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The Encoding of Spatial, Temporal, and Affective Dimensions in the Dentate Gyrus of the Hippocampus

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Neurosciences

by

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2012

The Dissertation of Lara Maria Rangel is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego

2012

DEDICATION

Sometimes I like to think of myself as an adult-born neuron growing up in a mature network. My original interests in science were quite broad and were narrowed through training into something quite specific and unique in its contribution to the scientific community. I would like to dedicate this dissertation to those who trained me, and more importantly, those who ensured my survival during this critical period in my career development.

I would like to dedicate this work to Andrea, Laleh, and Brad who encouraged and nurtured my wild speculations and demonstrated the importance of being raised as a scientist in a supportive and loving environment.

I would like to dedicate this work to my parents, my sister, and my husband for their unwavering love and confidence in me.

"This last group includes 15 units which…remained silent in spite of considerable, and sometimes drastic, attempts to fire them."

O'Keefe, J. and Dostrovsky, J., *The hippocampus as a spatial map, Preliminary evidence from unit activity in the freely-moving rat*, Brain Research, 34(1971): 171-175.

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LIST OF ABBREVIATIONS

DG: Dentate Gyrus

NG: Neurogenesis

CA1: Cornu Ammonis 1

CA3: Cornu Ammonis 3

TMZ: temozolomide

BrdU: 5-bromo-2'-deoxyuridine

$I_{Context}$: Contextual Information Score

ROC: Receiver Operating Characteristic

LFP: Local Field Potential

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sincere joy of working with one's colleagues, it is entirely due to these two individuals.

Chapter 2, in full, is a reprint of the material as it appears in the following manuscript being prepared fro submission: Rangel, L.M., Alexander, A.S., Aimone, J.B., Wiles, J., Gage, F.H., Chiba, A.A., Quinn, L. K., *Temporally Selective Contextual Encoding in the Dentate Gyrus of the Hippocampus*. The dissertation/thesis author was the primary investigator and author of this paper.

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ABSTRACT OF THE DISSERTATION

The Encoding of Spatial, Temporal, and Affective Dimensions in the Dentate
Gyrus of the Hippocampus

by

Lara Maria Rangel

Doctor of Philosophy in Neurosciences

University of California, San Diego, 2012

Professor Andrea A. Chiba, Chair
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Memory of an event must fundamentally include not only the time and

place of its occurrence but also a measure of its significance for an organism to

maintain and update the most relevant information in its world. It must then

follow that the brain structures necessary for learning provide a mechanism to

account for these details that cumulatively compose complex memories. To

investigate the degree to which the hippocampus accounts for spatial, temporal

and affective dimensions in its activity at the single cell and local field potential

levels, *in vivo* electrophysiological recordings were performed in the dentate

gyrus of rats during experiments explicitly designed to examine encoding along

temporal and affective dimensions. First, to test for the presence of time-dependent single cell activity in the dentate gyrus, rats were exposed to three distinct behavioral contexts presented either simultaneously or separated in time. In this experiment, single cell activity accounted for temporal segregation of contexts by demonstrating activity selective to a single context. In a subset of rats with reduced levels of hippocampal adult neurogenesis, temporally selective contextual encoding was reduced, suggesting that adult neurogenesis contributes to the encoding of temporal dimensions. In a second experiment designed to examine mechanisms by which affective associations are made in the hippocampus, local field potentials were acquired from the dentate gyrus during reinforcement learning. Robust decreases in the amplitude of theta frequency oscillations (4-12Hz) occurred concurrently with increases in the amplitude of beta frequency oscillations during time intervals key to associational learning. These changes in amplitude were observed only during learning epochs and in the presence of reinforcers, suggesting that these oscillatory changes are dedicated to facilitating the encoding of associations with an affective dimension. The results of these experiments further our understanding of how complex memories requiring associations across multiple dimensions are formed in the hippocampus.

CHAPTER 1: A brief history of time, space, and affect in the hippocampus: the hippocampus as a multidimensional terrain

Memories of events are far from simple. They are instead rich with intricate associations between features such as space, time, and affective relevance that can together compose a complex memory. It has long been understood that the hippocampus plays an important role in memory formation. How the activity of this brain region at the single cell level translates into the incorporation of rich contextual information into memory is not well understood. Although spatial, temporal, and emotional aspects of a memory are often considered as features encoded separately in the brain, here it is described how the hippocampus, and more specifically the dentate gyrus of the hippocampus, may have the ability to create distinct spatial representations that also incorporate time and salience.

To better understand why the brain would need to encode memories that include more than spatial information, it is important to know that space and time are linked and inseparable in nature. In fact, the physical dimensions of space and time are often considered together and referred to as *spacetime*, whereby they do not have separate existences. In extreme cases such as the realm of relativistic physics, this can mean that events occurring billions of years ago at the farthest reaches of the universe can occur simultaneously with your thoughts as you look up at the light from that event shining in the earth's sky. In our everyday lives, it can mean that the changes we observe in places we have

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known since childhood are measured by, and are indicators of the passage of time. In the latter case, which refers to our own egocentric view of the universe, we realize that memories of places ("where") from our childhood are dependent upon an inseparable temporal ("when") component. Since space and time are inseparable in nature, it should be tested whether the classical separation of space and time in the brain and in the encoding of memories is an artificial division.

As biological organisms, our egocentric view of space and time are additionally linked to an affective dimension. The addition of this dimension provides a measure of subjective importance to everything we encounter. Distinct places and key moments from our childhood can thus exist in our minds as highly positive or negative. In memory, this means that we have the ability to encode and remember what is most important to our lives. Here we explore the advantages of utilizing spatial, temporal, and affective dimensions at the single cell level in the hippocampus for the encoding of complex memories.

Revisiting the Hippocampus as a Cognitive Map:

Traditionally, the hippocampus has been viewed as a cognitive spatial map. This is largely due to the existence of *place cells* in the dentate gyrus, CA3, and CA1 regions that demonstrate reliable activity in restricted spatial locations, or *place fields*, of an environment. Together, these place cells are hypothesized to encode and create associations between the spatial components of our

allocentric universe. Indeed, the hippocampus has been shown to be essential to spatial learning [1–6]. This is especially the case when the formation of spatial associations is necessary to learn the behavioral significance of locations within a given context [7] such as navigation in the Morris Water Maze and freezing behavior in contextual fear conditioning [1,2,5,8–13]. Additionally, the strength of spatial learning has been correlated with the extent to which place cells demonstrate reliable and selective spatial activity [4,14]. Given this evidence, a wealth of research has been dedicated to understanding how single place cell activity translates to learning and performance in spatial tasks.

If place cells enable an internal spatial representation of the world, then testing the extent to which spatial firing properties of place cells account for learned features of an environment and changing behavioral conditions can help determine their ultimate contribution to learning and behavior. Numerous studies have attempted to examine how the hippocampus encodes more complex features of contexts and events that make memories distinct [15,16]. Experiments designed to test this hypothesis at the single cell level, however, restrict analyses to only spatial firing characteristics of place cells in the hippocampus, and are necessarily limited to investigating the role of the hippocampus in encoding and updating associations along spatial dimensions only. Yet, the hippocampus is known to be involved in the encoding of non-spatial memories. The question remains: what is the mechanism by which single cells in the hippocampus encode non-spatial features of memory, such as time and salience?

Early studies examining place specific cell activity in the hippocampus indicate that place cells may actually comprise a small subset of the principle cell population. In a study conducted by O'Keefe and Dostrovsky in 1971, the authors describe the response properties of 68 out of 78 recorded hippocampal cells in the following manner:

> 14 were classified as 'arousal' or 'attentional' units…21 'movement' units had patterns of activity directly related to the animal's behavior, firing briskly during some but not necessarily all of the following behaviors: orienting, sniffing, bar-pressing, and walking, and firing infrequently or not at all, during eating, drinking, grooming, quiet sitting, and slow wave sleep; two units had interesting properties related to the animal's expectations; and for the remaining 31, either no adequate stimulus could be found or their responses were inconsistent and uninterpretable.[17]

Although 8 of the cells in the above study did exhibit place specific firing, the passage above highlights the variable and still undetermined function of a large proportion of the hippocampal cell population. It is therefore worth investigating the function of the non-spatial cell populations in the hippocampus and testing the extent to which these cells might encode temporal and affective dimensions.

Introducing Time as a Dimension in the Hippocampus:

Just as spatial memories require organized associations between spatial features of an environment, the encoding of events in time also requires a temporal organization in the hippocampus that can account for similar and distinct temporal features. In short time scales, it has been demonstrated that the

temporal organization of place cell activity with respect to the phase of theta (4-12 Hz) frequency oscillations in the hippocampus is related to a rat's movement through space and may potentially facilitate memory formation [18–20]. Additionally, evidence also suggests that the single cells in the hippocampus can demonstrate reliable and selective activity for temporal segments during a given length of time in the same way that place cells demonstrate activity for distinct spatial locations over a given spatial environment [21,22]. These cells, labeled *time cells*, are the first evidence to suggest that the hippocampus may have a temporal organization for episodes that is very similar to its characterized mechanism for spatial organization of environments. More importantly, time cells are the first evidence that single cells in the hippocampus are dedicated to encoding a temporal dimension.

Although place and time cells provide a mechanism through which spatial and temporal associations can be made along two separate dimensions, it remains to be tested how associations are encoded across these dimensions long-term. How are spatial features of remote memories linked to events in time? The unity of these dimensions could be accomplished by place cells dependent upon temporal features or time cells with spatial contingencies.

A subregion of the hippocampus, the dentate gyrus (DG), has been hypothesized to combine both spatial and temporal dimensions at the single cell level. The DG is the only subregion of the hippocampus that demonstrates neurogenesis, or the continuous birth of new neurons, throughout adulthood.

Adult-born neurons are born in the sub-granular zone of the DG granule cell layer and demonstrate a characteristic development before becoming mature functional granule cells. Importantly, immature adult-born neurons exhibit a transient period of both intrinsic and synaptic hyperexcitability that is due to low membrane capacitance and less synaptic inhibition respectively [23–26]. This transient physiological difference between mature and immature granule cells may yield a unique role for adult-born cells in temporal encoding. Computational models from our laboratory demonstrate that temporally proximal events occurring within the transient period of hyperexcitability for a set of adult-born neurons elicit activity from common immature cells in an otherwise sparse firing mature network. There then exists a similarity in DG output for temporally proximal events that does not exist for events separated in time, a *temporal pattern integration* that is the direct result of adult-born neuron physiology during development [27,28]. This temporal pattern integration can then ultimately link disparate features in the spatial dimension through close proximity along the temporal dimension.

The transient period of hyperexcitability in adult-born neuron development is also a critical period for regulation of their survival and activity. Although a majority of adult-born cells die before becoming mature granule cells, exposure to learning paradigms or enriching environments during this critical period can greatly enhance their survival and bias their activity toward input received during their development [27,29]. This has led to the prediction that surviving adult-born

neurons provide dedicated and selective activity to temporally proximal events during their development and thus can create a new output from the dentate that is temporally distinct. Adult neurogensis in the dentate gyrus can therefore provide a mechanism for an additional type of pattern separation, a *temporal pattern separation*, through the continuous contribution of new temporal dimensions to distinguish between similar events separated in time. Place cells in dentate gyrus may thus separate similar or identical spatial locations that are far apart along the temporal dimension by exhibiting spatial activity that is dependent upon time.

The combined encoding of both spatial and temporal dimensions at the single cell level in the dentate gyrus can provide a mechanism for a more complete theory of how the hippocampus accomplishes associations between complex features of memories. In relativistic physics, *spacetime* describes everything in the universe as events that occur in space and time. The utility of combining these dimensions is that highly disparate locations in the universe can be linked in time, and highly overlapping locations can become more distinct in time. In other words, spacetime has the ability to reveal the relationship of events along both dimensions. In the dentate gyrus, the combination of these two dimensions means that associations can be made between spatial and temporal features of events. Multidimensional activity in the hippocampus may thus be mechanism through which the hippocampus accounts for relationships across spatial and non-spatial features of memories.

The Dentate Gyrus as Multidimensional Terrain:

The extent to which hippocampal cells encode dimensions other than space and time has been largely unexplored. If the hippocampus plays a role in encoding the importance of events in spacetime, it must be able to establish a relationship between events along an affective dimension. Fear, arousal, and salience are heavy influences upon the strength of our memories. Although there is no evidence to date suggesting the existence of "reward cells" in the hippocampus that encode reward exclusively, previous *in vivo* electrophysiological experiments have shown that ensembles of CA3 and CA1 place cells can demonstrate an overrepresentation or preference for salient locations in an environment [30]. This is the only evidence to date suggesting that place cells can account for affective information, and examines only spatial firing characteristics of these cells. To test whether hippocampal cells associate spatial or temporal features with their affective relevance, one would need to ascertain the extent to which hippocampal cells demonstrate similar or distinct activity due to proximity of these features along an affective dimension.

We propose that the hippocampus is more than a cognitive map and more equivalent to a multidimensional terrain. The large advantage of this multidimensional view is that it removes the hippocampus from the constraint of encoding complex features of memories along a single dimension. Instead, single cells are given the ability to reveal the relationships across spatial, temporal, and affective dimensions by demonstrating activity as events in *affective spacetime*.

By acknowledging that these dimensions exist in the hippocampus, we can begin to examine the exact dynamics of the relationships between these dimensions and determine the rules, if any, regarding how these relationships manifest themselves in the activity of single cells.

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CHAPTER 2: Temporally Selective Contextual Encoding in the Dentate Gyrus of the Hippocampus

Abstract:

Encoding and updating contextual information requires the ability to distinguish between subtle features and changes in a context. The dentate gyrus of the hippocampus has been shown to demonstrate very distinct firing rate patterns to describe similar environments. This ability has been described as *pattern separation*. Computational models predict that adult-born neurons in the dentate gyrus provide an additional mechanism for encoding contextual information by continually building and maintaining a population of neurons with dedicated and exclusive activity to information present during their development. There should thus exist a set of cells with activity selective to temporally segregated contexts. To test this, rats were exposed to one of three different temporal training paradigms. In these training paradigms, introductions to three behaviorally distinct environments were separated either by intervals greater than 3 weeks (extended timeline), intervals of exactly two weeks (condensed timeline), or not temporally separated (simultaneous timeline). Using the condensed timeline, we additionally administered an anti-mitotic agent, temozolomide (TMZ), to transiently knockdown neurogenesis levels in order to test for the contribution of these neurons in temporal pattern separation. At the end of training, *in vivo* electrophysiological recordings were performed in the dentate gyrus granule cell layer while rats were exposed to all three contexts on the same day for four

consecutive days. In all groups, we found that in addition to cells that were active in all three environments, there was a separate cell population that exhibited activity selective to only one or a subset of environments. The proportion of cells exhibiting this selectivity was highest in rats exposed to the extended timeline, and systematically reduced as temporal separation between context exposures was shortened. TMZ-treated rats exposed to the condensed timeline showed an additional reduction in context selectivity compared to their control counterparts. This finding corroborates theoretical predictions concerning the separation of temporal information in the dentate gyrus, and the role of adult-born neurons in encoding temporally specific events.

Introduction:

The hippocampus is a neural structure essential to the ability to form new memories. The function of the dentate gyrus is not fully understood. One predominant theory is that the dentate gyrus may play a role in discriminating between similar events [1-4]. This ability has been described as pattern separation, and tests of this function have primarily investigated discrimination of subtle changes in a spatial environment at the single cell level [5], or behavioral discrimination of spatial contingencies in a learning task [6-8]. The dentate gyrus of the hippocampus is one of the only brain regions to exhibit adult neurogenesis, the continual birth of new neurons throughout life [9]. Recent studies have begun to embrace the existence of adult neurogenesis in their tests of dentate gyrus

function and the potentially competing roles that adult-born neurons bring to the hippocampal circuit [10,11]. Importantly, immature adult-born neurons in the dentate gyrus exhibit a characteristic development that includes a transient period of hyperexcitability [12-15]. Computational models demonstrate that temporally proximal events elicit activity from common immature cells in an otherwise sparse firing mature network [16,17]. These immature cells, thus, may provide a similarity in dentate gyrus output for temporally proximal events that does not exist for events separated in time, a *temporal pattern integration* of inputs.

A potential outcome of such temporal pattern integration could be the creation of long-term *temporal pattern separation* of inputs. Surviving adult-born neurons would then provide a new output that distinguishes between contexts through their continuous contribution of new temporal dimensions generated by transient windows of hyperexcitability. The dentate gyrus as a whole may thus exhibit activity that describes both distinct spatial and distinct temporal aspects of a context.

To test the potential role of adult neurogenesis in temporal pattern integration and pattern separation, *in vivo* recordings of granule cells were obtained from the dentate gyrus of awake-behaving rats in three different temporal training paradigms. The paradigms spanned several time frames wherein contexts were introduced in such a way as to maximize or minimize integration. First, we designed an extended timeline paradigm in which rats were exposed to three distinct environments separated over long time periods. Using

this paradigm, we hoped to selectively recruit populations of neurons with dedicated activity to a single environment, and indeed found that the vast majority of cells were selective to only one of the three contexts.

Results:

A total of 76 cells were recorded from 3 rats exposed to the extended timeline in which the introductions to three behaviorally distinct contexts were separated by a period of greater than 3 weeks (Figure 2.1a). Temporal intervals greater than 3 weeks between behavioral context exposures in the extended timeline would ensure that the durations between contexts were long enough to recruit different populations of adult-born neurons but short enough to prevent age related reductions in adult neurogenesis proliferation. To determine the extent of context selectivity, we initially determined the mean firing rates for each cell in each context and utilized firing rate crtieria to categorize cells as active within a given context. All cells with a mean firing rate of 3Hz or greater in any context were removed from this analysis, to exclude interneurons that typically have high spontaneous firing rates between 5-80Hz [18]. To avoid imposing arbitrary firing rate thresholds, we used 200 firing rate thresholds between 0.01-2Hz in increments of 0.01Hz (Figure 2.1b, Figure S2.1). This measure highlighted the arbitrary nature of using a firing rate threshold, and revealed the dynamic range of cell selectivity by firing rate. The proportion of cells with activity selective to one behavioral context was greater than the proportion selective to two or all three behavioral contexts at all of the tested firing rate thresholds

greater than 0.02Hz. For instance, at a 0.4Hz firing rate threshold, 34% of cells exhibited activity selective to one context, 5% exhibited activity selective to two contexts, and 3% exhibited activity selective to three contexts (Figure 2.1b, inset). The remaining cells did not reach firing rate threshold for any context during the task.

Since firing rate may not be the most meaningful method for measuring contextual encoding, we further characterized the nature of context selectivity by utilizing a place field criterion. This criterion required a peak rate of at least 5Hz and a diameter greater than 10cm, a criterion well over 3 standard deviations above the mean firing rate for a given cell [19]. Field boundaries were defined as the points at which firing rate was reduced to 10% of peak. Again in this analysis, all cells with a mean firing rate of 3Hz or greater in any context were excluded. A total of 47 cells met place field criteria in at least one context. Of these 47 cells, 83% were selective to one context, 13% were selective to two contexts, and 4% demonstrated place field activity in all three contexts (Figure 2.1c).

Having found a robust selectivity to contexts separated in time, we then assessed the role of adult-born neurons on this context selectivity. We used an anti-mitotic chemotherapeutic agent, temozolomide (TMZ), to transiently knockdown levels of adult neurogenesis. In pilot studies, we determined that 3 injections of 12.5mg/kg each week produced an approximate 40% neurogenesis knockdown after 2 weeks that was fully recovered by 4 weeks (Figure S2.2). Given this narrow window of neurogenesis knockdown, we created a modified

version of the original paradigm that would create a temporal distinction between contexts but would also allow for the fullest extent of knockdown during contextual exposure. In this condensed timeline paradigm, rats were exposed to environments separated by a shorter interval of 2 weeks and injected with control or 12.5mg/kg TMZ three times per week for the duration of the experiment (Figure 2.2a). Adult born neurons have been shown to demonstrate action potentials as early as 1.5 weeks, but remain more plastic than their mature counterparts until 6 weeks. We predicted that transient neurogenesis knockdown would reduce context selectivity for the first two contexts, but that this selectivity would recover by the third context presented due to the greater availability of adult-born neurons. To enhance adult-born neuron survival and their potential contribution to dentate gyrus function, rats were given 30 minutes of enriched environment exposure daily throughout the course of the experiment.

A total of 86 cells were recorded from 6 control and 6 TMZ-treated rats exposed to the condensed timeline. The same firing rate and place field analyses performed for the extended timeline were utilized for the condensed timeline (Figure 2.2b, Figure S2.1). The proportion of cells with activity selective to one behavioral context was greater than the proportion selective to two or all three behavioral contexts at all of the tested firing rate thresholds greater than 0.3Hz. For instance, at a 0.4Hz firing rate threshold, 21% of cells exhibited activity selective to one context, 9% exhibited activity selective to two contexts, and 13% exhibited activity selective to three contexts (Figure 2.2b, inset). A total of 49 cells

from this group met place field criterion in at least one context. Of these 49 cells, 43% were selective to one context, 33% were selective to two contexts, and 24% demonstrated place field activity in all three contexts (Figure 2.2c). This distribution was significantly different from cells in the extended timeline (χ^2 p= 2.0×10^{-4}), and likely attributable to the longer inter-exposure intervals in the extended timeline.

A total of 70 cells were recorded from the 6 rats receiving TMZ and exposed to the condensed timeline. The same firing rate and place field analyses performed for the extended and condensed timelines were utilized for the TMZ group (Figure 2.2d, Figure S2.1). TMZ administration had an effect upon selective contextual encoding. At lower firing rate thresholds, a majority of the cells exhibited activity in two contexts. For instance, at a 0.4Hz firing rate threshold, 10% of cells exhibited activity selective to one context, 26% exhibited activity selective to two contexts, and 14% exhibited activity selective to three contexts (Figure 2.2d, inset). The remaining cells did not reach firing rate threshold for any context during the task. A total of 42 cells from this group met place field criterion in at least one context. Of these 42 cells, 21% were selective to one context, 60% were selective to two contexts, and 19% demonstrated place field activity in all three contexts (Figure 2.2e). This distribution was significantly different from cells in the condensed timeline that did not receive TMZ (χ^2 p= 0.029).

Lastly, to examine how the dentate gyrus distinguishes between environments in the absence of temporal segregation, we designed a simultaneous timeline paradigm. This paradigm removed temporal separation between the introductions to each context, but controlled for degree of context exposure and experiment time length (Figure 2.3a). A total of 53 cells were recorded from 5 rats exposed to the simultaneous timeline. The same firing rate and place field analyses performed for the extended and condensed timelines were utilized for the simultaneous timeline (Figure 2.3b, Figure S2.1). At lower firing rate thresholds, a majority of the cells exhibited activity in all three contexts. For instance, at a 0.4Hz firing rate threshold, 11% of cells exhibited activity selective to one context, 17% exhibited activity selective to two contexts, and 38% exhibited activity selective to three contexts (Figure 2.3b, inset). The remaining cells did not reach firing rate threshold for any context during the task. A total of 40 cells met place field criterion in at least one context. Of these 40 cells, 22.5% were selective to one context, 30% were selective to two contexts, and 47.5% demonstrated place field activity in all three contexts (Figure 2.3c). This distribution was significantly different from cells in the extended and condensed timelines (χ^2 p= 4.0x10^{-8}, χ^2 p= 0.047 for control, χ^2 p= 0.01 for TMZ-treated, respectively).

To quantify the extent to which cell activity could be predictive of the context the rat is in, we designed a contextual information score (See Methods, Figure 2.3d). Rats exposed to the extended timeline had a greater number of

cells with higher contextual information than rats exposed to the condensed or simultaneous timelines (Wilcoxon rank sum p= 1.9×10^{-5}, Komolgorov-Smirnov p= 3.5×10^{-5}, and Wilcoxon rank sum p= 3.2×10^{-6}, Komolgorov-Smirnov p= 7.0×10^{-6} respectively). Control rats exposed to the condensed timeline had a greater number of cells with higher contextual information than rats exposed to the same time receiving TMZ (Wilcoxon rank sum p= 0.03, Komolgorov-Smirnov p= 0.01). There was no significant difference in contextual information between control or TMZ-treated rats exposed to the condensed timeline and rats exposed simultaneous timelines (Wilcoxon rank sum p= 0.14, Komolgorov-Smirnov p= 0.39, comparing control condensed timeline rats with simultaneous timeline rats, and Wilcoxon rank sum p= 0.69, Komolgorov-Smirnov p= 0.14, comparing TMZ-treated condensed timeline rats with simultaneous timeline rats).

Discussion:

Our studies aimed to exploit the hypothesized roles of adult neurogenesis in the hippocampal circuit to better understand how the dentate gyrus incorporates spatial and temporal aspects of an environment into a memory. Given long timelines (temporal separation between contexts greater than 3 weeks), single granule cells preferentially exhibit a selective contextual encoding ability in which their activity is restricted to only one context. As we had no *a priori* knowledge of the type of activity that would constitute meaningful encoding of an environment, we designed three separate measures that confirmed this preferential activity for only a single context. By imposing a firing rate criteria from

0.01-2Hz upon cells from this group, we removed any potential bias in choosing

an arbitrary firing rate criterion and revealed that the highest proportion of cells

demonstrated activity selective to one context for all firing rate criteria above

0.02Hz. Using a place field criterion, we revealed that a vast majority of the cells

in this group demonstrated relevant spatially specific firing for only one context.

Lastly, using a measure that specifically addressed the degree to which activity of

a single cell could inform knowledge of which context the rat was in, it was shown

that this group had the highest proportions of cells with large contextual

information scores. This result is consistent with models, theories and

experimental tests of adult neurogenesis function in which mature adult-born

cells become selective and dedicated to environments experienced during their

development [16,17,20,21].

In the condensed timeline (temporal separation between contexts is 2

weeks), control rats show a reduction in the level of selective encoding to one

context in all three measures of analysis. This may be explained by the fact that

in the extended timeline, longer intervals between contexts recruit more distinct

populations of adult-born cells. Importantly, this group still maintains large

proportions of cells with activity selective to one or two contexts using a place

field criterion or firing rate criteria greater than 0.3Hz. The proportions of cells in

this group selective to one context at 0.1Hz and 0.2Hz criteria are similar to the

proportions found in previous *in vivo* electrophysiological recordings in the

dentate gyrus using these criteria [5]. A reassessment of context selectivity in

previous *in vivo* recording experiments in the dentate gyrus using a broader range of firing rate, place field, or IContext measures may reveal further similarities between the datasets [5,22].

The selectivity observed in control rats exposed to the condensed timeline is further reduced without the addition of adult-born neurons at the beginning of the condensed timeline. Specifically, TMZ-treated rats demonstrated a large proportion of cells selective to two contexts. This may indicate that in TMZ-treated rats, place cells are able to temporally discriminate at the two-context resolution (4 weeks), but less able at the single context (2 week) resolution. Interestingly, selectivity to a single context returns with the greater availability of adult-born neurons following TMZ recovery, as indicated by a high proportion of cells with place fields in only the third context of a training sequence (Figure S2.3). This suggests that the adult-born population may facilitate the selective contextual encoding ability.

The simultaneous context exposure timeline appears to shift the type of encoding to a non-selective type of encoding in which cells are active in all three contexts, which may indicate a lack temporal discrimination between contexts in this group. Thus, in each of the three timelines, firing selectivity of the neurons in control rats systematically reflected the nature of the timeline, revealing a new mechanism by which temporal pattern separation and integration is achieved in the dentate gyrus at the single cell level.

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Aimone, J.B., Wiles, J., Gage, F.H., Chiba, A.A., Quinn, L. K., *Temporally*

Selective Contextual Encoding in the Dentate Gyrus of the Hippocampus.

The dissertation/thesis author was the primary investigator and author of this

paper.

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Materials and Methods:

All procedures were performed in accordance with NIH and local IACUC guidelines. 14 adult male Long-Evans rats were used as subjects. The rats were housed individually and maintained on a 12-h light ⁄ dark cycle. They were acclimated to the colony room for 3 days and handled daily for at least 2 weeks prior to beginning the experiment, during which time they were placed on food restriction until they reached 85–90% of ad libitum weight. Rats were 3 months old at the time of surgery. Weight ranged from 300 to 350 g. Water was available at all times. All behavioral testing occurred during the rats' light cycle.

Behavioral Contexts: The three behavioral contexts were as follows: 1) a 24" x 24" square box containing rat bedding and randomly placed ¼ pieces of honey nut cheerios 2) a 48" diameter circular track with a 3" wide pathway and either ¼ pieces of honey nut cheerios or chocolate sprinkles in a reliably rewarded location that is shifted to a different track location up to three times per session 3) a 48" diameter circular cheeseboard with randomly placed chocolate sprinkles and three presentations of a large food reward (a 2" diameter weigh dish filled with chocolate sprinkles) at random time intervals and random locations for a period of >30 seconds (Figure S2.4). All three behavioral contexts were presented in the same location of the same recording room, containing constant external cues.

Timeline for Simultaneous and Temporally Distinct Exposure: Rats were exposed to the three behavioral contexts according to one of three timelines: an extended

timeline in which the initial exposure to each context was separated by greater than 3 weeks (Figure 2.1a), a condensed timeline in which the initial exposure to each context is separated by 2 weeks (Figure 2.2a), or a simultaneous timeline in which there was no temporal separation between initial context exposures (Figure 2.3a). Temporal intervals greater than 3 weeks between behavioral context exposures in the extended timeline would ensure a length of time between contexts long enough to recruit different populations of adult-born neurons but short enough to prevent age related reductions in adult neurogenesis proliferation. In the extended timeline (N=3 rats), rats were first trained to run around the circular track before undergoing surgery for microdrive implantation. After a recovery period of at least two weeks and during several weeks in which microdrive wires were slowly lowered to the granule cell layer of the dentate gyrus, rats were re-trained on the circular track and began exposure to the square box environment. During this time in training, they received exposure to both contexts each day. Once stable cell recording could be obtained from the granule cell layer and at least 3 weeks after initial exposure to the square box, rats were exposed to the circular cheeseboard environment. During this time in training, they received exposure to all three contexts each day. All analyses of cell activity were restricted days in which rats received exposure to all three contexts on the same day.

In the condensed timeline (N=6 rats), initial exposure of each behavioral context was separated by a fixed 2 weeks to provide an interval between

contexts long enough to recruit a new population of adult-born neurons, but short enough to allow for the fullest extent of knockdown during contextual exposure given the timeline of efficacy for the chosen knockdown procedure (Figure 2.2a). For the first context exposure, the rat was exposed to only a single behavioral context for 5 days followed by a 9-day rest period. In subsequent exposures to the second and third behavioral contexts, exposure to the new context also included exposure to previously presented contexts in the same recording day. Nine days after the end of training, rats received a 4-day test phase that included presentation of all three behavioral contexts. All analyses of cell activity were restricted to test days. The order of exposure to each of the three behavioral contexts was counterbalanced across groups.

In the simultaneous timeline (N=5 rats), rats were exposed to all three behavioral contexts in the same day for the first 5 days of recording. In subsequent exposure weeks, the numbers of exposures to each context were limited to two contexts per day and finally one context per day to equate time spent in each behavioral context across groups.

Microdrive Implantation Surgery: A microdrive consisting of four tetrodes of 17μm platinum iridium wire was surgically implanted using stereotaxic procedures (from bregma A/P: -4.0, M/L: +2.2mm, D/V: -2.2mm) and lowered into the granule cell layer of the dentate gyrus (D/V: ~2.7mm) until the appearance of place cell single units, "dentate spikes," and complex high frequency (16-90Hz) local field potential activity.

Neural Recordings: During targeting of the dentate gyrus granule cell layer, each wire of the microelectrode bundle was first checked for neural activity. The quietest wire was found and served as a reference electrode. If no activity was found on the recording wires the bundle was advanced in 10μm increments until activity was found, or until a total of 80μm was traversed. Wires were not turned within 24 hours prior to a recording session.

The activity on each wire was passed through a high-impedance OpAmp headstage that held a set of light-emitting diodes (Neuralynx Technologies, Bozeman, MT). A multiwire flexible cable connected the preamplifiers to a 32-channel commutator (Neuralynx Technologies, Bozeman, MT). The single-unit signal was filtered at 600 Hz (low pass)-6000 Hz (high pass), and amplified through Neuralynx Lynx-8 differential programmable amplifiers. The local field potential signal was filtered at 1Hz (low pass)-475 Hz (high pass). The amplifiers were integrated with the Cheetah data acquisition program (Neuralynx Technologies, Bozeman, MT), wherein the acquired analog signals were digitally converted at a rate of 32KHz (DT 2821 Data Translation, Marlboro, MA) prior to storage. The position of the rat was monitored by a set of light-emitting diodes placed on the headstage. A video tracking system (SA-2 Dragon Tracker, Boulder, CO) registered the position of the diodes with a sampling frequency of 30 Hz. All recording sessions began with 5 minutes of baseline recording activity in a home cage environment. Single cells were identified using Offline Sorter (*Plexon*, Dallas, TX) to compare relative amplitude of each spike across tetrode

wires. Final microdrive wire location was verified post-mortem in 40um sections using a Nissl stain (Fig. S2.5).

Firing Rate and Place Field Criterion Analyses:

The firing rate in each context was determined by dividing the total number of spikes per context by the time spent in the context. To assess context selectivity, the number of cells selective to one, two, or all three behavioral contexts was counted for every firing rate criterion between 0.01-2Hz in increments of 0.01Hz (Figure 2.1b, Figure 2.2b, Figure 2.2d, Figure 2.3b). To quantify differences in selectivity over multiple firing rate criteria across groups, a receiver operating characteristic (ROC) curve was made for the changing proportion of cells with activity in all three contexts compared to the proportion of cells selective to at least one context for all firing rate criteria from 0.01Hz-10Hz in increments of 0.01Hz (Figure S2.1). The area under the curve for each group was calculated using trapezoidal approximations.

To assess the presence of place field activity in each context, a place field criterion was used that required a peak firing rate of at least 5Hz and a diameter greater than 10cm. Place field boundaries were defined by the points at which firing rate was reduced to 10% of peak. The number of cells with place fields in one, two, or all three behavioral contexts was used to assess extent of context selectivity.

Informational Analysis of Context Selectivity (IContext): To assess dedicated activity to a given behavioral context, we developed a novel measure of contextual information using the following equation:

$$I_{Context} = (I_{C1} + I_{C2} + I_{C3})/3$$

$$I_C = (f_C/f_{Avg}) * \log_2(f_C/f_{Avg})$$

$$f_{Avg} = (f_{C1} + f_{C2} + f_{C3})/3$$

where f_C is the number of spikes occurring within a context divided by total time spent in a context, f_{Avg} = the average firing rate across all contexts, I_C = the information score of a given context in bits/spike, and $I_{Context}$ = the amount of information present in each spike regarding contextual inhabitance.

Adult Neurogenesis Knockdown: Temozolomide (TMZ) is an anti- cell proliferation drug that has been shown to reduce cell proliferation in the dentate gyrus by approximately 40% when given three injections of 12.5mg/kg each week for a total of two weeks. Although neurogenesis levels have been shown to recover through consistent use of this injection regime after four weeks, to prevent possible side effects from stopping treatment after the beginning of the experiment, three injections of 12.5mg/kg TMZ were performed every week for 10 weeks (beginning 3 weeks prior to the initial context exposure).

Immunohistochemistry: To quantify levels of neurogenesis in pilot rats receiving TMZ for 2 or 4 weeks, rats were injected with 50mg/kg BrdU for four consecutive days during week 2 or 4 of TMZ treatment, respectively (Figure S2.2). This measure indicates the number of dividing cells after 2 and 4 weeks of TMZ

administration. After 24hrs from the final injection of BrdU in each group, rats

were perfused with 0.1M PO_4 followed by 4% paraformaldehyde and rat brains

were removed from the skull to incubated at least 48hrs in 30% sucrose at 4°C.

Frozen brains were then cut into 40 μm coronal sections with a microtome.

Sections from every 240 μm spanning the whole dentate gyrus were selected.

The slices were incubated at 4°C for 72 hrs in the following primary antibodies:

goat anti-DCX (1:250) and mouse anti-NeuN (1:10). They were then incubated

for 2 hours at room temperature in the following secondary antibodies: Donkey

anti-Goat IgG Cy 3 (1:250) and Donkey anti-Mouse IgG Cy5 (1:250). Slices were

then postfixed for 50 min in 4% paraformaldehyde at room tempurature and

incubated for 30 min in 2N HCl at 37°C followed by 1N Borate Buffer at room

temperature. They were then incubated at 4° C in primary Rat anti-BrdU (1:100)

for 72 hrs. Lastly, they were incubated at RT in secondary Donkey anti-Rat IgG

Dylite 488 (1:250) for 2 hours.

Analysis of Theta Phase Relationships: To assess changes in the phase

relationship of single unit spikes to the local theta rhythm, the phase of each

spike for each cell was first determined from the filtered Fourier transform (5-

10Hz). The contribution of each spike phase to the overall vector of strength was

determined using the following equation:

$$R = \sqrt{X_w^2 + Y_w^2}$$

where $\quad X_w = \dfrac{\sum (w^x \cos \theta)}{\sum w}$

$$Y_w = \frac{\sum (w^x \sin \theta)}{\sum w}$$

and w is the power of theta at the time of a given spike divided by the maximum

theta power over the course of a recording session. Statistical significance of R

was defined as the 95% confidence interval in a distribution of one thousand

random R-values generated from random spike times equal in number to that of

each for each recording session.

Figure 2.1: Single granule cells preferentially exhibit a selective contextual encoding ability in the extended temporal training group. (A) Timeline of context exposures for the extended temporal training group in which the introduction of each context occured at intervals approximately 3 weeks apart (colored boxes = 1 week). All analyses were performed during a 4-day test phase in which rats received exposure to all three contexts in the same day. (B) The proportion of cells exhibiting activity selective to one blue), two (green), or three (red) contexts using a firing rate threshold between 0.1-2.0Hz and increasing at intervals of 0.1Hz. The percentage of cells selective to one, two, or three contexts at a 0.4Hz threshold is shown (inset). The remaining cells (gray) did not meet firing rate threshold in any context. (C) The percentage of cells selective to one (blue), two (green), or three (red) contexts using a place field criterion. (D) Two examples of cells exhibiting single context selectivity. Rat path within each context (gray) with superimposed spike locations (red dots) are shown to the left of rate maps for each of the three contexts. Both cells were recorded simultaneously from the same rat. Cell 1 exhibits activity selective to the circular track. Cell 2 exhibits activity selective to the cheeseboard.

A

Introduction to Context 1 — Circular Track

Surgery — ~ 2 week Recovery Period

Introduction to Context 2

Square Box · Square Box · Square Box · Square Box

Circular Track · Circular Track · Circular Track · Circular Track

Cheeseboard

Introduction to Context 3

Recording — All Contexts 4 Days

Training Phase — Test Phase

B

Proportion of Cells vs Firing Rate Criterion (Hz)

Selective to One Context
Selective to Two Contexts
Activity in All Three Contexts

34% · 58% · 5% · 3%

C

Selective to One Context
Selective to Two Contexts
Activity in All Three Contexts
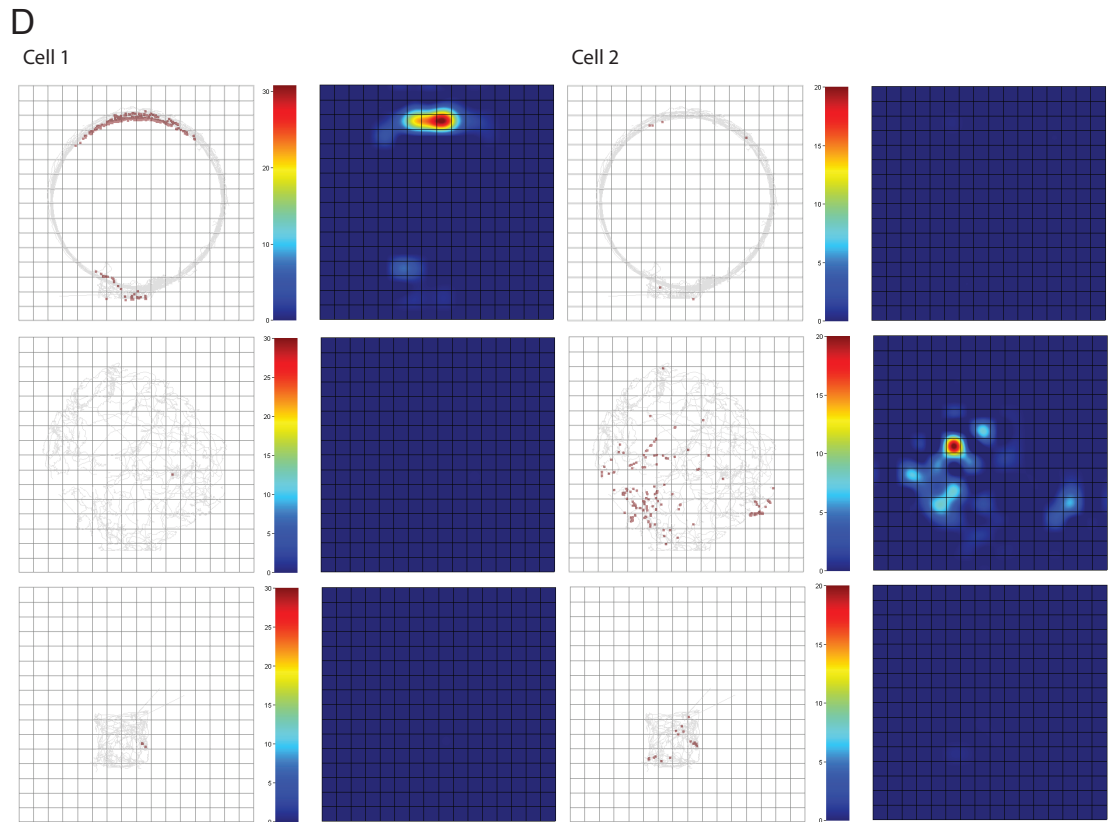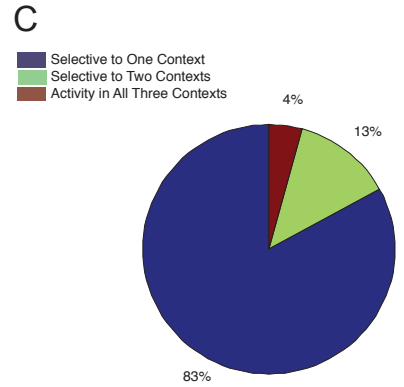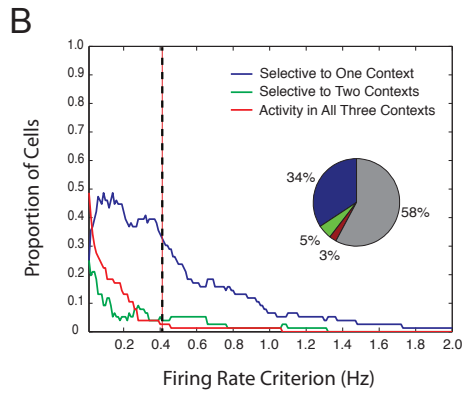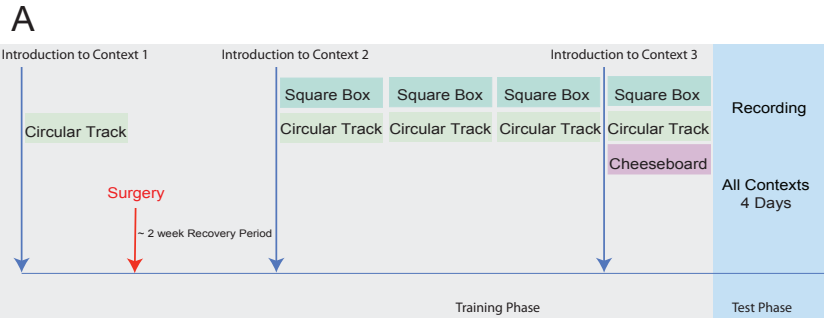
4% · 13% · 83%

D

Cell 1 · Cell 2

Figure 2.2: Single granule cells from TMZ-treated rats exposed to the condensed timeline exhibit selective contextual encoding to a lesser extent than cells from control rats exposed to the same timeline. (A) Timeline of context exposures for the condensed temporal training group in which the introduction to each context occurred at a fixed 2 weeks apart. All analyses were performed during a 4-day test phase in which rats received exposure to all three contexts in the same day. (B and D) The proportion of cells exhibiting activity selective to one blue), two (green), or three (red) contexts using a firing rate threshold between 0.1-2.0Hz and increasing at intervals of 0.1Hz for control (B) and TMZ-treated (D) rats. The percentage of cells selective to one, two, or three contexts at a 0.4Hz threshold is shown (inset). The remaining cells (gray) did not meet firing rate threshold in any context. (C and E) The percentage of cells selective to one (blue), two (green), or three (red) contexts using a place field criterion for control (C) and TMZ-treated (E) rats.
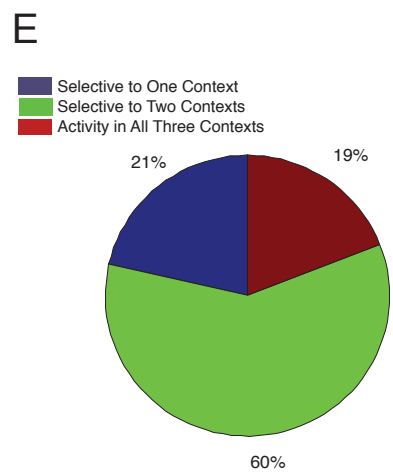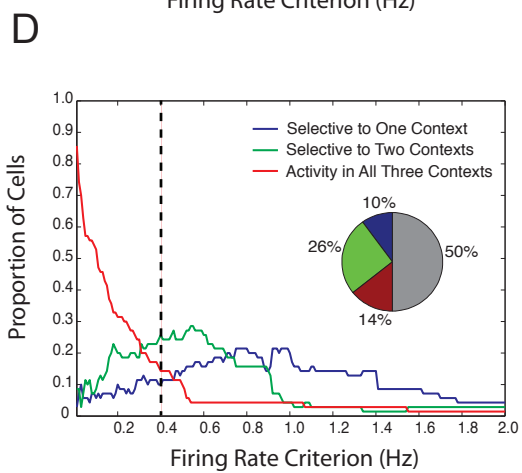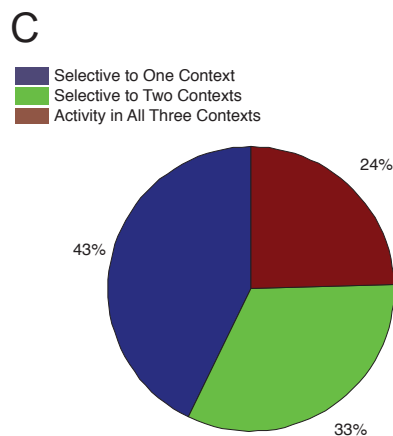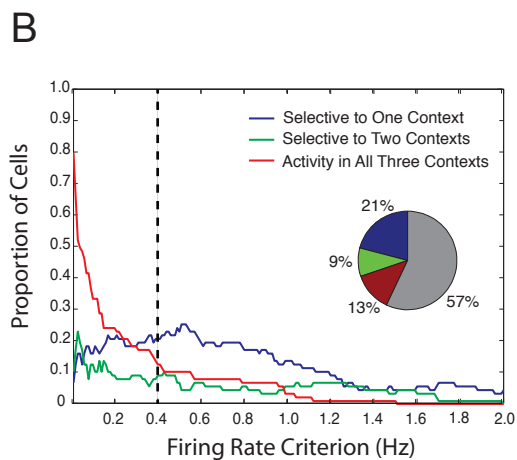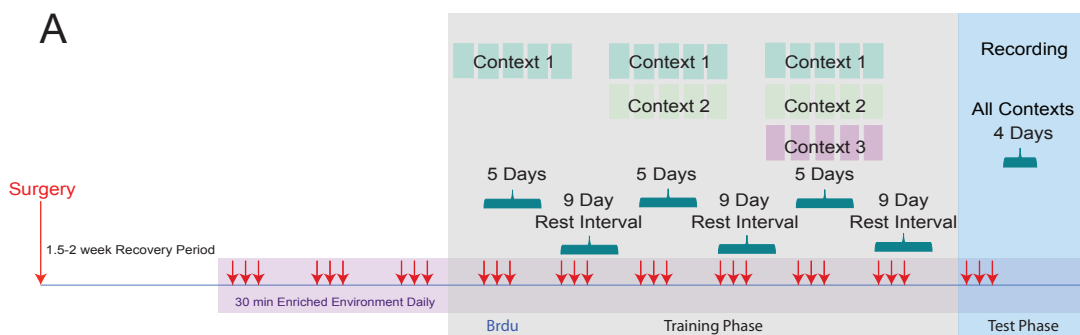
Figure 2.3: Systematic reduction in context selectivity reflects the nature of the training paradigm. Simultaneous temporal training paradigm reduces selective contextual encoding. (A) Timeline of context exposure for the simultaneous temporal training group in which there was no temporal segregation between initial exposures to each context occurred. All analyses were performed during a 4-day test phase in which rats received exposure to all three contexts in the same day. (B) The proportion of cells exhibiting activity selective to one blue), two (green), or three (red) contexts using a firing rate threshold between 0.1-2.0Hz and increasing at intervals of 0.1Hz. The percentage of cells selective to one, two, or three contexts at a 0.4Hz threshold is shown (inset). The remaining cells (gray) did not meet firing rate threshold in any context. (C) The percentage of cells selective to one (blue), two (green), or three (red) contexts using a place field criterion. (D) The proportion of cells with IContext scores between 0 -1.6 bits/spike in the extended (black), condensed control (light blue), condensed TMZ-treated (green), and simultaneous timeline (yellow) groups. The extended timeline group has the largest proportion of cells with high IContext scores (See Methods for IContext calculation).

A

Context 1
Context 2
Context 3

3 contexts/day
5 Days

2 contexts/day
5 Days

1 context/day
5 Days

9 Day
Rest Interval

9 Day
Rest Interval

9 Day
Rest Interval

Recording

All Contexts
4 Days

Surgery

Recovery Period and Dentate Gyrus Targeting

30 min Enriched Environment Daily

Training Phase

Test Phase

B

Selective to One Context
Selective to Two Contexts
Activity in All Three Contexts

11%
34%
17%
38%

Proportion of Cells

Firing Rate Criterion (Hz)

C

Selective to One Context
Selective to Two Contexts
Activity in All Three Contexts

23%
48%
30%

D

Extended Timeline
Condensed Timeline (Control)
Condensed Timeline (TMZ)
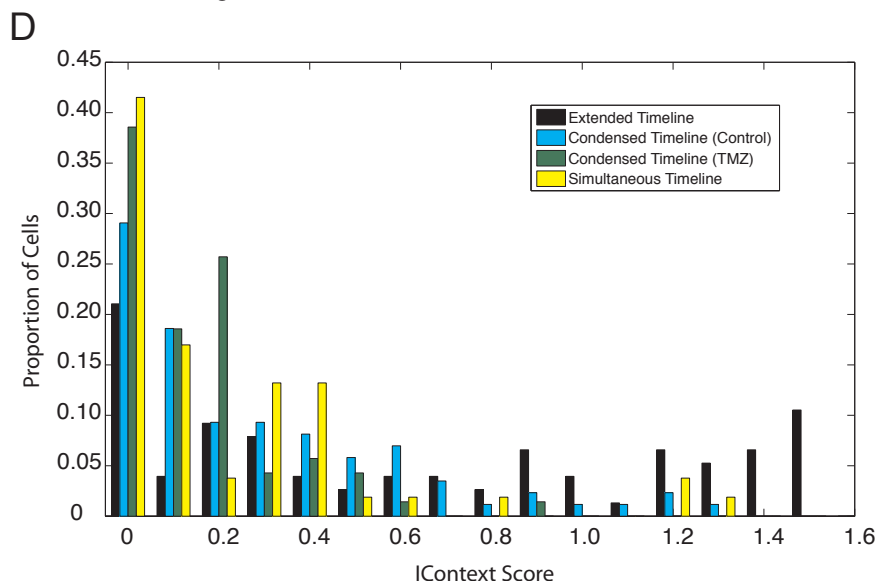Simultaneous Timeline
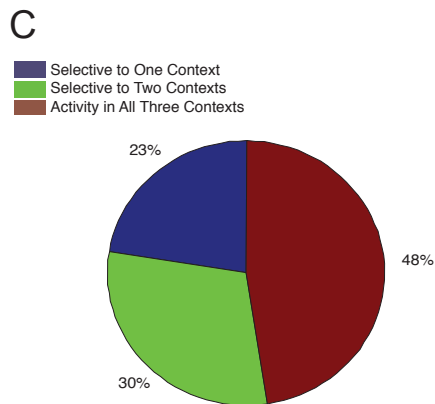
Proportion of Cells

IContext Score

Figure S2.1: ROC Analysis of Context Specificity in Each Group with Increasing Firing Rate Criterion. Receiver operating characteristic curves for the proportion of non-selective cells plotted against the proportion of cells selective to at least one context with increasing firing rate criteria from 0.01-10Hz (right to left). By comparing the proportion of cells active in at least one context to the proportion of cells active in all three contexts, the proportion of cells demonstrating selective activity (activity for one or two contexts exclusively) at each point is revealed.
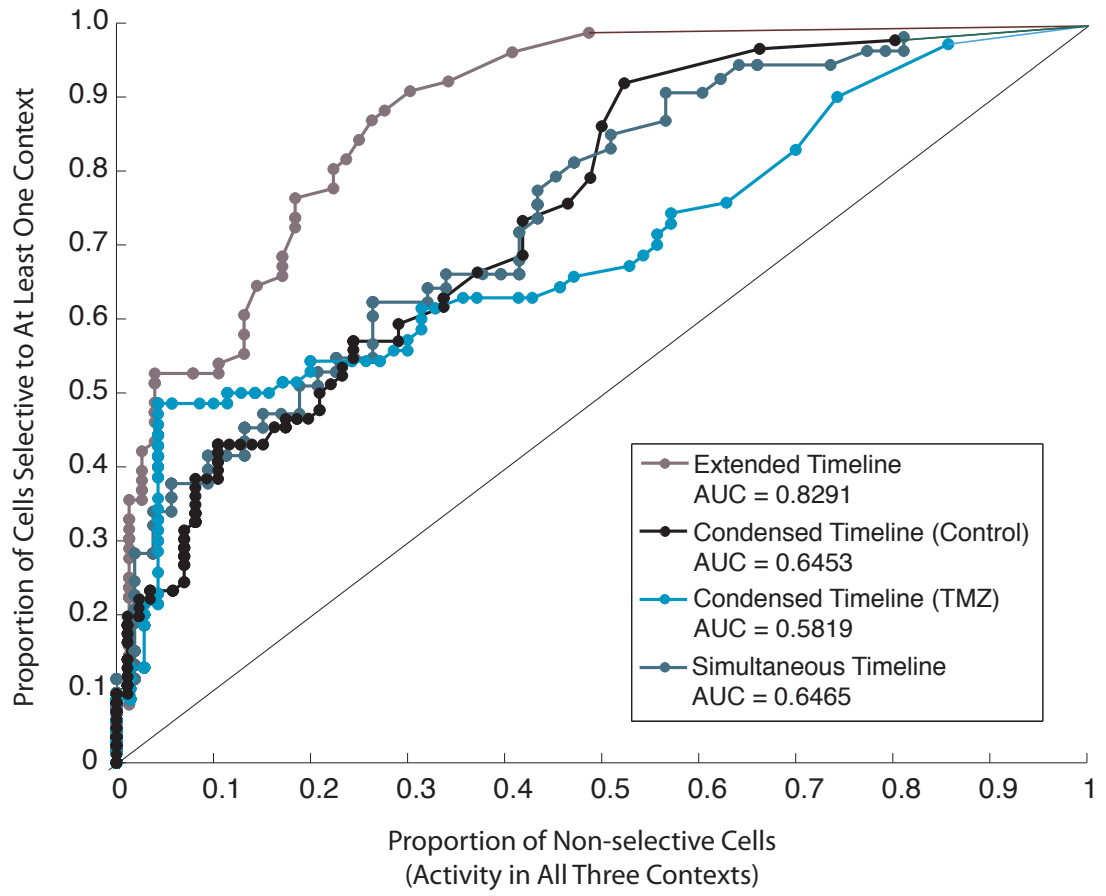
Figure S2.2: The Total Number of BrdU-labeled Cells after 2 and 4 weeks of TMZ administration. To test the timecourse of neurogenesis knockdown due to temozolomide (TMZ) administration, rats were given either control or 12.5mg/kg temozolomide (TMZ) injections three times a week for a period of 2 or 4 weeks (A and B, respectively). To label dividing cells at the end of the 2 or 4 week period, rats were given 50mg/kg injections of BrdU once a day for 4 days. The total numbers of BrdU-labeled cells from each group are shown in C.

A

B
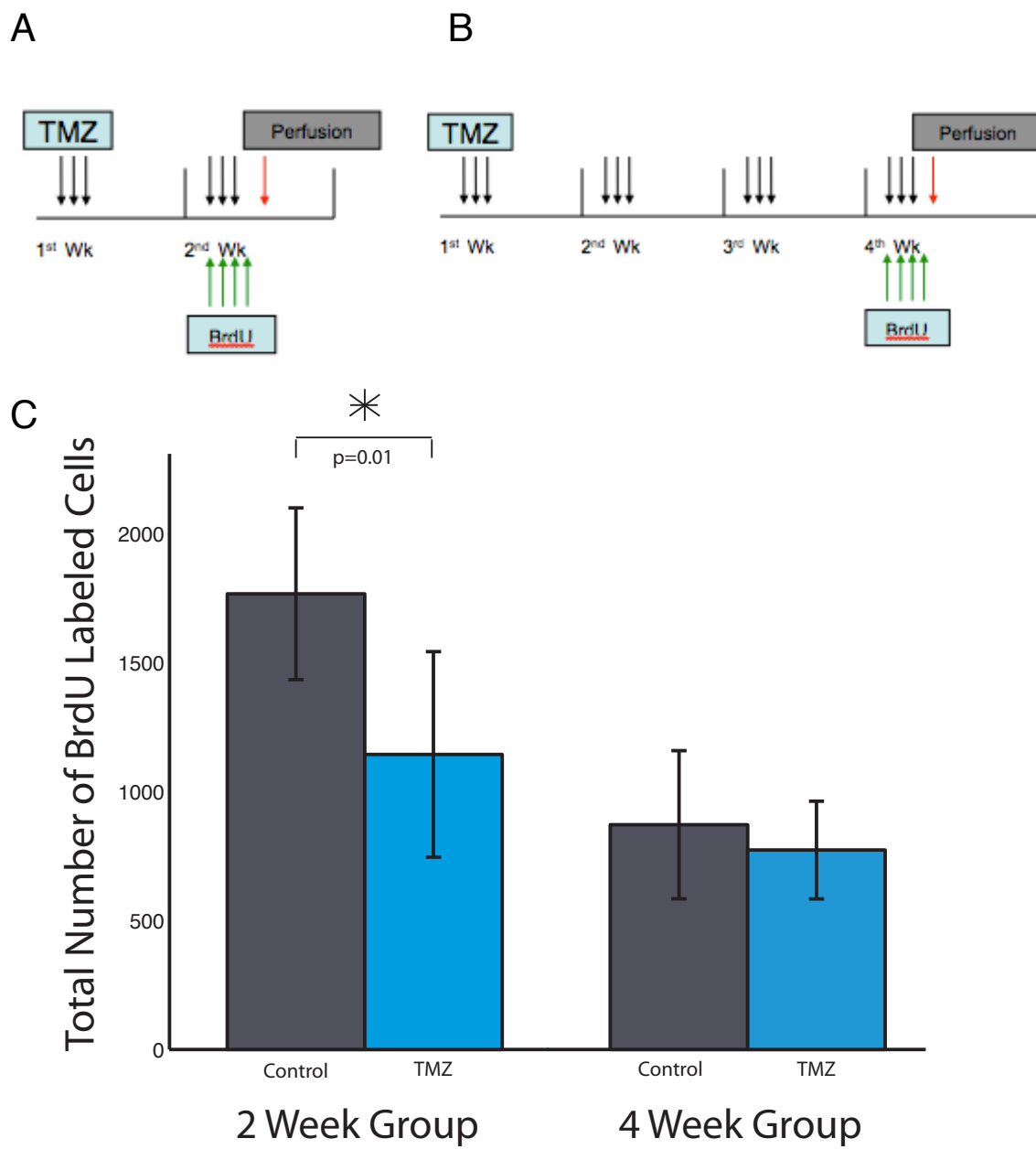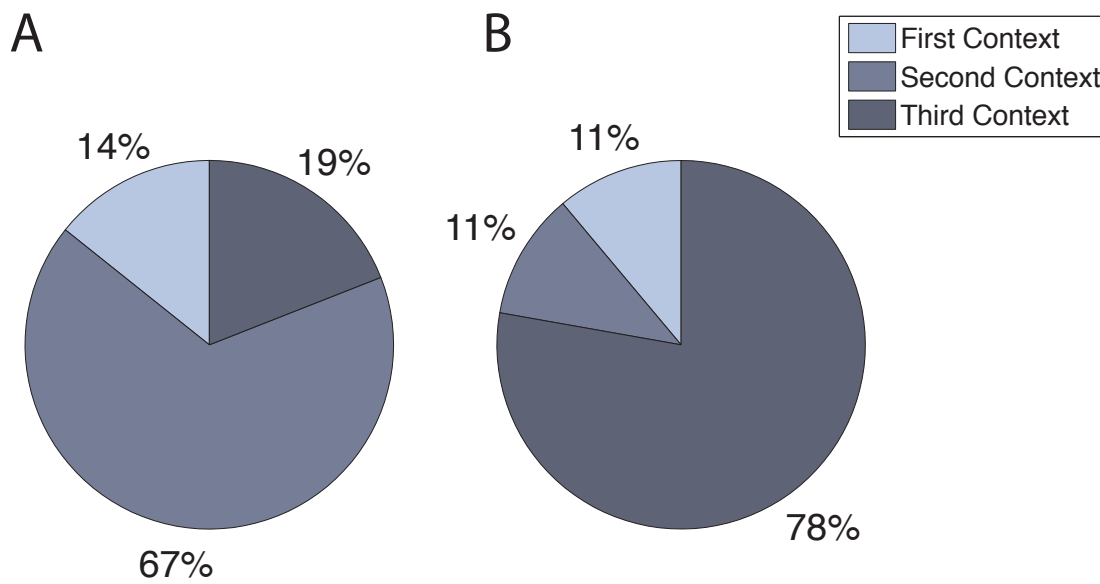
Figure S2.3: Context Specificity of Place Cells According to Single Contexts by Sequential Order of Contextual Exposure. Place cells from control (A) and TMZ (B) rats in the condensed timeline with activity selective to one context demonstrate selectivity that is temporally biased towards the second and third contexts presented, respectively.

Figure S2.4: Behavioral Contexts Used in each of the Temporal Training Paradigms. The three behavioral contexts were 1) a 24" x 24" square box containing rat bedding and randomly placed ¼ pieces of honey nut cheerios 2) a 48" diameter circular track with a 3" wide pathway and either ¼ pieces of honey nut cheerios or chocolate sprinkles in a reliably rewarded location that is shifted to a different track location up to three times per session 3) a 48" diameter circular cheeseboard with randomly placed chocolate sprinkles and three presentations of a large food reward (a 2" diameter weigh dish filled with chocolate sprinkles) at random time intervals and random locations for a period of >30 seconds.
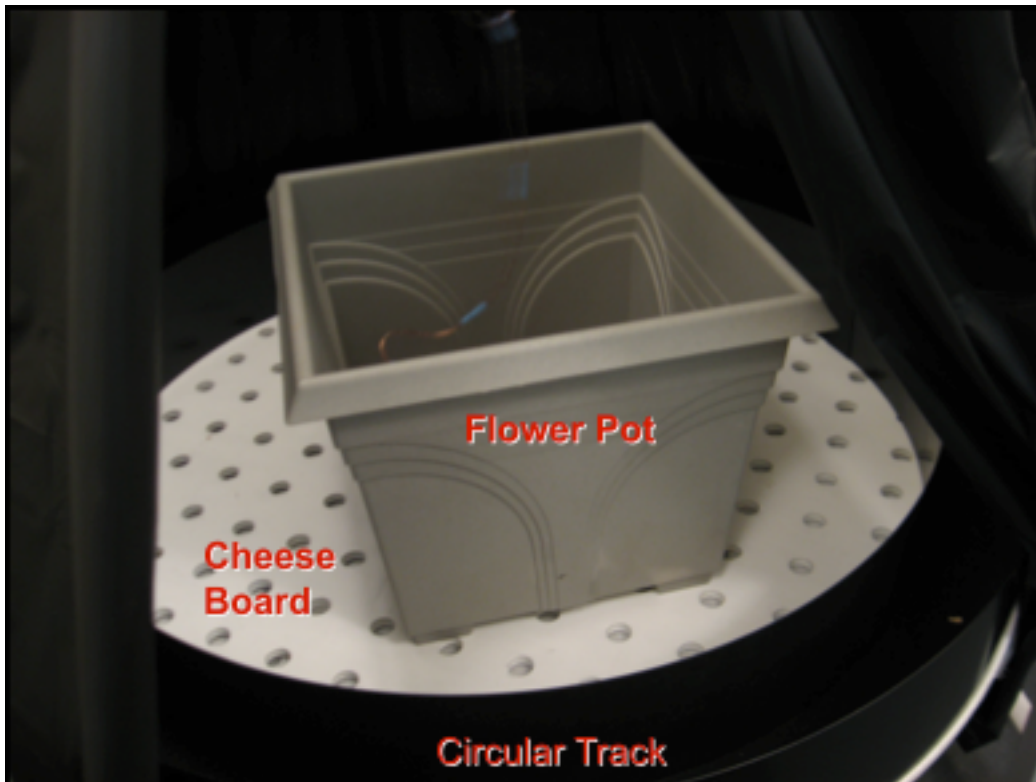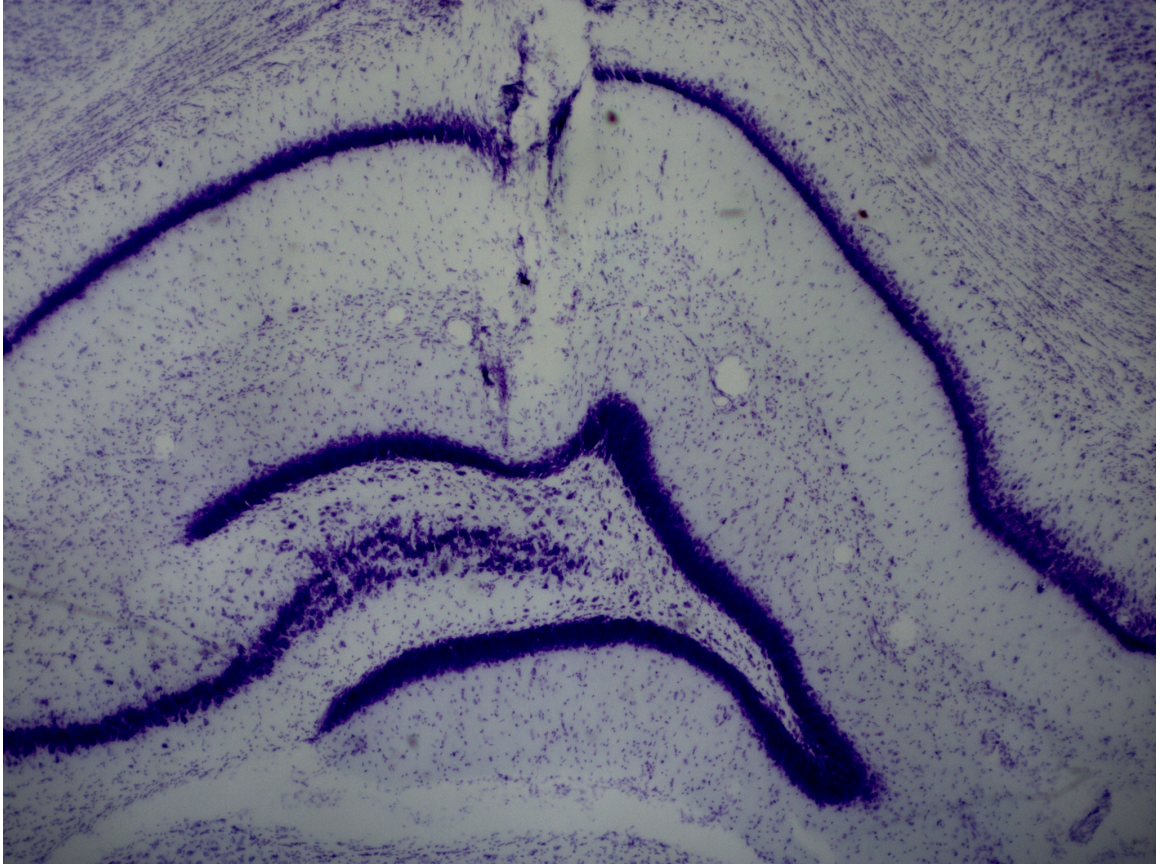
Figure S2.5: Representative Final Target Location of Recording Wires in the Dentate Gyrus. Final microdrive wire location was verified post-mortem in 40μm sections using a Nissl stain.

Figure S2.6: Single Cell Phase Relationships to Theta Rhythm in each of the Four Experimental Groups. The proportion of cells (y-axis) demonstrating significant single cell phase relationships to the local theta rhythm filtered from 5-10Hz at a given vector of strength (x-axis). One cell from a rat in the condensed timeline group receiving control injections was excluded from the figure for ease of visibility (vector of strength = 0.7685). Groups with higher context specificity (extended timeline, *purple* and condensed timeline with control injections, *black)* trended towards stronger phase relationships than groups with less context specificity (condensed timeline with TMZ injections, *light blue* and simultaneous timeline, *teal*).
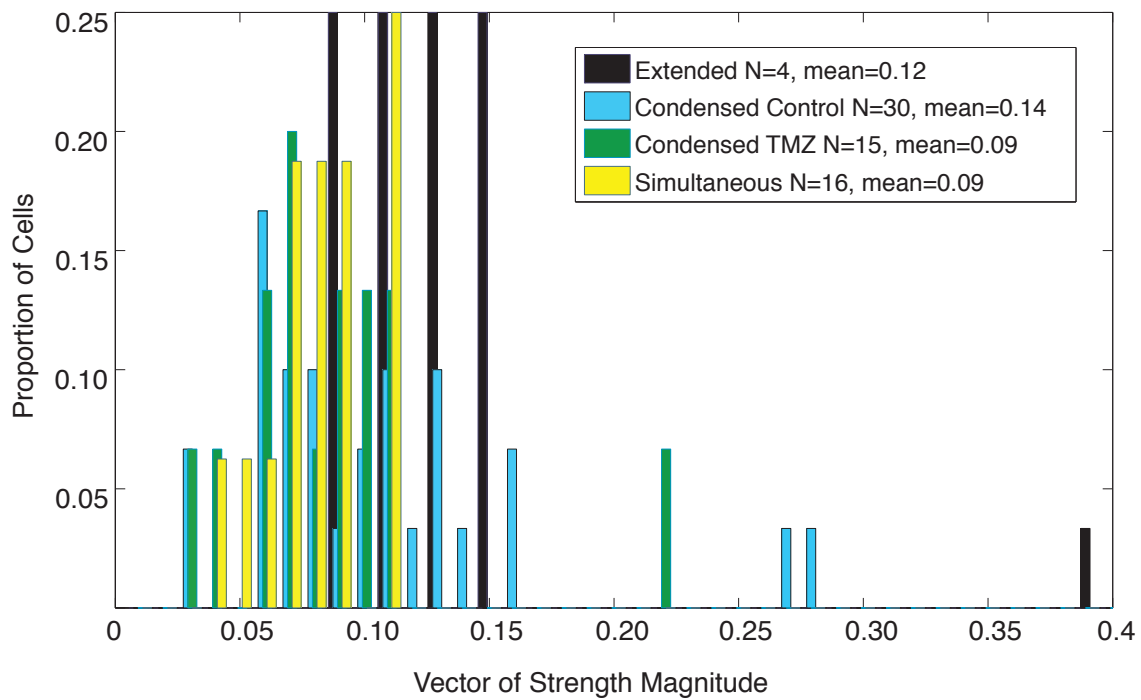
Table 2.1: The mean firing rate varied across groups but demonstrated a consistent decrease in mean firing rate for the square box context. One thing to note is that the different contexts were both behaviorally and spatially different; as a result the DG may be recruited to different extents. Indeed, we observed that the square pot with random foraging had a lower firing rate overall than the other contexts. While we do not believe this would have affected our comparisons across groups, it is likely that overall DG activation by different contexts is an independent factor to the IContext measure we described.

*Marks a significant difference in mean firing rate using one or more statistical tests.

	Circular Cheeseboard	Circular Track	Square Box
Extended Timeline	0.2921Hz std. ± 0.4338	0.2138Hz std. ± 0.3182	* 0.1341 std. ± 0.2156
Condensed Timeline (Control)	0.4272 std. ± 0.5893	0.3540 std. ± 0.5217	* 0.2582 std. ± 0.4420
Condensed Timeline (TMZ)	0.5454 std. ± 0.6142	0.4994 std. ± 0.6572	* 0.2882 std. ± 0.4915
Simultaneous Timeline	0.5228 std. ± 0.4073	0.6842 std. ± 0.6634	* 0.5366 std. ± 0.6157

CHAPTER 3: Theta and Beta Oscillatory Dynamics in the Dentate Gyrus of the Hippocampus During Associative Learning

The hippocampus is well known for containing strong rhythmic activity important for learning and memory. The theta rhythm in particular has been described as the dominating frequency during voluntary, exploratory behavior. The dynamics of theta activity during associative learning, and the possible introduction of other dominant frequencies at time points relevant to learning, has yet to be fully described. We performed *in vivo* electrophysiological recordings of local field potentials (LFP) in the dentate gyrus of the hippocampus as rats learned three conditioned reinforcement tasks. In each task, large decrements in theta-frequency (4-12Hz) amplitude were observed simultaneously with increases in beta-frequency (15-30Hz) amplitude. These dynamics were observed during time intervals surrounding conditioned stimulus-reinforcement pairings. The changes were not observed during behavior that did not require learning or contain reinforcement, suggesting that the modulations of theta and beta act in the service of associative learning.

Introduction:

The hippocampus is a locus of robust oscillatory activity. As indicators of large –scale synchronous activity, oscillations can provide meaningful insight into the temporal dynamics of input into the hippocampus during certain behaviors key to learning and memory. For example, the theta rhythm, which occurs in frequencies between 4-12 Hz, can dominate the hippocampal local field potential

during a range of behaviors including voluntary movement[1-3]. The theta rhythm has been shown to originate in large part from medial septal and enthorinal cortex inputs, both of which are necessary for hippocampal-dependent spatial learning [4-7]. Importantly, theta oscillations may be meaningful to behavior as suggested by evidence that encoding and retrieval are associated with different phases of the rhythm, and better learning can be achieved by driving the rhythm [8,9]. The dentate gyrus of the hippocampus can also exhibit higher frequency oscillations in the beta (15-30Hz) and gamma (30-60Hz) frequency ranges. For example, robust oscillatory bursts in the beta frequency range have been recorded in the dentate gyrus during behaviors such as odor sniffing. These bursts have been shown to demonstrate phase coherence with beta bursts in the olfactory bulb during an appetitive learning task, and are hypothesized to be important for olfactory learning [10-12]. The full range of behaviors that induce these beta bursts in the dentate gyrus has not yet been determined. Importantly, the degree to which reward is tied to this robust oscillatory activity is unknown.

The hippocampus has a well-established role in the encoding of learned associations. The mechanism through which these associations are made across disparate dimensions such as spatial, olfactory, or reward dimensions is unclear. The prevalence and dynamics of theta and beta rhythms during learning tasks that recruit these different dimensions may uncover valuable information regarding the separation or convergence of these different inputs into the dentate gyrus.

We designed three conditioned reward-learning tasks to observe changes in theta and beta frequency amplitude at different time points during learning. In one task, *in vivo* electrophysiological recordings were performed in rats trained to run laps along a circular track for a food reward in a reliably rewarded location. We observed a large reduction of theta amplitude and an increase in beta amplitude at points in which rats encountered a conditioned reward location. As these changes occur during time intervals in which associations must be made across spatial, olfactory, and reward dimensions, we examined similar time intervals during a conditioned cue task and an object instrumental learning task that removed spatial and olfactory contingencies. Lastly, to determine whether these changes could be observed in the absence of reinforcement learning, we examined theta and beta amplitude during random stopping behavior on the circular track.

Materials and Methods:

All procedures were performed in accordance with NIH and local IACUC guidelines. 13 adult male Long-Evans rats were used as subjects. The rats were housed individually and maintained on a 12-h light ∕ dark cycle. They were acclimated to the colony room for 3 days and handled daily for at least 2 weeks prior to beginning the experiment, during which time they were placed on food restriction until they reached 85–90% of ad libitum weight. Rats were 3 months old at the time of surgery. Weight ranged from 300 to 350 g. Water was available at all times. All behavioral testing occurred during the rats' light cycle.

Circular Track Training: On the first day of circular track training, rats were first allowed to explore the circular track with randomly spaced ¼ size honey nut cheerio rewards. After approximately 5 minutes of random exploration, they began receiving a food reward for successful laps in only one direction along the circular track. On all subsequent days after the first training day, rats received a food reward in a reliably rewarded location only for laps completed in only one direction. On several experiment days, the conditioned food reward location was shifted to a new reward location up to three times. Rats were required to complete at least 15 laps per food reward location. All data analysis was restricted to experiment days after at least 5 days of circular track training.

Conditioned Cue Reward Training: In this task, rats were exposed to a circular cheeseboard arena and allowed to explore for randomly spaced chocolate sprinkles for 20 minutes. At random time points during exposure to this context, a large weigh boat filled with chocolate sprinkles was placed in the arena at a random location. Over many training sessions, rats learned to approach weigh boats immediately after placement in the arena. The weigh boats thus acted as cue indicators of a large food reward.

Object Instrumental Learning Task: Two adult male Long-Evans rats were trained to leave a start box, traverse a space 25 cm in length, and push aside one of three distinct Lego objects in order to obtain a food pellet from a hole underneath. Each object covered one of three differently valued pellets (100% sucrose, 25% sucrose, or .002% quinine, Noyes/Research Diets).

A basic taste discrimination consumption test was performed to ensure that the rats had rank order preferences for the pellets. Custom pellets were designed, ordered, and manufactured at concentrations such that rats responded most preferentially to pellets with the highest sucrose concentration (100%), less preferentially to pellets with the lower sucrose concentration (25%), and least preferentially to the quinine HCl pellets (.002%). In order to test the ability of each rat to discriminate between the pellets and to determine their preferences, each rat had free access to two piles (equal in quantity) of the pellets. After 60 seconds the amount remaining in each pile was measured and compared. The piles of pellets were counterbalanced for side of presentation (left v. right) and presented on an exhaustive pair-wise schedule. All rats had equivalent discriminatory abilities and preferences.

As it was of great importance that the rats not rely on olfactory cues to determine the associative relationship between Lego object and pellet type, the holes under the objects were saturated with the three types of pellet to mask any olfactory cueing. In addition to this measure, each rat was tested at the end of the experiment for olfactory cueing by placing the wrong pellet under the object and testing choice behavior. The rats consistently chose on the basis of object association as opposed to pellet type. This indicates that the rats can use only the visual characteristics of the objects.

Microdrive Implantation Surgery: A microdrive consisting of 3-4 tetrodes of 17μm platinum iridium wire were surgically implanted using stereotaxic procedures

(from bregma A/P: -4.0, M/L: +2.2mm, D/V: -2.2mm) and lowered into the granule cell layer of the dentate gyrus (D/V: ~2.7mm) until the appearance of place cell single units, "dentate spikes," and complex high frequency (16-90Hz) local field potential activity.

Neural Recordings: During targeting of the dentate gyrus granule cell layer, each wire of the microelectrode bundle was first checked for neural activity. The quietest wire was found and served as a reference electrode. If no activity was found on the recording wires the bundle was advanced in 10μm increments until activity was found, or until a total of 80μm was traversed. Wires were not turned within 24 hours prior to a recording session.

The activity on each wire was passed through a high-impedance OpAmp headstage that held a set of light-emitting diodes (Neuralynx Technologies, Bozeman, MT). A multiwire flexible cable connected the preamplifiers to a 32-channel commutator (Neuralynx Technologies, Bozeman, MT). The single-unit signal was filtered at 600 Hz (low pass)-6000 Hz (high pass), and amplified through Neuralynx Lynx-8 differential programmable amplifiers. The local field potential signal was filtered at 1Hz (low pass)-475 Hz (high pass). The amplifiers were integrated with the Cheetah data acquisition program (Neuralynx Technologies, Bozeman, MT), wherein the acquired analog signals were digitally converted at a rate of 32KHz (DT 2821 Data Translation, Marlboro, MA) prior to storage. The position of the rat was monitored by a set of light-emitting diodes placed on the headstage. A video tracking system (SA-2 Dragon Tracker,

Boulder, CO) registered the position of the diodes with a sampling frequency of 30 Hz. All recording sessions began with 5 minutes of baseline recording activity in a home cage environment. Single cells were identified using Offline Sorter (*Plexon*, Dallas, TX) to compare relative amplitude of each spike across tetrode wires. Final microdrive wire location was verified post-mortem in 40um sections using a Nissl stain.

Analysis of Theta and Beta Amplitude Surrounding Conditioned Location/Object Encounters: To isolate theta and beta amplitudes surrounding food encounter on the circular track, local field potential activity was filtered between 5-10Hz (theta) and 15-30Hz (beta), respectively. The filtered signal was then rectified and filtered again using a .1-1Hz filter to examine large changes in amplitude over many seconds. The average signal during isolated intervals 6 seconds surrounding food encounters were then compared for every recording day for all laps. To further characterize changes in theta and beta amplitude over the course of the experiment, this same analysis was performed for the first three laps and last three laps only for each conditioned reward location on the circular track.

To isolate theta and beta amplitudes surrounding food encounter on the cheeseboard arena, the same analysis described above was performed for 6 seconds surrounding the start of weigh dish encounters. The mean theta and mean beta amplitudes of all dish encounters were calculated for every recording session. Similarly, the same analysis described above was performed for 6 seconds surrounding initial object encounter for each of the three object types in

the object instrumental learning task (best, good, and bad objects). The mean

theta and mean beta amplitudes of all trials were calculated for each object type

for a given recording session. For each of the three tasks, mean theta and beta

amplitudes at 2s prior to location/object encounters were compared to mean

theta and beta amplitudes at the time of encounter using a student's t-test.

Analysis of Theta and Beta Amplitude Surrounding Random Stops: To further

characterize the behavioral significance of changes in theta and beta amplitude

at conditioned food and object encounters in these experiments, theta and beta

amplitudes during intervals surrounding random stops in the circular track task

were examined. If the observed changes in theta and beta amplitude due to

conditioned location and object encounters is meaningful to the learning of

conditioned associations and not due to simple stopping behavior, then an

analysis of theta and beta rhythms surrounding the random stops should yield

random amplitude fluctuation of either frequency. Random stops on the circular

track were defined as stops made at least 30 inches away from the conditioned

reward location for durations of at least 3 seconds.

Results:

Circular Track Task: Local field potential recordings were acquired from the

dentate gyrus granule cell layer of 11 rats. These rats successfully learned to

complete full laps around a circular track in order to receive a food reward in a

reliably rewarded location. In recording sessions in which the reward location was

shifted, rats learned to stop at the new conditioned reward locations for food. In

recording sessions in which there was only a single conditioned reward location, theta amplitude was significantly reduced at the reward site compared to 2s prior to stopping at the reward location (Figure 3.1A, Figure 3.1B (*top*), t-test p=2.0x10^{-5}). Additionally, beta frequency oscillations showed a significant increase in amplitude (t-test p=4.4x10^{-5}). During the first three laps of circular track running, these changes were already present in theta but not beta frequencies (Figure 3.1B (*middle*), t-test p=2.0x10^{-4} for theta, and t-test p=0.08 for beta). In contrast, for the last three laps of track running, large decreases in theta amplitude and large increases in beta amplitude were observed (Figure 3.1B (*bottom*) t-test p=8.6x10^{-5}, t-test p=1.0x10^{-3}, for theta and beta, respectively).

In recording sessions in which there multiple conditioned reward locations, a reduction in theta amplitude and an increase in beta amplitude was observed at each of the conditioned reward locations (Figure 3.1C (*top*), t-test p=7.6x10^{-7} for theta, t-test p=7.1x10^{-4} for beta). Unlike the single reward location condition, this difference was apparent during even the first three laps for each reward location (Figure 3.1C (*middle*), t-test p=1.4x10^{-6} for theta, t-test p=6.4x10^{-4} for beta) as well as the last three laps (Figure 3.1C (*bottom*), t-test p=4.5x10^{-6} for theta, t-test p=1.6x10^{-4} for beta).

Conditioned Cue Reward Task: The same 11 rats used in the previous task were trained on a conditioned cue reward task in order to remove spatial requirements for learning. In this task, rats learned to randomly forage within a large circular arena and approach weigh boats filled with food reward when presented at

random times and at random locations. As spatial features of an environment were unimportant for learning this task, it allowed us to examine the dependence of changing theta and beta amplitudes upon spatial learning. As in the circular track paradigm, theta amplitude was significantly reduced at the weigh boat site compared to 2s prior to stopping at the weigh boat (Figure 3.2, t-test p=1.8x10^{-8}). Beta frequency oscillations also showed a significant increase in amplitude at the weight boat (t-test p=2.0x10^{-3}).

Object Instrumental Learning Task: Two rats that were not utilized in the previously described tasks were trained on the object instrumental learning task. Here the rats learned the associative value of three neutral Lego objects consistently paired with food pellets of three different reinforcement values. This task controlled for odor across reinforcement conditions and contained one negative as well as two positive reinforcers, thus providing a direct test of the role of olfactory input and reward value in modulating theta and beta amplitudes. Both rats learned to reliably approach and push over objects to receive food pellets of different valence underneath. The same theta and beta amplitude changes observed in previous tasks were observed at the rat's initial encounter with objects conditioned to 100% sucrose "best" (Figure 3.3b, t-test p=1.8x10^{-8} for theta, p=4.0x10^{-3} for beta), 25% sucrose "good" (Figure 3.3c, t-test p=3.0x10^{-8} for theta, p=7.0x10^{-3} for beta), and 0.002% quinine "bad" reinforcers (Figure 3.3d, t-test p=1.4x10^{-4} for theta, p=9.1x10^{-3} beta).

Random Stops on the Circular Track: Occasionally during running in the circular track task, rats would stop at random times and random locations. It is possible that the changes observed in theta and beta amplitudes during conditioned reward paradigms are solely due to stopping behavior and that these amplitude changes are not meaningful to learning. An analysis of 6 second intervals surrounding random stopping behaviors revealed not only a lack of decrease in theta amplitude at the time of the stop, but also a lack of increase in beta amplitude. Importantly, the observed changes in theta and beta amplitude during task behaviors were not seen during random stops (Figure 3.4, t-test p=0.42 for theta, and p=0.42 for beta).

Discussion:

In all of the tested learning tasks in this study, theta (4-12Hz) oscillations demonstrated a decrease in amplitude at time points when rats encountered conditioned locations and objects. As theta has been shown previously to be a dominant rhythm during sniffing behavior and olfactory learning, this pronounced decrease was particularly interesting in the tasks that likely contained sniffing and odor cues. This may suggest that the decrease in theta amplitude during the tasks was potentially the result of other prominent inputs that were active during this critical phase of reinforcement learning. Importantly, the only behavior tested that did not elicit a decrease in theta amplitude was random stopping behavior on the circular track, which was the only analyzed behavior not tied to the association epoch of a learning task. Indeed, these findings are also consistent

with previously reported decreases in theta amplitude at conditioned reward locations but not unrewarded locations [13]. However, since theta amplitude decreases were observed when the rat encountered objects conditioned to reinforcers of negative value, this study further demonstrates that a conditioning component is key to observing this change. Thus, the observed decreases in theta may be necessary or important for reinforcement learning.

In each of the learning tasks, the time frames in which theta amplitude decreases are intervals of known importance for learning. Specifically, in each task, these are epochs in which associations between conditioned stimuli and their reinforcement values must be made: an association window. During this critical time window, the potential contribution the beta frequency range that co-occurs with these large decreases in theta amplitude was tested. Here we found increases in beta (15-30Hz) amplitude in every learning task during these critical learning epochs.

To initially investigate the presence of beta during learning, we examined time intervals surrounding stops at conditioned reward locations during a circular track paradigm. In recording sessions in which there was only one conditioned location, increases in beta amplitude during the first three laps appeared after the rat reached the reward location. In contrast, increases in beta amplitude during the last three laps occurred earlier and was significantly greater at the initial stopping point. Given the persistence of these increases in beta amplitude throughout training sessions, this change is not due to novelty as has been

shown in previous *in vivo* electrophysiological recordings in mouse CA3 [14]

Importantly, in recording sessions in which there were multiple conditioned

locations, this increase in beta amplitude demonstrated flexible relocation to each

rewarded location. Interestingly, in the multiple location condition, increases in

beta amplitude were observed during even the first three laps of training,

suggesting that training at the first conditioned location of a recording session

was distinct from training in subsequent shifts of the reward location. Rats may

be maximally attentive to the food itself during the first three laps of a training

session rather than to the regularity of the associated location. Increases in beta

amplitude may thus require the recruitment of systems, perhaps sensory or

attentional, that are dependent upon initial orientation to the task at the beginning

of a recording session.

In recordings obtained from the circular track, these increases in beta

amplitude were observed during behaviors in which spatial, odor, and reward

features of the task are all likely present and necessary for learning. To

disambiguate the contributions of each of these dimensions to the observed

changes in beta amplitude, the remaining behaviors systematically removed or

isolated different combinations of these features. For example, in the conditioned

cue paradigm, an increase in beta amplitude was also observed when rats

encountered the weigh boat that had been tied to a reward. As associations with

spatial features of an environment were not necessary for learning in the cued

task, the observed increases in beta amplitude were not specific to spatial learning tasks.

In the object instrumental learning task, increases in beta amplitude were observed at each object, including objects conditioned to negative reinforcement. The increase in beta amplitude to conditioned objects of negative value indicates that, similar to the observed decrease in theta amplitude, beta amplitude changes were not limited to associations based on positive reinforcers. Moreover, as the increase in beta amplitude began at the point in which rats first encountered the objects, and lasted through the point at which the rat obtained (or refused) the reinforcer (the association window), it is likely that this was not simply a signal for the conditioned stimulus or unconditioned reinforcer, but instead acted in the service of associative learning. As spatial and olfactory cues were not necessary for learning in this task, these results further indicate that increases in beta amplitude are not restricted to spatial or olfactory learning, but rather generalize to a variety of conditions in which a cue must be associated with a reinforcer. Lastly, random stopping behavior did not elicit any change in beta amplitude, suggesting again that the observed changes are contingent upon reinforcement learning or learned relevance.

Oscillatory activity can provide an organizing principle across neural structures [1]. The observed reduction in theta amplitude may be permissive of a faster oscillatory rhythm that is better suited to association-formation and information transfer. Indeed, a large number of brain structures are involved in

associative learning, and long range synchrony of activity across these structures is likely required in order to achieve coordination of inputs. Notably, beta rhythmic activity during the same object instrumental learning task has been observed in the SI/Nbm region of basal forebrain, and in the multiple brain structures during similar associative learning tasks in monkeys and humans [15-18]. The beta rhythm has been hypothesized to be a facilitator of long-range coordination [19,20]. Importantly, it may be involved in linking multiple sensory and affective features during learning, but is not restricted only to spatial, olfactory, or reward dimensions. It instead allows for an associative window in the dentate gyrus of the hippocampus.

Acknowledgements:

Chapter 3, in full, is a reprint of the material as it appears in the following manuscript being prepared fro submission: Quinn, L. K., Rangel, L.M., Chiba, A.A., *Theta and Beta Oscillatory Dynamics in the Dentate Gyrus of the Hippocampus During Conditioning*. The dissertation/thesis author was the primary investigator and author of this paper.

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Figure 3.1: Decreases in Theta (4-12Hz) amplitude and Increases in Beta (15-30Hz) Amplitude in Response to Conditioned Reinforcement in a Circular Track Paradigm. (A) Raw EEG trace as the rat approaches at stops at the reward location. A dashed red line indicates a stop at the reward location. Mean theta (*red*) and mean beta (*blue*) amplitude (mV) for recording sessions in which there was one (B) or multiple (C) conditioned reward locations. The zero point marks the rat encounter with a conditioned reward location. Significant decreases in theta amplitude (*red*) are observed in the mean across all laps (B and C, *top*) and in the mean of the first three (B and C, *middle*) and last three (B and C, *bottom*) laps for both recordings sessions in which there were single and multiple reward locations. Significant increases in beta (*blue*) are observed in the mean across all laps (B and C, top) and the mean of the last three laps (B and C, *bottom*) for both recordings sessions in which there were single and multiple reward locations. No significant difference in beta amplitude was observed during the first three laps (B, *middle*) of single reward location recording sessions. In contrast, the first three laps (C, *middle*) of multiple reward locations recording sessions contained a significant increase in mean beta amplitude.

A

B
Single Reward Location
All Laps
First Three Laps
Last Three Laps

C
Multiple Reward Locations
All Laps
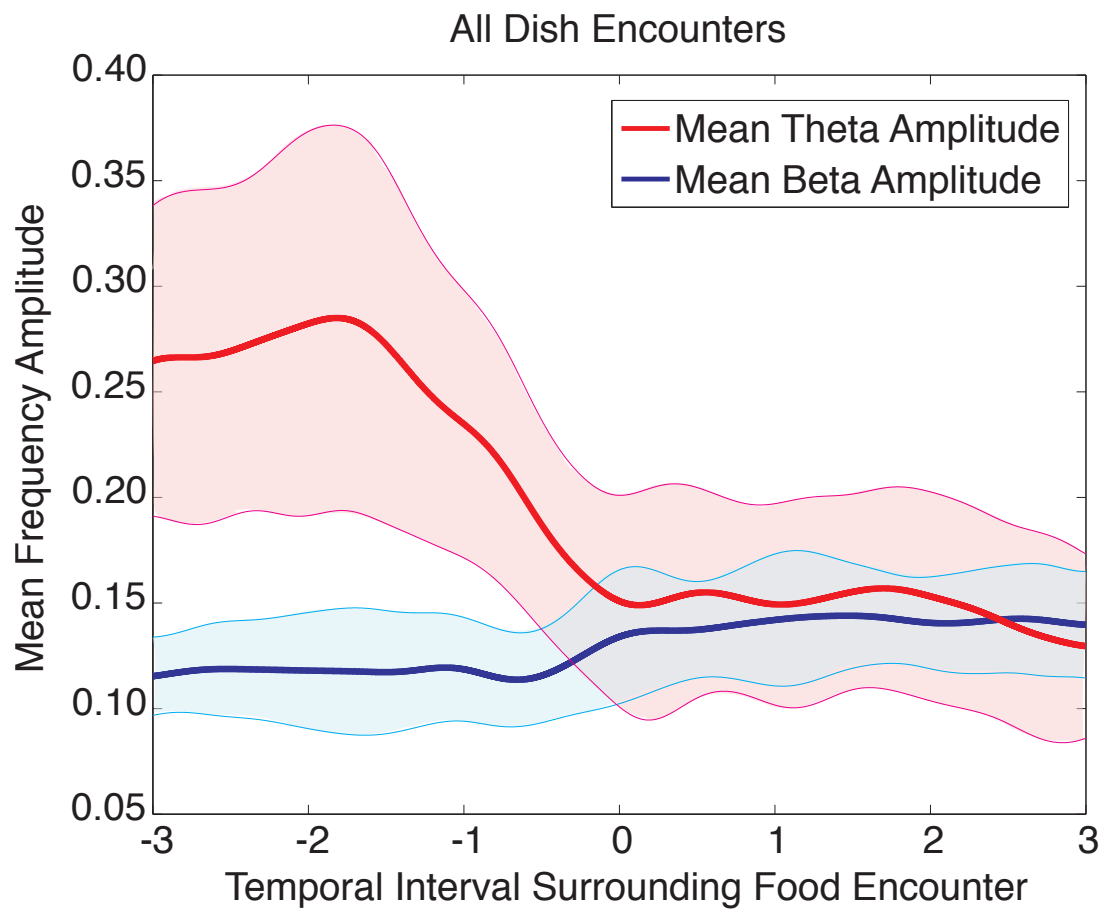#### First Three Laps
Last Three Laps
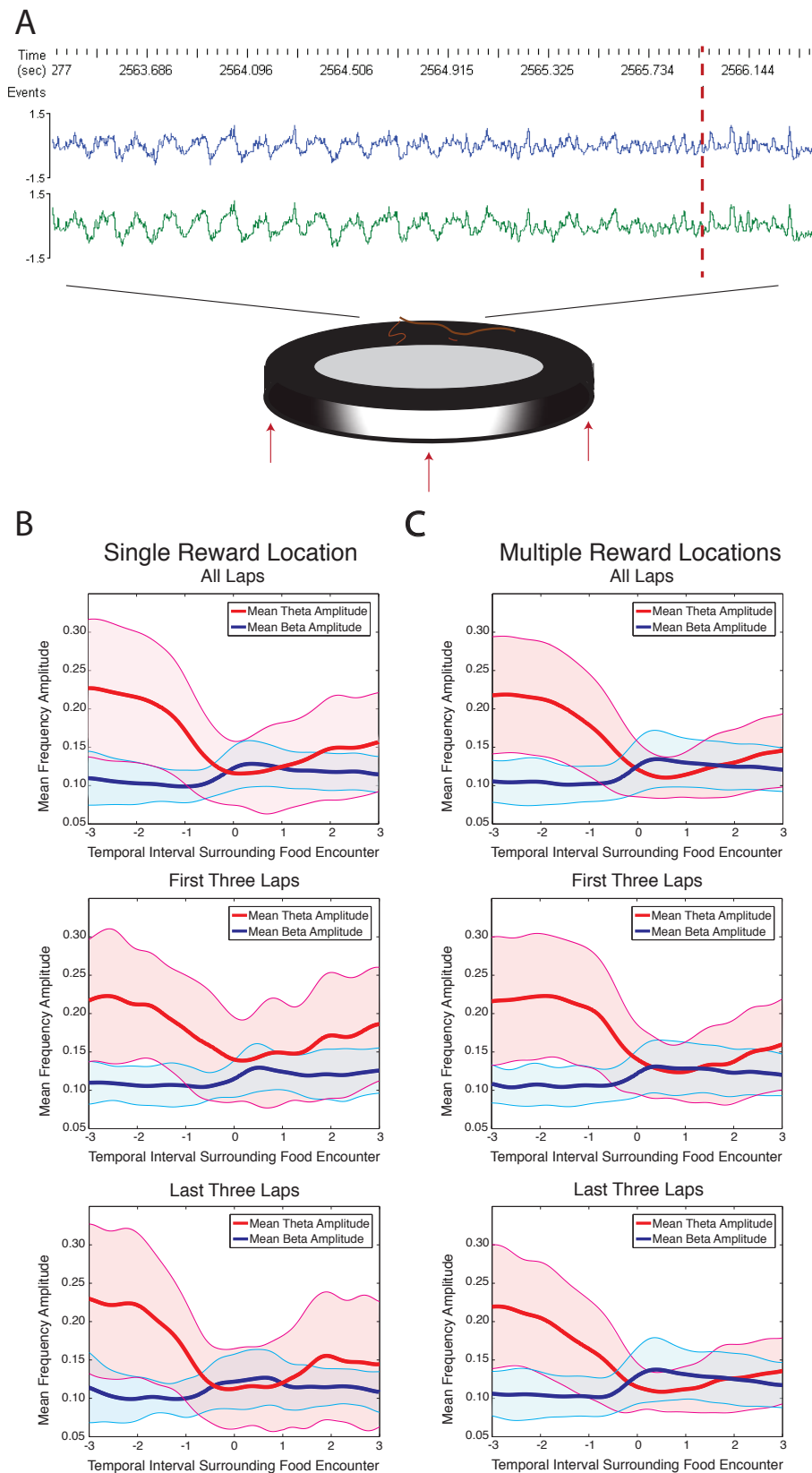
Figure 3.2: Conditioned Cue Paradigm Elicits Similar Changes in Theta and Beta Amplitude. Mean theta (*red*) and mean beta (*blue*) amplitude (mV) as the rat encounters the conditioned stimulus (weigh boat) at the zero time point.

Figure 3.3: Changes in Theta and Beta Amplitude during Encounters with Each Object in an Instrumental Learning Task. (A) Cartoon schematic of the task in which rats were trained to approach and push over three Lego objects in order to receive a 100%, (best), 25% (good), or 0.002% quinine (bad) pellet underneath. Each object was conditioned to one of the three pellet types. Mean theta (*red*) and mean beta (*blue*) amplitude (mV) for encounters with the best (B), good (C), and bad objects (D).
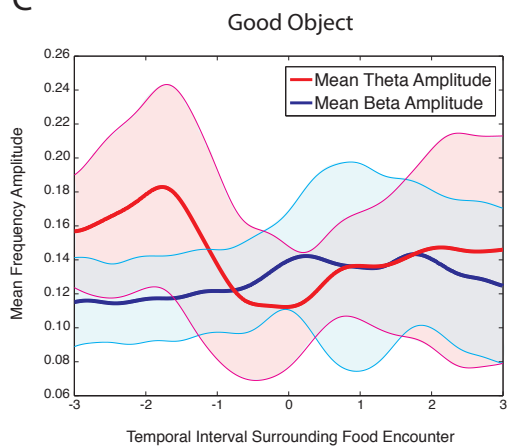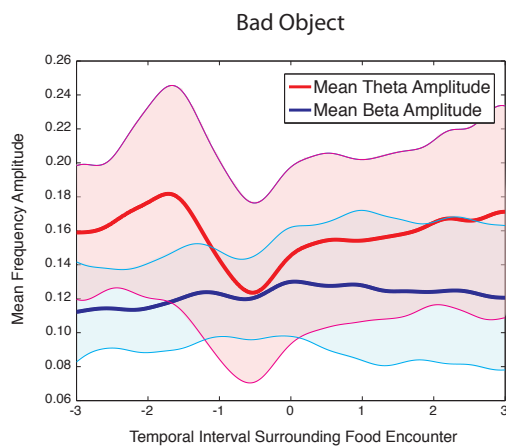
A

B

Best Object
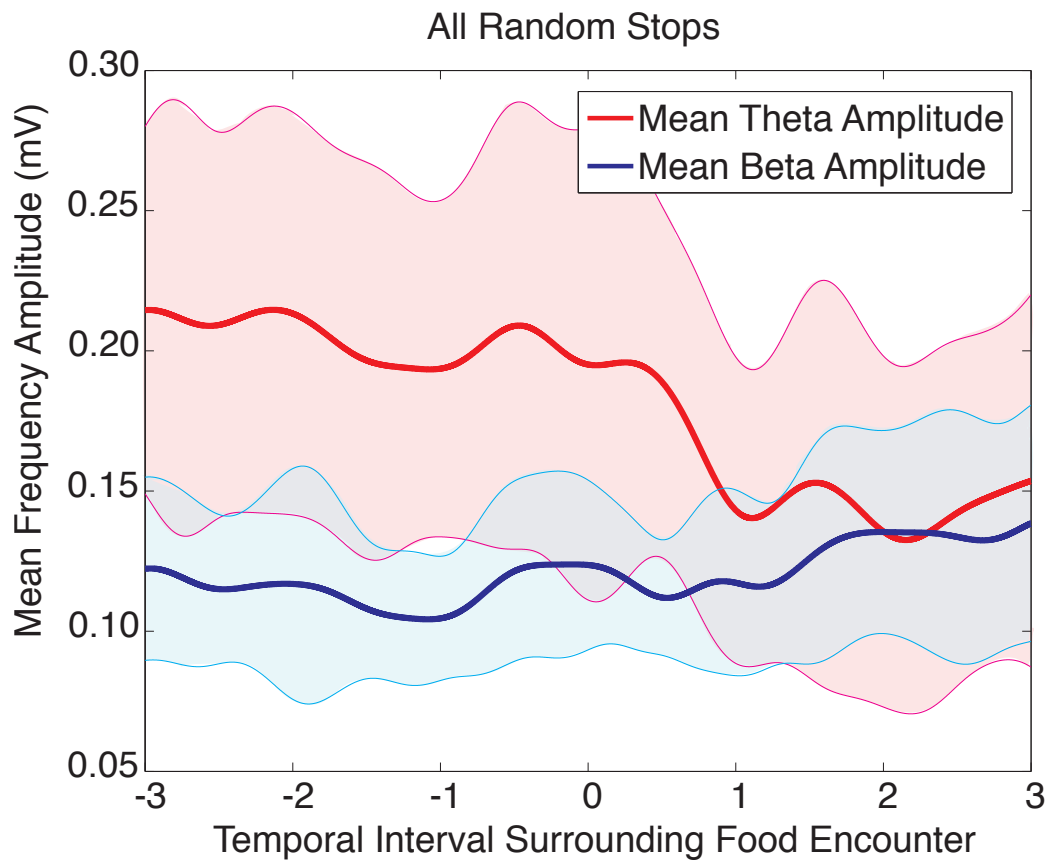
C

Good Object

D

Bad Object
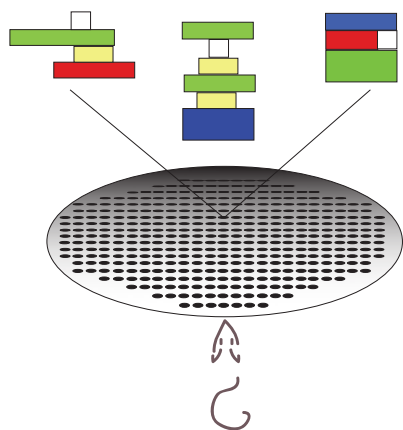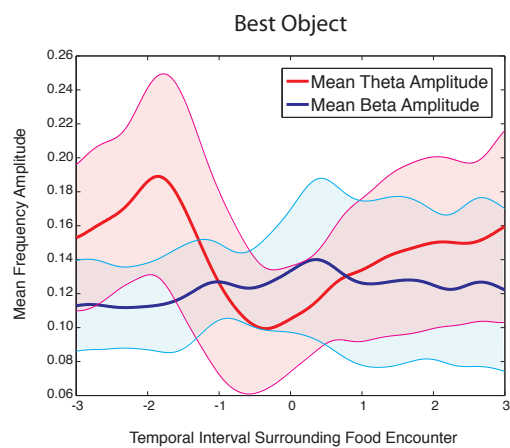
Figure 3.4: Random Stops on the Circular Track do not Elicit Changes in Theta and Beta Amplitude. Mean theta (*red*) and mean beta (*blue*) amplitude (mV) during 6-second intervals surrounding stops longer than 3 seconds. The zero point indicates the onset of a stop.

Figure S3.1: Single units in the dentate gyrus account for movement of conditioned reward locations in their firing rate activity. While there were few cells with activity near the conditioned reward locations on the circular track, sixteen cells were identified that exhibited 1-2 place fields during recording sessions in which there were multiple reward locations. Six of the sixteen cells maintained stable place fields in the presence of shifting reward locations (A). X-axis indicates position along the circular track in radians and y-axis indicates spatial information score for each 5cmx5cm pixel according to the following formula calculated for 30Hz time step: calculated for a 30Hz time step:
P_k is the probability of observing k spikes.
$P_{k|xi}$ is the conditional probability of observing k spikes in pixel x_i.

$$I_{pos}(x_i) \equiv \sum_{K \geq 0} P_{k|x_i} \log\left(\frac{P_{k|x_i}}{P_k}\right)$$

Single lines represent the spatial information score for each pixel when the conditioned reward site was located at the circle position of corresponding color. Five of the sixteen cells had place fields with a center of mass shift in the direction of new conditioned reward sites (B). Four of the sixteen cells had transient fields that appeared during training to only one conditioned reward location (C). One remaining cell (not shown) appeared to shift a distance proportional to the shift in conditioned reward location.
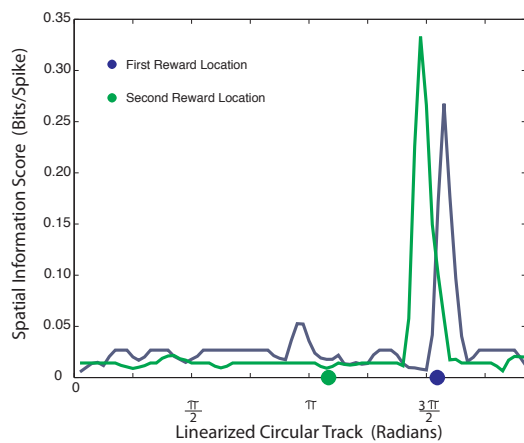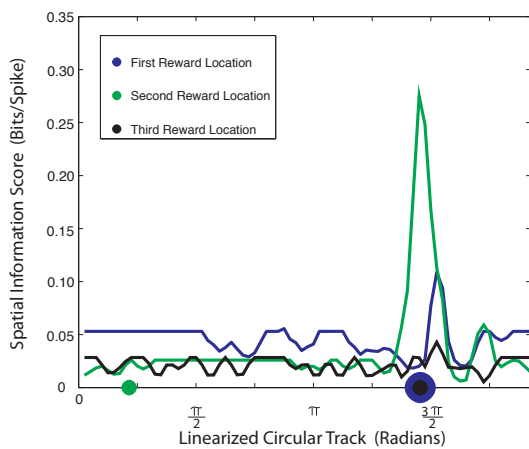
A   Stable
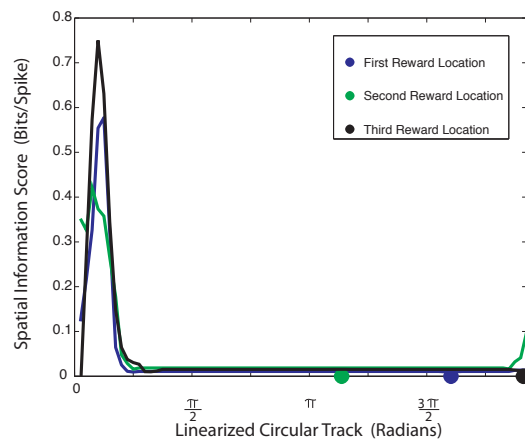
B   Shift Towards

C   Disappear

CHAPTER 4: Hippocampal Contributions to the Creation of Spatial Knowledge

States

One of the overarching goals of studying the role of the hippocampus in

learning and memory is to understand how associations made within this

structure contribute to knowledge. It is often difficult to determine how

hippocampal activity at the single cell level contributes to knowledge states as

complex as spatial cognition. Yet the discovery of place cells in some of the

earliest *in vivo* electrophysiological recordings in the hippocampus is a large

driving force in current hippocampal theory. This chapter reviews a history of

experimental evidence supporting the role of the hippocampus in spatial

knowledge according to early and modern theoretical mechanisms. In order to

realistically assess the extent to which the hippocampus is capable of creating or

embodying specific knowledge states, three specific definitions of spatial

knowledge are utilized.

A Map for Our Utility:

A cognitive map, as described by O'Keefe and Nadel in their foundational

1978 paper, arises from an innate knowledge of space [1]. Spatial cognition in this

case refers to a perception of the external world that is readily available and

usable in every organism. The process of cognitive mapping, theoretically

achieved by the hippocampus, can be described as:

> ... a construct that encompasses those processes that enable people to acquire, code, store, recall, and manipulate information about the nature of their spatial environment. It refers to the attributes and relative locations of people and objects in the environment, and is an essential component in the adaptive process of spatial decision-making-such as finding a safe and quick route to and from work, locating potential sites for a new house or business, and deciding where to travel on a vacation trip. Cognitive processes are not constant, but undergo change with age or development and use or learning [2].

If spatial cognition is a cognitive map for our utility that is an innate ability in all organisms, then spatial information should be available in the hippocampus during novel exposures to spatial environments and prior to any learning. Indeed, single cells in the hippocampus demonstrate spatially specific activity in the form of *place fields* during even the first few minutes of novel exposures to an environment [3]. These cells additionally demonstrate large coverage of spatial environments at predictable spatial resolution along the septo-temporal axis [4]. The hippocampus thus has the means to provide a spatial construct at the single cell level.

Place cell activity over the course of familiarity with a new environment is additionally reflective of increasing perception of space. A large body of experimental evidence suggests that stable place fields are highly contingent upon experience. Although these cells demonstrate place specific activity immediately, they remain unstable and flexible during initial encounters with an environment before demonstrating stable fields [5-8]. As further indication that the activity of these cells is linked to spatial perception, spatially specific activity of

these cells is closely associated with animal movement and perceived location, rather than absolute allocentric location. Specifically, the firing rate of place cells as an animal travels through its place field can be heavily modulated by speed, direction, and trajectory [9,10]. Moreover, rotations of spatial cues surrounding an environment cause predictable shifts in place field location relative to the degree of cue rotation [11,12]. Taken together, these finding suggest that place cells not only provide an internal representation of a spatial environment, but also encode these features in a behaviorally meaningful manner.

In order to contribute to spatial learning and memory, place cells must additionally be able to account for spatial changes of an environment. Experimental evidence has demonstrated that even cells with stable place fields can demonstrate changes in firing rate or location when fields are in close proximity to changing components of an environment [11,13]. Moreover, their long-term stability has been correlated with spatial learning performance, and their instability in aging is correlated with a decline in spatial learning ability [7,14]. Thus, in addition to providing a flexible spatial representation of an environment in a way that is behaviorally meaningful to an organism, these place cells may be utilized and required for learning.

Knowledge of a Map: Declarative Learning and Memory:

More recent theories of hippocampal contributions to spatial knowledge have emphasized that the hippocampus only plays a role in spatial cognition

when conscious spatial associations must be made. This type of knowledge has been referred to as declarative memory [15,16]. Specifically, lesions of the hippocampus result in a long-term inability to encode episodic memories, or the conscious knowledge of specific personal experience [17-19]. To the cognitive map definition of spatial knowledge, this requirement adds that an organism must have conscious knowledge of the map's appropriate utility.

In support of this new definition, rats and humans with lesions or inactivation of the hippocampus maintain an ability to navigate novel environments and perform spatial learning tasks over time. In rats, lesions of the hippocampus impair performance in spatial learning tasks [20-22]. Over a significantly longer period of time, however, successful performance in these tasks can be achieved, suggesting secondary mechanisms for forming semantic, or factual, spatial associations [23]. This is consistent with other studies demonstrating that the behavior of rats with hippocampal lesions reflects maintained perceptual learning of their spatial environment [26]. Moreover, rats with fimbria/fornix lesions that demonstrate spatial learning impairment, still exhibit the presence of place cell activity, suggesting that place cells alone are insufficient for spatial knowledge [24]. In clinical research, humans with lesions of the hippocampus additionally have no impairment in spatial perception and demonstrate an ability to navigate their current living environments [25]. These results suggest that in the absence of a hippocampus, an internal spatial map is available for use.

Yet, it is clear that humans and animals without a hippocampus lack conscious knowledge of their spatial environment or any explicit perception of changing environmental conditions. Whereas in the rat it is difficult to claim the presence of conscious spatial knowledge, previous research has used the ability to generate decisions (i.e. "declare") based on appropriate knowledge of spatial learning contingencies as evidence of declarative memory [27]. Even though rats with hippocampal lesions can perform spatial learning tasks over long periods of time, they demonstrate inflexibility in their ability to adapt to changing spatial conditions [28]. This is in high contrast to control rats, who instead demonstrate faster learning and adaptation to changing environmental conditions with increased experience [29]. Humans with hippocampal lesions demonstrate a similar inflexibility in being able to update their perception of changing environmental conditions. This is coupled by an inability to consciously recall the utility of their current spatial surroundings, suggesting a lack of knowledge for the appropriate use of any acquired semantic spatial associations [25]. Thus, in both rat and human examples the ability to assess the appropriate utility of a map is impoverished without a hippocampus.

Thus, although a cognitive map may be available for use in other areas of the brain in the absence of a hippocampus, the hippocampus appears to be essential for the conscious knowledge of this map. Indeed, cells in other areas of the brain demonstrate place specific firing in a manner analogous to place cells in

the hippocampus [30], and may account for the maintenance of semantic spatial memory encoding following hippocampal lesion.

Multi-dimensional Contributions to Spatial Knowledge: Contextual Learning and Memory:

In the introduction to this dissertation, it was suggested that space is so integrally linked with other dimensions that it would be difficult and potentially unmeaningful to examine it as encoded separately in the brain. Current hippocampal theory similarly suggests that the hippocampus creates relationships between important features across experiences and is thus essential for both spatial and non-spatial memory, or relational memory [31]. Here, consistent with the idea that spatial knowledge does not exist in isolation from related knowledge states, the definition of spatial knowledge is updated to include the relationships between space and other dimensions that together compose rich contextual knowledge.

At the single cell level, several studies have reported hippocampal neural activity associated with non-spatial stimuli and behaviors. Specifically, cells have demonstrated activity that is selective to time intervals, objects, salient locations, and the start and end of training trials [13,32-35]. In the latter case, the activity of hippocampal cells is reminiscent of marking the beginning and end of episodes. This is consistent with human experiments that have shown that hippocampal lesions result in an inability to encode and remember non-spatial as well as

spatial associations and, moreover, an inability to form any subsequent episodic memories. Thus, the role of the hippocampus in learning and memory is not limited solely to the spatial dimension.

Yet if spatial knowledge requires an understanding of space within the context of other dimensions, the deficits described above would be expected. Conscious knowledge of where you are is integrally linked to knowing when and why you are there. Regarding the other dimensions described in this dissertation, mechanisms of acquiring temporal and affective information in the absence of the hippocampus are additionally available through circadian and reward systems. The relationship between these dimensions in the formation of new episodic memories, however, is critically impaired without a hippocampus. Thus without an understanding of the temporal and affective context of spatial information, this definition of spatial knowledge cannot be achieved.

The primary goals of the experiments in this dissertation were to 1) demonstrate the existence of multiple non-spatial dimensions in the hippocampus and 2) provide evidence for potential mechanisms by which spatial and non-spatial dimensions are associated to create rich contextual memories. To this end, it was shown that single cells, and even place cells in the dentate gyrus of the hippocampus, demonstrate activity that is temporally dependent. These cells support the integration of both spatial and temporal information through activity that is selective to both space and time, thus revealing the relationship between these dimensions by encoding experiences as distinct events in *spacetime*. A

second experiment additionally demonstrated the presence of large-scale synchronous activity in the beta (15-30Hz) frequency range in response to conditioned reinforcement that was not specific to spatial or olfactory learning tasks. This activity not only demonstrates the presence of robust affective inputs into the dentate gyrus, but also suggests a mechanism by with dimensions are associated and converged in the hippocampus through long-range synchrony of input across structures. Thus the studies in this dissertation demonstrate that the dentate gyrus can account for information present not just in one dimension, but in at least two dimensions. Can single cells in the dentate gyrus account for spatial, temporal, and affective information? The proposed existence of *affective spacetime* in the dentate gyrus of the hippocampus would postulate that these mechanisms work together to create multi-dimensional constructs of rich contextual experiences in the dentate gyrus that ultimately inform knowledge.

In history, scientific theories have been shown to oscillate from complex to simple as we find new avenues of complexity in our discoveries and simplicity in the underlying principles that govern them. The work in this dissertation suggests that the role of the hippocampus in memory formation is more complex than the encoding of relationships along a single spatial dimension. This chapter additionally redefines theories of hippocampal function to propose that the hippocampus instead encodes relationships across multiple dimensions for the purpose of complex memory formation. In contrast, the proposed mechanism of multi-dimensional encoding in the hippocampus provides a simple and elegant

solution for single cells to associate and account for information across

dimensions in its activity. This dissertation thus takes a fitting place in scientific

history through its contribution to an evolving theory of hippocampal function.

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