLU-AA group (p

< 0.001), again showing the upregulation of IEG expression during exploration (Figure 24A). We also found that the NO-GLU-AA and the NO-GLU-AB groups differed significantly (p = 0.001) showing that *arc* expression was contextually mediated in this brain region. However, we did not find a difference between the NO-GLU-AA and the GLU-AA groups (p = 0.866), suggesting that unilateral infusions of glutamate in the LC did not induce remapping in lateral CA1 (Figure 24A). In medial CA1 there was a main effect of GROUP ($F_{4,28} = 32.423$, $p < 0.001$), a main effect of IEG_LOCATION ($F_{(2,56)} = 49.039$, $p < 0.001$), and a significant GROUP x IEG_LOCATION interaction ($F_{8,56} = 43.156$, $p < 0.001$). Post-hoc tests showed a significant difference between all groups in the proportion of DOUBLES except the NO-GLU-AB and the GLU-AB groups (p = 1.00) (Figure 24B). The consistency in the pattern of IEG expression between these two groups served as an important control demonstrating that the effect of glutamate was specific to the A/A group. Since the proportion of DOUBLES for the GLU-AA group falls in between and both the NO GLU A/A and NO GLU A/B group, the effect of unilaterally infusing glutamate into the LC is considered a partial remapping effect, or resetting of representations in the medial CA1 (Figure 24B).

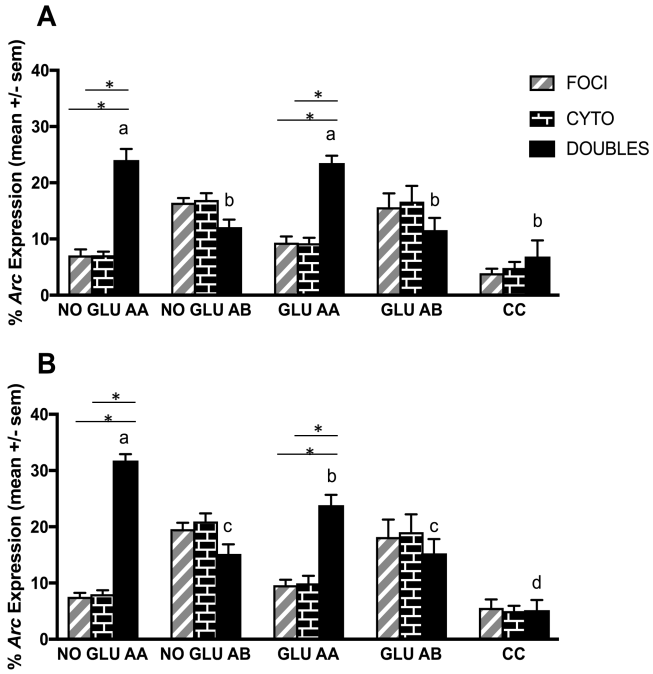


Figure 24. The proportion of pyramidal cells expressing *arc* solely within (FOCI) or outside (CYTO) the nucleus, as well as those containing *arc* mRNA in both cellular compartments (DOUBLES) across groups in the (A) lateral CA1 and (B) medial CA1. Following a 5-min exploration session in a novel context "A" and a 20-min home cage period, animals were given unilateral infusions of glutamate (GLU) or no infusions (NO-GLU) and then either placed back in the same context (AA condition) or in a new context "B" (AB condition). Letters (a,b,c) denote significant differences ($p<0.05$) between groups. Letters (a,b,c) denote significant differences ($p<0.05$) between groups.

Given the results of this experiment, we thought it was necessary to run a similar experiment involving bilateral infusions of the LC. We hypothesized that our effects in the HF would be more pronounced. We also employed more controls and utilized additional pharmacological treatments to assess the role of both phasic and tonic LC activation on hippocampal plasticity.

Table 3.

Experiment 1: Statistical analyses comparing [1]IEG expression across epochs and across groups for each hippocampal sub-region and [2]total IEG expression across groups for each hippocampal sub-region.

Brain Region	F	DF	p	Test Statistic	Result
[1]DGS	7.575 0.252	(4,28) (1,28)	<0.001 0.619	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]DGS	7.575	(4,28)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA3c	8.461 0.059	(4,28) (1,28)	<0.001 0.81	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]CA3c	8.461	(4,28)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA3b	11.287 2.480	(4,28) (1,28)	<0.001 0.127	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]CA3b	11.287	(4,28)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA3a	5.993 3.369	(4,28) (1,28)	<0.001 0.077	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]CA3a	5.993	(4,28)	0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA1 Lateral	13.793 1.102	(4,28) (1,28)	<0.001 0.303	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]CA1 Lateral	13.793	(4,28)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA1 Medial	32.374 0.817	(4,28) (1,28)	<0.001 0.372	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]CA1 Medial	32.374	(4,28)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP

Note: Interactions only reported if significant

2.3.2. Experiment 2: Bilateral Infusions

In this experiment, the large number of groups made data reduction necessary. In experiment 2, an additional control group has been added to control for the handling that is associated with the LC infusion and spatial exploration protocol. In experiment 1, the only control group (caged controls) remained undisturbed in their home cages. From here on in, these animals are referred to as "*Baseline*". In the second experiment, caged controls were picked up every 15

seconds in the same manner as the remaining groups. Since they differed from the controls in experiment 1, and were picked up in their home cages, this control group is referred to as "*Highly Familiar"*. Since there were no differences between the NO-GLU-AA group and the following A/A controls: NO-DRUG-AA, aCSF-AA, and CLON-AA, these four groups were combined to form a new group called "*Familiar*". We also found no differences between the three groups that received tonic LC activation: BETH-AA, ORX-AA, and CRF-AA, therefore these groups were also combined and called "*Familiar Tonic*". The unilateral phasic LC activation group GLU-AA (U) is now referred to as "*Familiar Phasic Unilateral*" and the bilateral phasic LC activation group; GLU-AA (B) is called "*Familiar Phasic Bilateral*". We attempted to block the natural remapping that occurs when animals visit two different contexts using clonidine, therefore, we termed the CLON-AB group "*Novel Inhibition*". Finally, since there were no differences between the NO-GLU-AB group and the important control GLU-AB, these animals that visited two different contexts were combined and are referred to simply as "*Novel*". These group combinations are depicted in Figure 25.

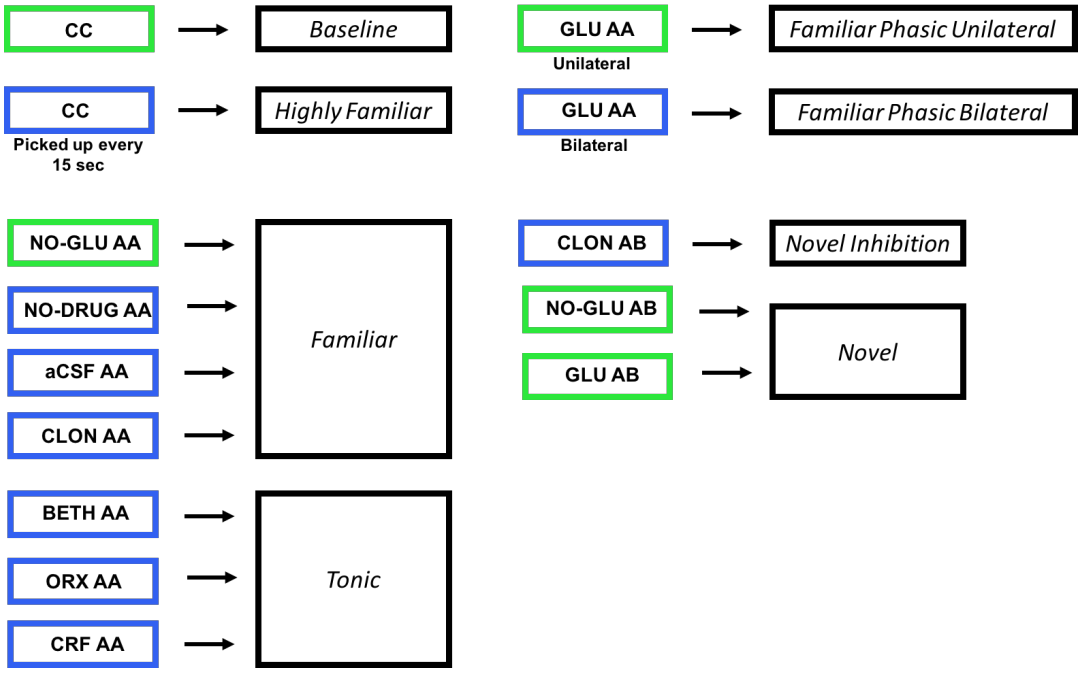


Figure 25. In experiment 2, additional controls pharmacological treatments were added, and several of the original groups from experiment 1 (green) and experiment 2 (blue) were combined to form new groups (black).

2.3.2.1 Total IEG Expression & Context Exploration

Similar to the results from experiment 1, we found significantly higher levels of IEG expression in the CA1 (M = 61.64%, SD = 25.83%) compared to the CA3 (M = 28.31%, SD = 12.86%), and very low expression in the DGS (M = 7.08%, SD = 3.33%) (Figure 26A-F). IEG expression was again driven by context exploration and was similar across both behavioural epochs (Figure 27A-F; Table 4).

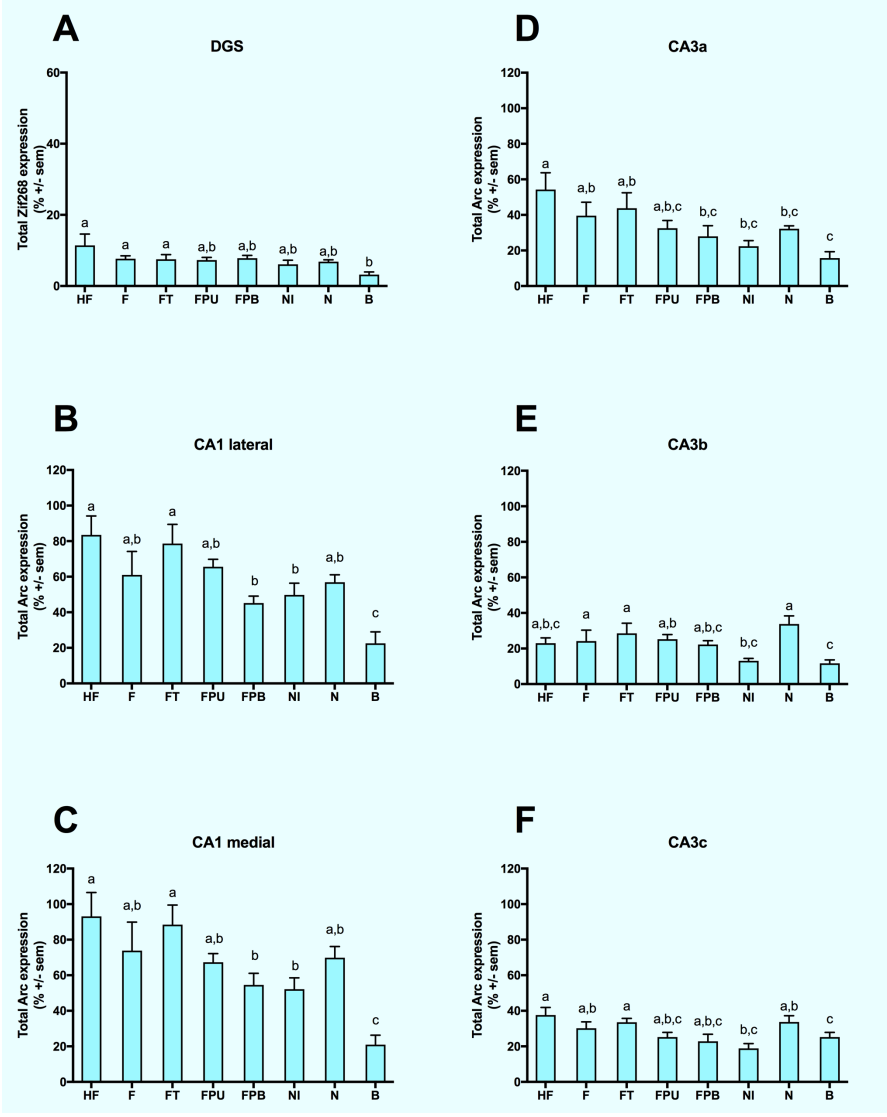


Figure 26. A-F) Total IEG expression across all groups for each brain region. IEG expression was higher in animals that explored a context compared to spending the same amount of time undisturbed in the home cage (A-F). Animals that remained in the home cage but were picked up every 15 seconds also demonstrated higher IEG expression (B-D). Letters (a,b,c) denote significant differences ($p < 0.05$) between groups.

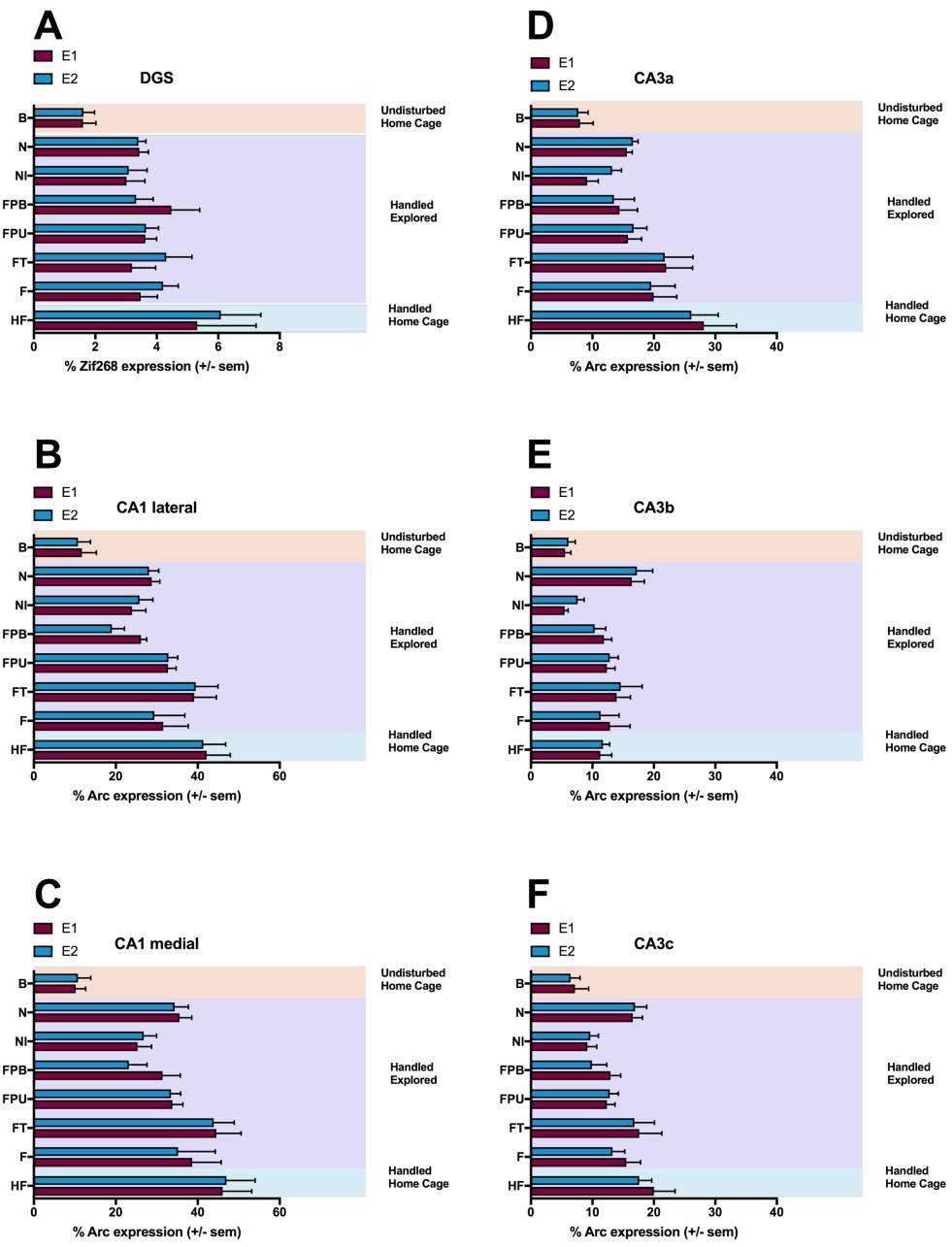


Figure 27. Total IEG expression was similar across both behavioural epochs (first and second context exposures) in each brain region. E1 (Epoch 1): proportion of cells labeled as IEG-positive solely within the cytoplasm (CYTO) plus the cells labeled within both the nucleus and the cytoplasm (DOUBLES); E2 (Epoch 2): proportion of cells labeled as IEG-positive solely within the nucleus (FOCI) plus the cells labeled within both the nucleus and the cytoplasm (DOUBLES).

Table 4.

Experiment 2: Statistical analyses comparing [1]IEG expression across epochs and across groups for each hippocampal sub-region and [2]total IEG expression across groups for each hippocampal sub-region.

Brain Region	F	DF	p	Test Statistic	Result
[1]DGS	339.554 0.884	(7,73) (1,73)	<0.026 0.35	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]DGS	4.044	(7,73)	0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA3c	475.695 3.817	(7,63) (1,63)	<0.001 0.055	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP; No effect of EPOCH
[2]CA3c	5.887	(7,63)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA3b	465.000 0.892	(7,63) (1,63)	<0.001 0.349	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]CA3b	7.342	(7,63)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA3a	429.163 0.671 2.922	(7,63) (1,63) (7,63)	<0.001 0.416 0.01	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch) Group x Epoch	Main effect of GROUP No effect of EPOCH Significant interaction
[2]CA3a	6.463	(7,63)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA1 Lateral	683.444 3.436	(7,63) (1,63)	<0.001 0.068	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]CA1 Lateral	9.553	(7,63)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
[1]CA1 Medial	708.470 2.523	(7,63) (1,63)	<0.001 0.117	Two-way RM ANOVA Between-subject (Group) Within-subject (Epoch)	Main effect of GROUP No effect of EPOCH
[2]CA1 Medial	11.557	(7,63)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP

Note: Interactions only reported if significant

2.3.2.2 Environment-Specific IEG Expression and Remapping in DGS

To assess whether contexts were contextually-mediated and whether our treatments affected remapping in the HF, we examined the differential expression of the proportion of CYTO, FOCI, and DOUBLES across groups for each HF sub-region. Using a two-way RM ANOVA, we found a main effect of GROUP ($F_{7,146} = 4.046$, $p < 0.001$), a main effect of IEG_LOCATION ($F_{2,146} = 5.072$, $p = 0.007$), and a significant interaction ($F_{14,146} = 2.372$, $p =$

0.005). There was a higher percentage of doubles in the *Highly Familiar* group compared to *Baseline* ($p < 0.001$) (Figure 27). These groups were identical with the exception that the experimenter picked up the animals in the *Highly Familiar* group every 15 seconds, therefore, this must have driven this effect.

In the DGS, as before, we did not detect a contextually-mediated pattern of IEG expression, as there was no difference in the percentage of doubles between the *Familiar* and *Novel* groups (Figure 28). However, administration of pharmacological agents into the LC did produce some effects in the DGS. Animals that received drugs targeted at tonic (*Familiar Tonic*) LC activation showed a higher percentage of FOCI-labelled cells compared to *Baseline* ($p = 0.039$) and animals that were bilaterally infused with glutamate in the LC (*Familiar Phasic Bilateral*) showed a higher percentage of cells labelled CYTO compared to the *Familiar* ($p = 0.03$) and *Baseline* ($p = 0.015$) groups (Figure 28A-B).

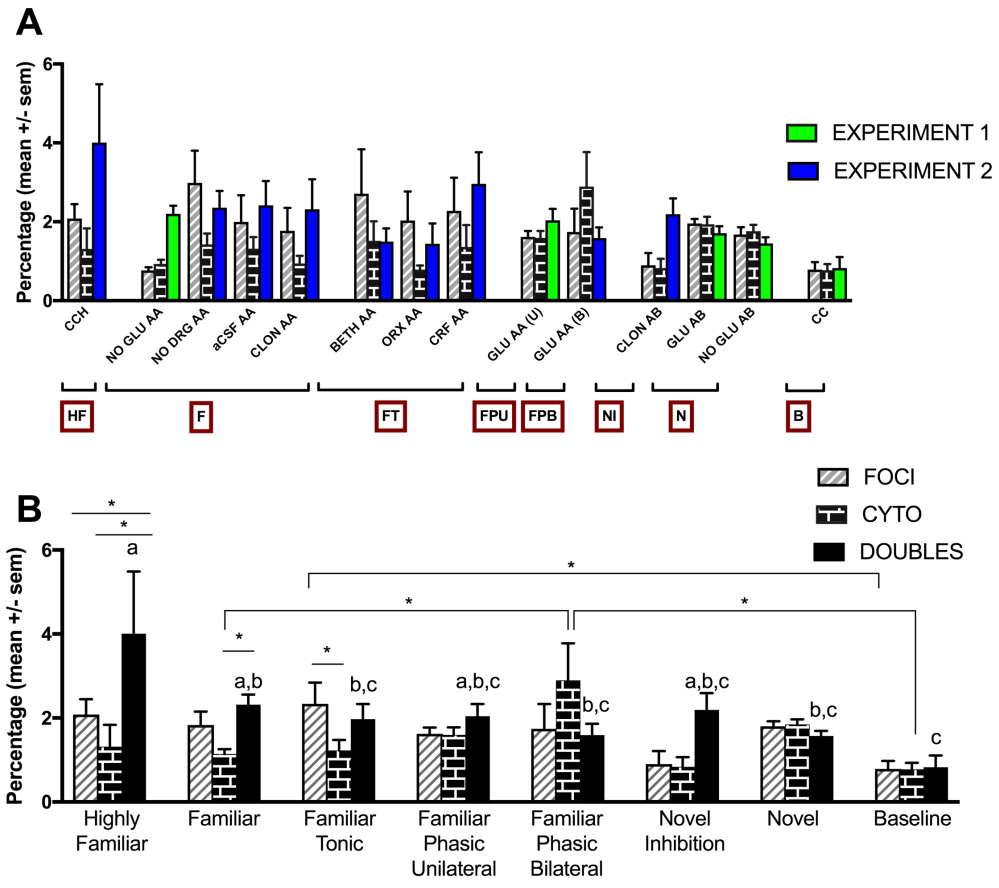


Figure 28. DGS: The proportion of granule cells expressing *zif268* solely within (FOCI) or outside (CYTO) the nucleus, as well as those containing *zif268* in both cellular compartments (DOUBLES) is shown. The proportion of DOUBLES for A) experiment 1 (green bars) & experiment 2 (blue bars) and then for B) the consolidated groups (black bars) are highlighted. Letters (a,b,c) denote significant differences ($p < 0.05$) in the proportion of DOUBLES between groups. Within-group differences in IEG-LOCATION are denoted by an asterix ($P < 0.05$).

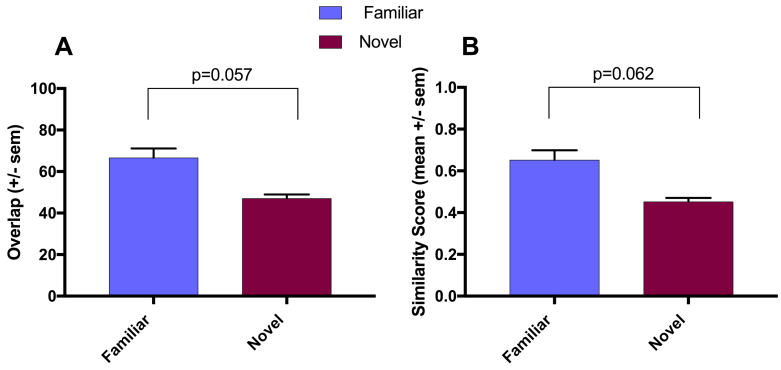


Figure 29. A-B) DGS: OVERLAP and SIMILARITY scores, alternative measures to the proportion of DOUBLES. 100% overlap or a similarity score of 1 indicates a complete overlap in the neuronal ensembles active during each behavioural epoch (E1 and E2) whereas 0% overlap or a similarity score of 0 indicates two completely discrete cellular populations. When using these normalized measures, *zif268* expression in the DGS appears to be environment-specific.

2.3.2.3 Environment-Specific IEG Expression and Remapping in CA3

Two-way RM ANOVAs revealed that for each sub-region within the CA3 there was a main effect of GROUP: CA3a ($F_{7,62} = 5.439$, $p < 0.001$), CA3b ($F_{7,62} = 9.481$, $p < 0.001$), CA3c ($F_{7,62} = 6.603$, $p < 0.001$), and a main effect of IEG_LOCATION: CA3a ($F_{2,62} = 47.333$, $p < 0.001$), CA3b ($F_{2,62} = 17.983$, $p < 0.001$], CA3c ($F_{2,62} = 27.068$, $p < 0.001$). For each CA3 sub-region, there was also a significant GROUP by IEG_LOCATION interaction in CA3a ($F_{14,62} = 11.748$, $p < 0.001$), CA3b ($F_{14,62} = 13.068$, $p < 0.001$), and CA3c ($F_{14,62} = 10.007$, $p < 0.001$).

In the CA3a, there was a contextually-mediated pattern of IEG expression with higher percentages of DOUBLES in the *Highly Familiar* and *Familiar* groups compared to the *Novel* group ($p < 0.001$). Moreover, there was a higher percentage of DOUBLES in the *Highly Familiar, Familiar Tonic, and Familiar* group compared to *Baseline* ($p < 0.001$) (Figure 30A-B). This observation is expected, since these animals visited the same context twice, however, we did hypothesize that tonic LC stimulation would perturb this pattern to reset the system and cause the recruitment of new cells but this was not the case. Tonic LC activation was not able to induce remapping (no significant difference between *Familiar Tonic* and *Familiar*, $p = 0.51$; and

significant difference between *Familiar Tonic* and *Novel,* $p < 0.001$). There was a higher percentage of DOUBLES in the *Familiar Phasic Unilateral* group compared to *Baseline* ($p = 0.06$) but not in the comparison between the *Familiar Phasic Bilateral* group and *Baseline* ($p = 0.47$), suggesting that unilateral infusions were only able to partially reset the system. Essentially, unilateral infusions resulted in a proportion of DOUBLE-labeled cells that fell in between levels associated with both Familiar and Novel groups; we have interpreted this as a "partial-resetting". Evidence for a partial remap is shown by no significant difference between the *Familiar Phasic Unilateral* and *Familiar* groups ($p = 0.093$), and no significant difference between the *Familiar Phasic Unilateral* and *Novel* groups ($p = 0.476$). In contrast, bilateral infusions fully reset the system demonstrated by no significant difference between the *Familiar Phasic Bilateral* and *Novel* groups ($p = 0.835$), and a significant difference between the *Familiar Phasic Bilateral* and *Familiar* groups ($p = 0.006$). Clonidine was not able to block remapping, as there was no difference in the percentage of DOUBLES between the *Novel* and *Novel Inhibition* group ($p = 0.866$).

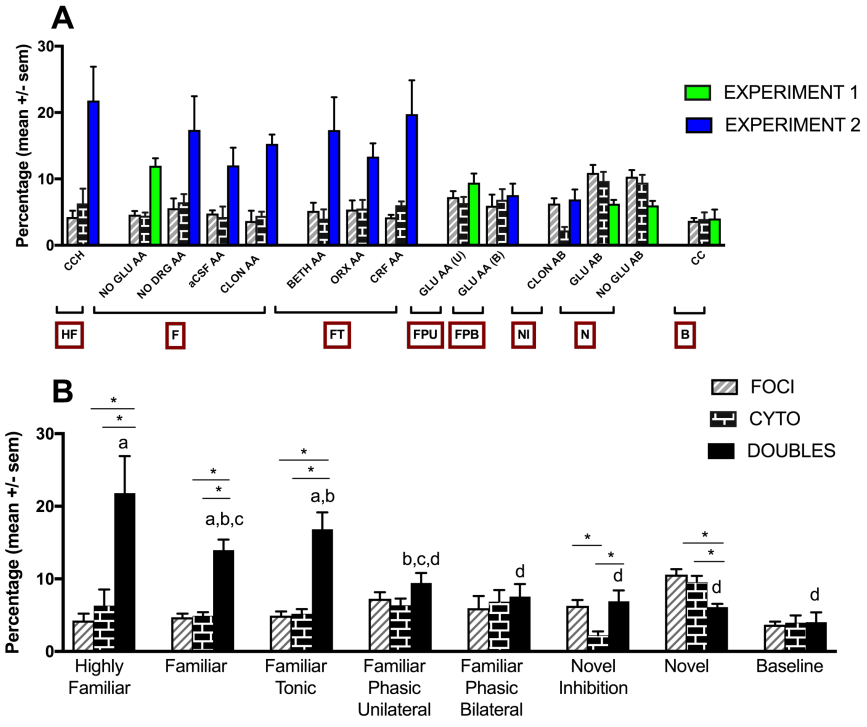


Figure 30. The proportion of CA3a pyramidal cells expressing *arc* solely within (FOCI) or outside (CYTO) the nucleus, as well as those containing *arc* in both cellular compartments (DOUBLES) is shown. The proportion of DOUBLES for A) experiment 1 (green bars) & experiment 2 (blue bars) and then for B) the consolidated groups (black bars) are highlighted. Animals that visited the same context twice (A/A; Familiar) as opposed to two different contexts (A/B; Novel) showed a higher proportion of DOUBLES showing that *arc* expression in the CA3a is contextually-mediated. Bilateral infusions of glutamate in the locus coeruleus (LC), associated with phasic activation, induced global remapping fully resetting contextual representations in the CA3a (Familiar Phasic Bilateral). Unilateral glutamate LC infusions (Familiar Phasic Unilateral) and tonic LC activation (Familiar Tonic) had no effect. Clonidine did not block natural remapping that occurs following exposure to two different contexts (Novel Inhibition). Animals that remained undisturbed in the home cage did not show much IEG expression (Baseline) until they were picked up by the experimenter (Highly Familiar). Letters (a,b,c) denote significant differences ($p < 0.05$) in the proportion of DOUBLES between groups. Within-group differences in IEG-LOCATION are denoted by an asterix ($P < 0.05$).

In the CA3b (Figure 31A-B) there was a higher percentage of DOUBLES in every group except the *Familiar Phasic Bilateral* and *Novel Inhibition* groups compared to *Baseline* demonstrating that in animals that explored an environment there was a higher level of cellular activity relative to animals that remained in the home cage, consistent with the rest of our results. There was a contextually-mediated pattern of IEG expression with a higher percentage of DOUBLES in the *Familiar*, compared to the *Novel* group (near significant effect, $p = 0.065$). Tonic LC activation was not able to induce remapping (no significant difference between *Familiar Tonic* and *Familiar*, $p = 1.000$). Unilateral infusions of glutamate only partially induced remapping as there was no significant difference in the percentage of DOUBLES between the *Familiar Phasic Unilateral* group and the *Familiar* group ($p = 0.904$) or the *Novel* group ($p = 0.916$). However, bilateral infusions of glutamate in the LC did induce remapping in the CA3b as there was a significant difference in the percentage of DOUBLES between the *Familiar Phasic Bilateral* group and the *Familiar* group ($p = 0.009$) and no significant difference between the *Familiar Phasic Bilateral* group and the *Novel* group ($p = 0.934$). In this sub-region, clonidine was also not able to block the natural remapping that occurs when animals visit two distinct contexts, as there was no difference in the percentage of DOUBLES between the *Novel* and *Novel Inhibition* group ($p = 0.657$).

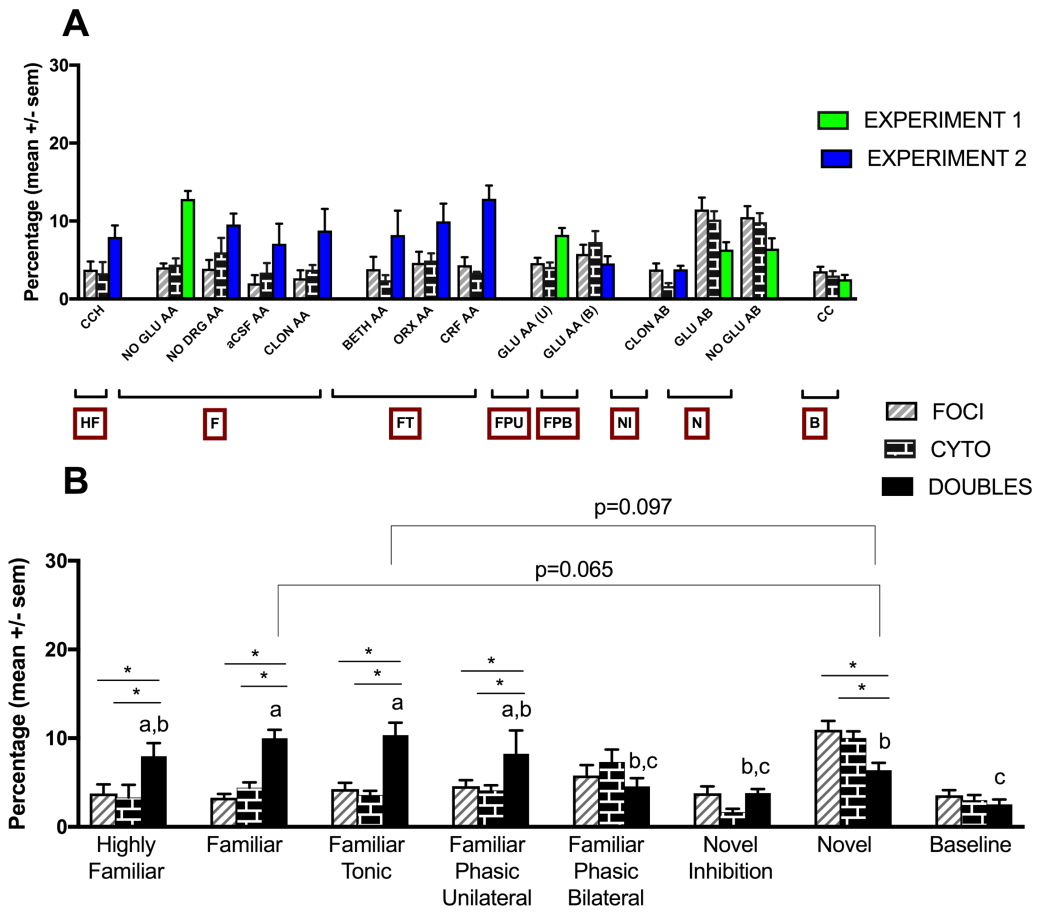


Figure 31. The proportion of CA3b pyramidal cells expressing *arc* solely within (FOCI) or outside (CYTO) the nucleus, as well as those containing *arc* in both cellular compartments (DOUBLES) is shown. The proportion of DOUBLES for A) experiment 1 (green bars) & experiment 2 (blue bars) and then for B) the consolidated groups (black bars) are highlighted. Animals that visited the same context twice (A/A; Familiar) as opposed to two different contexts (A/B; Novel) showed a higher proportion of DOUBLES showing that *arc* expression in the CA3b is contextually-mediated. Bilateral infusions of glutamate in the locus coeruleus (LC), associated with phasic activation, induced global remapping fully resetting contextual representations in the CA3b (Familiar Phasic Bilateral). Unilateral glutamate LC infusions (Familiar Phasic Unilateral) only partially reset the system while tonic LC activation (Familiar Tonic) had no effect. Clonidine did not block natural remapping that occurs following exposure to two different contexts (Novel Inhibition). Animals that remained undisturbed in the home cage showed very little IEG expression (Baseline) until they were picked up by the experimenter (Highly Familiar). Letters (a,b,c) denote significant differences ($p < 0.05$) in the proportion of DOUBLES between groups. Within-group differences in IEG-LOCATION are denoted by an asterix ($P < 0.05$).

In the CA3c (Figure 32A-B), exploration compared to remaining in the home cage was associated with activity greater number of cells engaging in IEG transcription. Moreover, the pattern of IEG expression was contextually-mediated with a higher percentage of DOUBLES in the *Highly Familiar* ($p = 0.044$) and *Familiar* ($p = 0.06$) groups compared to the *Novel* group. Again, tonic LC activation had no effect on remapping with no significant difference between the

Familiar Tonic and *Familiar* groups (p = 0.768) but a significant difference between *Familiar Tonic* and *Novel* groups (p = 0.003). Unilateral LC glutamate infusions resulted in partially induced remapping with no significant difference in the percentage of DOUBLES between the *Familiar Phasic Unilateral* group and the *Familiar* group (p = 0.734) or the *Novel* group (p = 0.981). Bilateral infusions resulted in a full remapping effect given that there was a significant difference in the percentage of DOUBLES between the *Familiar Phasic Bilateral* and the *Familiar* group (p = 0.029) and no significant difference between the *Familiar Phasic Bilateral* group and the *Novel* group (p = 0.993). Clonidine also had no effect in blocking remapping since there was no significant difference in the percentage of DOUBLES between the *Novel* and *Novel Inhibition* group (p = 1.000).

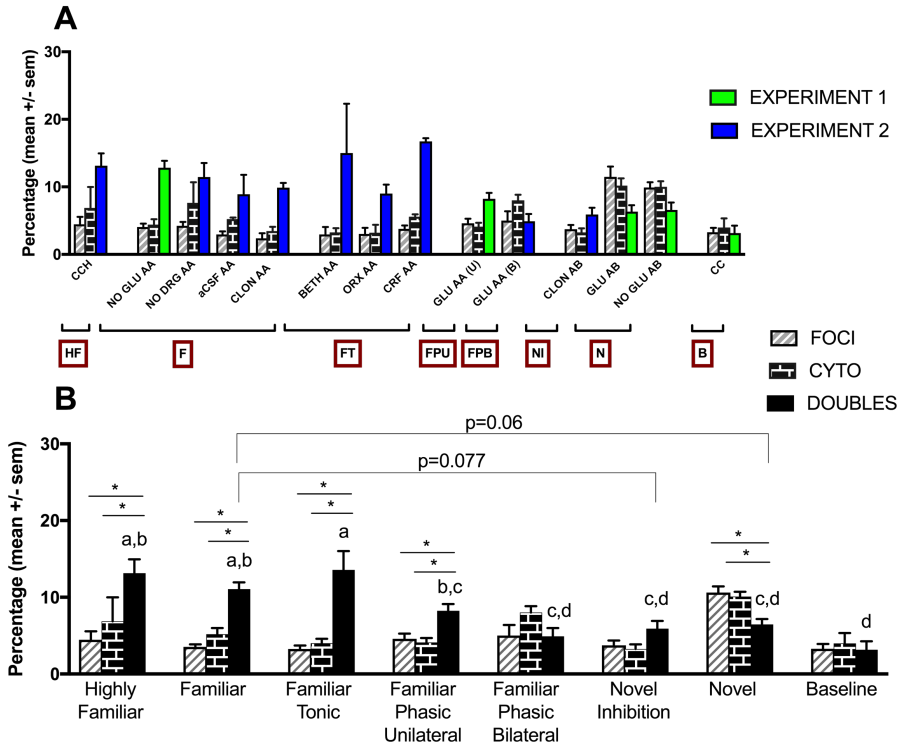


Figure 32. The proportion of CA3c pyramidal cells expressing *arc* solely within (FOCI) or outside (CYTO) the nucleus, as well as those containing *arc* in both cellular compartments (DOUBLES) is shown. The proportion of DOUBLES for A) experiment 1 (green bars) & experiment 2 (blue bars) and then for B) the consolidated groups (black bars) are highlighted. Animals that visited the same context twice (A/A; Familiar) as opposed to two different contexts (A/B; Novel) showed a higher proportion of DOUBLES showing that *arc* expression in the CA3c is contextually-mediated. Bilateral infusions of glutamate in the locus coeruleus (LC), associated with phasic activation, induced global remapping fully resetting contextual representations in the CA3c (Familiar Phasic Bilateral). Unilateral glutamate LC infusions (Familiar Phasic Unilateral) only partially reset the system

while tonic LC activation (Familiar Tonic) had no effect. Clonidine did not block natural remapping that occurs following exposure to two different contexts (Novel Inhibition). Animals that remained undisturbed in the home cage showed very little IEG expression (Baseline) until they were picked up by the experimenter (Highly Familiar). Letters (a,b,c) denote significant differences ($p < 0.05$) in the proportion of DOUBLES between groups. Within-group differences in IEG-LOCATION are denoted by an asterix ($P < 0.05$).

2.3.2.4 Environment-Specific IEG expression and Remapping in CA1

In the lateral and medial CA1, separate two-way RM ANOVAs revealed a main effect of GROUP (lateral: $F_{7, 63} = 10.294$, $p < 0.001$; medial: $F_{7, 63} = 13.267$, $p < 0.001$), a main effect of IEG LOCATION (lateral: $F_{2,63} = 86.688$, $p < 0.001$; medial: $F_{2, 63} = 73.554$, $p < 0.001$), and a significant GROUP x IEG LOCATION interaction (lateral: $F_{14,126} = 10.533$, $p < 0.001$; medial: $F_{14, 63} = 10.460$, $p < 0.001$). In both CA1 sub-regions (Figures 33A-B; & 34A-B) there was a higher percentage of DOUBLES in the A/A groups (except the *Familiar Phasic Bilateral* group) compared to *Baseline* showing not only that exploration drove IEG expression but that IEG expression is context-dependent. Both regions were contextually-mediated revealed by a significant difference between the *Familiar* and *Novel* groups (lateral: $p = 0.014$, medial: $p = 0.005$). Consistent with our previous results, tonic LC activation in the CA1 did not induce remapping. As in the CA3, unilateral glutamate infusions partial reset the system, shown by the absence of a significant difference in the percentage of DOUBLES between the *Familiar Phasic Unilateral* group and the *Familiar* group (lateral: $p = 1.000$; medial: $p = 0.807$) and no significant difference between the *Familiar Phasic Unilateral* and the *Novel* group (lateral: $p = 0.118$, medial: $p = 0.638$). In contrast, bilateral glutamate infusions had a pronounced effect on remapping in the medial CA1. There was a significant difference in the percentage of DOUBLES between the *Familiar Phasic Bilateral* and the *Familiar* group in the medial CA1 ($p = 0.034$), however, this effect did not reach significance in the lateral CA1 ($p = 0.116$). Furthermore, there was no significant difference between the *Familiar Phasic Bilateral* group and the *Novel* group (lateral: $p = 1.000$; medial: $p = 1.00$). Clonidine was again, unable to block natural remapping

(No significant difference between *Novel* and *Novel Inhibition* groups - lateral: p = 0.728; medial: p = 0.989).

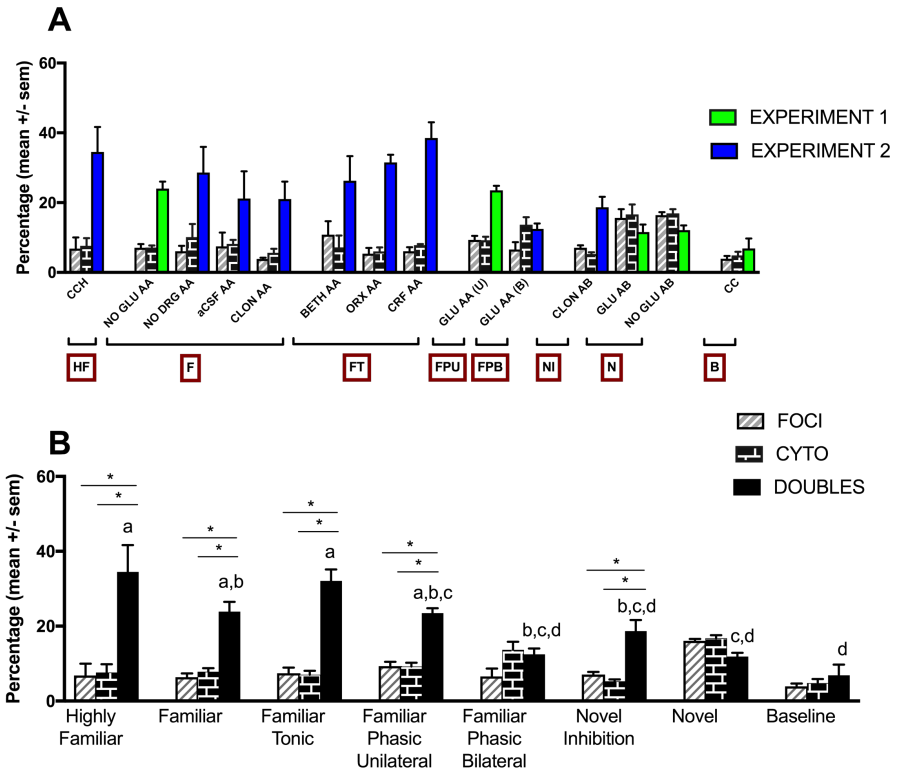


Figure 33. The proportion of lateral CA1 pyramidal cells expressing *arc* solely within (FOCI) or outside (CYTO) the nucleus, as well as those containing *arc* in both cellular compartments (DOUBLES) is shown. The proportion of DOUBLES for A) experiment 1 (green bars) & experiment 2 (blue bars) and then for B) the consolidated groups (black bars) are highlighted. Animals that visited the same context twice (A/A; Familiar) as opposed to two different contexts (A/B; Novel) showed a higher proportion of DOUBLES showing that *arc* expression in the lateral CA1 is contextually-mediated. Bilateral infusions of glutamate in the locus coeruleus (LC), associated with phasic activation, induced global remapping fully resetting contextual representations in the lateral CA1 (Familiar Phasic Bilateral). Unilateral glutamate LC infusions (Familiar Phasic Unilateral) and tonic LC activation (Familiar Tonic) had no effect. Clonidine only partially blocked the natural remapping that occurs following exposure to two different contexts (Novel Inhibition). Animals that remained undisturbed in the home cage showed very little IEG expression (Baseline) until they were picked up by the experimenter (Highly Familiar). Letters (a,b,c) denote significant differences (p < 0.05) in the proportion of DOUBLES between groups. Within-group differences in IEG-LOCATION are denoted by an asterix (P < 0.05).

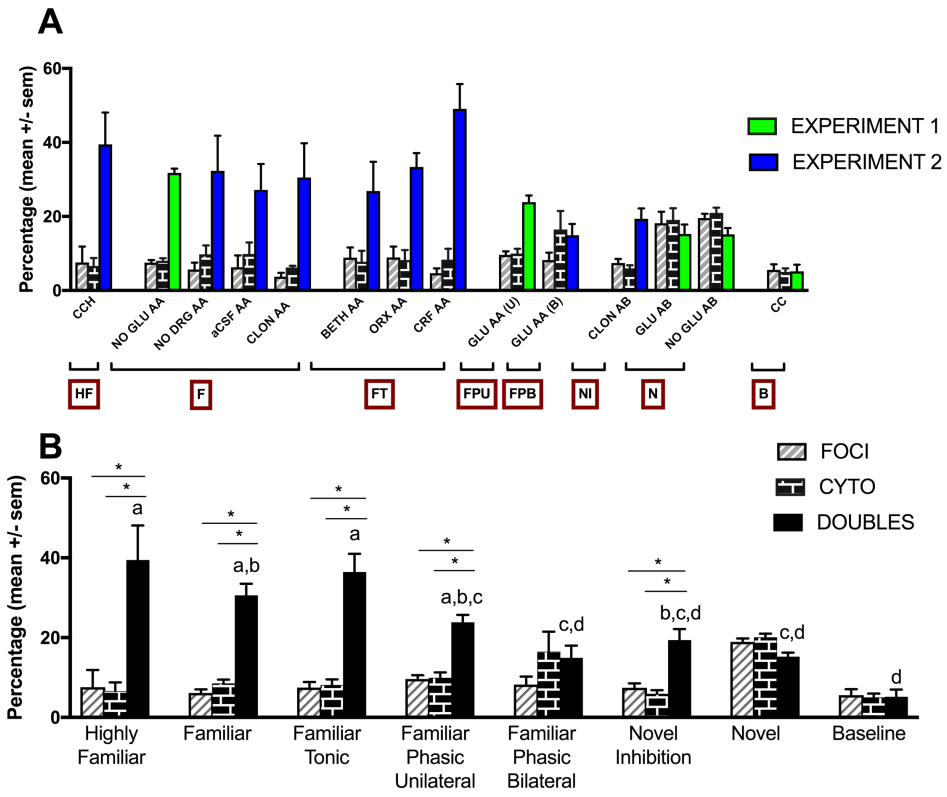


Figure 34. The proportion of medial CA1 pyramidal cells expressing *arc* solely within (FOCI) or outside (CYTO) the nucleus, as well as those containing *arc* in both cellular compartments (DOUBLES) is shown. The proportion of DOUBLES for A) experiment 1 (green bars) & experiment 2 (blue bars) and then for B) the consolidated groups (black bars) are highlighted. Animals that visited the same context twice (A/A; Familiar) as opposed to two different contexts (A/B; Novel) showed a higher proportion of DOUBLES showing that *arc* expression in the medial CA1 is contextually-mediated. Bilateral infusions of glutamate in the locus coeruleus (LC), associated with phasic activation, induced global remapping fully resetting contextual representations in the medial CA1 (Familiar Phasic Bilateral). Unilateral glutamate LC infusions (Familiar Phasic Unilateral) and tonic LC activation (Familiar Tonic) had no effect. Clonidine only partially blocked the natural remapping that occurs following exposure to two different contexts (Novel Inhibition). Animals that remained undisturbed in the home cage showed very little IEG expression (Baseline) until they were picked up by the experimenter (Highly Familiar). Letters (a,b,c) denote significant differences ($p < 0.05$) in the proportion of DOUBLES between groups. Within-group differences in IEG-LOCATION are denoted by an asterix ($P < 0.05$).

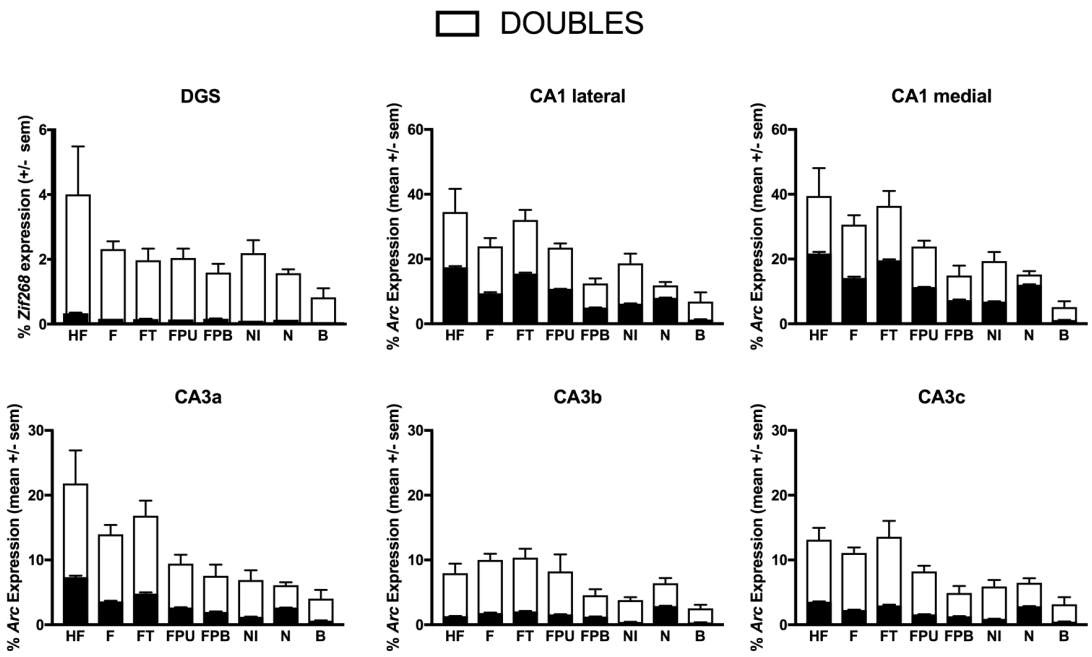


Figure 35. The proportion cells expressing *IEGs* in both cellular compartments (DOUBLES) is shown (white) and superimposed is the expected proportion based on random chance with replacement (black).

2.3.2.5 Environment-Specific IEG Expression and Remapping in Handled and Undisturbed Cage Controls

The *Highly Familiar* group and the *Baseline* group were both home cage-controls however, the *Highly Familiar* group was picked up every 15 seconds in a similar manner to the experimental groups while the *Baseline* group remained undisturbed. Interestingly, these groups differed in their IEG expression even though in both groups context exploration occurred in the home cage. This was not expected. Although IEG expression does not distinguish between new learning and memory retrieval (Chawla et al., 2005) since the level of IEG expression was equal across behavioural epochs and this relationship did not differ across brain regions, we decided to run a separate analysis that looked specifically at IEG expression during the first context exploration only to eliminate any effect of novelty or familiarity. We wanted to examine the relationship between spatial exploration and experimenter-handing on IEG expression. We compared the *Baseline* (Home Cage - Not Handled) and *Highly Familiar* (Home Cage-Handled) groups, to animals that had explored an environment but did not receive any drug treatments or

surgeries - NO-GLU-AA and NO-GLU-AB combined (these two groups did not differ in levels of IEG expression; Figures 26 & 27A-F). Separate one-way ANOVAs were run comparing IEG expression during the first behavioural epoch across groups for each hippocampal sub-region. In each sub-region, there was a main effect of GROUP (Table 5) and the effect grew stronger as the overall level of IEG expression increased (from DG→CA3c→CA3b/a & CA1). These results suggest that experimenter-handling may indeed play a role in the promotion of IEG expression since handled animals showed greater IEG expression than non-handled animals (Figure 36A-F).

Table 5.

Experiment 2: Statistical analyses comparing [1]IEG expression in the first epoch across groups for each hippocampal sub-region.

Brain Region	F	DF	p	Test Statistic	Result
DGS	5.773	(2,26)	0.009	One-way ANOVA Between-subject (Group)	Main effect of GROUP
CA3c	10.559	(2,26)	0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
CA3b	19.545	(2,26)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
CA3a	15.448	(2,26)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
CA1 Lateral	21.755	(2,26)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP
CA1 Medial	40.503	(2,26)	<0.001	One-way ANOVA Between-subject (Group)	Main effect of GROUP

Note: Interactions only reported if significant

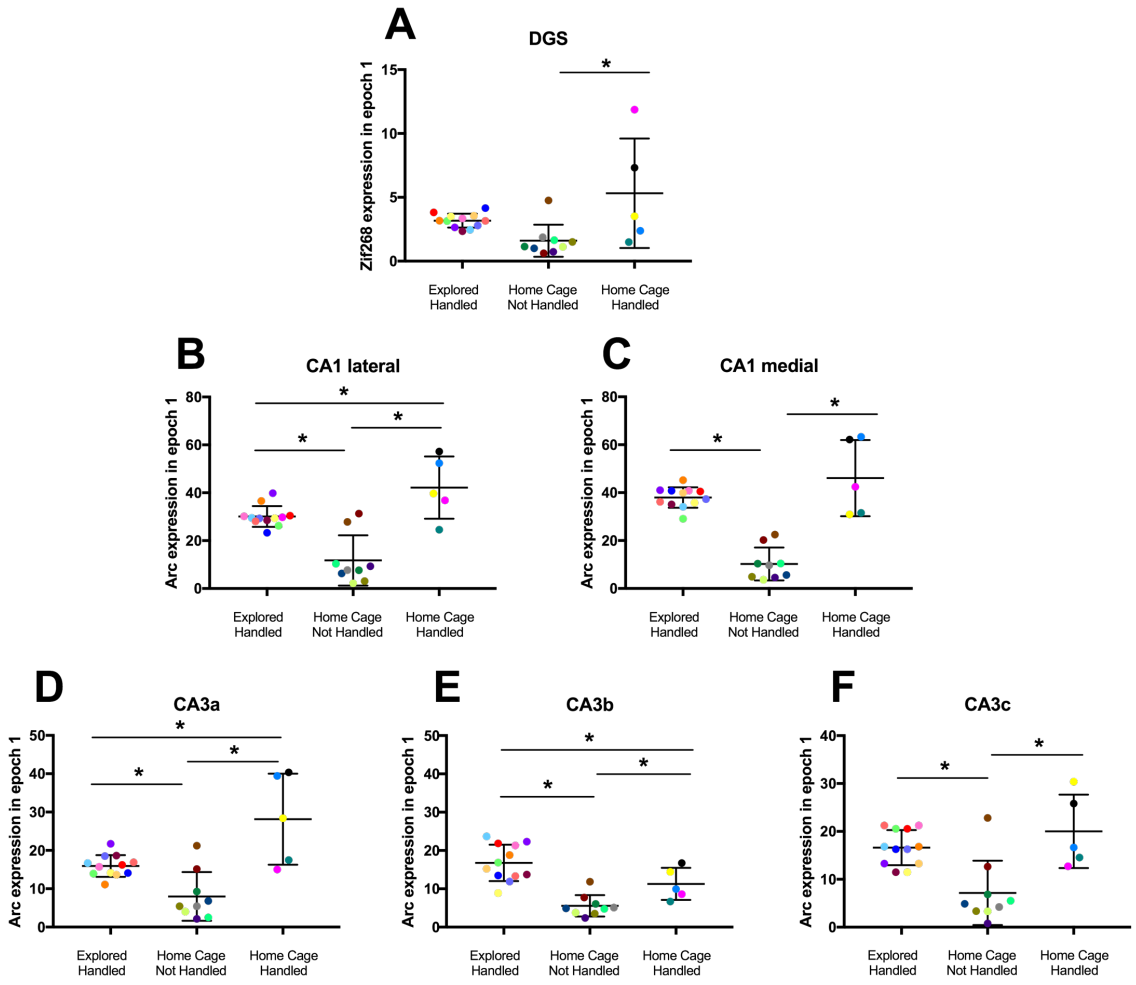


Figure 36. The effect of spatial exploration and experimenter-handing on IEG expression during Epoch 1. Animals in the *Baseline* (Home Cage - Not Handled) and *Highly Familiar* (Home Cage-Handled) groups were compared to animals that had explored an environment but did not receive any drug treatments or surgeries (NO-GLU-AA and NO-GLU-AB combined). Experimenter-handling is sufficient to drive IEG expression in the home cage. Significant differences are denoted with an asterix ($p < 0.05$).

2.4 Discussion

As memory is associated with the formation and modification of neuronal ensembles that are thought to encode or represent contextual information and also to mediate memory retrieval when reactivated (Holtmaat & Caroni, 2016), it is important to understand how these representations are updated. Experience and learning (e.g. exploration of an environment) induces plasticity-associated changes such as the transcription of IEGs such as *arc* and *zif268*, which is necessary for memory consolidation, and which contributes to structural and functional plasticity

through the selective strengthening of established synapses and the formation of new synapses (Hall et al., 2001; Holtmaat & Caroni, 2016). In line with previous studies, contextually-tuned neurons such as place cells respond by remapping when animals visit different environments. With the use of a gene-imaging approach, we have found that the noradrenergic pathway from the LC to the HF is involved in updating contextual representations through the recruitment of new neurons, or in other words, remapping.

2.4.1 Learning Drives IEG Expression

In previous studies, experience-dependent expression of *arc* and *zif268* has been shown to occur in the HF following spatial exploration (Guzowski et al., 2001, 2006; Vazdarjanova et al., 2002; Marrone et al., 2011; Satvat et al., 2011; Gheidi et al., 2013). Our results are consistent with these findings given that both *arc* and *zif268* expression were increased following context exploration compared to spending the same amount of time in the home cage. This effect was evident in all regions of the HF we examined, and with more activity observed in CA1 compared to CA3, and very little activity observed in the DG confirming previous indications of sparse firing in DG granule cells (Jung & McNaughton, 1993; Chawla et al., 2005; Leutgeb et al., 2007). The exception to this was our home cage controls that were picked up in the same manner as the experimental animals every 15 seconds. For all A/A and A/B groups this was done to ensure thorough sampling of the entire environment upon exploration given that place cell activation is thought to facilitate the induction of IEG transcription (Guzowski et al., 1999). However, these control animals did not explore environment A or B, yet our results suggest that picking the animals up every 15 seconds may be sufficient to drive *arc*/*zif268* expression. It is possible that even though IEG expression is contextually-mediated, stress may map onto this effect by increasing overall IEG expression. This should be considered in future studies. Animals that remained in the home cage showed very little IEG expression and likely experienced low levels of stress. Animals in the *Familiar Tonic* group demonstrated high IEG expression and one of the drugs used to induce tonic LC activation in the *Familiar Tonic* group was CRF which is known to

affect the mammalian stress response (Abe & Hiroshige, 1974; De Souza et al., 1991; Valentino et al., 1993; Turnbull & Rivier, 1997). It is possible that the animals in the *Highly Familiar* group which were picked up every 15 seconds in the home cage also demonstrated high levels of IEG expression as a result of stress. However, it is unclear why animals would find it stressful to be handled by the experimenter within the home cage but less so when exploring. Therefore, we believe it is more likely the result of salience and learning. During context exploration, animals are learning. When they are in their home cage and the experimenter is present (handling them every 15 seconds), this also involves learning while animals that are left undisturbed in the home cage are not undergoing learning. This learning is likely tied to IEG expression in a contextually-mediated manner. Consistent with the idea that animals in the home cage show low levels of gene expression, electrophysiological unit recordings in these animals also demonstrate large irregular activity (LIA) rather than theta frequencies typically associated with learning, a state characteristic of slow wave sleep (Jarosiewicz et al., 2002). In these animals, handling may provide enough of a novel/salient stimulus to give them reason to be vigilant, and thus upregulate gene expression. This suggests that in the wild, where there would always be salient/novel stimuli, (expect maybe in the nest/home base), that animals would constantly be in a state of heightened vigilance, and thus heightened IEG expression, and the low "basal" IEG expression that we see in our home cage controls may be a very artificial state that is for the most part specific to a sterile and consistent laboratory environment.

2.4.2 Environment-Specific arc and zif268 Expression

Consistent with findings from electrophysiology studies (Kubie & Ranck, 1983; Thompson & Best, 1989), we demonstrated that IEG expression in the HF was environment-specific (Guzowski et al., 2004; Chawla et al., 2005; Marrone et al., 2011; Satvat et al., 2011). This observation has been reported for *arc* in CA1 and CA3 (Guzowski et al., 1999, 2004; Vazdarjanova & Guzowski, 2004; Marrone et al., 2014) which is consistent with our results. However, similar to experiment 1, we did not find contextually-mediated *arc* expression in the

DGS when comparing the proportion of DOUBLES across groups. There are several things to consider here. While granule cells do exhibit place fields, most are silent due to the tonic inhibition that enables the DG to engage in orthogonal processes of separating memories through pattern separation (Engin et al., 2015). Furthermore, in comparison to mossy cells or pyramidal cells, granule cells have been shown to have only one place field and to remap less easily (Senzai & Buzsáki, 2017). Nevertheless, our results are inconsistent with other researchers such as Chawla et al. (2005), Vazdarjanova and Guzowski (2004), and Marrone et al. (2011). As mentioned earlier, we believe that these discrepancies result from an artifact of using a raw data measure in the DGS where firing is so sparse (Table 2). Although Chawla et al. (2005) reported contextually-mediated *arc* expression based on statistical comparisons across A/A and A/B groups on a raw measure as well, they used the proportion of cells labeled with *intranuclear foci* (FOCI) whereas the measure we used was the proportion of DOUBLES, or cells labeled with *intranuclear foci* and *cytoplasmic* signal. Moreover, we did not measure *arc* expression in the DGS but rather *zif268*. This is because neurons in the DG express *arc* mRNA for a sustained period of time (~8hr) following a single behavioral experience (Ramirez-Amaya et al., 2005, 2013; Marrone et al., 2012; Meconi et al., 2015). While this prolonged transcriptional response is thought to be important for long-term memory consolidation, for our purposes it obscures the temporal specificity of the catFISH technique. To get around this, it is possible to use a different method of double labelling for *Arc* and another IEG *homer1*, however the protocol we used involved single labeling of *zif268* which is not transcribed in a sustained manner and is the simplest method to use. In 2011, Marrone et al. showed that rats indeed exhibited location-specific *zif268* expression in the DGS. In this study, they used OVERLAP rather than the proportion of DOUBLES [and in Vazdarjanova and Guzowski (2004) they used SIMILARITY]. If we calculate OVERLAP (Figure 29A) and SIMILARITY (Figure 29B), then statistically speaking, we get a near significant effect demonstrating that granule cells in the DGS are in fact exhibiting an environment-specific pattern of *zif268* expression.

Given that there was a location-specific pattern of cellular activity in the HF where more neurons were reactivated following exposure to the same context twice as opposed to a dissimilar activation profile (Guzowski et al., 1999) in animals that visited distinct contexts, this supports the notion that IEG transcription in the HF is tied to processing contextual or spatial information rather than simply an artifact of a non-specific response such as stress. One might expect a loss of spatial selectivity from CA3 to CA1 which was observed given the increased overall IEG expression, however, our effects were equally strong in the CA1 compared to the CA3.

2.4.3 Phasic LC Activation Induced Global Remapping

As we are specifically interested in how the LC-NE pathway can serve as a potential mechanism involved in the remapping of hippocampal contextual representations, we investigated how a previously established representation could remap in a familiar environment. We found that bilateral infusions of glutamate, which cause phasic activation of the LC, induced a full reset of representations in the CA3 and CA1 (while unilateral infusions only partially reset the system). These results are in accordance with previous studies showing that LC activation can induce plasticity in the HF (Mueller et al., 1981; Lacaille & Harley, 1985; Stanton & Sarvey, 1987; Heginbotham & Dunwiddie, 1991; Dunwiddie et al., 1992; Harley & Sara, 1992; Klukowski & Harley, 1994; Harley, 1998; Brown et al., 2005; Jurgens et al., 2005), and suggests that the LC-NE system is involved in updating or globally remapping contextual representations in the HF. Unfortunately, we could not properly assess the role of phasic LC activation on remapping in the DGS since IEG expression was not contextually-mediated here (see above). We did find a significant difference in the number of cells labeled with *cytoplasmic* signal between the *Familiar* group and the *Familiar Phasic Bilateral* group (Figure 28B). Reasonably, we cannot interpret this to suggest that the glutamate-induced phasic LC discharge, which caused the release of NE, had an effect on IEG transcription that occurred 30 minutes prior, however, this may be evidence for the induction of metaplasticity where NE participates in the regulation of synaptic plasticity processes (Abraham & Bear, 1996). One possibility is that in addition to causing IEG

transcription to occur in different cells than the cells that were previous activated (global remapping), NE affected the stability of micro RNAs (miRNAs) that interact with IEG mRNA to keep the window of time during which protein can be translated open longer (mRNA stability) as miRNAs are one of the most important regulators at the post-transcriptional level (Chen et al., 2017). Mature miRNAs are short non-coding transcripts (18-25 nucleotides) which can bind to target mRNAs to negatively regulate their expression (Sun et al., 2015). A potential candidate miRNA is miR-124 which is abundantly found in the mammalian brain (Sun et al., 2015) and normally inhibits *zif268* (Yang et al., 2012). It is plausible that NE can inhibit miR-124 expression thereby enhancing the stability of *zif268,* a relationship yet to be characterized. In oysters (*Crassostrea gigas*), a NE-responsive miRNA has been found (Chen et al., 2017). And miR-124 downregulation has been observed following administration of xenobiotic compounds such as cocaine, fluoxetine, and methadone (Rodrigues et al., 2011) as well as endogenous compounds such as estrogen (Katchy & Williams, 2016), corticosterone (Dwivedi et al., 2015) and key biogenesis enzymes such as Dicer and Drosha (Gulyaeva & Kushlinskiy, 2016). Since *zif268* is an important transcriptional regulator required for the maintenance of LTP and long-term memory (Hall et al., 2001; Jones et al., 2001), it is possible that NE could facilitate the expression of *zif268* in this manner. One of the ways to test this would be to examine whether NE affects the levels of other genes regulated by mir-124 and / or looking at mir-124 levels directly. Furthermore, following memory retrieval, the expression of *Zif268* protein in the DG is more prolonged compared to *Arc* (Lee et al., 2004; Besnard et al., 2014). Our manipulation engaging noradrenergic circuitry may have enabled some mechanisms related to memory retrieval warranting further testing.

Unilateral infusions of glutamate in the LC were only able to partially reset hippocampal representations. This is likely due to the fact that the left and right hippocampi are interconnected (Fanselow & Dong, 2010), therefore inconsistency from converging information from each hemisphere resulted in only a partial reset. There are contralateral hippocampal projections from

the CA3 to the CA1 present (Shinohara et al., 2012) and in the rat (but not in mice) (van Groen et al., 2003) these projections also exist from the EC to the DG, CA3, and CA1 (Witter, 2007). Computational models of hippocampal function tend to focus on network coherence and partial remapping represents a state of incoherence (Jeffery, 2011) or engram-instability. HF representations likely transition to a more stable state with time and continued learning until consistency is achieved and interference overcome (Colgin et al., 2008). We were unable to examine what these representations would have looked like at a future time point, although this would have been interesting to investigate.

2.4.4 Tonic LC Activation Failed to Induce Global Remapping

Given that both phasic and tonic LC activity can effectively induce plasticity-related changes within the HF in a β-adrenergic manner, we hypothesized that infusion of pharmacological agents that cause tonic LC discharge would also induce global remapping. While phasic LC activation fully reset hippocampal contextual representations, increasing tonic LC discharge failed to induce remapping. This is consistent with the fact that detection of novel stimuli in the environment is associated with phasic rather than tonic LC activation, and it plausible that remapping would occur only when new learning is taking place to promote behavioural adaptation. For instance, remapping has been shown to occur in the HF when mice are contextually fear-conditioned, and then again during extinction learning (Wang et al., 2012c, 2015). Furthermore, if phasic LC activity can act as a modulatory switch recruiting new neurons to create new contextual representations or update existing ones, then tonic LC discharge may be providing a gating mechanism that tunes the phasic LC signal. The interaction between optimal responding to salient stimuli (phasic) and moderate LC tonus to promote focused attention has already been demonstrated (Aston-Jones and Cohen, 2005). One possible explanation put forth by Sebastian Bouret (personal communication) is that an increase in tonic discharge could potentially reset the system in a way where it is continuously being reset repeatedly but is not

able to settle into a final resting state that drives remapping. This is an interesting hypothesis, if this were the case, from a theoretical perspective we could imagine that the subsequent NE release that occurs following LC activation is not necessarily driving remapping effects but instead, the suppression of discharge that occurs following activation that is only associated with phasic responses. However, this is unlikely for two reasons, 1) animals that were given clonidine in the LC and placed in the same environment twice experienced a suppression of discharge in the LC but no associated remapping; 2) the argument itself is tautological since our operational definition of reset is equated with remapping, and therefore a constant stream of network-reset activity would hypothetically involve the recruitment of many different cells and the subsequent transcription of IEGs in these cells which we did not detect. If the brain were indeed a computer we could easily define what resetting the system means but in terms of neural coding, this has proven a challenging task.

2.4.5 Clonidine Failed to Block Remapping

We were able to partially block novelty-induced remapping in the CA1, but not the CA3, by administration of clonidine in the LC. The LC responds to sudden changes in the environment and is thought to promote adaptive behaviour in the face of these changes. Changes in the environment are associated with increased arousal and NE release, and these effects are opposed / regulated by presynaptic α-2 adrenergic receptors which exert tonic inhibitory control over NE release (Langer, 1981; Schoffelmeer & Mulder, 1983; Quintin et al., 1986; Washburn & Moises, 1989; Berridge et al., 1993; Sara et al., 1993; Kawahara et al., 1999) yet few studies have explored the topic of autoreceptor-mediated inhibition of NE-mediated synaptic plasticity in the brain (Washburn & Moises, 1989; Jedema et al., 2008). If remapping can be induced by activation of the LC-NE system then it stands to reason that suppression of LC firing using the α-2 adrenergic agonist clonidine should be able to block this effect (Svensson et al., 1975; Warsh et al., 1981). Surprisingly, our results do not support this.

At low doses, clonidine diminishes NE levels (Svensson et al., 1975) but at higher doses, clonidine can increase NE by producing agonist effects at post-synaptic α-1 adrenergic receptors (Andén et al., 1970; Svensson et al., 1975; Grant & Redmond, 1981). In rabbits, intracerebral ventricular administration of a low dose (0.5ug) of clonidine reduced theta rhythmicity in the MS and hippocampal electroencephalographic (EEG) (Kitchigina & Kutyreva, 2002; Kitchigina et al., 2003) whereas a higher dose (5ug) had the opposite effect (Kitchigina and Kutyreva, 2002). It is possible that we used a dose that was too high to inactivate the LC. Berridge et al. (1993) showed that unilateral inactivation of the LC with clonidine did not affect hippocampal EEG activity, but bilateral clonidine infusions were able to fully suppress neuronal discharge in the LC and EEG activity. In this study, they used a molar concentration of 4.35mM and 35-150nl of infusate. This is very close the concentration (3.75mM) and volume we used (200nl) therefore it is unlikely that the reason we were not able to block remapping was due to the dose we used. Another possibility why clonidine failed to block remapping may be that inhibition of spatial or contextual inputs does not simply promote the strengthening of connections that expressed the original place map (Schoenenberger et al., 2016). Inhibition may induce desynchronization which in turn, could promote the assembly of new maps. For instance, inhibitory neurons show stronger firing when an animal is first presented with a new environment and less firing later on suggesting that inhibition is a critical first component of the remapping process (Wilson & McNaughton, 1993; Schoenenberger et al., 2016). However, clonidine administered to the A/A group did not induce remapping. We believe the most parsimonious explanation may be that clonidine abolishes tonic LC activity (not associated with remapping), but not necessarily phasic LC activity (which drives remapping). More specifically, clonidine has a global inhibitory effect on LC firing but if the animal is stimulated (e.g. loud sound), LC neurons can still be activated transiently (Saunier et al., 1993). Therefore, exposure to a new context can still be processed by the LC even after clonidine administration. It is also possible that contextual / spatial changes were likely detected through parallel inputs such as sensory pathways that can potentially shape HF representations despite

inhibitory influence from the LC. Our results regarding clonidine are not entirely surprising since it is notoriously difficult to block remapping (personal communication with Jill Leutgeb). Muscimol inactivation of the MS failed to prevent the emergence of new spatial representations which suggests that the persistence of new place fields can occur despite aberrations in theta. Our findings add to this, demonstrating that new contextual representations can emerge after inactivation of the LC.

Interestingly, impairments in *rate remapping*, where place fields are retained but cells alter their firing properties in response to small changes in the environment (e.g. color / shape) (Fyhn et al., 2007), have been observed ipsilaterally following lesions to the lEC (Lu et al., 2013). If global remapping is largely driven in a feed forward manner from grid cell inputs originating in the mEC (Brandon et al., 2014), then hypothetically, reduced input via inactivation of the mEC may be able to disrupt global remapping. However, Miao et al. (2015) and Rueckemann et al. (2016) recently showed that inactivation of the mEC induced place cell remapping rather than blocking it. It has also been found that NE can facilitate GABAergic transmission in the EC. Together these findings suggest that phasic activation of the LC appears to contribute to inactivity in the mEC possibly driving a desynchronization effect in the CA3 and CA1 through direct projections from the mEC to both these regions (van Groen et al., 2003) and causes the release of NE in the DG, in both cases promoting global changes in map stability. Future experiments will be focused on verifying that this is indeed a BAR-dependent effect with the use of drugs such as propranolol, to block the recruitment of new neurons.

2.5 Summary

Through the activation of the LC during important events, NE is involved in the acquisition of new information and therefore, plays a crucial role in the encoding of new memories. The current study shows that the LC-NE system is involved in the recruitment of new neurons during memory encoding whereby post-encoding neuronal activity and plasticity promotes the consolidation of hippocampal-dependent memory (Takeuchi et al., 2016) and the

stabilization of hippocampal contextual representations. When new information is encountered or contingencies in the environment are detected, these representations require updating through global remapping of these contextual representations in the HF. As the mechanisms by which remapping occurs remain elusive, we have shown that phasic activation of the LC can induce plasticity in the HF resulting in the recruitment of new neurons and a global reorganization of hippocampal representations. Several recent studies have shown that structures within the brain such as the HF are heterogeneous in nature comprised of many subpopulations of cells projecting to different regions, supporting different faculties. For instance, cells within the CA1 that project to layer V of the EC are involved in memory encoding while those projecting from CA1 to the subiculum are involved in retrieval (Ritchey, 2017). Our results show a relationship between phasic, bilateral LC activation and the recruitment of new neurons; it is possible that activation of the LC-NE system does this in such a way where it directs activity away from the CA1-subiculum pathway implicated in retrieval and instead along this CA1-EC pathway thought to support encoding. Our results are in accordance with Seymour Kety's hypothesis (1970) regarding biogenic amine-facilitated memory through the enhancement of neuronal activity during affectively important events, and Bouret & Sara's (2005) *network-reset hypothesis* which suggests that the LC-NE system induces a ‘‘reset’’ in its target structures, by interrupting existing functional networks and facilitating the emergence of new ones. We have demonstrated that the LC-NE system, implicated in shifting attention to environmental imperatives (Sara, 2009), is a potential mechanism for global remapping through the promotion of plasticity acting as a modulatory switch recruiting new neurons to create new contextual representations when necessary and likely updating existing representations in the presence of new information.

3.0 Norepinephrine as a Memory Reset Signal: Switching the System from Retrieval Back to Encoding During a Spatial Memory Task can be Both Adaptive and Maladaptive

3.1 Introduction

The LC responds to a number of salience cues, including novelty in the environment, and sends a major noradrenergic projection to the HF (Aston-Jones & Bloom, 1981; Vankov et al., 1995; Berridge & Waterhouse, 2003; Harley, 2007b; Aston-Jones & Waterhouse, 2016). Activation of the LC causes the release of NE (Blackstad et al., 1967; Fuxe et al., 1968; Ungerstedt, 1971; Pickel et al., 1974; Ross & Reis, 1974; Lindvall & Björklund, 1974; Morrison et al., 1978; Dahl & Winson, 1985; Harley & Milway, 1986; Harley et al., 1989; Babstock & Harley, 1992; Frizzell & Harley, 1994; Klukowski & Harley, 1994; Walling & Harley, 2004; Lemon et al., 2009) and induces downstream plasticity effects in the HF resulting in increased excitability (Lacaille and Harley, 1985; Stanton and Sarvey, 1985; Harley, 1991; Hagena et al., 2016) and LTP (Bliss et al., 1983; Neuman & Harley, 1983; Gray & Johnston, 1987; Hopkins & Johnston, 1988; Walling & Harley, 2004; Almaguer-Melian et al., 2005; Lashgari et al., 2008; Lim et al., 2010; Walling et al., 2011; Hagena et al., 2016) both of which are BAR-dependent in these circumstances (Kitchigina et al., 1997). It has been proposed that activation of the LC-NE system induces changes in network dynamics occurring at critical times when learning is necessary to promote adaptive behaviour (Sara et al., 1994; Bouret & Sara, 2005; Hagena et al., 2016). These network configurations function to "reset" the system and as such, this hypothesis has been referred to as the *network-reset hypothesis* (Bouret & Sara, 2005). Given the direct projections from the LC to the HF, and the abundance of BARs in the HF, we are interested in how the LC-NE system exerts modulatory influence over hippocampal contextual representations. More specifically, to what degree is the LC-NE system involved in the formation of new episodic memories, and in updating existing memories to incorporate new information?

We hypothesize that the LC-NE system can bias the memory system towards the process encoding. *Encoding* is the first step in memory formation. It is the process that takes place following episodes of learning, which recruits populations of neurons in the HF that form representations of the contextual elements pertaining to those episodes, that can later facilitate memory retrieval processes (Guzowski et al., 1999; Chawla et al., 2005; Ramirez-Amaya et al., 2005; Vazdarjanova et al., 2006; Rowland et al., 2011; Pevzner et al., 2012; Josselyn et al., 2015; Tonegawa et al., 2015a, 2015b; Eichenbaum, 2016). As memories are acquired, they can either last a short time (i.e. short-term memory or working memory), or they can gain permanence through consolidation (Frey et al., 1988; Meiri & Rosenblum, 1998; Schafe et al., 1999; Miyashita et al., 2008). Tasks that involve memory retrieval require reactivation of the representations formed during encoding (Garner et al., 2012; Liu et al., 2012, 2013, 2014; Ramirez et al., 2013).

Novelty-associated activation of the LC can induce alterations in hippocampal contextual representations. We recently showed that following placement in a familiar environment, a situation in which established representations are nearly always reactivated, phasic activation of the LC via bilateral glutamatergic infusions can fully remap these representations. This observation is consistent with the idea that NE provides a "*reset*" signal causing the HF to recruit distinct populations of neurons in the presence of new information suggesting that the LC-NE system is involved in the mechanism by which global remapping occurs. The way in which this "switch" between encoding and retrieval may separate memories and promote adaptive behaviour remains poorly understood. This is particularly true since a dysregulation in memory "updating" may underlie the pathology of anxiety disorders such as PTSD (Maren et al., 2013; Morrison & Ressler, 2014; Giustino et al., 2016; Liberzon & Abelson, 2016; Elsey & Kindt, 2017; Lee et al., 2017; Sheynin & Liberzon, 2017).

In the current study, we sought to investigate the role of NE in encoding and retrieval processes of the HF. There is an abundance of research to show that the NE system mediates

different stages of memory through a neuromodulatory effect (McGaugh et al., 1990; Do Monte et al., 2008) but how does this fit with the network reset hypothesis? We hypothesize that NE resets the memory system in such a way that it causes the HF to move from a state of retrieval back to a state of encoding when it is necessary, when novel information needs to be incorporated. This hypothesis suggests that the effect of modulating NE on memory will critically depend on the stage of training. To further understand how NE modulation of hippocampal circuits affects spatial memory, we tested whether infusions of the BAR-agonist isoproterenol would *impair* working and reference memory retrieval (i.e. switching the system back to encoding when it is maladaptive) and whether infusions of the BAR-antagonist propranolol would have the opposite effect. Given that LC neurons exhibit plasticity as a function of environmental contingency changes (Sara & Segal, 1991) to promote adaptive behaviour, we also tested whether isoproterenol could, in contrast, *enhance* cognitive flexibility thus improving reversal learning (i.e., switching the system back to encoding when it is adaptive).

3.2 Materials and Method

3.2.1 Animals

Experiments included 98 adult, male Fischer-344 rats (Harlan Indianapolis, IN). Sixty-seven of these rats were 16 weeks old at the start of the study and weighed in the range of 325-375g. Fifty-two of these animals were tested on Delayed Non-Match to Positon (DNMP), while 15 animals were tested on the Elevated Plus Maze (EPM). A further 21 were ten weeks old at the start of the study weighing in the range of 300-350g which were tested on the Barnes Maze (Barnes, 1979). Rats were housed in standard transparent Plexiglas cages (47.6cm L x 26.0cm W x 20.3cm H), pair-housed initially and then single-housed after surgery. They were kept on a 12:12hr reverse light cycle (lights ON at 7pm) and were provided with food and water *ad libitum* until they recovered from surgery after which the animals in experiment 3 were food restricted to 90% of their free fed body weight and the animals in experiments 4 & 5 remained on an *ad*

libitum diet. All procedures were approved by the Wilfrid Laurier University Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

3.2.2 Apparatuses

3.2.2.1 Delayed Non-Match to Position (DNMP) Task

For the DNMP task, we used a radial arm maze (122cm in diameter; Stoelting Co., Wood Dale, IL), which consisted of 12 grey, equidistantly spaced, polyethylene arms (50cm L x 10cm W x 13cm H) that radiated from a small circular rotating central platform. The maze rested on a table, elevated 84cm from the ground, located in the center of the room (2.44m L x 2.24m W x 2.95m H) and extra-visual cues (geometric shapes) were positioned on the walls. Other visual cues included a computer in one corner of the room.

3.2.2.2 Elevated Plus Maze (EPM)

Anxiety and locomotor activity was measured using a grey polyethylene EPM (Stoelting Co., Wood Dale, IL) consisting of two open and two closed runways (50cm L x 10cm W x 40cm H) elevated 40cm from the ground. EPM testing took place in a smaller room (1.83m L x 1.78m W x 2.95m H) where the maze was positioned in the center.

3.2.2.3 Barnes Maze

The Barnes maze consisted of a grey circular polyethylene disk (122cm in diameter) with 20 circular equidistant holes (10cm in diameter; 9.65cm between holes) located around the perimeter of the maze (1.3cm from the edge). The maze was elevated 90cm from the ground and beneath each hole was a slot where an escape box (35.56 L x 13.34cm W x 10.16cm H) or a "false" escape box (11.43cm L x 13.34cm W x1.9cm H) could be inserted. For any given trial 19/20 holes were connected to a false escape box and only 1 hole lead to the true escape box. The false escape boxes were significantly smaller than the true escape box; therefore, rats could not escape the maze via these boxes. Their main purpose was to conceal any visual cues that may be apparent from a distance or through an open hole. Four bright white lights (150W) were mounted

above the maze, which illuminated the entire maze area. The rest of the room was dark when testing. The maze was located in the center of a larger room (4.5m L x 3.35m W x 2.95m H) and extra-visual cues (geometric shapes) were positioned on the walls. Other visual cues included several desks and cabinets. To record behaviour in all three testing rooms, a webcam connected to a computer running ANY-maze tracking software (Stoelting Co., Wood Dale, IL) was mounted above each apparatus on the ceiling and behaviour was tracked using ANY-maze software (Stoelting Co., Wood Dale, IL).

3.2.3 Surgery

For four consecutive days prior to surgery animals were weighed, handled for 15 minutes, and given 20g of a nutritionally complete dietary supplement containing trimethoprim / sulfamethoxazole antibiotic (MediGel® TMS; ClearH20, Westbrook, ME) in addition to their regular diet in their home cage. On the following day, rats underwent implantation of a bilateral guide cannula. Several days prior to surgery, two 22-gauge stainless steel guide cannulas (Plastics One, Roanoke, VA) were cemented together to form a bilateral cannula and then left to dry. The next day they were autoclaved and again left to dry. At the start of surgery, rats were deeply anesthetized with 5% isoflurane and 70% oxygen, (induction) and maintained at a level of 2-3% isoflurane for the duration of the surgery. They were anchored in a stereotaxic frame with ear bars to ensure a flat skull surface and prepped for aseptic surgery. Rats were administered a sub-cutaneous (s.c.) injection of ketoprofen (Anafen®; Sigma Aldrich, Oakville, ON; 0.15ml of a 10 mg/mL solution) for general analgesia, and 3ml of sterile physiological saline (0.9%; s.c.) for fluid replacement in case of blood loss. A midline incision was made on the scalp and six holes were drilled. Each rat was implanted with the bilateral cannula (8mm in length, Plastics One, Roanoke, VA) aimed at the dentate gyrus with the coordinates: AP -3.3mm, ML +/-2.1mm, DV -4.2mm (from skull) relative to Bregma (Paxinos & Watson, 2005). Cannulae were anchored to the skull with four skull screws (0-80, Plastics One, Roanoke, VA) and dental acrylic. At the end of surgery stainless steel stylets (flush with guide) were screwed into the cannulae to ensure

patency and rats were placed on a heating pad for 1 hour. They were given an additional 0.15ml injection (s.c.) of ketoprofen 24 hours later and allowed 7 days for recovery undisturbed except for daily weighing. During the first four days of recovery rats continued to receive 20g of TMS in their home cage and were given their regular diet mixed with water in mashed form in addition to regular chow pellets.

3.2.4 Drugs and Infusions

Rats received either (-)-isoproterenol bitartrate (ISO; 10ug/ul dissolved in sterile saline; Sigma Aldrich, Oakville, ON) or (+/-) -propranolol hydrochloride (DL) (PRO; 3ug/ul dissolved in sterile saline; Sigma Aldrich, Oakville, ON). Given that few experiments have targeted the DG with these specific drugs in awake, freely-moving animals, the doses we chose were based on an exhaustive literature search (Table 6). We decided to infuse 5ug in the DG of each hemisphere since Geyer and Masten, (1989) found that infusion of a similar amount resulted in an increase in diversive exploration. For PRO, no studies had targeted the DG specifically. Ji et al. (2003) and Chai et al. (2014) found impaired memory consolidation and a blockade of NE-facilitated memory enhancements when they targeted the CA1 (5ug per side), however, Hatfield and McGaugh (1999) and Barsegyan et al., (2014) found spatial memory impairments and diminished NE-facilitated memory enhancements with smaller amounts (0.3, and 1ug) when targeting the BLA. Therefore, we decided to use 1.5ug for PRO. For each infusion, stylets were unscrewed from each rat's cannulae and a 30-gauge infusion cannula (1mm below pedestal) connected via polyethylene tubing (PE-10) to a 10-µL Hamilton syringe mounted onto a microfluidic infusion pump (Harvard Apparatus, model: 70 -2000, Holliston, MA) was inserted into the guide cannulae. Rats were infused with 0.5µL on either side of the brain at a rate of 0.5µL/min and the infusion cannula was left in place for 1 minute post-infusion to ensure that the liquid had diffused from the injection site.

Table 6.

Authors	Drug	Manufacturer	Concentration (mM)	Concentration (ug/uL)	volume infused (per side, uL)	infusion rate (ul/min)	Duration (min)	Total mass (per side; ug)	Target
Geyer & Masten, 1989	L-isoproterenol hydrochloride	Sigma	0.4	0.1	20	0.333	60	2	DG (bilateral)
Geyer & Masten, 1989	L-isoproterenol hydrochloride	Sigma	1.2	0.3	20	0.333	60	6	DG (bilateral)
Sun et al., 2006	L-isoproterenol hydrochloride	Sigma	400	99.088	2	0.0667	30	198.176	CA1 (bilateral)
Qi et al., 2008	L-isoproterenol hydrochloride	Sigma	40.3682	10	1	0.5	2	10	CA1 (bilateral)
Alsene et al., 2011	L-isoproterenol hydrochloride	Sigma	12.1104	3	0.5	0.5	1	1.5	dorsal HF (bilateral)
Alsene et al., 2011	L-isoproterenol hydrochloride	Sigma	40.3682	10	0.5	0.5	1	5	dorsal HF (bilateral)
Alsene et al., 2011	L-isoproterenol hydrochloride	Sigma	121.1045	30	0.5	0.5	1	15	dorsal HF (bilateral)
Lethbridge et al., 2014	L-isoproterenol hydrochloride	Sigma	0.0001	0.0000247	1	0.08	12	0.0000247	DG (bilateral)
Lethbridge et al., 2014	L-isoproterenol hydrochloride	Sigma	0.001	0.000247	1	0.08	12	0.000247	DG (bilateral)
Lethbridge et al., 2014	L-isoproterenol hydrochloride	Sigma	0.01	0.00247	1	0.08	12	0.00247	DG (bilateral)
Lethbridge et al., 2014	L-isoproterenol hydrochloride	Sigma	0.1	0.0247	1	0.08	12	0.0247	DG (bilateral)
Hansen & Manahan-Vaughan, 2015	L-isoproterenol hydrochloride	Tocris	16.1473	4	5	1	5	20	ICV (single injection)
Garrido Zinn et al., 2016	L-isoproterenol hydrochloride	Sigma	80.7363	20	0.5	0.5	1	10	BLA (bilateral)
Garrido Zinn et al., 2016	L-isoproterenol hydrochloride	Sigma	40.3682	10	1	1	1	10	CA1 (bilateral)
Current Study	isoproterenol-bitartrate	Sigma	47.335	10	0.5	0.5	1	5	DG (bilateral)
Hatfield & McGaugh, 1999	propranolol hydrochloride	Sigma	5.0708	1.5	0.2	0.5	0.4	0.3	BLA (bilateral)
Ji et al., 2003	propranolol hydrochloride	Sigma	16.9027	5	1	0.5	2	5	CA1 (bilateral)
Straube et al., 2003	propranolol hydrochloride	Sigma	0.00676	0.0019996	5	1.25	4	0.009998	ICV (single injection)
Walling & Harley, 2004	propranolol hydrochloride	Sigma	20.284	6	5	1	5	30	ICV (single injection)
Berlau & McGaugh, 2006	propranolol hydrochloride	Sigma	8.4517	2.5	0.2	0.38	0.5333	0.5	BLA (unilateral)
Qi et al., 2008	propranolol hydrochloride	Sigma	50.7082	15	1	0.5	2	15	CA1 (bilateral)
Barsegyan et al., 2014	propranolol hydrochloride	Sigma	1.6903	0.5	0.2	0.4	0.5	0.1	BLA (bilateral)
Barsegyan et al., 2014	propranolol hydrochloride	Sigma	5.071	1.5	0.2	0.4	0.5	0.3	BLA (bilateral)
Barsegyan et al., 2014	propranolol hydrochloride	Sigma	16.9033	5	0.2	0.4	0.5	1	BLA (bilateral)
Chai et al., 2014	propranolol hydrochloride	Sigma	33.8055	10	0.5	0.5	1	5	CA1 (bilateral)
Hansen & Manahan-Vaughan, 2015	propranolol hydrochloride	Tocris	1.3522	0.4	5	1	5	2	ICV (single injection)
Current Study	propranolol hydrochloride	Sigma	10.142	3	0.5	0.5	1	1.5	DG (bilateral)

(Geyer & Masten, 1989; Hatfield & McGaugh, 1999; Straube et al., 2003; Ji et al., 2003; Walling & Harley, 2004; Sun et al., 2005; Berlau & McGaugh, 2006; Qi et al., 2008; Alsene et al., 2011; Barsegyan et al., 2014; Lethbridge et al., 2014; Hansen & Manahan-Vaughan, 2015b; Garrido Zinn et al., 2016)

3.2.5 Experiment 3: DNMP Procedure

The DNMP task consisted of four stages: (1) Habituation (2) Pre-training (3) Acquisition-training and (4) Testing. A timeline of the procedure is outlined in Figure 36.

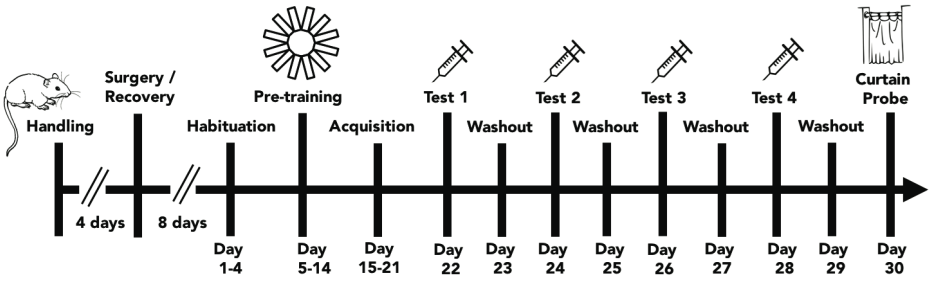


Figure 37. Schematic depicting the experimental timeline of the DNMP study. There were four major stages: (1) Habituation (2) Pre-training (3) Acquisition training and (4) Testing 3.2.5.1 Habituation. Habituation lasted for 4 days. On day 1, rats were given one 20-minute habituation trial where they freely explored the maze. All 12 arms were open and baited with a reward placed in a small plastic grey cup at the end of the arm. The next day rats were given two 10-minute trials (inter-trial interval = 1 hr) in the maze with 6 arms open and baited. For the next two days, they were given two 5-minutes trials a day with 3 arms open and baited. On day 5 rats began Pre-training.

3.2.5.1 Pre-Training

Pre-training lasted for 10 days. During this phase rats were given two trials a day with only one arm open and baited. The goal was to train the rats to retrieve the reward in less than 2 minutes. By the tenth day all rats were able to do this. On day 14, rats began acquisition training.

3.2.5.2 Acquisition Training

During acquisition, animals received 4 trials a day. Each trial consisted of two phases: the *sample phase* and the *choice phase*, which were separated by a 10-minute delay. During the sample phase, all arms except the sample arm were blocked off. The rat was placed in the center of the maze and permitted to visit the sample arm and obtain half a Froot Loop®. The time it took to obtain the reward was recorded. Once the animal retrieved the reward, he was left in the maze for an additional 10 seconds to promote memory for the sample arm location using extra-maze cues. The rat was then placed back in his home cage and 10 minutes later tested on the choice phase. During the 10-minute delay, the maze was rotated to eliminate the possibility of odour being used as an intra-maze cue. This rotation allowed the preservation of the arm location but the arm itself was a different arm.

During the choice phase, the previously rewarded sample arm was now “unrewarded”. An additional correct arm was open and “rewarded”. The cups in each of the two arms appeared identical from afar and in fact they both contained half a Froot Loop®, but the cup in the unrewarded arm contained a mesh overlay that did not allow the rat access to the reward. When a rat chose the incorrect arm (i.e., entered the sample/unrewarded arm), he was permitted to self-correct this behaviour. If the rat re-entered the incorrect arm it was considered an additional error. When a rat made a correct choice, he was given an additional full Froot Loop® in the home cage immediately after the trial ended. Correct arms varied in distance from the sample arm by a spatial separation of 2 (S2, Low), or 5(S5, High) arms (Figure 37 schematic), see section below entitled: *3.2.6 Pilot Experiments*. For the choice phase, the time it took to reach the reward was

recorded along with whether the animal made the correct choice and if not, how many errors were made.

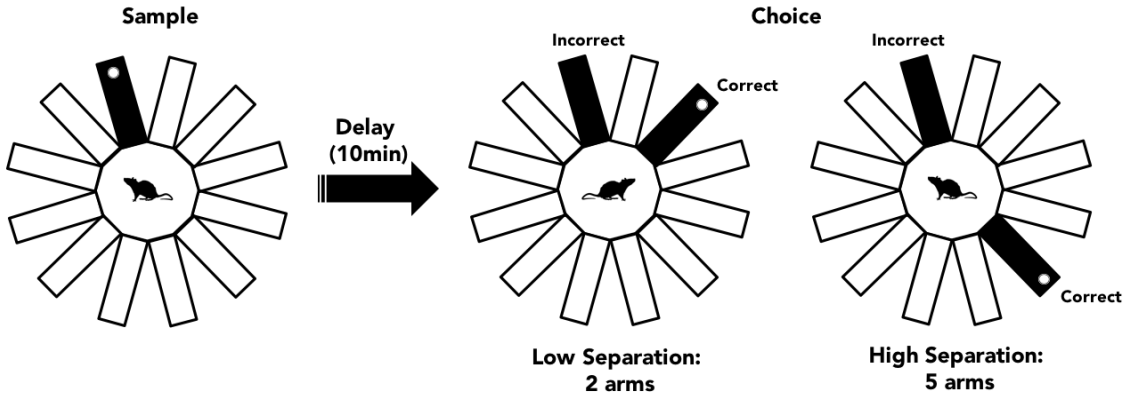


Figure 38. Schematic showing the DNMP task in the radial arm maze. In the Sample phase one arm is open and baited and animals are trained to obtain a reward from this arm. After a delay of 10 minutes, in the Choice phase, animals are placed back in the maze and presented with a choice between the previously rewarded arm and the new arm. These two arms are separated by either 2 (low separation, difficult, DG-dependent) or 5 (high separation, easy, DG-independent) arms. For successful performance, animals must remember the location of the previously rewarded arm, and instead enter the new arm to receive a reward.

Rats were given 4 trials (sample + choice phases) per day (2 Low & 2 High) of pseudo-randomly presented combinations of sample + correct arms (counter balanced for clockwise and counter clockwise permutations) (Tables 7 & 8). All rats were tested on trial 1 before trial 2 began with an inter-trial interval of 90 minutes. They were trained until a criterion of 4/6 correct choices were made on S5 trials across 3 consecutive days, with rats reaching this criterion within 6-7 days of acquisition training.

One hour after the last acquisition training trial, stylets were unscrewed from each rat's cannulae, and the infusion cannula was inserted to make sure that the cannula was not blocked. The infuser was left in the cannula for two minutes on each side of the brain to simulate what would occur during testing but no fluid was delivered. This was done in attempts to reduce the elicitation of a nonspecific stress response on test day. Following this, the dust caps were screwed back in and then animals were returned to their home cage. The following day testing began.

Table 7

Combinations of Arm Separations used for Acquisition and Washout

5 CW		5 CCW		2 CW		2 CCW		
Sample Arm	Choice Arm	Sample Arm	Choice Arm	Sample Arm	Choice Arm	Sample Arm	Choice Arm	Day
2	7	11	6	3	5	12	10	A1
11	4	8	3	7	9	6	4	A2
6	11	9	4	12	2	5	3	A3
7	12	10	5	6	8	11	9	A4
9	2	4	11	8	10	7	5	A5
3	8	12	7	9	11	4	2	A6
12	5	3	10	5	7	2	12	W1
4	9	7	2	10	12	9	7	W2
10	3	5	12	4	6	10	8	W3
5	10	2	9	2	4	8	6	W4

Note: Animals were tested with an arm separation of 2 or 5 counterbalanced for clockwise and counter-clockwise directions. (define abbrevs) The testing order is shown in Table 8.

Table 8.

Training Schedule: Order of S2 and S5 Trials During Acquisition and Washout

Rat	Trial 1	Trial 2	Trial 3	Trial 4
1	5R	2L	2R	5L
5	5R	2L	5L	2R
9	5R	2R	2L	5L
13	5R	2R	5L	2L
17	5R	5L	2R	2L
21	5R	5L	2L	2R
2	5L	2L	2R	5R
6	5L	2L	5R	2R
10	5L	2R	2L	5R
14	5L	2R	5R	2L
18	5L	5R	2L	2R
22	5L	5R	2R	2L
3	2R	2L	5R	5L
7	2R	2L	5L	5R
11	2R	5L	2L	5R
15	2R	5L	5R	2L
19	2R	5R	2L	5L
23	2R	5R	5L	2L
4	2L	2R	5R	5L
8	2L	2R	5R	5L
12	2L	5R	2R	5L
16	2L	5R	5L	2R
20	2L	5L	2R	5R
24	2L	5L	5R	2R

Note: Rats received 4 trials / day. R = Clockwise, L = Counter-clockwise

Table 9.

Balanced Latin Square Design Used for Testing Schedule

Rat	Test 1	Test 2	Test 3	Test 4	Pattern
1	PS5	PC5	PS2	PC2	DCBA
2	PC5	PS2	PC2	PS5	CBAD
3	PS2	PC2	PS5	PC5	BADC
4	PC2	PS5	PC5	PS2	ADCB
5	PC2	PS2	PC5	PS5	ABCD
6	PS2	PC5	PS5	PC2	BCDA
7	PC5	PS5	PC2	PS2	CDAB
8	PS5	PC2	PS2	PC5	DABC
9	PS2	PS5	PC2	PC5	BDAC
10	PS5	PC2	PC5	PS2	DACB
11	PC2	PC5	PS2	PS5	ACDB
12	PC5	PS2	PS5	PC2	CBDA
13	PC5	PC2	PS5	PS2	CADB
14	PC2	PS5	PS2	PC5	ADBC
15	PS5	PS2	PC5	PC2	DBCA
16	PS2	PC5	PC2	PS5	BCAD
17	PC5	PS5	PS2	PC2	CDBA
18	PS5	PS2	PC2	PC5	DBAC
19	PS2	PC2	PC5	PS5	BACD
20	PC2	PC5	PS5	PS2	ACDB

Note: PC2=A; PS2=B; PC5=C; PS5=D. Infusions made Pre-Choice = PC, infusions made Pre-Sample = PS. Animals were tested with an arm separation of 2 or 5.

3.2.5.3 Test Day

Using a counterbalanced Latin Square design (Table 9), animals were tested on four different conditions on four different days with each test day separated by a washout period of one day (Figure 36). We used a 2x2x2 design with a between-subject factor of DRUG treatment, a within-subject factor of INFUSION TIME, and a within-subject factor of ARM-SEPARATION. Rats received either ISO, (n=16) or PRO, (n=17). Rats were assigned to a drug treatment following acquisition, and this remained constant throughout testing. On test day, rats were infused 3 minutes prior to either the sample phase (Pre-Sample, PS) or the choice phase (Pre-Choice, PC) and were tested with an arm separation of 2 (S2, Low) or 5 (S5, High). Therefore, the four conditions tested were as follows: PS-S2, PS-S5, PC-S2, and PC-S5. In contrast to acquisition training, on each test day, instead of receiving two S2 and two S5 trials, animals received all four trials in the condition they were being tested (all S2 or all S5). This allowed us to include TRIAL as an additional within-subject factor making the design a 2x2x2x4 design. Trial 1 was considered a habituation trial where, like the previous day, stylets were removed and the infusion cannula was inserted but no fluid was infused. Trial 2 served as a baseline trial where animals were infused with sterile saline (0.9%NaCl) and trial 3 was the test trial where animals received the drug treatment they were assigned to. Stylets were then replaced and animals were placed back in their home cages. Trial 4 was a 90-minute post-test trial. The next day after the final test day, rats were given one more washout session and the day after that they received a curtain probe test.

3.2.5.4 Washout Sessions and Curtain Probe

The procedure for both the washout sessions and the curtain probe were identical to acquisition training. For the curtain probe, the exception was that a curtain was placed around the maze to obscure visual access to any extra-maze cues. The purpose of the curtain probe was to

demonstrate that the rats were relying on extra-maze cues to complete the DNMP task rather than intra-maze or interoceptive cues.

3.2.6 Pilot Experiments

The DNMP procedure used was adapted from Clelland et al. (2009) and Morris et al. (2012). In their study, it was determined that spatial discrimination was dependent on the DG when stimuli were presented with little separation in an eight-arm radial maze but not when stimuli were presented more widely apart. Therefore, we ran a pilot study in rats that did not undergo surgery (n=12) to specifically determine which arm separations in a twelve-arm radial maze would yield the most comparable results. Animals received habituation and pre-training trials and then 6 acquisition-training sessions. Each acquisition-training session consisted of 6 trials (sample + choice) a day to assess performance on arm separations 1-6 (order counterbalanced). Given the results of our pilot experiments (Figure 41B-C), we decided to use a separation of 2 arms (S2, Low, 60 degrees) as the difficult, DG-dependent separation, and 5 arms (S5, High, 150 degrees) as the less difficult separation (Figure 37). We found that these separations were comparable to those used in the Clelland et al. (2009) study in terms of angular distance. Furthermore, Clelland et al. (2009) found that during their pilot experiments, mice were performing at chance levels when a one-arm separation was used (45 degrees) and the use of a six-arm separation (180 degrees) in our maze would not allow us to control for clockwise and counter clockwise permutations.

3.2.7 Experiment 4: Elevated Plus Maze

To assess the effects of ISO and PRO on locomotion and anxiety, a separate group of rats were tested in the EPM. A separate group of animals was used since there were no drug-naïve animals in the DNMP experiment to serve as the vehicle group for EPM testing. Rats underwent similar handling and surgical procedures as above. Following recovery, rats were split into 3 groups: ISO (n=5), PRO (n=5), and vehicle (n=5). Using the same doses as above, rats were

given a bilateral intra-DG infusion of either ISO, PRO, or vehicle and then 3 minutes later tested in the EPM. Rats were placed at the junction of the four arms at the beginning of the session. Their behaviour was monitored for 5 minutes. Anxiety-like behaviour was assessed by measuring the percentage of time spent in the open arms of the maze compared to the closed arms and the number of entries into the open and closed arms. General locomotor activity was assessed by measuring the total number of arm entries (Figure 38A).

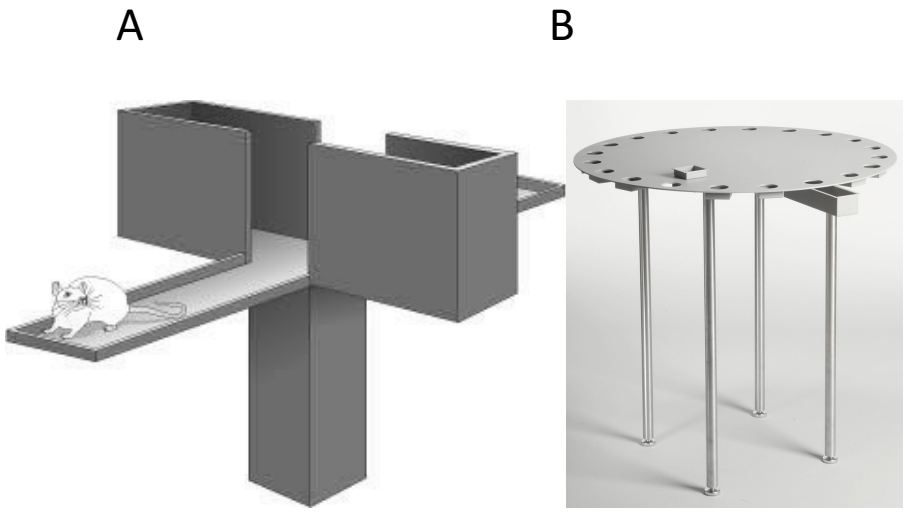


Figure 39. Depiction of the (A) Elevated Plus Maze and the (B) Barnes Maze.

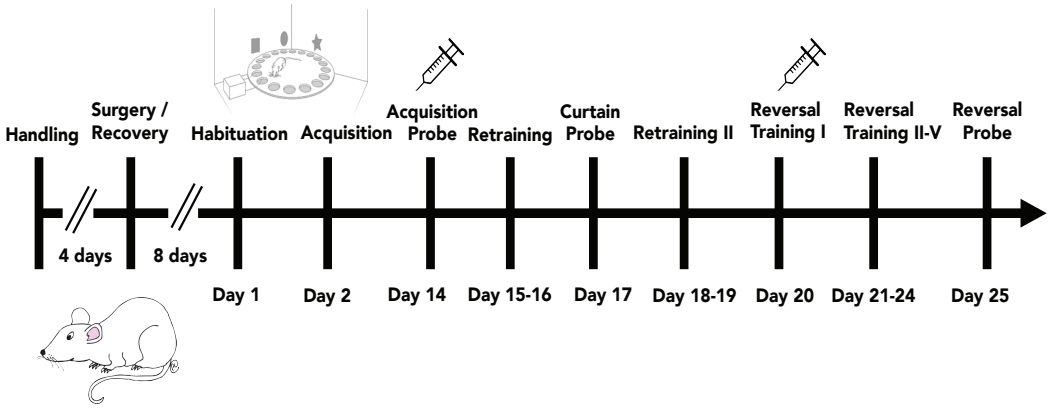


Figure 40. Schematic depicting the experimental timeline of the Barnes Maze study. There were nine major stages: (1) Habituation (2) Acquisition training (3) Acquisition probe (4) Retraining I (5) Curtain probe (6) Retraining II (7) Reversal training I (8) Reversal training II-V and (9) Reversal probe.

3.2.8 Experiment 5: Barnes Maze

Before testing began rats were pseudo-randomly assigned to one of four possible escape locations. These locations were equidistant positioned at 90-degree intervals (North, West, South, East). This was to prevent odour cues from becoming saturated around any one hole, although the maze was cleaned with 10% ethanol between trials to eliminate any odours (Figure 38B). The experiment consisted of nine distinct phases: (1) Habituation (2) Acquisition Training (3) Acquisition Probe Test (4) Retraining (5) Curtain Probe Test (6) Retraining II (7) Reversal Training I (8) Reversal Training II-V and (9) Reversal Probe Test (see experimental timeline Fig 36). For all training trials, rats were grouped into squads of 3-4 where all members of a squad completed a given trial before subsequent trials were run. Each trial (except habituation) began with a 5-second acclimatization period during which the rat was being held in the start box in the center of the maze. Trials began automatically after the 5-second delay and the start box was lifted. Animals were motivated to escape from the brightly lit, open platform into the dark, recessed escape box due to their natural tendency to seek out dark, closed spaces.

3.2.8.1 Habituation

Rats were given one 5-minute habituation trial where they freely explored the maze and could descend into the escape box. Once they entered the escape box, they were permitted to stay in the box for 30 seconds and were then removed and placed back into the center of the maze until the end of the 5-minutes period.

3.2.8.2 Acquisition Training

During acquisition training the animal learned the spatial location of the escape box, as this was consistent from trial to trial. Acquisition training lasted for 12 days. For the first 4 days (A1-A4), rats were given three trials per day and during the following 8 days (A5-A12) this was reduced to two trials per day for a total of 28 trials with an inter-trial-interval (ITI) of 2 hours on all days. Including the habituation trial animals received a total of 29 trials prior to the

Acquisition Probe Test. Since each trial began with the rat in the start box, it was not possible to choose which direction the rat would be facing at the start of the trial. For this reason, we scored all the videos afterwards to determine if the direction the rats were facing at the start of the trial was counterbalanced. This was necessary to assess whether the rats were using a fixed motor response to find the escape hole.

Each trial lasted up to 5 minutes. If the rat did not find the escape hole in that time, it was gently guided to the escape box. Once the rat was inside the escape box, it was left there for 30 seconds before removing the rat and returning it to its home cage. During each trial, ANY-maze software recorded the rat's latency to reach the escape hole (seconds), total distance travelled (cm), and path efficiency. Path efficiency is represented as an index of the efficiency of the path taken by the rat to get from the first position in the test (start) to the last position (escape hole). A value of 1 is indicative of perfect efficiency (e.g. the animal moved in a straight line from the start to the escape hole). It is calculated by dividing the straight-line distance between the first and the last position by the total distance traveled by the rat. This measure was not used during probe sessions, as it cannot be analyzed across time. The experimenter recorded the number of reference errors the animal made prior to reaching the escape hole and the number of hole-deviations there were between the first hole the animal visited and the escape hole. Reference errors were recorded as a rat dipping its head into any hole other than the escape hole. Repeated dips into the same hole were considered a single error. Hole deviations were quantified as the number of escape holes (10 maximum) between the true escape hole and the location in which the animal's head first entered a false escape hole. This ranged between 0-10. The experimenter also measured the search strategy that was used to find the escape hole. There were three possible search strategies: (1) Random (RD) – this occurred when the animal moved about the maze in a random, un-systematic manner, searching the same hole more than once and moving into the center of the maze often. (2) Serial (SE) – Animals that used a serial search strategy first visited a hole more than two hole deviations away from the escape hole and then in a serial fashion systematically

checked adjacent holes until reaching the escape hole. The animals search path was classified as serial even if he did not make any errors but visited a location at the edge of the maze more than two holes away. (3) Spatial (SP) This search strategy occurred when a rat moved directly from the center of the maze to the correct escape hole or any hole within two hole deviations away on either the left or right side of the escape hole.

One hour after the last acquisition training trial stylets were unscrewed from each rat's cannulae, and the infusion cannula was inserted to make sure that the cannula was not blocked. The infuser was left in the cannula for two minutes on each side of the brain to simulate what would occur during testing but no fluid was delivered. This was done in attempts to reduce the elicitation of a nonspecific stress response during the acquisition probe test. Following this, the stylets were screwed back in and the animals were returned to their home cage.

3.2.8.3 Acquisition Probe

The day following acquisition training rats were given a 5-minute probe test where the escape box was removed and replaced with a false escape box. The maze was rotated to ensure that the animals were using extra-maze visuospatial cues to find the escape hole instead of relying on any intra-maze cues. The maze was divided into 20 zones and the time spent in each zone was recorded. Other measures included latency to reach the escape hole, number of reference errors, hole deviations, spatial strategy, and distance traveled.

Fifteen minutes prior to the test, rats were given an infusion of either sterile physiological saline or PRO and then placed back in their home cages. Seven minutes prior to the test rats were given another infusion of either vehicle (saline) or ISO. Infusion volume, rate, and procedure were the same as the previous infusion. Rats were then placed back in the home cage and tested 3 minutes later. This resulted in four groups: vehicle-vehicle (VV; n=6), vehicle-ISO (VI; n=5) PRO-vehicle (PV; n=3), PRO-ISO (PI; n=3). Following the acquisition probe animals received two days of retraining (2 trials per day, ITI = 2hrs) to reduce any extinction learning that may have occurred during the acquisition probe trial.

3.2.8.4 Curtain Probe

The purpose of the curtain probe was to assess whether rats were using intra or extra-maze cues to locate the escape box. The procedure for this test was identical to the acquisition probe except that animals did not receive any infusions and a brown plastic curtain was hung around the maze from the ceiling effectively blocking all visual access to the rest of the room. After this test animals received an additional two days of retraining (2 trials per day; ITI = 2hrs) to reduce any extinction learning that may have occurred during the curtain probe trial.

3.2.8.5 Reversal Training

Reversal training was similar to acquisition training with the same dependent measures except that the location of the escape box was moved to the opposite side of the maze (180-degree rotation). Rats were given 5 days of reversal training with one trial on the first day and two trials per day (ITI = 2hrs) after that. Similar to the acquisition probe, one hour after the last retraining trial stylets were unscrewed from each rat's cannulae, and the infusion cannula was inserted and left in the guide cannula for two minutes on each side of the brain, stylets were then screwed back in and the animals were returned to their home cage. The following day animals received their first reversal training trial. Fifteen minutes prior to the first reversal training trial rats were given an infusion of either saline or PRO. Seven minutes prior to the test rats were given another infusion of either saline or ISO. Rats were then placed back in the home cage and 3 minutes later given the first reversal training trial. The groups were the same as the acquisition probe test (VV, n=6; VI, n=5; PV, n=3; PI, n=3). Therefore, if a rat was in a specific group during the acquisition probe test then that rat remained in that group for the reversal training trial. One hour later, rats in the VV group were split in half and were either returned to their home cages (VV; n=3) or given an infusion of ISO (VVI; n=3) and then returned to their cages. The remainder of the reversal training trials occurred in the absence of any infusions.

3.2.8.6 Reversal Probe

Following reversal training, a probe trial was given to measure how well animals remembered the new location of the escape hole or if perseveration of the previous response would occur due to the older memory of the previous escape location. The procedure for the reversal probe was the same as the curtain probe but without a curtain. The same measures were recorded.

3.3 Histology

Cannula placements were confirmed histologically at the end of the experiments. Rats were infused bilaterally with 1% methylene blue (0.5μL per side; 0.5μL/min) and the infusion cannula was left in place for 1 minute post-infusion to ensure that the liquid had diffused from the injection site. Rats were then decapitated under isofluorane anesthesia. Brains were removed and flash-frozen in a beaker of 2-methyl butane bathed in dry ice/ethanol and then placed at -80 degrees. Coronal sections (35 μm) were cut on a cryostat and mounted to gelatin-subbed slides. Two sets of slides were taken for each brain with every other slice mounted onto slides labeled "A" and the next slice onto slides labeled "B". Sections labeled "A" were left unstained and showed the blue dye to visualize the total area (in μm) where the drug diffused to whereas the corresponding slides labeled "B" were stained with nuclear fast red to visualize the cannula tracks. Slides were then imaged under a microscope at 4x and images from the A slides were superimposed onto the images of the B slides to confirm placement and diffusion site. In the DNMP experiment four animals in the ISO group and three animals from the PRO group were excluded because one or more of the cannulae were not in the correct position. For the Barnes Maze experiment four animals were excluded due to incorrect cannula placements in the DG.

3.4 Data Analysis

Statistical analyses were conducted using SigmaPlot™ version 11.0 (Systat Software, San Jose, CA). For the DNMP task, the dependent measures were: latency to obtain reward,

number of errors, and the percentage of trials where a correct choice was made. Latencies were collected using a timer, and the experimenter recorded the number errors, which was later used to calculate the percentage of correct trials. Pilot data were analyzed using one-way analysis of variance (ANOVA) to compare arm separations 1 through 6. Body weights, habituation, and pre-training data were analyzed using two-way (GROUP x DAY) repeated measures (RM) ANOVAs. Acquisition data was analyzed using two-way (ARM-SEPARATION x DAY) RM ANOVAs. Test data were analyzed using three-way (GROUP x ARM-SEPARATION x TRIAL) ANOVAs and washout, and curtain probe data were analyzed using three-way (GROUP x ARM-SEPARATION x DAY) ANOVAs. Pairwise comparisons were made when necessary using Tukey's HSD test. In all cases, $p<0.05$ was accepted as significant. Error bars in graphs represent +/- sem; *$p < 0.05$.

In quantifying the EPM data we measured distance traveled, mean speed, time spent immobile, line crossings, time spent in each zone of the maze, and the number of entries into the zones. The locomotor measures (distance, speed, line crossings, and immobility) were analyzed one-way ANOVAs, and the time spent in each zone, as well as the number of entries, were analyzed using two-way (GROUP x ZONE) RM ANOVAs. Pairwise comparisons were made when necessary using Holm-Sidak tests. In all cases, $p < 0.05$ was accepted as significant. Error bars in graphs represent +/- sem; *$p < 0.05$.

For the Barnes maze data, we measured path efficiency, total distance traveled, latency to reach the escape hole, the number of hole deviations, reference errors, and characterization of the search path used to find the escape hole. Using a one-way analysis of variance (ANOVA) these data were compared across days during acquisition training. Difference scores in these measures were calculated between the acquisition probe and the last day of acquisition training. For each measure, one-way ANOVAs were then conducted to measure group differences. During each of the probe sessions the maze was divided into 20 equal zones and the time spent in each zone was recorded. Group differences in the time spent in the escape zone, and the escape quadrant (which

included the escape zone as well as the two zones to the left and right of the escape zone) were compared using a one-way ANOVA. To demonstrate whether differences in performance existed between the acquisition probe and the curtain probe a two-way repeated measures (RM) ANOVA with the between-subject factor of GROUP and the within-subject factor of SESSION was conducted. On the first day of reversal training group comparisons in latency to reach the escape hole, hole deviations, and reference errors were calculated using a one-way ANOVA. Performance across the rest of the reversal training days was assessed with a two-way RM ANOVA (GROUP by DAY). During the reversal probe, behaviour across groups was analyzed with a one-way ANOVA. Pairwise comparisons were made when necessary using Tukeys HSD test. In all cases, $p < 0.05$ was accepted as significant. Error bars in graphs represent +/- sem; *$p < 0.05$.

3.3 Experiment 3 (DNMP): Results

3.3.1 Body Weight

Using a two-way RM ANOVA with the between-subject factor of GROUP and the within-subject factor of DAY we saw a significant interaction ($F_{1,44} = 2.145$, $p < 0.001$). Rats gained weight throughout the experiment (data not shown).

3.3.2 Habituation & Pre-Training

During habituation, animals were permitted to obtain a reward in each of the arms that were open. Initially, all 12 arms were open, and this was gradually reduced to 3 arms across the 4 days of habituation. Using a two-way RM ANOVA, we found that the percentage of Froot Loops® collected increased across days, with no group differences observed (main effect of DAY: $F_{3,93} = 9.421$, $p < 0.001$) (Figure 40A).

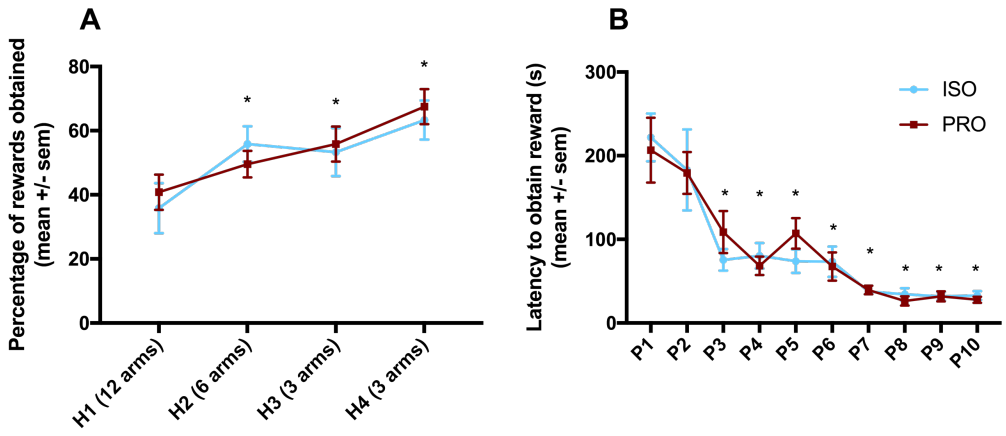


Figure 41. A) Habituation lasted for 4 days. On day 1, rats were given one 20-minute habituation trial where they freely explored the maze with all 12 arms open and baited. On day 2, rats were given two 10-minute trials (inter-trial interval = 1 hr) in the maze with 6 arms open and baited. Days 3 and 4 they were given two 5-minutes trials with 3 arms open and baited. On day 5 rats began Pre-training. B) Pre-training lasted for 10 days. During this phase rats were given two trials a day with only one arm open and baited. The goal was to train the rats to retrieve the reward in less than two minutes. By the tenth day all rats could do this. On day 14 rats began Acquisition training.

During the pre-training phase only one arm of the maze was open and baited and the time it took to obtain this reward was measured. Rats were given two trials per day; a two-way RM ANOVA revealed that there were no group differences in the time it took to learn this behavioural response and that over the course of this training period, animals were able to obtain the reward more quickly with significantly shorter latencies emerging by pre-training day 3 (P3) [main effect of DAY: ($F_{9,279} = 17.446$, $p < 0.001$) (Figure 40B).

3.3.3 Pilot Experiments

To determine what number of arm separations should be used for the highly similar (difficult, DG-dependent) and widely separated (easy, DG-independent) low similarity conditions (in terms of the visual cues that would be used to discriminate them) we ran a pilot study. Animals in this study received habituation and pre-training, and then 6 days of acquisition training. We measured latency to obtain the reward, the number of errors made and the percentage of trials where a correct response was made. Three separate one-way ANOVAs were conducted on the means of the last 3 days. Animals learned to obtain the reward more quickly

across time (data not shown). On the last 3 days of training, there were no differences in the time it took to obtain the reward across all arm separations (Figure 41A). However, there were differences in terms of the number of errors made and the percent of correct trials. A main effect of ARM SEPARATION ($F_{5,66} = 3.498$, $p = 0.007$) showed that rats in the 2-arm separation group made significantly more errors compared to when the separation was 5 and 6 arms (Figure 41B). Likewise, a main effect of ARM SEPARATION $F_{5,66} = 3.178$, $p = 0.012$) also showed that rats in the 2-arm separation group had the lowest percentage of correct trials, which differed significantly from the 5-arm separation group (Figure 41C). Based on the fact that the mice in the Clelland et al., (2009) study were performing at chance levels when a 1-arm separation was used and the fact that clockwise and counter-clockwise permutations cannot be counter-balanced for a 6-arm separation in a 12-arm radial maze we decided to use the 2-arm separation for the difficult, DG-dependent high similarity condition (S2, high) and the 5-arm separation for the easy, DG-independent, low similarity condition (S5, low).

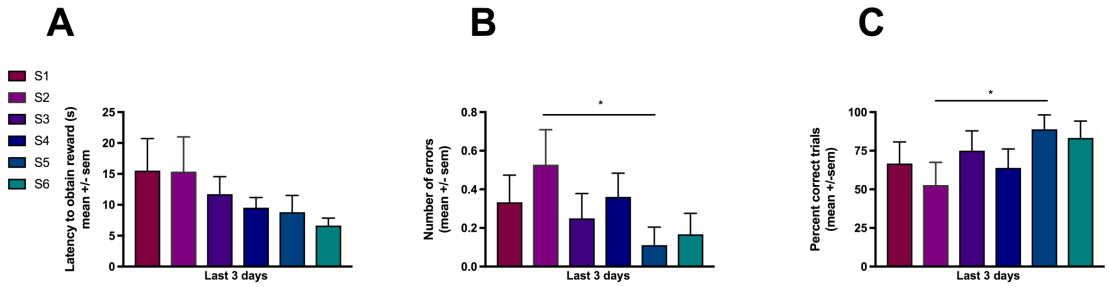


Figure 42. A pilot study was run to determine which arm separations in a 12-arm radial maze would yield the most comparable results to Clelland et al. (2009) from which the DNMP task was adapted. Animals received habituation and pre-training trials and 6 acquisition-training sessions. Each acquisition-training session consisted of 6 trials (sample + choice) a day to assess performance on arm separations 1-6 (order counterbalanced). We assessed A) Latency to obtain the reward B) Number of errors made, and C) Percentage of correct trials. Given that animals made the most errors, and the least percentage of correct trials, in the 2-arm separation when compared to the 5-arm separation, we decided to use these: 2-arm separation (S2, Low, 60 degrees, difficult, DG-dependent) and 5-arm separation (S5, High, 150 degrees, less difficult separation, DG-independent). These separations were also comparable to those used in Clelland et al. (2009) in terms of angular distance.

3.3.4 Acquisition Training

During acquisition, animals were given 4 trials/day (sample + choice), two S2 and two S5. To reach criterion, animals were required to make a correct choice on 4 out of 6 easy (S5)

trials over a period of 3 consecutive days. By the 6th day of acquisition training 62.5% of the rats reached this criterion; the other 37.5% reached this criterion by day 7. For the analysis, we included the first 3 days and the last 3 days of training data for a total of 6 training days. Therefore, the data is inclusive for rats that took 6 days to reach criterion, and for the rats that took 7 days, the set excludes the data from acquisition day 4.

A two-way RM ANOVA (ARM SEPARATION x DAY) was conducted on the latency to obtain the reward in the choice phase. A main effect of DAY demonstrated that animals were able to complete the trials more quickly across acquisition ($F_{5,159} = 8.582$, $p < 0.001$). Post hoc tests revealed that significantly lower latencies began to emerge by acquisition day 3 (A3). There was also a significant main effect of ARM SEPARATION ($F_{1,159} = 13.186$, $p < 0.001$) with animals taking longer to complete the trial when faced with a closer arm separation (S2) (Figure 42A). A two-way RM ANOVA (ARM SEPARATION x DAY) was also conducted to assess differences in the percentage of trials where a correct choice was made. There was a significant main effect of DAY ($F_{5,159} = 9.035$, $p < 0.001$) and a significant main effect of ARM SEPARATION ($F_{1,159} = 10.368$, $p = 0.003$) (Figure 42B). At the start of acquisition training all rats were performing at approximately 50% error rate. As they learned the DNMP task, a difference in performance emerged by acquisition day 5 (A5) with rats improving in the S5 condition to 94% by day 6 and only 65% in the S2 condition. A similar pattern emerged when we ran a two-way RM ANOVA on the number of errors made in the choice trials. There was a significant main effect of DAY ($F_{5,159} = 8.928$, $p < 0.001$) and ARM SEPARATION ($F_{1,195} = 14.144$, $p < 0.001$) (Figure 42C). Toward the end of acquisition training (A5), rats in the S5 condition performed the task with very few errors while rats in the S2 condition continued to find the task difficult.

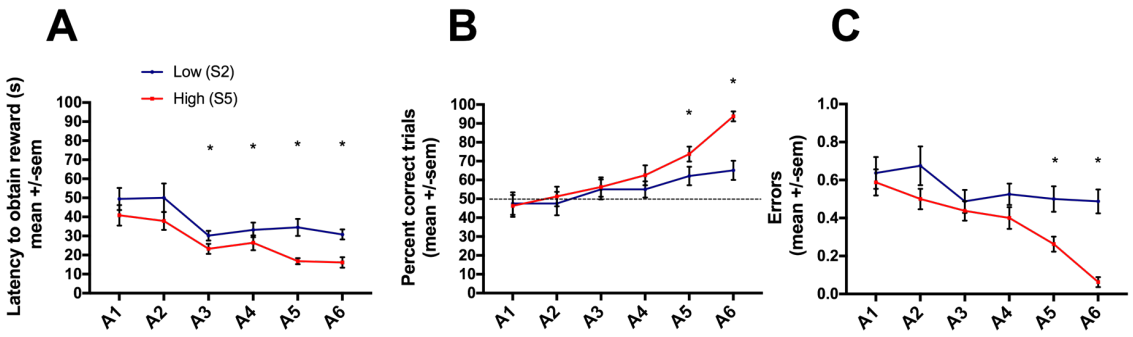


Figure 43. Acquisition Training (A1-A6). Rats took 6-7 days to reach criterion. For rats that took 7 days, the set excludes the data from acquisition day 4. During acquisition animals received 4 trials a day, each data point represents the mean for those 4 trials. A) By A3, animals took significantly less time to obtain the reward, with rats in the 5-arm separation (S5) condition (red) outperforming the rats in the 2-arm separation (S2) condition (blue). All animals began training performing successfully in approximately 50% of the trials. B) By A5, animals in the S5 condition were demonstrating successful performance on 95% of the trials whereas animals in the S2 condition remained at 65% demonstrating that the task is more difficult in the S2 condition. C) This same pattern was also evident in the number of errors made. Significant differences are denoted with an asterix ($p < 0.05$).

3.3.5 Test Day

Rats were either assigned to the ISO group or the PRO group. This remained constant throughout testing. All rats were tested on 4 conditions: PS-S2, PS-S5, PC-S2, and PC-S5, which took place across four test-days (each separated by a washout day). During testing, the latency to obtain the reward, the number of errors, and the percent correct trials were measured. PS and PC data were analyzed separately. Separate three-way ANOVAs (GROUP x ARM-SEPARATION x TRIAL) were conducted for each dependent measure.

3.3.5.1 Pre-Sample Infusions

Latency to obtain the reward. For the trials where the drug was infused 3 minutes prior to sample (PS) (Figure 43A), there were no significant main effects or interactions.

Errors and percent correct trials. A three-way ANOVA revealed that rats tested in the PS condition made more errors (fewer correct choices) when they were administered PRO compared to ISO on both easy (S5) and difficult (S2) trials demonstrated by a significant main effect of DRUG on the number of errors ($F_{1,248} = 4.56$, $p = 0.034$) and percent correct trials ($F_{1,248} = 6.486$, $p = 0.011$) (Figure 43 B-C).

3.3.5.2 Pre-Choice Infusions

Latency to obtain the reward. For the trials where the drug was infused 3 minutes prior to the choice phase (PC) (Figure 43D), there was a main effect of ARM SEPARATION ($F_{1,248}$ = 4.947, p = 0.027) with S2 trials taking longer than S5 trials, and a significant TRIAL x DRUG interaction ($F_{3,248}$ = 3.192, p = 0.024). Post hoc tests revealed that animals in the ISO group demonstrated longer latencies to obtain the reward on the test trial compared to baseline (p = 0.008) and compared to PRO animals (p = 0.022) in the difficult (S2) condition. In the easy (S5) condition, post hoc analyses showed that ISO animals did not exhibit longer latencies during the test, but took significantly longer during the post-test compared to baseline (p = 0.002) and the test (p = 0.034) and that during the post-test there was a significant difference between ISO and PRO animals (p = 0.006).

Errors and percent correct trials. When the same analysis was conducted on the number of errors made during the choice phase, there was a main effect of DRUG ($F_{1,248}$ = 4.609, p = 0.033). Animals made more errors when they were administered ISO in both S2 and S5 trials, however, this effect was more pronounced in the difficult (S2) condition. For percent correct trials, there were no significant main effects or interactions however, there was a parallel trend observed (Figure 43 E-F).

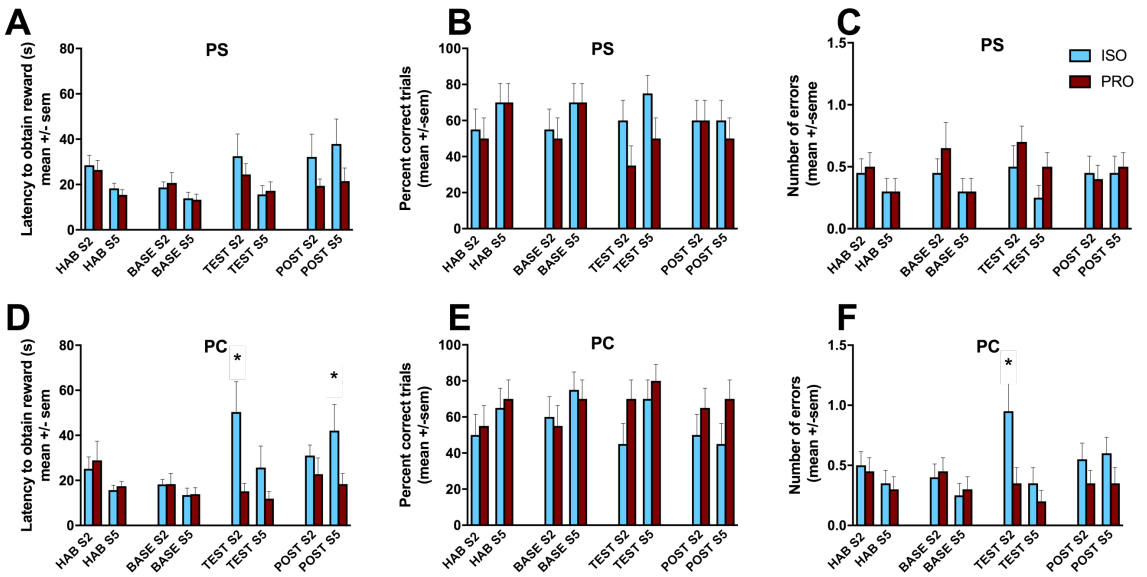


Figure 44. Animals were tested on conditions on 4 different days with each test day separated by a washout period. Rats were assigned to either the ISO, (n=16) or PRO (n=17) group. They received a drug infusion either 3 minutes prior to the sample phase (Pre-Sample, PS) or the choice phase (Pre-Choice, PC) and were tested in the 2-arm separation (S2) or 5-arm separation (S5) condition. The 4 conditions were as follows: PS-S2, PS-S5, PC-S2, and PC-S5. In contrast to acquisition training, on each test day, instead of receiving two S2 and two S5 trials, animals received all four trials in the condition they were being tested (all S2 or all S5). This allowed us to include trial as an additional within-subject factor. Trial 1 was a habituation trial (HAB) where, like the previous day, stylets were removed and the infusion cannula was inserted but no fluid was infused. Trial 2 served as a baseline trial (BASE) where animals were infused with saline and trial 3 was the test trial (TEST) where animals received the drug treatment they were assigned to. Stylets were replaced and animals were placed back in their home cages. Trial 4 was a 90-minute post-test trial (POST). We measured A&D) Latency to obtain the reward B&E) Percent correct trials and C&F) Number of errors. Data from the PS condition (A-C) shows that PRO given PS (B) causes a reduction in the percentage of correct trials. Data from the PC condition (D-F) shows that ISO given PC causes D) an increase in latency during the test and this carries over to the post-test as well as, F) an increase in the number of errors which also carries over and is more pronounced in the S2 condition. Significant differences are denoted with an asterix ($p < 0.05$).

3.3.6 Washout

Between each test day, animals were given a washout day that was identical to acquisition training to allow the drug to clear before recommencing testing. Using a three-way ANOVA (GROUP x ARM SEPARATION x DAY) across all four washout days on the latency to obtain the reward, we found a main effect of ARM SEPARATION ($F_{1,249} = 17.333$, $p < 0.001$). Like acquisition, rats took longer in the S2 trials compared to the S5 trials (Figure 44 A-B). The latencies were also similar. The same analysis yielded a significant main effect of ARM SEPARATION on the number of errors ($F_{1,249} = 16.882$, $p < 0.001$) and the percent correct trials

($F_{1,249} = 13.522$, $p < 0.001$) with animals in the difficult (S2) condition making more errors than those in the easy (S5) condition (Figure 44 C-F).

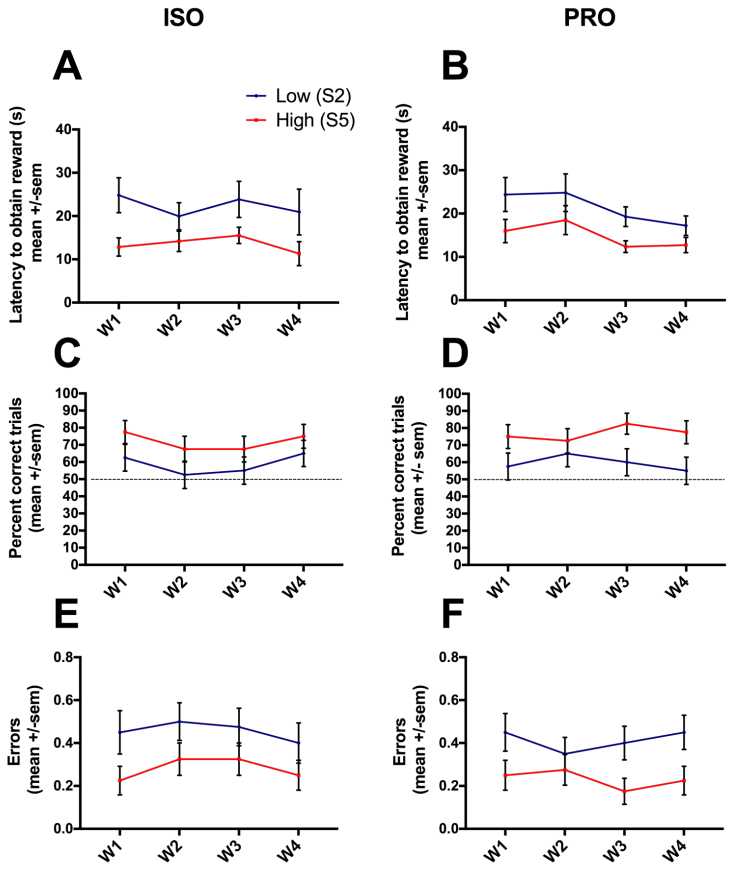


Figure 45. Washout Days (W1-W4). Between each test day rats were given a washout day to ensure there were no carry over effects from drug infusions (left=ISO, right=PRO). In all measures (A-B) Latency to obtain reward, (C-D) Percent correct trials and (E-F) Number of errors, performance was similar to acquisition and consistent across all washout days. Consistent with acquisition training, animals performed better in the S5 condition (red) compared to the S2 condition (blue).

3.3.7 Curtain Probe

To ensure that animals were using extra-maze cues rather than intra-maze or interoceptive cues to complete the DNMP task, following the last washout day animals were given a curtain probe. This day was identical to acquisition training and washout except that a blue curtain was hung from the ceiling in a circular fashion, surrounding the maze such that animals could not see any of the cues in the room except for the webcam above and a partial view of a few ceiling tiles. We compared the data from the curtain probe to the data from the previous

washout day. Using a three-way ANOVA (GROUP x ARM SEPARATION x DAY) run on latency to obtain the reward, errors and percent correct trials, we found a main effect of DAY on latency ($F_{1,124} = 15.170$, $p < 0.001$), errors ($F_{1,124} = 31.703$, $p < 0.001$), and on the percent correct trials ($F_{1,124} = 31.391$, $p < 0.001$) with rats taking much longer to complete the trials, and making significantly more errors in both drug conditions on both S2 and S5 trials during the curtain probe (Figure 45 A-B).

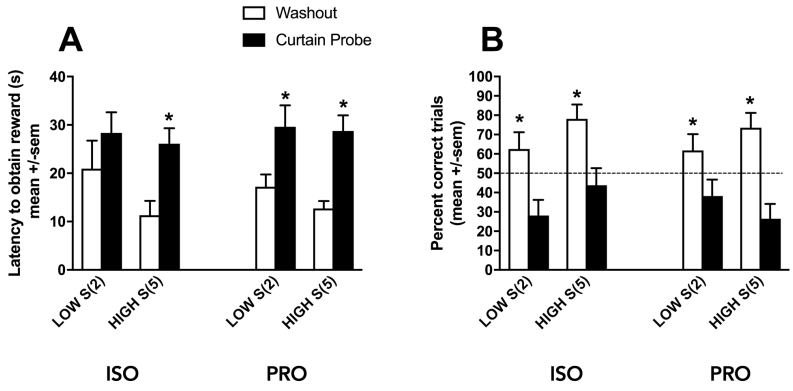


Figure 46. To ensure that rats were using extra-maze cues, we gave them a curtain probe trial where a curtain was placed around the maze so that the cues on the wall were no longer visible. We compared performance on this test to the previous washout session. As expected, there were no group differences and all animals preformed worse on the curtain probe compared to washout. A) Rats took longer to obtain the reward and demonstrated B) fewer percent correct trials during the curtain probe test. Significant differences are denoted with an asterix ($p < 0.05$).

3.4 Experiment 4 (EPM): Results

To determine whether the aforementioned effects were related to any possible anxiolytic / anxiogenic or locomotor properties of the drugs used, we ran a separate experiment using the elevated plus maze. Dependent measures related to locomotor activity included distance traveled, mean speed, number of line crossings, and time spent immobile. For each of these measures separate one-way ANOVAs were conducted. There were no group differences (Figure 46 A-D). We also measured time spent in the open arms, closed arms, and start area for each rat, as well as the number of entries made into the open and closed arms. Using two-way RM ANOVAs with the between-subject factor of GROUP and the within-subject factor of ZONE, we found a main

effect of ZONE. All animals spent more time in the open arms compared to the closed arms or the start area of the maze ($F_{2,47}$ =28.043, p < 0.001) (Fig 10A) and there were significantly more entries into the open arms compared to the closed arms ($F_{2,47}$ =14.758, p = 0.002) (Figure 47A-B).

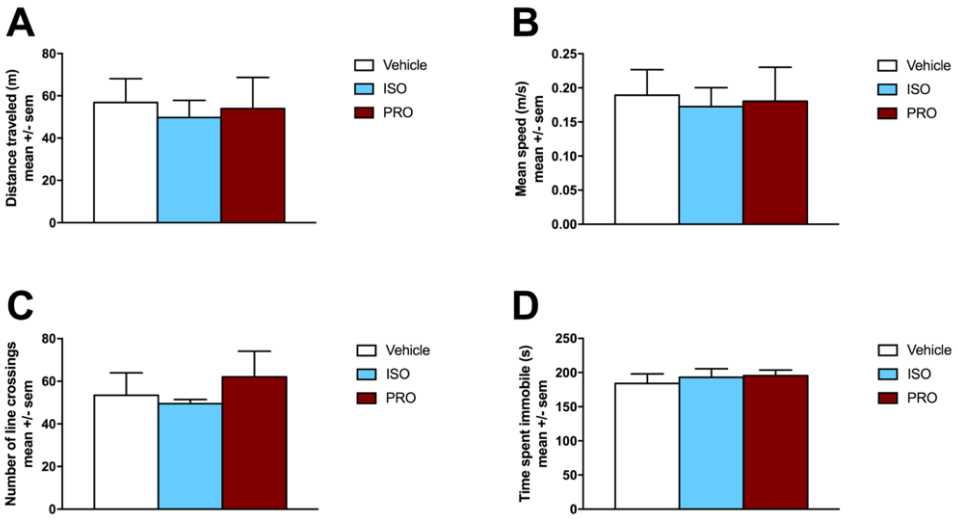


Figure 47. Rats were tested in the elevated plus maze to determine if either ISO or PRO affected locomotor behaviour. We measured A) Total distance traveled B) Speed C) Number of line crossings and D) Time spent immobile and found no effect of either ISO (blue) or PRO (red) on locomotion.

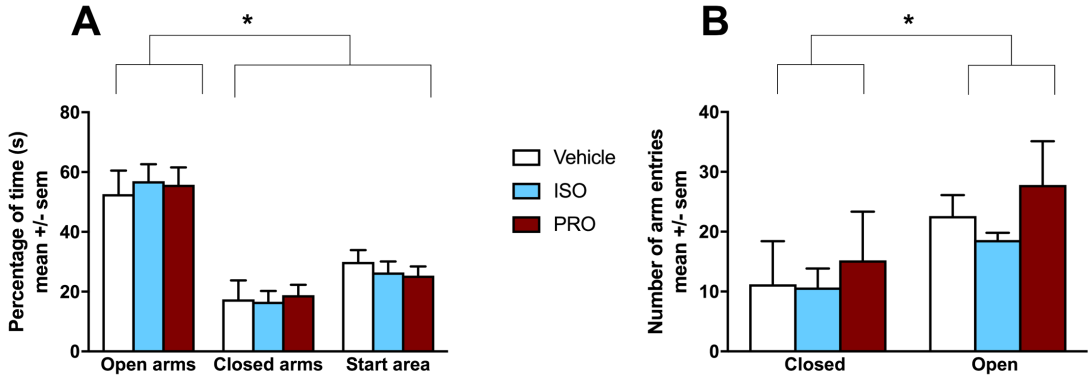


Figure 48. Rats were tested in the elevated plus maze to determine if either ISO or PRO affected anxiety-like behaviour. We measured A) Percentage of time the animals spent in the open arms, closed arms, and the start area. We also measured B) the number of arm entries into the open and closed arms. We found no group differences and no effect of ISO (blue) or PRO (red) on anxiety-like behaviour. All animals spent more time in the open arms. Significant differences are denoted with an asterix (p < 0.05).

3.5 Experiment 5 (Barnes Maze): Results

3.5.1 Body Weight

A two-way RM ANOVA was run with the between-subject factor GROUP and the within-subject factor DAY to assess if there were any differences in body weight. There were no group differences, and as expected, a main effect of day as rats gained weight throughout the experiment ($F_{23,276} = 17.489$, $p < 0.001$) (data not shown).

3.5.2 Cardinal Direction at Start

Given that each rat was placed in a holding box for a 5-second acclimatization period at the start of each trial, we could not choose the direction the rat would be facing when the trial began. To ensure that this was counterbalanced for north, west, south and east directions, the videos were scored (n = 850) by a researcher blind to the conditions of the experiment. The results are listed in Table 10.

Table 10.

Cardinal Direction Rat Faced at Start of Trial

Direction	Number of Trials	Percentage of Trials (%)
North	236	27.76
West	227	26.71
South	191	22.47
East	196	23.06

Note: Total number of trials=850 (does not including habituation trial).

3.5.3 Acquisition Training

During the 12 days of acquisition training (A1-12) animals learned the location of the escape hole. Compared to the first day of acquisition, latency to reach the escape hole was significantly lower by day 9 (Figure 48A). By this day, animals also traversed less distance in the maze per trial (Figure 48F) as they exhibited a more direct heading towards the escape hole and spent less time exploring the maze. This was further demonstrated by a significant increase in the

percentage of animals using a spatial search strategy compared to a serial search strategy (very few animals used a random strategy at any point in the experiment) (Figure 48B), and by the significant increase in path efficiency (Figure 48D), both responses emerging by day 7. Rats also made significantly fewer reference and hole deviation errors by day 7 (Figures 48C & 48E).

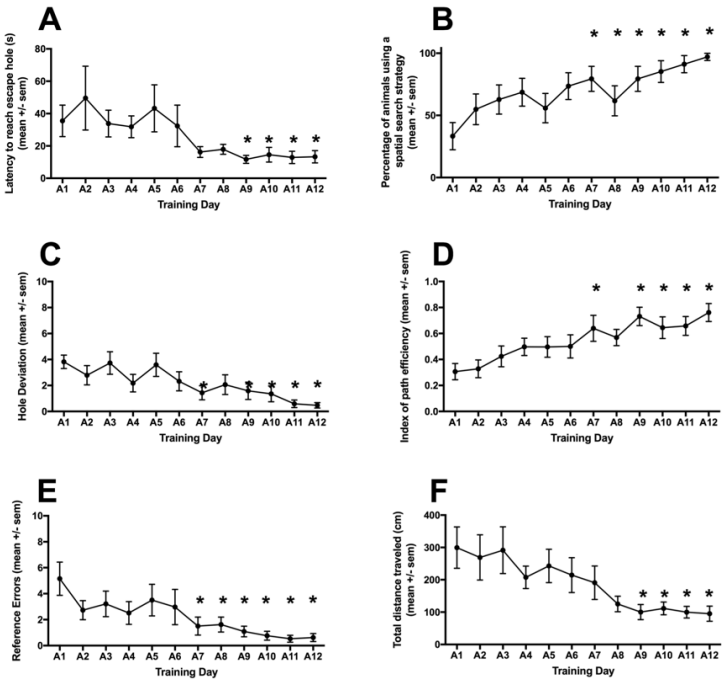


Figure 49. During the 12 days of acquisition training (A1-A12) rats learned the spatial location of the escape box, which was consistent from trial to trial. For the first 4days (A1-A4) rats were given three trials per day and during the following 8 days (A5-A12) this was reduced to two trials per day for a total of 28 trials with an inter-trial-interval (ITI) of 2 hours on all days. During each trial, ANY-maze software recorded the rat's A) latency to reach the escape hole, F) total distance travelled, and D) path efficiency. Path efficiency is represented as an index of the efficiency of the path taken by the rat to get from the first position in the test (start) to the last position (escape hole). A value of 1 is indicative of perfect efficiency (e.g. the animal moved in a straight line from the start to the escape hole). It is calculated by dividing the straight-line distance between the first and the last position by the total distance traveled by the rat. This measure was not used during probe sessions, as it cannot be analyzed across time. The experimenter recorded E) the number of reference errors the animal made prior to reaching the escape hole and the C) number of hole-deviations there were between the first hole the animal visited and the escape hole. Reference errors were recorded as a rat dipping its head into any hole other than the escape hole. Repeated dips into the same hole were considered a single error. Hole deviations were quantified as the number of escape holes (10 maximum) between the true escape hole and the location in which the animal's head first entered a false escape hole. This ranged between 0-10. B) The experimenter also measured the search strategy that was used to find the escape hole. There were three possible search strategies: (1) Random (RD) – this occurred when the animal moved about the maze in a random, un-systematic manner, searching the same hole more than once and moving into the center of the maze often. (2) Serial (SE) – Animals that used a serial search strategy first visited a hole more than two hole deviations away from the escape hole and then in a serial fashion systematically checked adjacent holes until reaching the escape hole. The animals search path was classified as serial even if he did not make any errors but visited a location at the edge of the maze more than two holes away. (3) Spatial (SP) This search strategy occurred when a rat moved directly from the center of the maze to the correct escape hole or any hole within two hole deviations away on either the left or right side of the escape hole. By A7, all animals were demonstrating better performance than on the first day (A1). Significant differences are denoted with an asterix ($p < 0.05$).

3.5.4 Acquisition Probe Test

We compared the last day of acquisition training to performance during the acquisition probe (AP) across groups using a difference score for latency (Figure 49A), search strategy (Figure 49B), hole deviations (Figure 49C), and reference errors (Figure 49D). A one-way ANOVA showed a main effect of GROUP on latency to reach the escape hole ($F_{3,13} = 4.041$, $p = 0.031$). Post hoc analyses revealed that this effect was attributed to an increase in the latency to reach the escape hole during the AP in the group that received ISO (VI). While the VI group also made more reference errors, larger hole deviations, and fewer of these animals used a spatial search strategy, these effects were not significant.

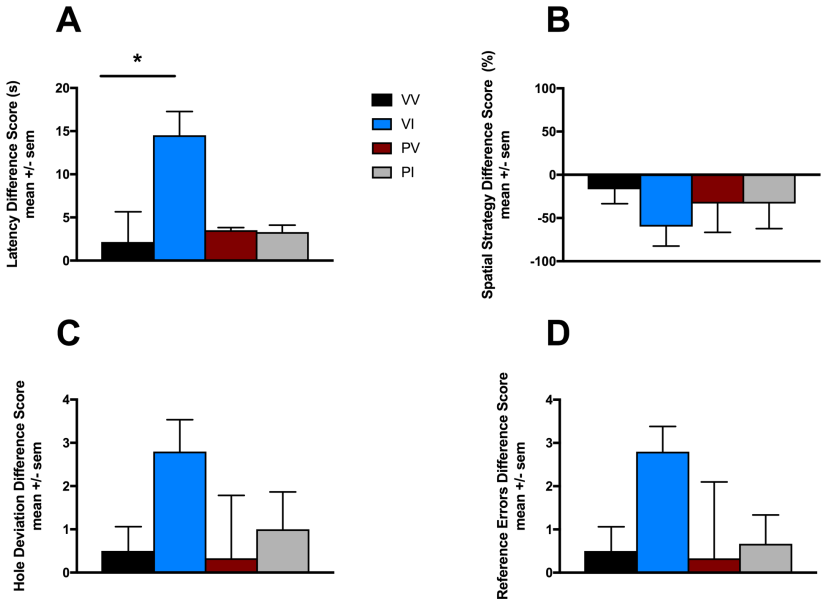


Figure 50. Animals were given a 5-minute acquisition probe test where the escape box was removed and replaced with a false escape box. The maze was rotated to ensure that the animals were using extra-maze visuospatial cues to find the escape hole instead of relying on any intra-maze cues. 15 min prior to the test, rats were given an infusion of either saline (V) or PRO and then placed back in their home cages. 5 min prior to the test rats were given another infusion of either saline (V) or ISO. Therefore, there were 4 groups: VV (black), VI (blue), PV (red), and PI (grey). We compared the last day of acquisition training (A12) to performance during the acquisition probe (AP) across groups using a difference score for A) latency to reach the escape hole, B) percentage of animals using a search strategy, C) hole deviations, and D) reference errors made. While the VI group showed the greatest reference memory impairments compared to the other groups, the only measure that was significant was latency. Significant differences are denoted with an asterix ($p < 0.05$).

During the probe trials, the maze was divided into 20 equal zones. The *escape zone* (ZC) contained the escape hole, and the *escape quadrant* (ZC, ZQ-2, ZQ-1, ZQ+1, ZQ+2) contained

the escape zone plus the two zones to the left and right of the escape zone. The amount of time spent in the escape zone the escape quadrant was calculated, and compared across groups (Figure 50). Animals in the VI group spent greater than chance levels of time in the escape zone but less time compared to the other groups ($F_{3,13} = 3.128$, $p = 0.062$). These animals also spent significantly less time in the escape quadrant ($F_{3,13} = 3.8$, $p = 0.037$). These results demonstrate that the ISO infusion given 3 minutes prior to the AP trial, impaired spatial performance in the maze. This effect was not observed in the other groups.

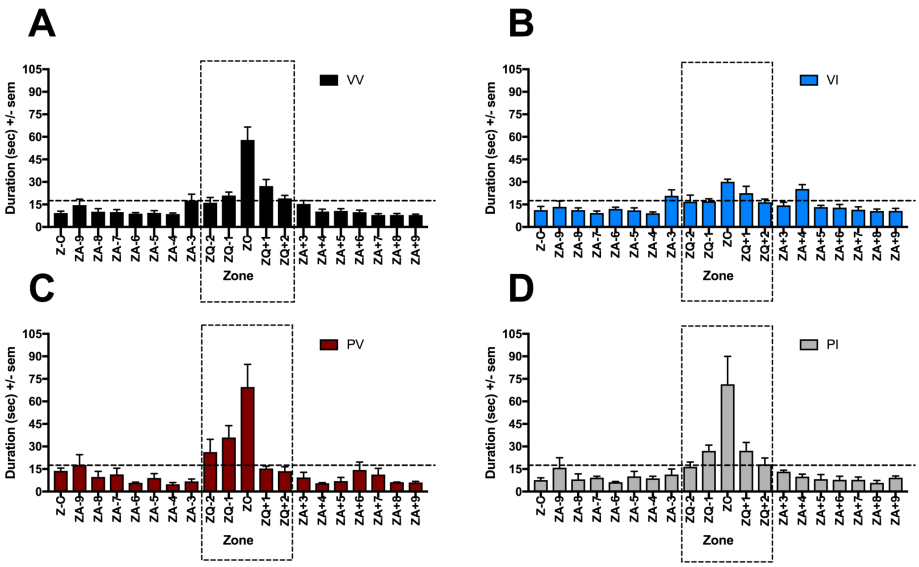


Figure 51. Acquisition probe: During the probe trials, the maze was divided into 20 equal zones. The *escape zone* (ZC) contained the escape hole, and the *escape quadrant* (ZC, ZQ-2, ZQ-1, ZQ+1, ZQ+2) contained the escape zone plus the two zones to the left and right of the escape zone. The amount of time spent in the escape zone the escape quadrant was calculated, and compared across groups. Animals in the VI (blue) group spent greater than chance levels (dotted line) of time in the escape zone but less time compared to the other groups (VV=black, PV=red, PI=grey). These animals also spent significantly less time in the escape quadrant. These results demonstrate that the ISO infusion given 3 minutes prior to the AP trial, impaired spatial performance in the maze. This effect was not observed in the other groups.

3.5.5 Curtain Probe Test

Animals were retrained following the AP and then given a curtain probe trial (CP) to determine if they were indeed using extra-maze cues to locate the escape hole. We compared the previous trial (RT2) to the CP for latency, search strategy, reference errors, and hole deviation (Figure 51) and calculated a difference score. Separate one-way ANOVAs were conducted across groups. As expected, there were no group differences, but all groups showed impaired

performance including increased latency ($F_{3,13}$ = 14.851, p = 0.002), hole deviation ($F_{3,13}$ = 16.916, p = 0.001), and reference errors ($F_{3,13}$ = 14.031, p = 0.002), as well as a significant decrease in the percentage of animals using a spatial search strategy ($F_{3,13}$ = 21.994, p < 0.001) on the CP compared to the previous training day (Figure 51A-D).

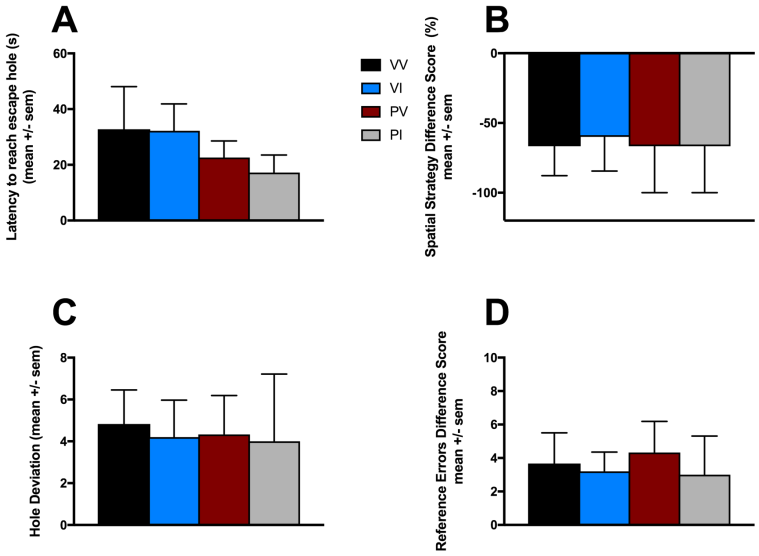


Figure 52. Animals were given a curtain probe trial CP to ensure that they were using extra-maze cues to locate the escape hole. We compared the previous retraining trial (RT2) to the curtain probe trial and calculated a difference score for A) latency to reach the escape hole, B) percentage of animals using a spatial search strategy, C) hole deviations and D) number of reference errors. All animals showed impaired performance including increased latency, hole deviations, and reference errors, as well as a decrease in the percentage of animals using a spatial search strategy demonstrating that they were using extra-maze cues to solve the task.

The time spent in each zone during the CP is shown in Figure 52. We compared the time spent in the escape zone across groups during the CP and the AP using a two-way RM ANOVA and found a significant GROUP x DAY interaction ($F_{3,13}$ = 3.869, p < 0.001). Post hoc analyses revealed that this effect was due to rats spending less time in the escape zone during the CP compared to the AP, except the rats in the VI group, which showed impaired performance on this measure during both probe tests (Figure 53A). We also conducted a two-way RM ANOVA looking at the time spent in the escape quadrant; there was a main effect of GROUP ($F_{3,13}$ = 5.103, p = 0.015) driven by the difference between the VI and PI group, and a main effect of DAY ($F_{3,13}$ = 40.809, p < 0.001). All rats spent significantly more time in the escape quadrant

during the AP compared to the CP (Figure 53B). From these results, we can infer that the rats were relying on extra-maze cues, rather than interoceptive or intra-maze cues to locate the escape hole.

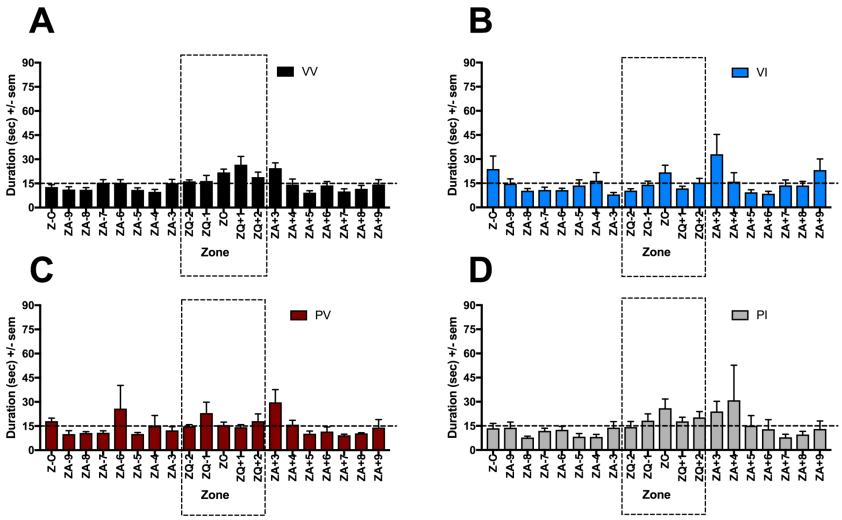


Figure 53. Curtain probe: During the probe trials, the maze was divided into 20 equal zones. The *escape zone* (ZC) contained the escape hole, and the *escape quadrant* (ZC, ZQ-2, ZQ-1, ZQ+1, ZQ+2) contained the escape zone plus the two zones to the left and right of the escape zone. The amount of time spent in the escape zone the escape quadrant was calculated, and compared across groups. Animals were equally impaired and spent the same amount of time in all zones of the maze. Time spent in the escape zone or quadrant was not greater than chance (dotted line) suggesting that animals use extra-maze cues to locate the escape hole.

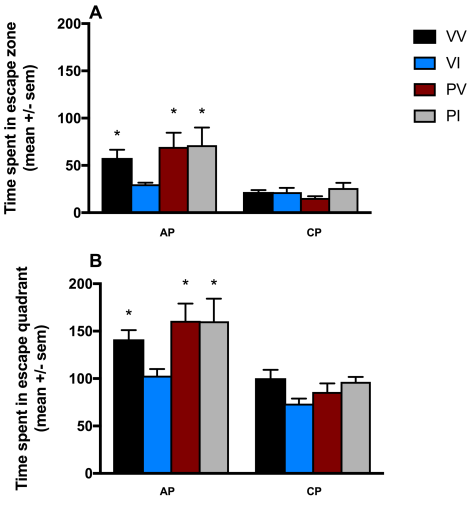


Figure 54. The time spent in the A) escape zone and the B) escape quadrant was compared across group during the acquisition probe (AP) and the curtain probe (CP) to show that the level of impairment induced following administration of ISO (VI, blue) during the acquisition probe, was equal in magnitude to the impairment induced if there were no extra-maze cues present to successfully perform the task. In both cases, the animal's "map" needed to solve the task was compromised. Significant differences are denoted with an asterix ($p < 0.05$).

3.5.6 Reversal Training

We assessed the effect of moving the location of the escape hole to the opposite side of the maze during reversal training. To investigate group differences during the first reversal trial where animals received another drug treatment prior to the trial, we used separate one-way ANOVAs to examine latency, hole deviation and reference errors. Search strategy was not analyzed, as all animals on this trial used a serial search strategy. The one-way ANOVA on latency showed a main effect of GROUP ($F_{3,13} = 5.48$, $p = 0.012$) (Figure 54A), and no group differences in reference errors (Figure 54B) or hole deviation (Figure 54C).

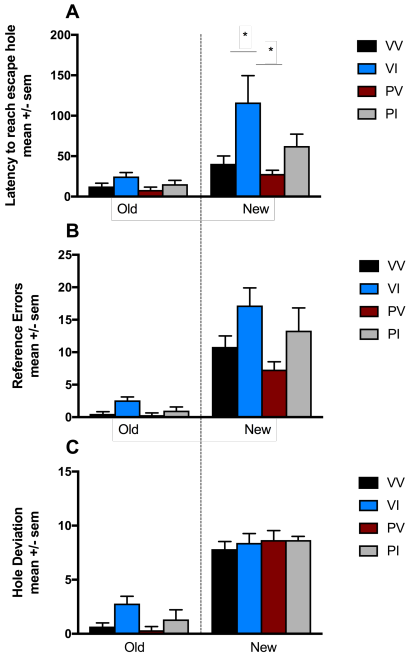


Figure 55. Reversal Training Day 1(RV1): We assessed the effect of moving the location of the escape hole to the opposite side of the maze during reversal training. 15 minutes prior to the first reversal training trial rats were given an infusion of either saline (V) or PRO. 5 min prior to the test rats were given another infusion of either saline or ISO. Rats were then placed back in the home cage and 3 minutes later given the first reversal training trial. The groups were the same as the acquisition probe test (VV= black, VI=blue, PV=red, PI=grey). We calculated A) latency to, B) hole deviations from, and C) reference errors for both the old and new escape holes. Animals in the VI group tool the longest to find the new escape hole. Significant differences are denoted with an asterix ($p < 0.05$).

For the remainder of the reversal training days (RV2-RV5), rats received two trials per day. We took the mean of the two trials and analyzed learning for the new location of the escape by comparing performance across days. Using a two-way RM ANOVA with the between-subject

factor of GROUP and the within-subject factor of DAY we looked at latency (Figure 55A), search strategy (Figure 55B), hole deviation (Figure 55C), and reference errors (Figure 55E). In addition, similar to acquisition training, we looked at total path efficiency (Figure 55D) and distance traveled (Figure 55F). For all analyses, we did not find any group differences but found a main effect of DAY for each measure. Overall, animals took less time to find the new escape hole on RV3-5 compared to RV2 ($F_{3,36} = 6.669$, $p = 0.001$) and began using a spatial search strategy by RV4 ($F_{3,36} = 5.533$, $p = 0.003$). The number of hole deviations ($F_{3,36} = 11.948$, $p < 0.001$) and reference errors ($F_{3,36} = 13.64$, $p < 0.001$) made on RV4-5 were significantly lower than RV2-3. Moreover, animals became more efficient in the path they took to reach the escape hole by RV4 ($F_{3,36} = 7.731$, $p < 0.001$) and traveled less distance by RV4 compared to RV2-3 ($F_{3,36} = 10.097$, $p < 0.001$).

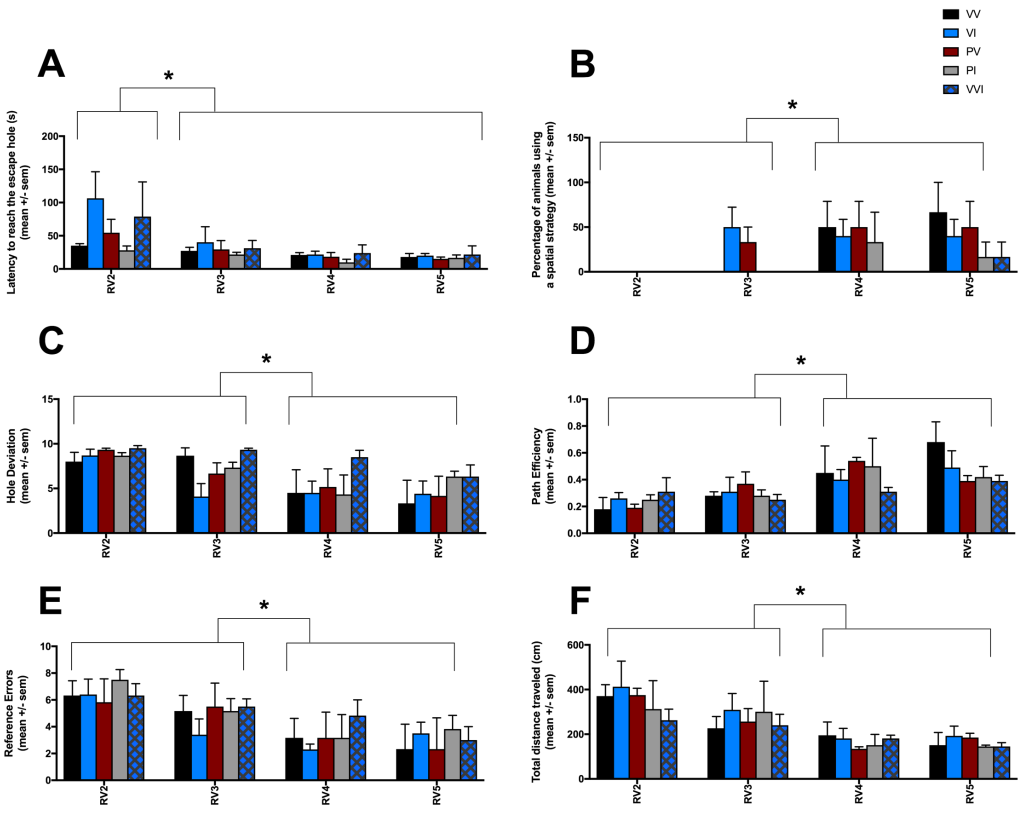


Figure 56. Reversal Training Days 2-5 (RV2-5): For the subsequent reversal training days' animals received two trials a day (mean of both trials reported) and six measures were taken with respect to the new escape hole A) Latency to reach the escape hole B) Percentage of animals using a spatial search strategy, C) Hole deviations D) Path efficiency, E) Reference errors, and F) Total distance traveled. No group differences were found, but all

animals performed better on the last two reversal training days compared to the first two. Significant differences are denoted with an asterix ($p < 0.05$).

3.5.7 Reversal Probe Test

To assess memory for the new escape hole location, as well as investigate whether activation of BARs in the DG immediately prior to reversal training, conferred any mnemonic advantage we used separate one-way ANOVAs to compare groups on latency, search strategy, hole deviation, and reference errors in the reversal probe (RP). There were no group differences in latency (Figure 56A), search strategy used (Figure 56B), or reference errors made (Figure 56D). In terms of hole deviation (Figure 56C) there was a main effect of GROUP ($F_{4,12} = 4.828$, $p = 0.015$). Post hoc tests showed that this effect was attributed to a difference between the VI group and the PV and VVI groups.

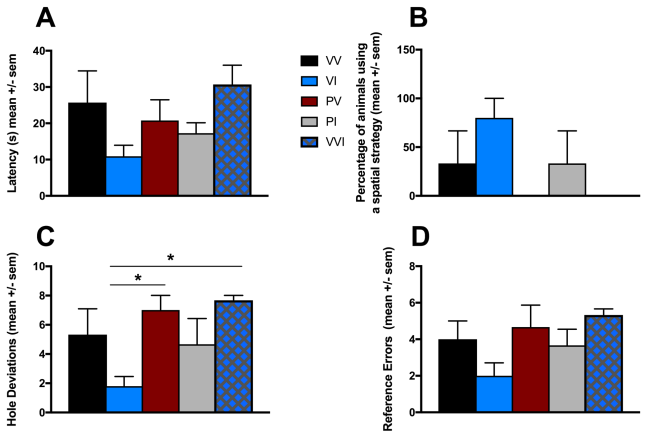


Figure 57. Animals were given a 5-minute reversal probe test where the escape box was removed and replaced with a false escape box. The maze was rotated to ensure that the animals were using extra-maze visuospatial cues to find the escape hole instead of relying on any intra-maze cues. 15 minutes prior to the test, rats were given an infusion of either saline (V) or PRO and then placed back in their home cages. Five minutes prior to the test rats were given another infusion of either saline (V) or ISO. Therefore, there were 4 groups: VV (black), VI (blue), PV (red), and PI (grey). We measured A) latency to reach the escape hole, B) percentage of animals using a search strategy, C) hole deviations, and D) reference errors made. The VI group demonstrated the greatest cognitive flexibility compared to the other groups, however, the only measure that was significant was hole deviations. Significant differences are denoted with an asterix ($p < 0.05$).

The distribution of time spent in each of the 20 zones during the RP test is shown in Figure 57. For the time spent in the escape zone across groups, Levene's test for homogeneity of variance revealed unequal variances ($p = 0.007$) and thus we ran a nonparametric Kruskal-Wallis one-way ANOVA on ranks and found no significant group differences. For the time spent in the

escape quadrant, we ran a one-way ANOVA and found a near significant main effect of GROUP ($F_{3,13} = 3.319$, $p = 0.054$). From the trend of these data we can infer that animals in the VI group showed enhanced performance compared to the other groups, when tested in the RP. This may be the result of enhanced cognitive flexibility imparted via the activation of BARs in the HF, immediately prior to learning a new escape hole location.

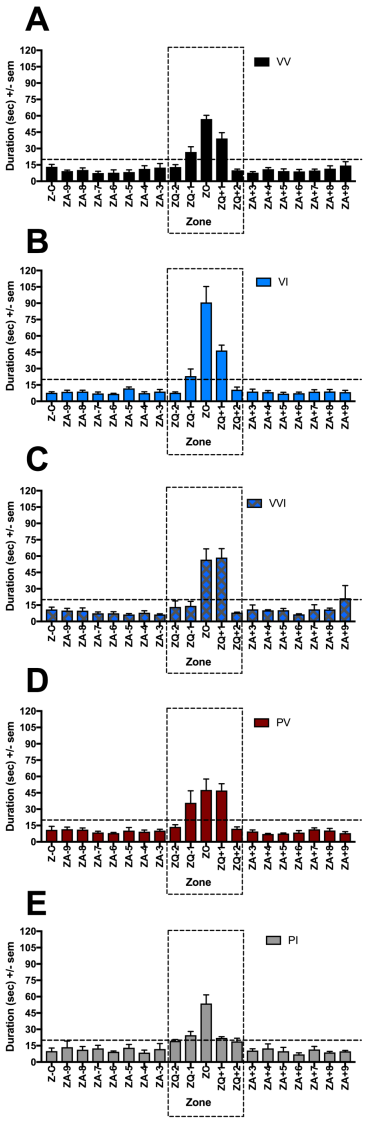


Figure 58. Reversal probe: During the probe trials, the maze was divided into 20 equal zones. The *escape zone* (ZC) contained the escape hole, and the *escape quadrant* (ZC, ZQ-2, ZQ-1, ZQ+1, ZQ+2) contained the escape zone plus the two zones to the left and right of the escape zone. The amount of time spent in the escape zone the escape quadrant was calculated, and compared across groups. All animals spent greater than chance levels (dotted line) of time in the escape zone but the VI (blue) spent more time compared to the other groups (VV=black, PV=red, PI=grey). These results demonstrate that the ISO infusion given 3 minutes prior to the first reversal training trial, promoted cognitive flexibility and improved spatial performance in the maze.

3.5.8 Locomotion

To ensure that the drug treatments we administered did not have any effect on locomotor behaviour, we compared the total distanced traversed in the maze during each of the probe sessions. Using a two-way RM ANOVA with the between-subject factor of GROUP and the within-subject factor of DAY, we found no differences in any of the means compared (Figure 58).

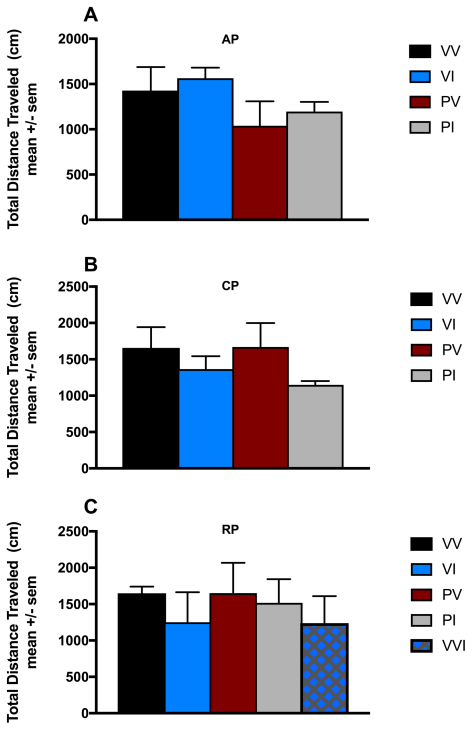


Figure 59. Total distance was measured in each of the probe tests A) Acquisition probe B) Curtain probe and C) Reversal probe. There were no group differences and the distance traveled was consistent across each trial.

3.4. Discussion

In the Chapter 2, we demonstrated that phasic activation of the LC was associated with hippocampal plasticity involving the recruitment of new neurons and a global reorganization of hippocampal representations. This is in line with the fact that phasic LC activity, and subsequent NE release is associated with the detection of novelty. Given that our previous study implicates the LC-NE system in the remapping of contextual representations, we ran the current set of experiments to assess whether behavioural evidence for this could be obtained via activation of

BARs in the DG. These data complement the IEG expression data in Chapter 2 to show that activation of BARs in the DG may facilitate remapping of contextual representations. Together, these data suggest that the presence of this molecular switch biases the memory system towards encoding, while its absence promotes retrieval. Here we show intra-DG infusions of ISO given prior to retrieval (when theoretically a contextual map formed during encoding needs to be reactivated) caused deficits in working and reference memory retrieval, an effect blocked by pre-treatment with PRO.

Consistent with the results of Clelland et al. (2009), animals performed better when the distance between the arms in the radial maze was greater. Curtain probe trials demonstrated that animals relied on extra-maze cues to complete the task and these cues tend to be more disparate when the distance between arms is greater. In contrast, when the arms are closer together in space, the distal cues used to orient potentially overlap causing interference in the representations for the arms. The DG is considered a pattern separator and can orthogonolize these representations even when the arms are close, thus the closer the sample and choice arm are in the task, the more the task becomes dependent on the DG for processing. Pre-choice administration of ISO resulted in greater impairments in the S2 condition compared to the S5 condition, consistent with the role of the DG in pattern separation. Given the effects of ISO on latency, it is possible that ISO caused an increase in exploratory behaviour, which is in line with previous research demonstrating the involvement of NE in exploration (Flicker & Geyer, 1982) and more specifically, the ability of ISO to promote diversive exploration (Geyer & Masten, 1989). Accuracy was only affected in the S2 condition; therefore, it is possible that HF-dependent recollection processes were impaired and performance in this condition relied solely on HF-independent familiarity mechanisms (Eichenbaum et al., 2012).

Importantly, these effects were not due to changes in locomotor activity as there were no group differences in the total distance travelled, the mean speed, or the number of line crossings in the EPM. Similarly, these effects were not the result of changes in anxiety-related behaviour

since there were no group differences in the time spent in the open or closed arms of the EPM. All animals spent more time in the open arms of the maze, suggesting that these drugs do not impart anxiogenic effects.

PRO given prior to the choice phase had no effect. We were unable to find a study done in rats in which PRO was infused into the DG to establish an effective dose. Therefore, it is possible that the dose we used was too low. However, Qi et al. (2008) used a high dose of PRO (15ug) in the CA1 to assess whether BARs played a role in contextual fear memory retrieval. They found that that PRO produced no effect on retrieval after 1 or 7 days after contextual fear learning occurred. Interestingly, they also found that ISO (10ug) infused 30min before the retention test severely disrupted retrieval of 7-day contextual fear memory, which is consistent with our findings. It is also important to note that during the choice phase in the current study, animals were required to reactivate a previously formed representation. Since the task did not require the recruitment of a new ensemble, our hypothesis in fact predicts that infusion of PRO prior to the choice phase would not impair this ability. Thus, it is not surprising that we did not find an effect with PRO, consistent with the findings of Qi et al. (2008).

Many studies investigating the role of NE on memory have focused on mechanisms of consolidation and reconsolidation. More specifically, these studies largely focus on the emotional modulation of memory due to the potential application to understanding PTSD (van Stegeren et al., 1998; Przybyslawski et al., 1999; Cahill & Alkire, 2003). In humans, activation of BARs results in the augmentation of memory consolidation (specifically emotional memory) and this is thought to be mediated via receptors in the BLA (Cahill et al., 1994; Cahill & McGaugh, 1998; van Stegeren, 2008; Chamberlain & Robbins, 2013; Barsegyan et al., 2014; Kuffel et al., 2014; for reviews see Ferry et al., 1999; McGaugh, 2000; Roozendaal et al., 2009; Roozendaal & McGaugh, 2011), an effect which disappears if participants are pretreated with PRO (Cahill et al., 1994; van Stegeren et al., 1998; Maheu et al., 2004) or if given PRO post-learning (Sara et al., 1999; Tronel et al., 2004; Roozendaal et al., 2008; Barsegyan et al., 2014). This has greatly

expanded our understanding of the pathogenesis of anxiety disorders such as PTSD. However, fewer studies have examined the role of NE on mechanisms of encoding. Although NE is generally thought to enhance encoding, it is difficult to differentiate whether activation of the noradrenergic system is affecting encoding or consolidation per se in situations where emotional memories are remembered better than neutral memories. Furthermore, these effects have been typically shown to be dependent on the BLA and are not necessarily mediated by the HF.

While many studies have been conducted to assess the role of NE in consolidation and reconsolidation, fewer studies have examined the way in which BARs regulate memory retrieval (Brown & Silva, 2004; Chamberlain et al., 2006; Thomas, 2015). Experimental manipulations that enhance NE activity have been typically shown to facilitate memory retrieval, and manipulations that inhibit NE release to impair retrieval. These effects were observed when memory retention was tested at least 24 hours after learning and drug manipulations were given prior to testing. Mice deficient for dopamine β-monoxygenase (enzyme which catalyzes the reaction of dopamine to NE) (DBM -/-) showed deficits in contextual and spatial memory; these deficits were recovered when mice were administered a BAR agonist (Murchison et al., 2004). Researchers also found that temporarily depleting NE in a rat's brain by injecting a DBM inhibitor resulted in impaired memory retention in a passive avoidance task when administered 30 minutes prior to test. This occurred after 1, 3, 5, or 7 days following the initial training (Hamburg and Cohen, 1973). In addition, Cohen and Hamburg (1975) were able to replicate their results, again producing amnesia for the passive avoidance task using PRO. Animals were injected with PRO 1 or 3 days following training, and memory retention was impaired when tested 2 hours after the injection. In rats, PRO also led to deficits in spatial reference memory in the water maze (Ji et al., 2003), caused the disruption of retrieval of a cocaine-associated memory (Otis & Mueller, 2011), and abolished the expression of a cocaine conditioned place preference following co-blockade of β1- and β2-ARs (Fitzgerald et al., 2016). However, in humans, PRO given prior to a test of memory retrieval had no effect (Rimmele et al., 2016) and in rats, reversibly inactivating

the LC with lidocaine had no effect on spatial reference memory retrieval (Khakpour-Taleghani et al., 2009). BARs may also mediate the reinstatement of previously extinguished memories since activation of BARs by NE has been shown to induce reinstatement of fear memories (Morris et al., 2005). Here, we report that PRO had no effect on memory retrieval, suggesting that activation of BARs may not be necessary for retrieval. However, one important difference between these studies and ours is that we did not look at memory retrieval post-consolidation but instead examined the role of BARs on working memory.

The literature with respect to working memory is much less consistent. In rodents, Khakpour-Taleghani et al. (2009) showed that inactivation of the LC had no effect on spatial working memory. Administration of PRO in rats also had no effect on working memory (Kobayashi et al., 1995; Ohno et al., 1997). In Rhesus monkeys, moderate doses of PRO (0.01, 0.05 and 0.1 mg/kg) impaired spatial working memory, while a low dose (0.005 mg/kg) and high dose (0.5 mg/kg) had no effect (Wang et al., 2012a). In humans, a low (25 mg) dose of PRO impaired numerical working memory in subjects with low arousal levels (Müller et al., 2005), and repeated administration of a high (160 mg) dose impaired working memory (Frcka & Lader, 1988). However, several other studies utilizing a moderate dose (40 mg) found no effect at all of PRO on working memory in humans (Bodner et al., 2012; Becker et al., 2013; Ernst et al., 2016). Therefore, it appears that the role of the noradrenergic system on memory that has not been consolidated is still unclear. Here we show that inactivating BARs prior to encoding, and activating BARs prior to retrieval can impair spatial working memory. If NE promotes the recruitment of new neurons to form contextual representations then, it follows that PRO given prior to encoding would disrupt this process and that when these representations must be reactivated upon memory retrieval, that perturbing the system in a bias towards encoding mechanisms that involve recruiting new neurons following administration of ISO would also impair memory.

In the Barnes maze, we tested reference memory during the acquisition probe. ISO

infusions administered prior to this test resulted in longer latencies to reach the location of the escape hole, as well as less time spent in the escape quadrant and zone. Animals in the ISO group also made more errors and the number of hole deviations was greater, however, given the high variability in our data, we did not see a significant effect for these measures. These effects were blocked by PRO. During the curtain probe, performance was impaired across groups on all measures demonstrating that animals were using extra-maze cues to orient themselves. These results are consistent with the DNMP data suggesting that BAR activation in the DG immediately prior to a test of memory retrieval impairs memory. Together with the results from the IEG data in Chapter 2, these findings suggest that BAR activation in the HF facilitates the recruitment of new neurons biasing the system towards encoding rather than retrieval. When animals were infused with ISO a second time immediately prior to reversal training, although it was not a significant effect, they exhibited longer latencies to reach the new escape hole. This may be attributed to an increase in exploratory behaviour.

In contrast, ISO administered prior to reversal learning led to improved performance during the reversal probe, specifically in terms of the number of hole deviations, and the time spent in the new escape zone. In reversal training days following, there was also a trend in the direction that demonstrated these animals were learning more quickly. These effects were blocked by administration of PRO and were not due to differences in locomotor activity given there were no group differences in the total distance travelled across all probe tests. This is consistent with previous work (Segal & Edelson, 1978) and the hypothesis that the modulating effect of NE depends on the stage of training where NE may serve as a novelty signal involved in updating contextual representations, promoting cognitive flexibility. The LC-NE system has been previously implicated in reversal learning. For instance, in a cognitive-behavioural task called the *oddball-task,* performed by monkeys, Aston-Jones et al. (1994) showed that the LC neurons selectively responded to a cue when it was the target and not when it was the distractor. They also showed that during reversal training when the distractor became the target and vice versa, LC

responding switched to the new cue (Aston-Jones et al., 1994, 1997; Rajkowski et al., 1994; Aston-Jones & Cohen, 2005). Sara et al., (1994) also showed that neurons in the LC were responsive to a stimulus when it was novel, and when it acquired salience during conditioning, but also during reversal training and during extinction. The authors noted that the most striking and consistent observation in the study was that LC neurons responded immediately to any change in stimulus-reinforcement contingency with respect to both appetitive and aversive stimuli, and that these responses were even stronger than the initial responses to novel stimuli encouraging the view that the NE system is implicated in shifting attention to environmental imperatives (Sara, 2009). Reversal training constitutes a situation where there is a contingency change and remapping is likely necessary. Therefore, it is reasonable to assume that activation of BARs during this type of learning would confer an advantage to the animal promoting a disengagement from established representations and the recruitment of new representations towards an enhancement of processes that promote the incorporation of new information (Bouret & Sara, 2005; Harley, 2007a).

This work highlights the involvement of hippocampal BARs in the process of remapping contextual representations to promote new learning in a way that supports flexible and adaptive behaviour. It is well-established that the pathophysiology of anxiety disorders such as PTSD are characterized by noradrenergic dysregulation (Hendrickson & Raskind, 2016). Furthermore, it is hypothesized that PTSD also involves impairments in memory "updating" mechanisms where the incorporation of new information (e.g. safety signals) is not effectively encoded at a functional level that may represent an inability to remap (i.e. patients bringing up trauma-related representations rather than incorporating safety signals into existing memories through remapping processes) (Maren et al., 2013; Morrison & Ressler, 2014; Giustino et al., 2016; Liberzon & Abelson, 2016; Elsey & Kindt, 2017; Lee et al., 2017; Sheynin & Liberzon, 2017). A more extensive understanding of the underlying neurobiological mechanisms involved in updating

memories, or in learning new contingencies may provide insight into target systems, and novel drug and intervention strategies for treatment.

4.0 General Discussion

Fifty years ago, Seymour Kety introduced the idea that NE could act as a neuromodulator to promote memory, specifically during affectively important events (Kety, 1967, 1970). Since then, several studies have shown that NE facilitates memory consolidation and retention for emotional memory (McGaugh et al., 1990; Do Monte et al., 2008), effects which are realized over the span of hours to days given the time frame of the consolidation process, and have been shown to be dependent on BARs (Devauges & Sara, 1991; Lemon et al., 2009). Post-encoding activation of the LC, which is the major source of NE in the brain, also promotes plasticity (Harley & Sara, 1992; Klukowski & Harley, 1994) and the consolidation of hippocampal dependent memory (Takeuchi et al., 2016). Fewer studies have investigated whether NE plays a role in the encoding of new memories, updating existing memories, or the modulation of memory over shorter time scales such as in working memory. Here we asked these questions, and consistent with Kety's hypothesis, proposed that through the activation of the LC during important events, that NE is involved in the acquisition of new information and therefore, plays a crucial role in the encoding of new memories. We sought to determine whether the noradrenergic pathway from the LC to the DG is involved in modulating memory in a way that it helps to sculpt hippocampal contextual representations. We proposed that activation of the LC-NE system would cause a disengagement from established representations and an enhancement of processes that promote the incorporation of new information (Bouret & Sara, 2005; Harley, 2007a), with the hopes of possibly elucidating a mechanism by which place cell remapping occurs. Based on the network reset hypothesis, we extended this to include a behavioural component suggesting that LC activation would bias the memory system towards encoding rather than retrieval when adaptive, promoting cognitive and behavioural flexibility (Devauges & Sara, 1991; Sara et al., 1994; Aston-Jones & Cohen, 2005; Bouret & Sara, 2005; Yu & Dayan, 2005; McGaughy et al., 2008).

The data presented here support these hypotheses. In experiments 1 and 2, using the catFISH protocol, we showed that animals create distinct hippocampal representations when exposed to two different environments while reactivating a previously formed representation when presented with the same environment twice. This is consistent with previous findings (Guzowski et al., 1999; Marrone et al., 2011). We manipulated the LC-NE system pharmacologically to induce tonic and phasic LC discharge. In animals that visited the same environment twice, phasic but not tonic LC activation fully reset the animal's representation of that environment causing the recruitment of new neurons rather than reactivation of the previously formed representation. These animals possessed a cellular profile that was indistinguishable from the animals that had visited two different contexts. This implicates the LC-NE system in memory encoding processes which include the formation of new memories and the updating of existing memories through mechanisms of remapping. In experiments 3 and 4, we also showed that NE release in the DG can cause impairments in spatial working and reference memory. We argue that the infusions of the BAR-agonist ISO, caused a similar (artificial and experimenter-induced) reset of representations and since performance on the task is dependent on the reactivation of previously formed representations, the animal's performance was compromised. This is a situation where a reset of representations would be considered maladaptive. In a situation where the recruitment of new neurons would instead be adaptive, such as in reversal learning, we hypothesized that NE release in the DG would, in contrast, impart an advantage to the animal and improve this type of learning and memory. We showed that administration of ISO prior to reversal learning did in fact improve cognitive flexibility and promoted adaptive behaviour as animals performed better during the reversal probe.

One theoretical consideration relates to the distinction between tonic and phasic LC firing. We know that the LC is important for regulating arousal, mediating responses to stress, as well as attention and flexible behaviour. We also know that the LC responds to infrequent, novel, and salient (e.g. conditioned) stimuli. In fact, a recent, high-resolution fMRI study conducted in

humans demonstrated that the LC codes for relative saliency, and prioritizes its responses towards novel or unexpected information with no additional modulatory effect of emotional content (Krebs et al., 2017). So how does the LC coordinate different firing patterns to subserve all these roles? We have been operating under the assumption, mainly adopted from previous papers that two modes of LC discharge exist. *The tonic mode*: The LC exhibits higher, sustained discharge when an animal is awake, and even more so when an animal is stressed. *The phasic mode*: The LC exhibits a brief train of action potentials in a burst, followed by a period of hyperpolarization or suppression in response to novel or salient stimuli. In our first experiment, we used different pharmacological agents to mimic these firing patterns based on previous studies that recorded from the LC with simultaneous application of these drugs. Based on the network reset hypothesis by Bouret & Sara (2005), and the fact that both phasic and tonic activation of the LC cause downstream plasticity effects to occur (Mueller et al., 1981; Lacaille & Harley, 1985; Stanton & Sarvey, 1987; Heginbotham & Dunwiddie, 1991; Dunwiddie et al., 1992; Harley & Sara, 1992; Klukowski & Harley, 1994; Harley, 1998; Brown et al., 2005; Jurgens et al., 2005a, 2005b) and the release of NE (Dahl & Winson, 1985; Harley & Milway, 1986; Harley et al., 1989; Babstock & Harley, 1992; Frizzell & Harley, 1994; Klukowski & Harley, 1994; Walling et al., 2004; Lemon et al., 2009) we originally hypothesized that both phasic and tonic manipulations would induce a reset. We found that only phasic LC activation induced a reset of representations and concluded that this was likely attributed to the fact the LC responds in phasic bursts to novel stimuli and that increases in tonic discharge are not necessarily associated with the detection of novelty, therefore only a phasic signal would induce a reorganization of the memory system. Recently, there has been some debate in the literature as to what exactly constitutes these two modes, and whether they should be considered “distinct modes” per se?

The theoretical construct of LC output and attention put forth in Aston-Jones and Cohen's (2005) computational *adaptive gain model*, suggests that performance on a task is optimal when tonic discharge is moderate which allows for phasic LC responses to occur in

response to behaviourally relevant stimuli. One potential problem with this theory relates to a principle characteristic of LC firing whereby previous spontaneous activity affects the probability that subsequent firing will occur. Therefore, one would predict that higher tonic discharge would be optimal and higher tonic discharge is associated with more NE release in terminals (Chandler, 2016). However, if we consider phasic LC responses to salient events (that may require a shift in behavioral strategy) to be our "signal", and basal LC activity, or tonic discharge to be "noise", then lower tonic discharge would be optimal in achieving a high signal to noise ratio and may be particularly important when it is necessary to filter out irrelevant information (Bremner et al., 1996; Berridge, 2008). Aston-Jones and Cohen (2005) suggest that moderate tonic LC discharge is optimal based on the fact that monkeys in their oddball study performed best when this was the case. However, it is currently unclear whether high, moderate, or low tonic discharge facilitates attention, novelty-detection or cognitive shifts.

What is a cognitive shift? Hypothetically this would occur during a change in contingency when a relevant stimulus becomes no longer relevant or *vice versa*. We tested reversal learning where an animal was required to shift its attention from one location (or stimulus) to another. This can be considered a cognitive shift (Sara, 2009; Hagena et al., 2016). However, any situation where an animal goes from being focused on a task, to less focused and more exploratory, or vice versa, is also considered a cognitive shift. Berlyne (1966) used the terms *diversive* to describe when an animal is in an *exploratory*-state and *inspective* to describe when the animal is focused on a task. In the *adaptive gain model* (Aston-Jones & Cohen, 2005), they correlate phasic LC signaling with exploitive, inspective, task-oriented behaviour and suggest that high tonic LC firing causes a shift in that the animal loses focus for the task at hand and switches to an exploratory behavioural state. However, phasic to tonic shifts during changes in task contingencies have not always been reported (Kalwani et al., 2014). In the current set of experiments, we found that administration of ISO in the DG promoted exploratory behaviour. We inferred this given the increased latency to make a correct choice in the DNMP task and to find

the escape hole in the Barnes Maze. We assumed that our infusion was comparable to what would have been observed following phasic LC activation and thus, would reset the system. However, we did not measure the amount of NE that was released following our intra-LC glutamate infusions nor did we measure the amount of NE that was released following our intra-LC ORX, BETH or CRF infusions in both experiments 1 and 2. Therefore, it is possible that the ISO infusions we delivered were in fact more comparable to the level of BAR activation that would occur following tonic LC firing rather than phasic. If so, our results would in fact be in accordance with the *adaptive gain model* given that our infusions induced an exploratory state.

In the DNMP task, the increase in latency was accompanied by an increase in the number of errors; this suggests that ISO may have caused animals to perseverate and one could argue that this is what may occur following a very large phasic burst (McGaughy et al., 2008; Chandler, 2016) where the animal would be hyper-focused on a task or stimulus. In the Barnes Maze however, ISO infusions during the first reversal training session did not cause animals to perseverate. While they were impaired, they spent an equal amount of time exploring all the holes (data not shown) but made more reference errors and took longer to find the new escape hole suggesting they were in a true state of exploration. During the acquisition probe, we saw a similar trend where animals tended to explore their environment following the infusion of ISO. Since there were only two arms open in the DNMP task, it is possible that we could not accurately measure exploratory behaviour in this task. Future experiments should involve phasic activation of the LC followed by behavioural testing to adequately answer these questions.

It may be more accurate to view LC firing in terms of temporal windows rather than distinct modes. Imagine an animal is placed in an environment where it quickly makes a contextual hippocampal representation of its surroundings. The animal may be mildly stressed, the environment is novel. There will likely be moderate to high tonic discharge with superimposed phasic responses to the novelty of the context that quickly habituate. Sometime into the session, the animal receives a foot shock. This activates the LC, causing a large phasic burst

in relation to the shock. The animal must quickly update the hippocampal contextual representation of the environment with the newly learned information that this location now poses a threat. This is followed by sustained high tonic activation because the animal is now more stressed. Since these things are happening simultaneously, it seems that referring to them as *modes* or *states* may be misleading. LC discharge is characterized by the level of firing that occurs over a given period. Phasic firing can only be examined in short time frames and tonic firing can only be viewed over longer temporal windows. But within any given time-frame, an animal possesses a certain sensitivity to novel or salient stimuli and it is this sensitivity that we are concerned with. Therefore, when we talk about phasic and tonic firing in the LC, we should strive to include a measure that speaks to the interaction between them.

One thing that has been consistently reported is that the LC exhibits phasic responses to task-related stimuli, and in particular, to rule changes during reversal learning (Sara & Segal, 1991; Aston-Jones et al., 1994, 1997; Rajkowski et al., 1994; Sara et al., 1994; Aston-Jones & Cohen, 2005; Bouret & Sara, 2005; Bouret & Richmond, 2009). These data prompted Bouret and Sara (2005) to propose the *network reset model* where cognitive shifts are under the influence of NE and its ability to interrupt neural network activity (Chandler, 2016). We specifically looked at whether phasic LC activation could reset hippocampal networks, however, there is evidence to suggest that contextual representations are also formed in the PFC and it is reasonable to assume that phasic LC activation could also perturb network dynamics in this region as well. LC neurons send direct projections to the PFC (Arnsten, 2000) and NE is released in the PFC following LC activation. Impairments in the Attentional Set Shifting Task, which is a measure of attention and cognitive flexibility (Heisler et al., 2015) are typically associated with deficits in the PFC. However, previous research shows that it is LC-NE afferents to the PFC that are implicated (McGaughy et al., 2008). The ability to detect and ultimately to react in a behaviourally adaptive manner to salient stimuli is critical to survival, especially in circumstances that deliver a certain degree of uncertainty. Our data supports a role of the LC in reversal learning and cognitive shifts

in attention. Given the involvement of the LC in shifting attention, and its ability to filter out relevant information, it is not surprising that many studies are now focusing on LC target projections in models of Attention Deficit / Hyperactivity Disorder (ADHD) (Brennan & Arnsten, 2008; Arnsten & Pliszka, 2011; Berridge & Devilbiss, 2011; Darcq & Kieffer, 2015). A better understanding of LC network dynamics and projections could potentially reveal mechanisms to counteract pathological conditions such as ADHD (Chandler, 2016).

One interesting discrepancy in the literature concerns the rate at which phasic LC responses habituate. The discrepancy arises when comparing results from rodents to primates. In primates, responses to target stimuli did not habituate even after 100 presentations (Aston-Jones et al., 1991; Rajkowski et al., 1994; personal communication with Sebaestien Bouret) and were greatest when stimuli elicited an orienting response (Aston-Jones & Cohen, 2005; Sara & Bouret, 2012). In contrast, LC responses did habituate when observed in rats, with different populations of LC neurons habituating at different rates (Sara & Segal, 1991), and in some cases, this occurred very rapidly (Sara et al., 1994; Vankov et al., 1995). Habituation of LC neurons has also been shown to occur in mice exposed to a novel environment (Takeuchi et al., 2016). This discrepancy has never been resolved in the literature. The LC codes for relative saliency for instance, a target that is presented infrequently does not elicit a response from the LC if it presented along with many *novel* or other *task-related* stimuli. However, if presented without those competing stimuli, LC responses may be observed (Krebs et al., 2017). Therefore, this discrepancy in habituation may be explained in terms of relative saliency. When a tone stimulus preceded a shock, LC cells responded when the tone was novel, but these responses habituated rapidly when the tone was no longer followed by shock. Similarly, in the holeboard task performed by rats, LC responses habituated rapidly when they were not reinforced. In monkeys (Aston-Jones et al., 1991; Rajkowski et al., 1994), the target stimulus was associated with a reward. Therefore, this discrepancy may be attributed to stimulus context and reinforcement, as well as relative saliency (Krebs et al., 2017).

One of the reasons it is difficult to resolve this issue is that very few people record from the LC in the first place. This is due to the fact that the LC is a small structure and somewhat difficult to target (George et al., 2013). Given how small it is, it has previously been assumed that the populations of cells within are homogenous, yet in fact, it is quite a heterogenous structure (Loughlin et al., 1986; Sara and Bouret, 2012; Schwarz & Luo, 2015; Schwarz et al., 2015) (Figure 59). One potential methodological consideration of this work is that activation of LC projects to many areas, many of which are projecting back onto the LC and may also project to the HF. Moreover, we are stimulating different subpopulations of neurons that may possess divergent functions (Schwarz & Luo, 2015; Schwarz et al., 2015). Previous studies have also shown that atrophy in the LC is ubiquitous in Alzheimer's and Parkingson's disease (Mann et al., 1980; Haglund et al., 2006; Isaias et al., 2012; Hammerschmidt et al., 2013). In aging populations, memory disorders such as Alzheimer's disease and Dementia are prevalent and the LC-NE system is clearly implicated, therefore, a greater understanding of the anatomical distribution of LC neurons could help elucidate the mechanisms by which the LC-NE system interacts with memory which offers therapeutic value to the study of neuropsychiatric and neurodegenerative disease states (Chandler, 2016).

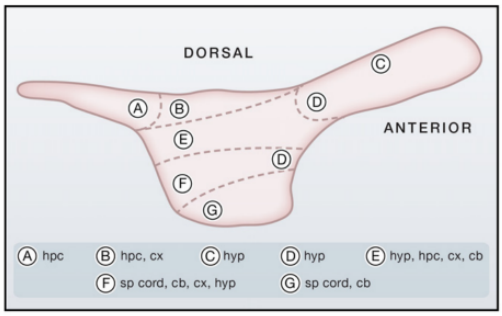


Figure 60. Sagittal schematic of the rodent LC. Subdivisions and legend proposed by Loughlin, Foote, & Grzanna (1986) from which the figure has been adapted. Reprinted from Neuron 76(1), Sara SJ & Bouret S, Orienting and reorienting: the locus coeruleus mediates cognition through arousal. 130-141., Copyright (2012), with permission from Elsevier.

As the field continues to advance technologically, more effective ways of targeting the LC have become available. Selectively inhibiting or activating LC neurons can be achieved using combinatorial, synergistic chemogenic and optogenetic strategies. These techniques involve the delivery of site specific adeno associated viruses (AAV) viruses containing Cre-recombinase under the control of a cell type-specific promoters enabling the expression of a Cre-dependent transgene in a cell-type (e.g. TH+) specific manner. The delivery of Cre-dependent vectors in local and retrograde projecting populations of neurons (Oyibo et al., 2014; Gompf et al., 2015; McCall et al., 2017) allows for the isolation and manipulation of specific pathways. For example, to inhibit the entire LC for a sustained period of time, A Cre-dependent adeno-associated virus (AAV) containing the $G\alpha_i$-coupled hM4Di DREADD (Armbruster et al., 2007) can be infused directly into the LC of Cre driver lines such tyrosine-hydroxylase (Th) or DBH-Cre mice where LC inhibition can be induced chemogenically using clozapine-N-oxide (Figure 60). To inhibit the LC for a shorter period, the green light-sensitive opsin archaerhodopsin-3 (Arch) can be substituted for hM4Di and the LC can be inhibited using pulses of light at a wavelength of 542nm. Similarly, to activate LC neurons for a sustained period, the excitatory hM3Dq DREADD can be used, or more precise temporal targeting can be achieved by selectively targeting the blue light-sensitive opsin channelrhodopsin-2 (ChR2) to LC-NE neurons of Th-Cre mice which can later be activated by pulses of light at a wavelength of 473nm. Cell body photostimulation can be applied in the LC itself, or light can be applied to the terminals in the HF depending on the specific question we are trying to answer. Moreover, if we are interested in a specific subpopulation of cells such as those active during a particular experience, we can tag specific neurons in the LC or the HF and later reactivate only those cells using inducible tamoxifen- or tetracycline-controlled systems (Garner et al., 2012).

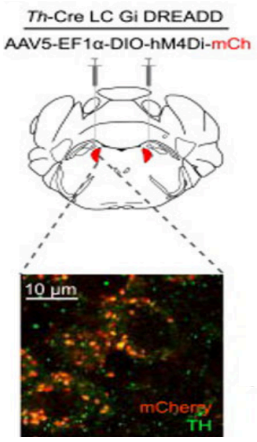


Figure 61. LC-NE neurons selectively targeted with the inhibitory designer receptor exclusively activated by designer drug (DREADD) via injection of a Cre-dependent AAV into the LC of tyrosine hydroxylase-IRES-Cre mice (Th-Cre). Reprinted from: Neuron, 87(3), McCall JG, Al-Hasani R, Siuda ER, Hong DY, Norris AJ, Ford CP, & Bruchas MR, CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. 605-620, Copyright (2015) with permission from Elsevier.

Future experiments should also include a design where we activate the LC either pharmacologically or optogenetically, to emit a phasic burst and simultaneously record electrophysiologically, in the HF to replicate our findings that phasic LC discharge can remap hippocampal representations while examining activity in place cells. In addition, given that we were unable to block remapping using clonidine, we should attempt to block remapping using a BAR antagonist such as PRO to assess if remapping is indeed dependent on BAR activation. Employing technologies such as the use of in vivo calcium imaging would also afford the opportunity to view remapping effects in real time. Other methodological factors that we should also take into consideration include the fact that our n-values for experiment 5 are quite low and another cohort should be run.

In the past 15 years, we have seen an explosion in the number of papers investigating pattern separation. And while there is evidence to show that the DG subserves this function, there is much we do not understand regarding the way in which memories are separated, and even less concerning how they are updated. For this reason, the work presented here is highly novel. Few studies have examined the role of the LC-NE system on memory encoding and to date no studies

that we are aware of have looked at the role of the LC-NE system in remapping. From a clinical perspective, the current experiments are particularly significant with respect to gaining insight to the etiology of anxiety disorders such as PTSD. The LC is particularly vulnerable to stress (Bremner et al., 1996) and NE is involved in the neural mechanisms of conditioned fear (Davis, 1986; Bremner et al., 1996). Conditioned-fear is the process that occurs when an organism learns to fear a specific stimulus within its environment (Pavlov, 1927). The contextual element of fear conditioning is also dependent on the HF (Gewirtz et al., 2000; Anagnostaras et al., 2001; Maren et al., 2013). PTSD involves the recall of traumatic events in a way that seems to dominate over other types of memories and this may involve the release of NE in the HF (Bremner et al., 1996). In animals, models of PTSD are typically associated with impairments in extinction learning which involves a cognitive shift (Bremner et al., 1996; Yamamoto et al., 2008; Knox et al., 2012; VanElzakker et al., 2014). We hypothesize that individuals with PTSD may be impaired in their ability to engage in cognitive shifts, and rather than remapping contextual representations in the presence of new information (e.g. safety signals), they reactivate old (fear-related) representations thus interfering with their ability to demonstrate adaptive behaviour (Strawn & Geracioti, 2008; Sara, 2016). Given that PTSD is characterized by a sensitization to stress (George et al., 2013) and a dysregulated LC-NE system (George et al., 2013; Pietrzak et al., 2013), extending this work to include animal models of PTSD could be useful. For instance, animals that have undergone *single prolonged stress* (SPS) which is considered to model PTSD in rodents, show significantly lower baseline levels of tonic LC discharge, but in response to paw compression, their evoked responses are significantly higher than animals which did not receive SPS (George et al., 2013). Furthermore, anxiety disorders are more prevalent in females (Freedman et al., 2002; Kessler et al., 2005, 2012; Tolin & Foa, 2006; Breslau et al., 2017), and sex differences in the LC-NE system have been shown to emerge during development (Guillamón et al., 1988; Luque et al., 1992; Pinos et al., 2001; Bangasser et al., 2016; Bangasser & Wicks, 2017). Given that the LC has been identified as a candidate region of interest in anxiety disorders and depression (Austin et

al., 2003), it would be beneficial to assess sex differences and include both male and female subjects in prospective studies (Beery & Zucker, 2011; Bangasser et al., 2016). Understanding the interaction between stress-induced LC-NE activation and memory is also important for the development of treatment interventions for anxiety disorders such as PTSD and panic disorder (Bremner et al., 1993).

In summary, there has been an interest in the relationship between the LC-NE system and memory for many years (Bremner et al., 1996; Harley, 2007b; Sara, 2009, 2016). The LC responds to salient and novel cues and innervates a vast number of brain regions. Early brain slice electrophysiology studies demonstrated that LC-induced NE release in these regions, specifically the HF, caused plasticity-associated effects. These effects were later shown to occur *in vivo* and were also correlated with changes in learning and behaviour. Changes that have been associated with the enhancement of consolidation and retrieval, especially when memories were emotional in content (Tully & Bolshakov, 2010) and can be considered somewhat global in the sense that neuromodulation occurs over longer time scales rather than fast-acting, small molecule neurotransmitters (e.g., glutamate and g-Aminobutyric acid). Here, we probed the brain in a way that examined the role of NE in a targeted fashion, on the micro-circuitry of mnemonic processes such as encoding and retrieval over shorter time scales. Combining the use of genetic and molecular technologies we examined different subpopulations of HF cells across time to demonstrate that NE not only plays a crucial role in memory encoding, but is implicated in mechanisms underlying memory-updating and network remapping. NE provides a "*reset*" signal causing the HF to recruit distinct neural populations, thereby providing a molecular switch to dictate if hippocampal circuits should generate new representations or update existing ones to incorporate novel information. We have shown that novelty-associated LC activation helps to sculpt contextual representations in the HF in an adaptive manner, causing the HF to move from a state of retrieval back to encoding when novel information needs to be incorporated and can promote cognitive flexibility thus improving reversal learning (i.e., switching the system back to

encoding when it is adaptive). Understanding how this system becomes dysregulated, either when the system switches from retrieval to encoding when it is maladaptive (e.g., in situations where retrieval is necessary to perform a task, or when irrelevant information should be filtered out) or when NE is unable to act as a reset switch in the presence of novel information, may be implicated in disorders of attention and anxiety respectively. To this end, our goal is to further illuminate the relationship between the LC-NE and systems of memory through an understanding of the network resetting in hippocampal cell populations.

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