Spatial novelty detection and consolidation mechanisms in CA1 and CA2 in a dynamically changing environment.

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GUNCHA BHASIN

National Brain Research Centre,
(Deemed University)
Manesar, Gurugram
Haryana-122052, India.

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ट्रीय मस्तिष्क अनुसंधान केंद्र
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न० एच०–08, मानेसर-122 052 (हरियाणा), भारत

National Brain Research Centre
NBRC

National Brain Research Centr
(Deemed University)
(An Autonomous Institute of Deptt. of Biotechnology,
Ministry of Science & Technology, Govt. of India)
NH-08, Manesar - 122 052 (Haryana), INDIA

CERTIFICATE

This is to certify that the thesis entitled **"Spatial novelty detection and consolidation mechanisms in CA1 and CA2 in a dynamically changing environment"** is the result of work carried out by **Guncha Bhasin**, in the **Division of Systems and Behavior Neuroscience** at National Brain Research Centre, Manesar, Haryana, India, towards the requirements for Integrated-Ph.D. degree.

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Dr. Arpan Banerjee
SUPERVISOR
NBRC

Prof. Pravat Mandal
DIRECTOR IN CHARGE
NBRC

PLACE: Manesar

DATE: 12.10.2021

दूरभाष / Telephone : +91-124-2845200 फैक्स / Fax : +91-124-2338910
ई-मेल / E-mail : info@nbrc.ac.in Website : www.nbrc.ac.in

DECLARATION

I, **Guncha Bhasin**, hereby declare that the work presented in the form of this thesis entitled **"Spatial novelty detection and consolidation mechanisms in CA1 and CA2 in a dynamically changing environment"** was carried out by me under the guidance of Dr. Arpan Banerjee, Additional Professor/ Scientist V, **National Brain Research Centre (Deemed University)**, Manesar, Haryana, India.

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Guncha Bhasin

Guncha Bhasin

Place: Manesar

Date: 12/10/2021

DEDICATED TO

MY PARENTS

Without their love, support and patience, none of this would have been possible

A journey of a 1000 miles begins with a single step.

-Lao Tzu

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Being an agnostic, I do not believe in the entity of “God” but I do believe in a higher power that resides within and above us all, so I would like to start by expressing my gratitude towards that power.

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Somewhere along this journey, I lost my grandparents, who were my biggest security blanket and source of strength. I miss them dearly, but I know they are still showering me with blessings from beyond.

CONTENTS

LIST OF ABBREVIATIONS:

MTL	medial temporal lobe
LFP	local field potential
SWR	sharp wave ripple
REM sleep	rapid eye movement
nREM sleep	non rapid eye movement
PoS	post subiculum
SuM	supra mammillary nucleus
LDN	lateral dorsal thalamus
ADN	anterior dorsal thalamus
CA	cornu ammonis
DG	dentate gyrus
EC	entorhinal cortex
LEC	lateral entorhinal cortex
MEC	medial entorhinal cortex
LIA	large irregular activity
HD	head direction
EEG	electroencephalogram
STDP	spike-timing-dependent plasticity
PVN	paraventricular nuclei
SO	stratum oriens
SP	stratum pyramidale
SR	stratum radiatum
SLM	stratum lacunosum moleculare
EPSP	excitatory postsynaptic potential
IPSP	inhibitory postsynaptic potential
CB	cannabinoid

PV	parvalbumin
OLM	oriens-lacunosum/molecular
CCK	cholecystokinin
HCN	hyperpolarization-activated cyclic nucleotide
LTP	long term potentiation
LTD	long term depression
TREK	two pore potassium channel
A1 receptor	adenosine receptor
Avpr1b	vasopressin 1b receptor
NMDA	N-Methyl-D-aspartic acid
ERK	extracellular regulated kinase
MAPK	mitogen-activated protein kinase
RGS-14	regulator of G-protein – 14
KO	knock-out
A1Rs	adenosine 1 receptor
cAMP	cyclic adenosine monophosphate
V1b	vasopressin 1b receptor
AVP	arginine vasopressin
FGF2	fibroblast growth factor 2
EGFR	epidermal growth factor receptor
PCP4	purkinje cell protein 4
NTF3	neurotrophin 3
CACNG5	calcium voltage-gated channel auxiliary subunit gamma 5
TARP	transmembrane AMPA receptor regulatory protein
AD	alzheimer's disease
MCI	mild cognitive impairment

SC	schaffer collateral pathway
NMDAR	N-methyl-D-aspartate receptor
EIB	electrode interface board
HS-27	head stage-27
CCD	charged couple device
TIFF	tag image file format
Fam	familiar
PCA	principle component analysis

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3. SPATIAL NOVELTY DETECTION IN CA1 AND CA2:

SYNOPSIS:

Spatial novelty detection and consolidation mechanisms in CA1 and CA2 in a dynamically changing environment

The spatial component of episodic memory i.e. spatial memory is encoded as a "cognitive map" of the external environment which may be used for efficient navigation, orientation, direction and successful interpretation of external sensory cues. This ability is one of the most fundamental cognitive properties of mammals for survival, as it helps form a spatial relation between an organism and its environment. The hippocampus, has been herald as the locus of a cognitive map in the brain, as was first proposed by Tolman in 1948 and subsequently established by several studies (O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978). Hippocampal place cells are the functional units of spatial navigation and are present in all subregions- CA1, CA2, CA3 and CA4.

In the past decade or so, CA2 has been acknowledged as an important part of the hippocampal circuitry, rendering it much more than a mere 'transition zone' between CA3 and CA1. Its unique connections and striking differences in its biophysical and synaptic properties regarding synaptic plasticity (Zhao *et al.*, 2007) and resistance to LTP (Chevaleyre & Siegelbaum, 2010), along with different morphological characteristics and patterns of gene and protein expression. make it a very important and strategic region, poised to modulate both CA3 and CA1 firing as well as their synchrony with theta rhythm, thereby heavily influencing place cell firing and spatial navigation mechanisms within the hippocampus.

Moreover, CA2 receives direct input from the supramammillary nucleus (Haglund *et al.*, 1984; Berger *et al.*, 2001) and has been shown to be the initiator of SWRs in awake immobile and sleep, acting as a trigger and initiator for sleep replays (Oliva *et al.*, 2020). It also plays a role in coding for current location during immobility and sleep through brief periods of desynchronization in slow wave sleep (SWS) (Kay *et al.*, 2016) Further, the N units/ramping cells in CA2 may help in choosing and selecting which particular experience will be re activated during a subsequent sharp wave ripple in sleep (Kay *et al.*, 2016; Stöber *et al.*, 2020). Therefore, CA2 not only influences other hippocampal sub fields during spatial memory acquisition but also during its subsequent spatial memory consolidation during sleep.

While CA3-CA1 pathway has been extensively studied with respect to spatial navigation and memory, with some even looking at CA3-CA2 place cell topography (Lee *et al.*, 2015; Lu *et al.*, 2015) CA2-CA1 studies are far and few in between (Mankin *et al.*, 2015; Alexander *et al.*, 2016). Many studies have thoroughly established that multiple independent circuitries co-exist within the hippocampus: mainly the classic trisynaptic pathway of EC-CA3-CA1 and the recently discovered disynaptic loop of EC-CA2-CA1(Chevaleyre & Siegelbaum, 2010). These circuitries are activated simultaneously or alternately for different functional optimization; with maximum influence being exerted on the most downstream subfield of the hippocampus, i.e. CA1. Furthermore, axons of CA2 basket cells extend to all 3 CA fields of the hippocampus, providing feedback inhibition to CA3 and feed-forward inhibition to CA1, making it uniquely poised to co-ordinate and control the hippocampal circuitry (Mercer *et al.*, 2007).

My PhD thesis focuses on elucidating and characterizing neurophysiological properties of CA2 neurons and their comparison with CA1 neurons, with respect to

their role and contribution to spatial memory and navigation mechanisms. In particular, the primary goal of the present study is to tease out the specific contributions of CA1 and CA2 towards mnemonic processes such as detection of novel spatial stimuli and it's subsequent consolidation. To meet this end, in vivo multiunit electrophysiological recordings (spike + LFP) from CA1 and CA2 were performed simultaneously in rat hippocampus (*Long evans*) to examine and distinguish the neuronal firing responses in both ensembles with respect to spatial novelty detection and consolidation.

A novel 3-day paradigm, where the rat experienced varying degrees of spatial familiarity and novelty with each successive day, as the track was elongated and geometrically reshaped, was specifically designed for this aim. This paradigm enables the rat to experience complete novelty on the first day and varying degrees of familiarity and novelty on day2 (50% novel, 50% familiar) and 3 (33% novel, 67% familiar) as the track is extended in length each day. Thus, not only does the animal experience varying degrees of novelty across days but also within the same lap on days 2 and 3. The track was elongated from a square track on day 1, to a rectangle on day 2 and to an L-shaped track on day 3.

A dual bundle microdrive, made entirely in the lab, containing 20 tetrodes (9+1 tetrode in each bundle) was implanted in each rat's right hemisphere aimed at CA2 and CA1. Post implantation surgery, the animals were allowed to recover and the tetrodes were gradually lowered each day over a period of 2 weeks till they reached the desired recording area (the hippocampal pyramidal layer). They were also trained to run clockwise on a plain black circular track for chocolate sprinkles in the behaviour room, which was devoid of any sensory cues. The animals were introduced to the

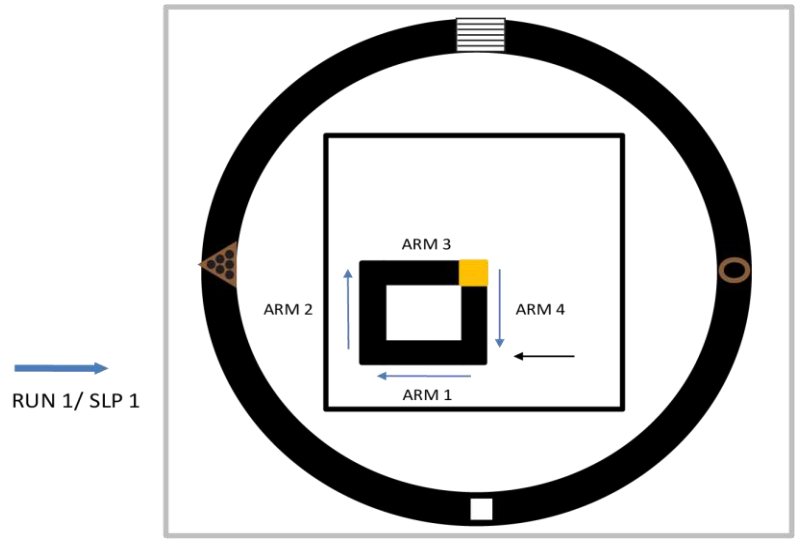
experimental tracks only on the day of the experiment, and the behaviour room now also had 4 distinctly shaped visual cues hung perpendicular to each other.

The 3-day paradigm consisted of the animal running on each particular track for that day for 4 sessions, consisting of 20 clockwise laps each, where it received one chocolate sprinkle on the sandpaper area after each lap. Post behaviour, the rat was allowed to sleep for 3-4 hours, and its spike and LFP were recorded.

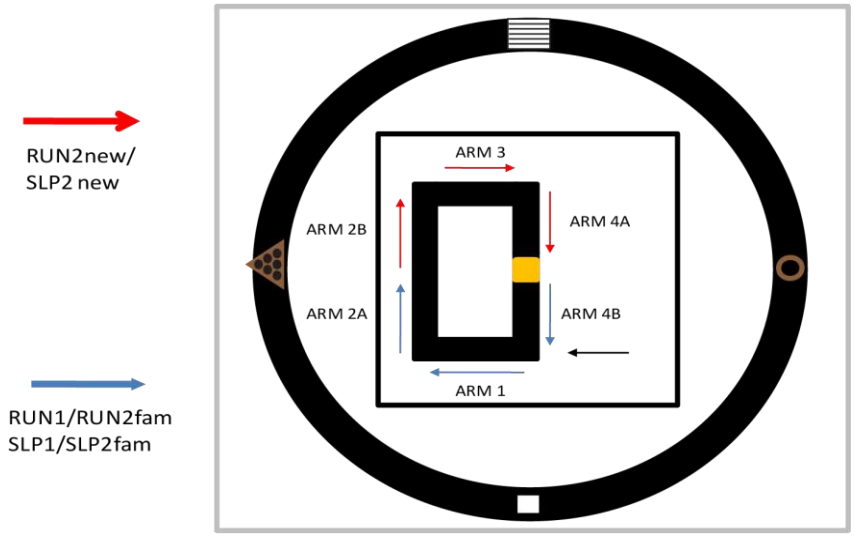
On day 1, although the rat ran in a clockwise fashion on a square track, only place cells from arm1, 2 and 4 were considered for appropriate comparison and analysis. This is termed as RUN1. Since these arms are present on both day 2 (arms1, 2a and 4b) and 3 (arms1, 2a and 6) as well, though numbered differently to maintain arm count continuity each day, they are the only common arms between each track, across all days. They are termed RUN2fam and RUN3fam respectively on day 2 and day 3. Correspondingly, the added/novel arms on day 2 (arms 2b, 3 and 4a) and day 3 (arms 3b, 4 and 5) are termed RUN2new and RUN3new, respectively. All the above mentioned C-shaped, 3-arm tracks: RUN1, RUN2fam, RUN2new, RUN3fam and RUN3new are similar in shape and size and are thus used for comparative analysis with each other. The middle arms (arms 2b and 3a) on day 3 are excluded from the same to maintain track length accuracy for analysis. Therefore, not only can RUN1 be compared across days with RUN2fam and RUN3fam, as this area becomes more and more familiar from being completely novel across days; it can also be compared with RUN2new and RUN3new, since all 3 are novel areas of the track when first introduced in the environment on their respective days. Furthermore, comparison within the same day between familiarity and novelty can also be done on day 2 (RUN2fam v/s RUN2new) and day 3 (RUN3fam v/s RUN3new). The same comparisons have been applied to sleep analysis as well.

A total of 4 implanted rats were used for this study. A total of 427 CA1 and CA2 place cells were recorded on all 3 days of the experiment: 121 cells on the 1st day, 155 cells on the 2nd day and 151 cells on the 3rd day. After excluding cells that fired for the reward area, a total of 105 place cells were recorded on day1, 136 place cells on day 2 and 141 on day 3 (total number: 382 cells). Out of these 382 place cells, 288 were recorded from CA1 and 94 from CA2. Following the RUN session each day, a total of 275 place cells were recorded in SLEEP: 69 place cells on day1, 103 on day 2 and 102 on day 3. After excluding cells that fired for the reward area in sleep, a total of 269 place cells remained: 192 from CA1 and 77 from CA2.

Across days (days1 v/s day2 v/s day3 : RUN1 v/s RUN2fam v/s RUN3fam) and within days comparison (days 2 and 3: novel v/s familiar: RUN2fam v/s RUN2new and RUN3fam v/s RUN3new) between CA1 and CA2 ensemble populations were made with respect to place cell counts, average firing rates and pairwise cross correlations between cell pairs, separately for place cells that fired on the elongated portion of the tracks on day2 and 3 and for the common portion of the track from day1 that was present on all days. Furthermore, spike activity from CA1 and CA2 place cells within sharp wave ripples (SWRs) in nREM sleep during replay events from each day were analyzed to understand how the consolidation of an ever changing track/environment as it gets modulated in shape, form and size occurs across days.



DAY 1



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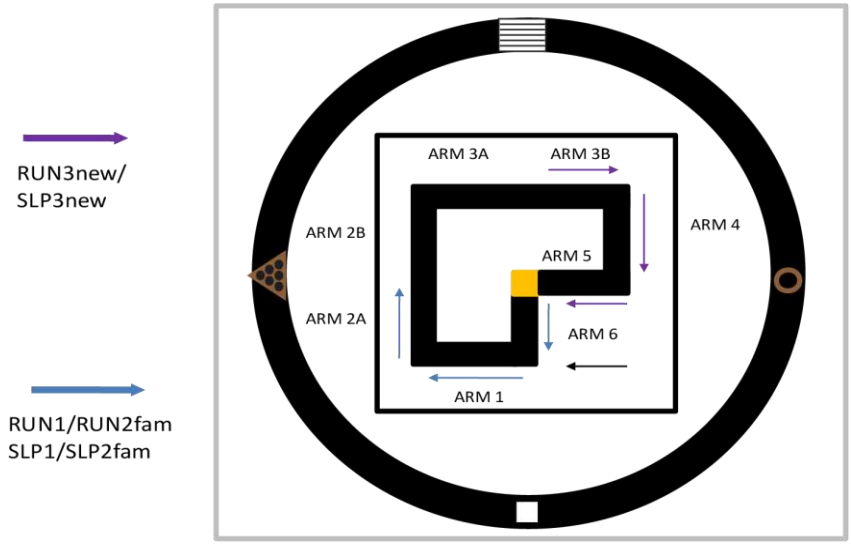


DAY 3

3 DAY NOVEL PARADIGM

It was observed that the first introduction of novelty in a relatively familiar environment (termed early novelty detection) on day2 resulted in a skewed distribution of both CA1 and CA2 place cells, with majority coding for the novel part, but not when novelty was introduced on day 3 (termed subsequent novelty detection). Therefore, as familiarity of the track increased from 0% on day1 to 50% on day2 and 67% on day3, it was correspondingly observed that 0% of place cells fired for familiarity on day 1 (RUN1), 35% of place cells fired for familiar track on day 2 (RUN2fam) and 70% on day 3 (combining RUN3fam, arm 2b, and arm 3a). Similarly, as novelty of the track decreased from 100% novelty on day1, 50% on day2 and 33% on day3, the corresponding % of place cells firing for novel track portion also decreased from 100% on day 1 (RUN1), to 65% on day 2 (RUN2new) to 30% on day 3 (RUN3new). Therefore, only on day 2, a skewed place cell distribution was observed for both CA1 and CA2 ensemble populations, as 65% of the place ells fired for the novel part of the track (50% of total area) while only 35% of place cells fired for the remaining 50% track area (familiar part of the track). This distribution did not extend to day 3, where 30% place cells fired for 33% novel track area while 70% place cells fired for 67% familiar track area.

However, place cells coding for the novel area on both days 2 and 3 from both ensembles had a higher average firing rate and pairwise cross correlation than place cells coding for familiar area (RUN2new>RUN2fam and RUN3new>RUN3fam). In addition, it was also observed that average firing rates and pairwise cross correlations for place cells coding for a particular area decrease as a function of increasing familiarity across days but remain similar and comparable while coding for novelty across days (RUN1>RUN2fam>RUN3fam). This could indicate that novelty detection may be rooted in specific characteristics of novel place cells such as higher

mean firing rate and pairwise cross correlation rather than higher place cells counts each time novelty is introduced in a relatively familiar environment.

Finally, the individual spikes firing in each of the significant replay sequences were classified further based on their peak firing positions on RUN for that particular day, and divided further into SLP1 (corresponding to RUN1), SLP2fam, SLP2new (corresponding to RUN2fam and RUN2new from day2) and SLP3fam and SLP3new (corresponding to RUN3fam and RUN3new from day3). Cumulative spikes fired in sleep sequences from all place cells belonging to either familiar or novel arms of the track on both day 2 and 3 were compared within the corresponding days. Each of the spikes firing within each sleep SWR, were classified as 1.CA1fam 2.CA1new 3.CA2fam and 4.CA2new for days 2 and 3. For day1, spikes were identified on basis of whether they belonged to CA1 or CA2. All the spikes belonging to one category were summed across and their cumulative totals were compared.

For within day comparison on day2, when CA1fam and CA1new were compared across all rats and it was observed that more spikes from place cells belonging to the novel track fired in SWR during sleep compared to their familiar counterparts. Similarly, for day 3, it was observed that a higher number of spikes fired in sleep from familiar part of the track (SLP3fam) across all rats when compared to novel part of the track (SLP3new) Thus, for CA1, on both day 2 and 3, whichever part of the track in RUN had higher number of place cells firing for it, had a higher number of spikes firing in sleep from those place cells belonging to that part of the track. Therefore, a ‘coherent response’ from CA1 place cells was observed in RUN and SLEEP spike firing responses for a particular day

Conversely, the same was not observed for CA2 spikes in sleep. For both day 2 and 3 when the cumulative total for SLP2fam v/s SLP2new and SLP3fam v/s SLP3new were compared, there was no clear, conclusive trend seen for CA2 cells firing in sleep replays, and they showed no preference for any particular part of the track: familiar or novel, irrespective of how many place cells fired for each of those parts in RUN.

Thus, it was observed that while for CA1 spatial coverage in prior behaviour is closely reflected in subsequent sleep for that particular day; the same 'coherent response' is not observed in CA2. Therefore, although CA1 and CA2 showed the same response to novelty detection, they differed in their response to its subsequent consolidation.

Previous studies have shown that both CA3 and CA1 place cells are less stable in novel v/s familiar environments (Leutgeb, 2004), whereby CA1 input is largely dominated by the powerful disyanptic pathway of EC-CA2-CA1, and is only later on taken over by the classic trisynaptic pathway, over days (post 24 hours) to stabilize CA1 place fields (Karlsson & Frank, 2008). Lesion studies of EC-CA1 pathway have impaired spatial coding (Brun *et al.*, 2008), indicating that CA2 directly influences spatial acquisition and learning driven processes in CA1. Further, changes in an environment such as shape (Mankin *et al.*, 2015) affect CA2 place fields the least when compared to CA1 or CA3 place fields. On the other hand, their sensitivity to smaller contextual changes and local cues, suggests that CA2 activity is an indicator of novelty signal to its downstream areas, i.e. CA1. Thus, during the development of novel place fields, the input to CA1 is primarily dominated by CA2, which in itself codes for a novelty signal, and hence may explain why both ensembles show the same characteristic response to both types of novelty detection (early and subsequent).

Many studies have indicated that the competitive, alternate and independent circuitries via CA3 (trisynaptic pathway) and CA2 (disynaptic pathway) to CA1 as well as excitatory projections from CA2 to CA3 (Kohara *et al.*, 2014) modulate the flow of spatial information in the hippocampus depending on different behavioural states (sleep v/s awake). It has been proposed that CA2 drives sensory based representations in awake state while CA3 drives memory based representation in sleep (Middleton & McHugh, 2020) via adenosine; which allows CA3 to control sleep replay content. Therefore, CA3 takes over (via the classical trisynaptic loop) and influences CA1 firing in sleep replays more for memory consolidation of prior experience. The influence of CA3 over CA1 could explain the coherent response seen in CA1 for both day2 and 3's SLEEP, which was not observed in CA2.

This 'switch' between dominance of influence over CA1 from CA2 in RUN/behaviour and CA3 in subsequent SLEEP could explain why CA1 and CA2 show the same response to spatial novelty detection but not in subsequent consolidation. This could be the first step towards better understanding what each of these hippocampal subfields independently contribute to during memory acquisition and consolidation of space.

CHAPTER 1

INTRODUCTION

HIPPOCAMPUS - SPATIAL MEMORY AND NAVIGATION

Introduction:

The hippocampus and related medial temporal lobe regions play a pivotal role in encoding, consolidation, and retrieval of associations responsible for episodic memory (O'Keefe & Nadel, 1978; Brun, 2002). The term 'episodic memory' was coined by Tulving (Tulving, 1972) and was described as having perceptible properties and a spatio-temporal relative relation to other experienced events. Thus, it is used to capture the memory of a singular event that might occur when a previous particular occasion is recalled. That event (which happens at a particular time) might include both spatial and nonspatial components, including where the event occurred, who was present, and which objects were seen (what, when, and where) (Rolls *et al.*, 2002). It is distinct and separate from 'semantic memory' which involves facts or general knowledge about the world. The spatial component of episodic memory (spatial memory) is encoded as a "cognitive map" of the external environment which may be used for efficient navigation, orientation, direction and successful interpretation of external sensory cues. The locus of this 'cognitive map' is the hippocampus. This ability is one of the most fundamental cognitive properties of animals for survival, as it helps form a spatial relation between an organism and its environment.

With the discovery of place cells (hippocampal pyramidal cells) in rats by John O'Keefe and Dostrovsky (O'Keefe & Dostrovsky, 1971), the basis of this cognitive map was attributed to the hippocampus. Subsequent discoveries of head direction cells in many brain areas such as lateral dorsal thalamus (LDN) (Mizumori & Williams, 1993), striatum (Wiener, 1993), lateral mammillary nuclei (Stackman & Taube, 1998), retrosplenial cortex (Chen *et al.*, 1994) ADN (Blair & Sharp, 1995; Taube, 1995) and Post subiculum (Taube *et al.*, 1990a, 1990b) along with the discovery of

grid cells in dorsomedial entorhinal cortex (Hafting *et al.*, 2005) and more recently in pre and para subiculum (Boccara *et al.*, 2010), has led to believe that the formation of this cognitive map is not restricted just to the hippocampus, but in fact encompasses parahippocampal areas as well.

1.1 Hippocampus and memory:

The bilateral resection of the medial temporal lobe (MTL) of the world famous patient H.M. resulted in his complete inability to form new memories, along with severe retrograde amnesia (Scoville, 1954; Scoville & Milner, 1957), determining MTL's importance for maintaining certain forms of memory: specifically, declarative memory, including episodic memory, while non-declarative memory (such as skill-learning, routine functions, motor learning etc.) remained unaffected (Squire, 1992). The MTL comprises of the hippocampal formation (the dentate gyrus, the CA fields, and subiculum), the entorhinal cortex, the parahippocampal cortex, and the perirhinal cortex. Many lesion studies in rodents, non-human primates as well as converging studies from human patients (involving ischemic damage to various portions of the MTL), have helped distinguish the role of the hippocampus v/s the rest of the medial temporal lobe. While working memory is largely independent of the MTL and remains intact in patients with damage similar to that of H.M (Squire, 2009; Jeneson *et al.*, 2012), long-term memory is primarily reliant upon the MTL, particularly the hippocampal formation, and is disrupted in such patients (Squire, 2009).

The hippocampus-proper is known to play a critical role in binding together the content and context of an episodic memory (Squire, 1992; Zola-Morgan & Squire, 1993). Rats with hippocampal lesions are impaired on a wide array of memory tasks,

including spatial tasks such as the Morris water maze, radial arm maze tasks, and spatial alternation tasks, as well as non-spatial tasks such as odor discrimination, cue relationships, and novel object preference (Squire, 1992). Additional to its pivotal role in formation of spatial cognitive map in the brain, the hippocampus has also been known to provide important contextual information that helps choose the best stimulus-driven response available (Hirsh, 1974). Rats with hippocampal lesions are unable to pair their motivational context (hungry or thirsty) with external cues for where to find food or water (Kennedy, 2004). The hippocampus is also required for the expression of freezing in contextual fear conditioning (Penick & Solomom, 1991; Kim & Fanselow, 1992; Phillips & LeDoux, 1992), and thus for binding context and shock into a single representation.

Further, the hippocampus also appears to be required for some temporal context tasks, especially when timing information must be combined with other information. For example, rats with hippocampal lesions can recognize previously experienced odors but cannot maintain a representation of the order of their presentation (Fortin *et al.*, 2002). Similarly, mice with hippocampal lesions are impaired on a *what-where-when* task designed to assess episodic-like memory with minimal training. While intact mice explore "old" objects more than "recent" objects and "displaced" objects more than "stationary" objects at a test session 50 minutes later (Dere *et al.*, 2005a), while those with hippocampal impairments do not show these tendencies (Good *et al.*, 2007; DeVito *et al.*, 2009).

These observations support the idea that the hippocampus is critically involved in binding together information from various modalities (time, space, odors, visual cues, internal motivational state, etc.) in order to make the most appropriate behavioral

choice for any given context (based on episodic-like memory for similar events). Thus, the hippocampus is necessary for a broad range of memory tasks that involve the organization and flexible expression of memories. Correspondingly, hippocampal networks map many non-survey spatial and non-spatial organizations, including mapping of multiple intersecting routes (e.g., the continuous alternation task), distinct spatial mappings for different cognitive operations in the same environment (e.g., delayed vs. continuous alternation), mapping of temporal organizations (e.g., remembering the order of events in episodes), and abstract relations between events that form a continuity of associations (e.g., hierarchical organizations, social space).

1.2 Hippocampus and navigation:

According to Gallistel "Navigation is the process of determining and maintaining a course or trajectory from one place to another" (Gallistel, 1990). Navigational strategies are distinguished into two domains: one involving local navigation, where one can approach a directly observed goal location in the currently perceived environment, and the other by wayfinding, where the goal must be reached by moving beyond immediately perceived space via learned routes or by referring to an internalized cognitive map (Trullier & Meyer, 1997; Franz & Mallot, 2000). Both navigational strategies involve multiple cognitive and behavioral mechanisms that involve the hippocampus.

In an experiment by Packard and McGaugh (Packard & McGaugh, 1996) rats were trained for different numbers of days and then probed which navigational strategy would be used by them after rotating the maze 180° and allowing a free choice. If the animals used a route-based strategy, then in the probe test they should follow the turn-

left route even as it takes them to the east rather than the west goal location, but if they used survey navigation, they should employ the cognitive map that guides them to the west goal even as it requires taking a right-turn route. After 1 week of training, normal rats in the probe test turned right at the choice point and approached the west goal, consistent with flexible survey navigation in a cognitive map to the remembered goal location, and this performance was dependent on the hippocampus. After an additional week of training, rats turned left in the probe test, consistent with a rigid route-following strategy, and this performance was dependent on the striatum and not the hippocampus. These findings strongly support O'Keefe and Nadel's notion that navigation using a cognitive map requires the hippocampus while route following does not. Consequently, hippocampal lesions do not impair learning to turn in a particular direction in a T-maze, presumably because learning can be accomplished using a recognition-triggered response that does not require a cognitive map. However, imposing a time delay in these alternating choice paradigms results in task impairment, as the memory demand imposed by the addition of a delay engages performance driven by a cognitive map supported by the hippocampus. Similarly, the Morris water maze task is successfully learned in animals with hippocampal damage when they can guide navigation along a single route (Eichenbaum *et al.*, 1990) but not when the task involves concurrent learning of multiple routes, and animals with hippocampal damage fail under this protocol (Morris, 1981).

Thus, the hippocampus is essential to survey navigation, which is required in tasks that demand remembering goals and recent experiences, where integration of multiple paths into an organization of places that compose a survey map is required, as well as for flexible expression of navigation guided by this map. Correspondingly, in these

situations, even when survey mapping is not required, hippocampal networks map environments by parsing locations into place fields and can predict paths through these maps as reflected in sequences of place cell firings.

1.3 Hippocampal place cell system:

Successful navigation requires the understanding of one's ideothetic position/location in a given environment, and place cells do just that. Discovered by John O Keefe and Dostrovsky in 1971, place cells are found in the hippocampus. Each place cell fires in response to a particular location, and subsequently covers the entire environment as the animal traverses it, but without any topographic representation (Bostock *et al.*, 1991; Wilson & McNaughton, 1993; O'Keefe *et al.*, 1998).

The hippocampus, has often been herald as the locus of a cognitive map in the brain, as was first proposed by Tolman in 1948 and subsequently established by several studies (O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978). This cognitive map does not produce any navigational demands on the animal and simple curiosity/exploratory behaviour acts as a sufficient motivational attribute towards its formation (O'Keefe & Nadel, 1978). Tolman suggested that an important function of the cognitive map was to allow animals to mentally explore all possible outcomes of multiple courses of action before committing to one. Hippocampal place cell sequences during different behavioural states (LIA and theta) help form representations that could be used to perform mental simulations over a multitude of possible actions, coupled with valuation signals that balance the costs and benefits associated with each outcome. This makes the hippocampus immensely valuable for

planning, decision making, rewarded outcomes and successful navigation in the external world.

More than 50% complex spike cells in the hippocampus (rodent) are found to be place cells (Taube, 1999), indicating the primary nature of hippocampal activity is spatial / navigatory. Place cells are the pyramidal firing neurons in the hippocampus, and are found in all its sub-fields: CA1, CA2, CA3 and DG. Each place cell fires only within its own specific place field at a specific location in any given environment and is virtually silent outside it, but the entire environment is represented in the activity of the local cell population within the hippocampus (O'Keefe, 1976; Wilson & McNaughton, 1994). When exposed to a new environment, the place field of each place cell will be established within minutes, and the resulting representation of space usually remains stable for days. When transferred to another environment, resulting in a new map (Colgin *et al.*, 2008) or when the experimental environment is somehow altered (e.g. elongated etc.), the place cells are known to ' remap' ; either globally or locally (Derdikman & Moser, 2010).

The same place cell can also participate in the representing different environments, where it fires for different places in different environments, but the relationship between the firing fields differs from one setting to the next (O'Keefe & Nadel, 1978; Pavlides & Winson, 1989). Despite the non-topographical representation of space by hippocampal place cells, the brain is (probably) able to determine the position of the animal by reading out the sequential activity of a local population of place cells (Redish *et al.*, 2001). In multi cellular in –vivo hippocampal recording experiments, the rat's movement can be reconstructed quite successfully and accurately by the

experimenters (Wilson & McNaughton, 1993). All the above evidence indicates that hippocampal place cells form a spatial map of the environment (Derdikman & Moser, 2010). Spatially selective cells are also found in the entorhinal cortex and the subicular complex (Quirk *et al.*, 1992; Sharp & Green, 1994; Taube, 1995).

Although most hippocampal in vivo place cell recordings have focused on dorsal hippocampus, place cells have also been found in the ventral part of the hippocampus (Jung *et al.*, 1994; Poucet *et al.*, 1994) indicating they exist throughout the length of the structure. Dorsal and ventral hippocampal regions are distinct functional modules, with the dorsal hippocampus involved in more cognitive operations and the ventral hippocampus playing a greater role in affective processes (Moser & Moser, 1998; Fanselow & Dong, 2010). Recent studies have also indicated that social memory information flows from dorsal CA2 to ventral CA1a to the NAc shell in a single serial circuit, with ventral CA3 inputs to ventral CA1 providing a parallel but necessary path (Meira *et al.*, 2018). The study also found that ventral CA1 and dorsal CA2 are important for social memory recall, it is likely that ventral CA1 participates in social memory retrieval as a result of its input from dorsal CA2, rather than from ventral CA3, since it is not necessary for social memory recall (Chiang *et al.*, 2018).

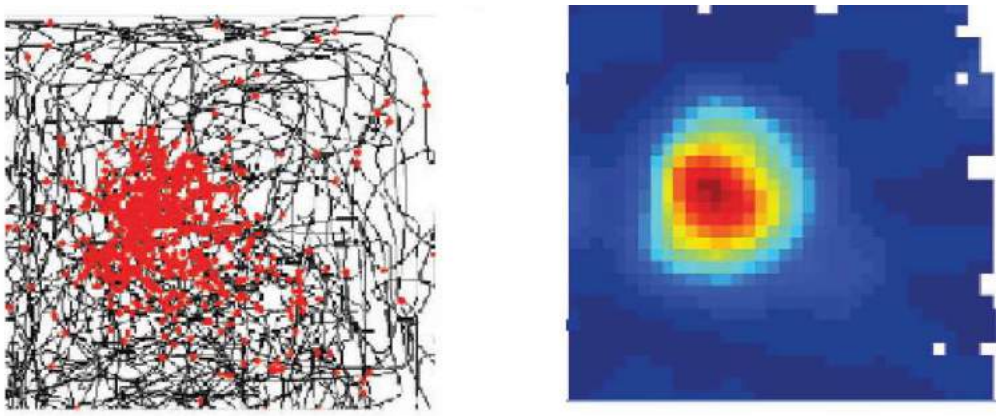


Figure 1.1: Hippocampal place field
Trajectory of rat (in black) in an open field exploration with place cell firing (in red) (left)
Color-coded rate map of the same cell (right)
(Moser *et al.*, 2015)

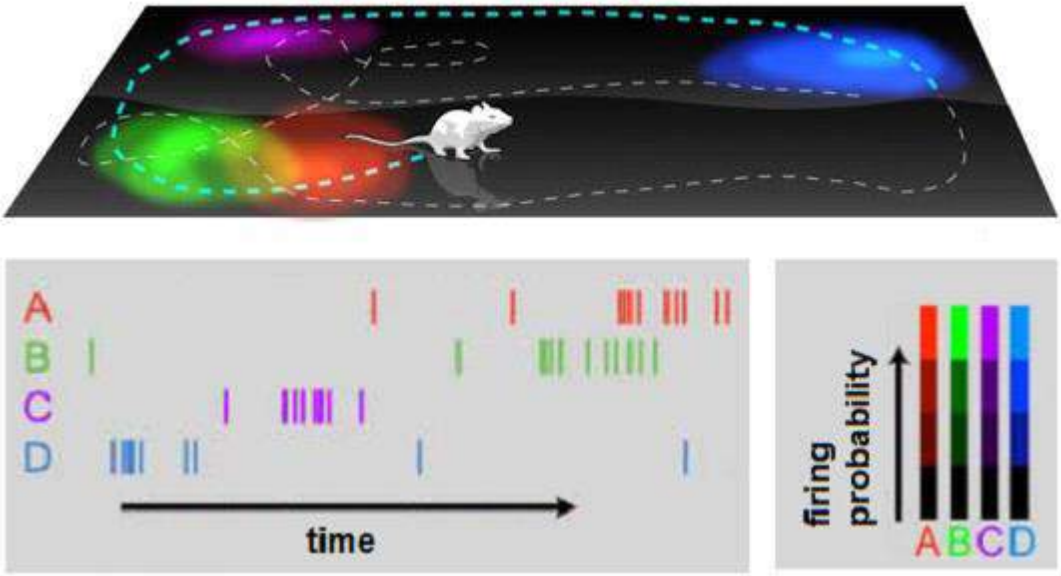


Figure 1.2:
Four hippocampal place cells firing in a rectangular environment (top). The same place cells are depicted as a raster plot during an exploration sequence (bottom left) and their firing probability (bottom right) (O'Neill *et al.*, 2010).

1.3.1 Firing properties of place cells:

The firing of hippocampal place cells is highly location-specific, independent of head direction, and is virtually zero outside its firing field (anywhere else in that environment). Unlike head direction cells, there are many (almost 50%) cells that are silent in a given particular environment, indicating that there are different 'active subsets' of place cells for different environments (O'Keefe, 1976; Wilson & McNaughton, 1993). A place cell can have different representations in different environments as well, indicating that each time a novel environment is entered; a new subset becomes active, irrespective of the firing status of those cells in previously encountered environments (O'Keefe & Conway, 1978). Similar to head direction cells, place cells are also anchored to salient cues, and are found to rotate with respect to the rotation of a cue card, without any change in its firing; provided it is a familiar environment. While encountering a novel environment however, ideothetic information (vestibular system, proprioceptive system, motor efference copy (Gothard *et al.*, 1996) is first relied upon to update the firing of place cells, with the visual input coming into play for re-calibration purposes to eliminate any cumulative error that arises from path integration strategy (Mittelstaedt, 1983; Gothard *et al.*, 1996).

Although place cells are location specific, their firing is not related to a particular behaviour of the animal in a given apparatus/environment, since the amount of time spent at a particular locus in a field does not result in preferential place fields at those loci (Muller, 1996). Instead, some studies have indicated that it is the task that seems to shape up the representation of space by the place cells, with firing seen for each

aspect of the task, including the environment (Wiener *et al.*, 1995) as well as each reference frame of the environment having a signature representation (Gothard *et al.*, 1996). Such different representations of the same environment based on landmarks and reference frames then combine together for successful navigation. Another study indicated that a change in task (random to directed search) resulted in a change in the firing location of 1/3rd of place cells, and this change was rapid and paralleled the change in behaviour, while a constant task performance resulted in stable fields over months together (Markus *et al.*, 1995).

Place cells in CA1 and CA3 are involved in pattern suppression and pattern completion, in that they are able to completely "re map" the representation of an environment if there are changes in the sensory input, as well as recognize a previously encountered environment as the same even if some vital cues/landmarks are removed from it; respectively (Leutgeb *et al.*, 2005). A change in rate firing as well as place firing in these cells, leads to a new representation of an environment, and is known a global remapping; while some changes in rate firing for some cells, with the place firing remaining largely constant is known as rate remapping. The mode of remapping chosen by place cells depends on the input from grid cells in MEC. Whereas rate remapping is associated with stable grid fields, global remapping is always accompanied by a coordinate shift in the firing vertices of the grid cells (Fyhn *et al.*, 2007). Moreover, a conflict in placement of cues of more than 45 degrees results in complete/global remapping by the place cells, indicating that they treat this new placement of cues as a new environment; while a mismatch of less than 45 degrees, leads to the rotation of existing place fields in sync with rotation of the cues (Skaggs & McNaughton, 1998).

1.3.2 Directionality in place cell firing:

Despite a stable and robust head direction system for navigation in rats, there seem to be place cells which are directional in nature, and fire with the learning of a new task. With a directed search task, new directional place cells emerge, along with the already existing non-directional place cells, indicating their sensitivity to the behavioural aspects of the task (Markus *et al.*, 1995). For example, place cells show strong directional tuning in a radial arm maze and other symmetrical environments (O'Keefe & Recce, 1993), but not while foraging in an ambiguous environment (Barnes *et al.*, 1990; Muller *et al.*, 1994). Directional differences seem to be maximal in clockwise v/s anti-clockwise directions (Markus *et al.*, 1995) and in environments with constant distal cue representation, indicating that they may rely more on distal cues than proximal cues as a stable, reliable landmark. The directionality is maintained even in dark (McNaughton *et al.*, 1989; Markus *et al.*, 1994) and is not altered by visual cue complexity of the environment. Different studies by Wan and Gothard (Wan & Touretzky, 1993; Gothard *et al.*, 1996) suggest that this directionality in place cell firing may be due to the reliance of the place cells on path integration; and that directed tasks require a shift in reference frame points during navigation resulting in directionality in place cells whereas random foraging on a circular (symmetrical) platform has only one frame of reference and hence place cells are found to be omni-directional in such tasks. Thus, the primary drive for the 'direction-ness' of place cells seem to be the vestibular system , and the visual cues come into play as stable references only after a stable mapping has already taken place with respect to the environment (McNaughton *et al.*, 1991).

1.3.3 Interactions between head direction (HD) cell system and place cell (PC) system:

The sufficient evidence from literature seems to point at the critical interactions between the various areas of the limbic system known to have HD cell firing activity and the CA3/CA1 areas of the hippocampus, known to have place cell activity. A strong coupling between place cells and head direction cells is known to exist, and both systems are controlled by a complex interaction between self-motion ideothetic cues and external visual cues (Knierim *et al.*, 1995, 1998; Blair & Sharp, 1996; Goodridge *et al.*, 1998; Zugaro *et al.*, 2003). A rotation of a prominent visual cue card results in an equal shift in the firing activity of both place cells and head direction cells, indicating that they respond to external sensory cues in the same way (Knierim *et al.*, 1995, 1998). Also, neither type of system requires a constant visual input to maintain their firing, after initially establishing a map of the environment beforehand. Thus, both cells are capable of stable firing over minutes in darkness after correctly placing themselves with respect to the environment (Quirk *et al.*, 1990; Taube *et al.*, 1990a; Chen *et al.*, 1994; Markus *et al.*, 1994). Apart from the above common similarities, both systems require a vestibular input to fire (Stackman & Taube, 1997) and reduce their firing drastically if restrained/ hand held tightly (Knierim *et al.*, 1995; Taube, 1995). Both systems are known to remain coupled to one another even when they completely uncoupled from external landmarks (Knierim *et al.*, 1995).

However, while place cells are found to rely on proximal cues (local apparatus cues), head direction cells on the other hand are found to rely more on distal cues, which relatively remain constant with respect to the external environmental representation

(Knierim & Rao, 2003). A mismatch between proximal and distal cues in an environment may result in a CA1 remapping but the HD representation maintains its coherent representation bound to distal landmarks (Yoganarasimha, 2006). However, centrally placed objects are not able to exert cue control over place cell system, even though it could be treated as another proximal cue, which do exert a strong control over them (Cressant *et al.*, 1997).

A lesion to brain areas containing HD activity result in a decrease in the stability of firing of place cells, further displaying the inter- dependence of the two systems (Taube *et al.,* 1992; Warburton *et al.,* 2001). When HD cell system is lesioned, it results in loss of control of distal cues over place cell firing, making them insensitive to distal cue card rotations. This effect was much more prominent after PoS lesions, in comparison to ADN lesions (Calton *et al.*, 2003). In another study, a loss of cue card control over HD cells resulted in hippocampal re-mapping (Knierim *et al.*, 1995, 1998). However, bilateral lesions of the hippocampus do not disrupt HD cell activity in PoS and ADN, indicating that the hippocampus is not required for initiating or maintaining HD activity, nor for the establishment of a new preferred direction in a novel environment (Golob & Taube, 1997).

1.3.4 Path integration v/s sensory cues:

Animals navigate by using cues (external and internal) to familiarize themselves of their position and direction and build their own cognitive representation of the surroundings, around these cues. There are primarily two mechanisms that animals use to achieve this. One mechanism involves using one's own inherent sense of

representation of space and is called path integration (Mittelstaedt & Mittelstaedt, 1980; Gallistel, 1990) while the other involves the use of external sensory cues, more prominently visual, to orient oneself called landmark navigation (O'keefe & Conway, 1980; Collett *et al.*, 1986).

Path integration or self-motion navigation involves the use of proprioceptive cues, motor efference copy, vestibular cues etc, and is the primary mechanism of navigation that the animal adopts when it first encounters a novel environment. This process is also called 'dead reckoning', whereby the animal constantly updates itself on the basis of its previously encountered location. It is so named after the fact that the animal constantly integrates its own movements over time to calculate track distance. After many trials, and repetitive encounters with the same environment, they start treating the external cues as reliable and use it in addition to path integration to form a cognitive map of the environment, provided the cues do not change their position in any way with respect to one another and to the environment (Alyan & Jander, 1994). These cues may be somatosensory, auditory and/or visual in nature.

Both head direction cells and place cells are influenced controlled by path integration and visual landmarks. While some studies show that visual landmarks override ideothetic cues (Mizumori & Williams, 1993; Goodridge & Taube, 1995), others show the opposite (Chen *et al.*, 1994; Wiener *et al.*, 1995). Many models have been proposed whereby the animal relies on path integration and uses landmark navigation only to re calibrate its own internal sense of navigation. Thus, landmark calibration is an experience- dependant process which is bound to path integration by associative learning (McNaughton *et al.*, 1996). Also, in a mis-match between visual cues and

ideothetic cues, visual cues have very little control over place cells (Chen *et al.*, 1994) and the extent of control of visual cues depends on the degree of mis-match between external and internal cues (Blair & Sharp, 1995; Gothard *et al.*, 1996; Rotenberg & Muller, 1997). For small rotations (mis-match) e.g. 45 degrees, place cells and head direction cells strongly align with visual cues; but for larger rotations, they align with the inertial frame and dis regard landmark information (Skaggs & McNaughton, 1998). Further evidence comes from the fact that place cells remain stable if in the animal's presence, most visual landmarks are removed or even if lights are extinguished (McNaughton *et al.*, 1996). Although, some studies do show that place fields may be independent of visual cues and instead spatial firing is related to changes in spatial attention (Markus *et al.*, 1995).

Furthermore, a number of elegant models have demonstrated how the physiology of the entorhinal cortex and hippocampus allow the hippocampus to update its representations based on self-motion cues, suggesting that the hippocampus performs primarily as a path-integrator (McNaughton *et al.*, 1996; Samsonovich & McNaughton, 1997), such that the ensemble response of hippocampal cells may give high-precision information about the current position of the rat (Wilson & McNaughton, 1993).

1.4 Hippocampal sequences and memory:

The 'two stage' memory formation hypothesis (Buzsáki, 1989, 2015) indicates that in the initial stage, memory formation occurs in the hippocampus occurs in which a short-lasting trace is weakly encoded, followed by subsequent stage in which a long-lasting trace is consolidated—and that these two stages correspond to neural activity

during theta (locomotor) and SWR (sharp-wave ripple) periods, respectively. Neural activity in the hippocampus can be divided into two states: theta (6-12Hz) state, characterized by a slow but strong rhythmic firing of principal neurons, alongside an oscillation in the local field potential (LFP); and LIA (large irregular activity), characterized by a lack of coherent oscillations with brief bursts of activity (~100ms) in principal neurons. In rodents, theta is associated with locomotion, attention and arousal and shallow REM (rapid eye movement) sleep, while LIA is associated with quiescent periods of grooming, sitting, eating, resting and non-REM deep sleep states.

1.4.1 Phase precession and theta:

EEG recordings in lower animals such as rats, rabbits and cats show different signature waves corresponding to a specific behaviour of the animal. One such wave is a slow sinusoidal rhythm of 6-12 Hz, in rats, known as theta wave. Theta wave is driven by pulsed impulses from medial septum and the diagonal band of Broca in the brain. A theta wave is characteristic of the investigation of a new/novel environment or of changes in an already existing environment. Thus, it signifies the exploratory/ curiosity behaviour of an animal, and is thus an important waveform related to spatial encoding of the environment by it. The amplitude of theta is proportional to the concurrent motor activity of the animal (O'Keefe & Nadel, 1978). There are 2 distinct forms of theta oscillations- Type I (7-12Hz): occurs during "voluntary," primarily movement related behaviors and Type II (5-7Hz): occurs when animal is stationary, but alert/attentive to their surroundings (e.g., during presentation of conditioned stimuli, preparation of a motor response, fearful freezing).

A place cell fires in relation to the current phase of a theta cycle that is from 0 degrees to 360 degrees. Initially it corresponds to the peak of the hippocampal oscillation, but as the number of cycles increase, the cell discharges at an earlier phase than the preceding one till it reaches the trough of the rhythm. Thus, the place cell produces a rhythmic discharge of a slightly higher frequency than the ongoing theta oscillation and this is called phase precession. This phenomenon allows a temporal code for location, since many fields overlap and at a given time, each place cell will fire with respect to a different phase of theta depending on the distance covered in each field. It also results in compression of temporal sequences of firing, thus facilitating synaptic plasticity (Skaggs & McNaughton, 1996; Dragoi & Buzsáki, 2006; Foster & Wilson, 2007). Further, at path bifurcations, theta sequences have been shown to encode either one or the other upcoming path (Johnson & Redish, 2007; Redish, 2016). These paths represented by theta sequences correlate with the paths taken in subsequent navigational behavior (Wikenheiser & Redish, 2015), suggesting that theta sequences also contribute to or reflect a spatial planning process. More recently, it has been proposed that phase precession could also impart temporal structure to episodic memories so that the order of events may be recalled successfully (Buzsáki & Moser, 2013; Howard & Eichenbaum, 2013).

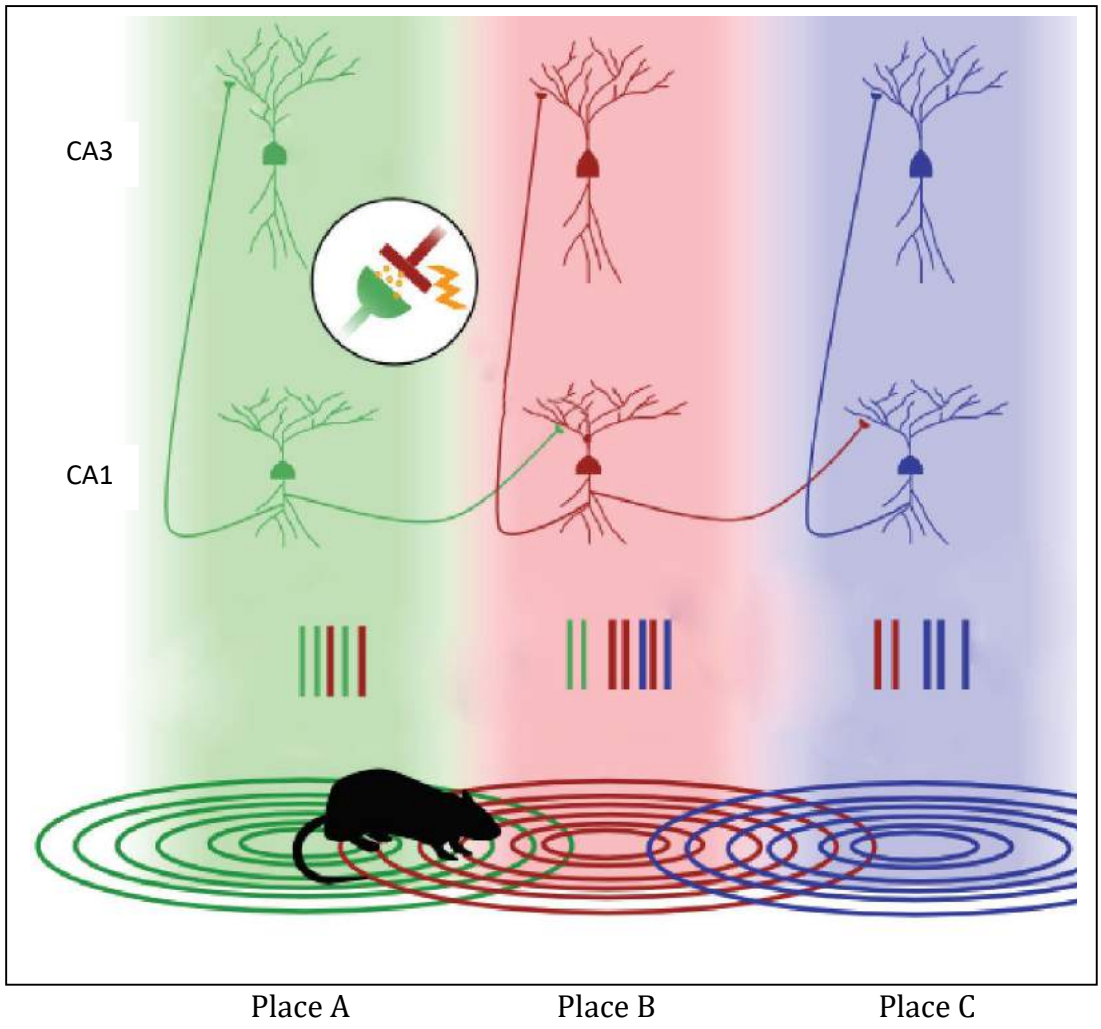


Figure 1.3: Hippocampal modulation by theta during exploration
Three overlapping hippocampal place fields from CA1 and CA3, labelled Place A, B and C firing in a sequential pattern. This firing occurs during the same theta cycle, that establishes conditions necessary for synaptic plasticity to occur between CA3-CA1 place fields via Schaffer Collateral pathway, during strong theta modulation (4-7Hz) (Sadowski *et al.*, 2011)

1.4.2 LIA and sharp wave ripple activity:

The brain pattern during LIA remains largely asynchronous but is interrupted by sudden acute deflections called sharp waves. Theses sharp waves display a fast superimposed oscillation called as a ripple. Superimposed on these sharp waves are several (~5-10) cycles of smaller amplitude high frequency (140-250Hz) ripple oscillations and together, they constitute the sharp wave ripples (SWRs). These SWRs reflect synchronized firing of hippocampal pyramidal cells, that are phase locked to this oscillation. During rest/sleep, SWRs occur between .1 to 1 times per second, accompanied by pairs, triplets or short trains of neuronal activity (O'Keefe & Nadel, 1978; Buzsáki *et al.*, 1983). The order of this neuronal activity is highly correlated with the order in which they were activated during prior behavioural experience (forward or reverse order).

The 2-stage memory formation hypothesis states that activity during SWRs in LIA and sleep reflect an internally generated re-expression of memory trace. This re-activation might support learning at network level in the brain, whereby activity during SWRs would be broadcast to further areas of the brain, beyond the hippocampus, leading to long term memory stores. Experiments involving rats running on a linear track (that limits ensemble activity and re-activity to simple linear sequences), have shown that the place cell sequences that fire during behaviour, also fire as temporally structured sequences (called "replay") during SWRs in SWS (slow wave sleep) (Lee & Wilson, 2002) and pauses in exploration (Foster & Wilson, 2006; Csicsvari *et al.*, 2007; Diba & Buzsáki, 2007). While forward reactivation is presumed to rely on synaptic mechanisms, reverse replay, has been suggested to rely on an

activity-based mechanism: proximate-field place cells remain more excitable than distant-field cells upon arrival of a post-movement rising excitation wave (SWR), thus advantaging replay in the reverse order (Buzsáki, 1989). Moreover, the temporally compressed SWR replay (20 fold compression) allows for the temporal structure experienced on a behavioural timescale of seconds to be re-expressed at a faster timescale, appropriate for expression for synaptic plasticity (milliseconds) (Mehta *et al.*, 2002). Therefore, replay of place cell activity during sleep-SWRs contributes to memory consolidation of prior experience and successful future navigation to returned paths and novel environments.

Further, place cells that were active during behaviour, fire at a higher rate during sleep (Pavlides & Winson, 1989), and pairwise correlations between these coactive cell pairs during exploration are enhanced during post-behavior sleep (Wilson & McNaughton, 1994). The enhancement of strength of these correlations reflects the order in which cells were activated during behavior (Skaggs & McNaughton, 1996), suggesting ensemble-level coordination of place cell "reactivation" during sleep. In aged animals, the temporal patterning of LIA sequences is altered, and the extent of sequence alteration predicts spatial memory impairment (Gerrard *et al.*, 2008). Recent experiments have causally linked SWR events during sleep and memory consolidation as well. Using precisely timed electrical stimulation to the hippocampus, spiking sequences have been disrupted during SWRs, sparing otherwise normal ensemble activity. Using this technique, it has been found that stimulation contingent on SWRs during post-behavior sleep impairs learning (Ego-Stengel & Wilson, 2009; Girardeau *et al.*, 2009) although sequences are less tightly correlated with SWRs than previously thought, raising the possibility that some non-sequence process disrupted by

stimulation causes the memory impairment. Nevertheless, these experiments support the evidence that SWR-associated hippocampal process plays an important role in memory consolidation during sleep.

Eventually, the hippocampal sequences generated during both LIA and theta states contribute to the construction of mutable, behaviorally relevant representations of the external world. Wikenheiser and Redish (Wikenheiser & Redish, 2015) propose that the hippocampus creates representative ‘chunks’ by enhancing connections between sequence items close to each other in representational space, during theta sequences, thereby parsing behavioral experience (spatial and nonspatial aspects). The hippocampus then strings together all the ‘chunks’ of information acquired in theta sequences, during offline and online LIA states, forming integrative representations that capture meaningful relationships between distant/non sequential segments of the environment. By recombining information in such configurations that differ from actual experience (e.g., backward sequences), the hippocampus can generate representations of never-experienced paths, which can then be integrated with the rest of the cognitive map, providing animals access to representations necessary for planning ahead and for flexible behavior.

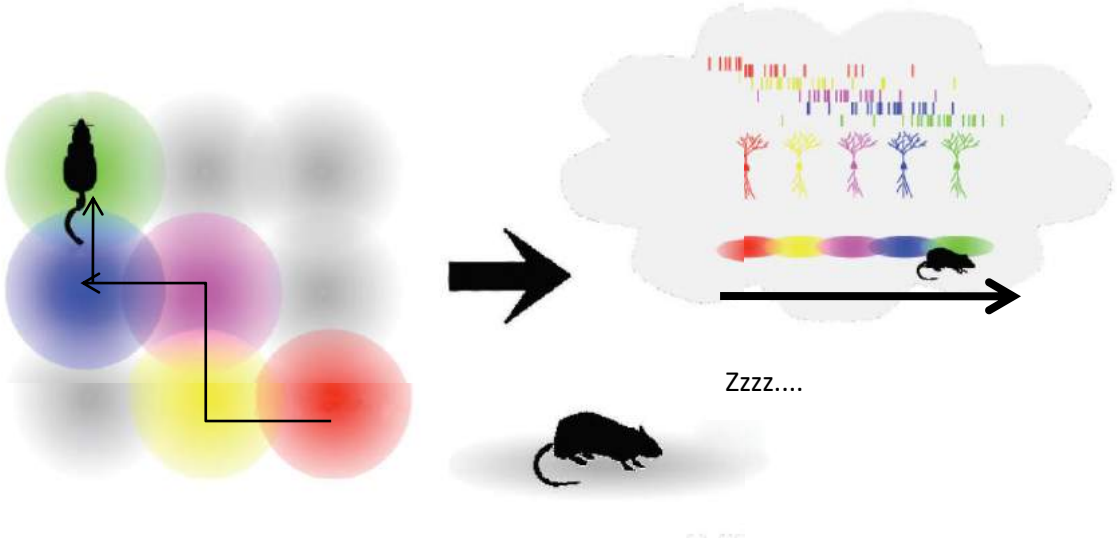


Figure 1.4: Offline reactivation of place cell assemblies during nREM sleep for memory consolidation
Patterns of activity reflecting spatial exploration during the online state are recapitulated during offline epochs including quiet waking immediately following activity, and nREM (slow wave sleep) during SWRs. The greatest degree of temporal overlap in firing patterns occurs between cells with overlapping place fields (Sadowski *et al.*, 2011)

1.5 Spatial novelty detection in the hippocampus:

Natural exploratory behaviour of most animals (including rats) includes the tendency to respond specifically to novel stimuli by increasing their exploratory behaviour, and decreased exploration of familiar stimuli or space. This would require active maintenance, up gradation and successful integration or comparison of familiar configuration with current contextual cues; to avoid exploration redundancy and to instead focus on and distinguish novel stimuli. The hippocampus is actively involved in encoding novel information and retrieving old information, match-mismatch between internal representation and external sensory stimuli etc. Neural representations of novel (i.e., previously not encountered) environments form rapidly in the rodent hippocampus (Wilson & McNaughton, 1993; Frank, 2004; Leutgeb, 2004) and place field activity stabilizes (i.e., becomes more similar across successive traversals of the environment) with experience (Wilson & McNaughton, 1993; Leutgeb, 2004; Cacucci *et al.*, 2007).

The critical comparison between the external sensory environment and the internal representation of the environment, is made in CA1, as it is uniquely poised to receive information simultaneously from both from the DG–CA3 network and directly from the entorhinal cortex (Hasselmo & Schnell, 1994; Lisman & Otmakhova, 2001; Hasselmo *et al.*, 2006). Similarly, CA3 receives converging afferents originating from multiple brain areas (e.g., DG, entorhinal cortex, and medial septum) and could act as a potential comparator (Mizumori *et al.*, 1999; Vinogradova, 2001). CA3 is also important for rapid formation of spatial or contextual memory, especially in the acquisition of novel information (Kesner *et al.*, 2002; Lee & Kesner, 2003; Nakazawa *et al.*, 2003). DG, on the other hand, seems to be an essential player in pattern

separation (Treves & Rolls, 1994; McClelland *et al.*, 1995), by orthogonalizing similar input patterns and amplifying the differences in those patterns. Pattern separation may be essential in determining the intra-hippocampal representation of the environment. Thus DG is essential for filtering neocortical inputs to CA3, and the DG-CA3 network is essential for processing novel spatial information, but not novel object information (Lee *et al.*, 2005).

There are relatively little studies that have focused on hippocampal activity during the initial exploration of a new environment, with most focusing on place cell activity in familiar environments where the animals has been exposed to repeatedly while training before neural activity is recorded (Muller, 1996). Previous studies focusing on novel exposures have suggested that hippocampal spatial responses develop on the very first traversal of a new place (Hill, 1978) and that hippocampal population activity accurately represents the position of the animal once it has had ~10 min of experience in the new place (Wilson & McNaughton, 1993). They also reported that 10 out of 15 interneurons decreased their rates in a novel open field environment, whereas Fyhn (Fyhn *et al.*, 2002) reported that alterations in the escape platform location in a familiar water maze caused a minority of inhibitory interneurons to temporarily decrease their rates. Later studies like the one done by Frank (Frank, 2004), traced hippocampal plasticity across days in a novel environment and hypothesized that the rapid development of place fields (in ~5 seconds of first exposure) could not be attributed to STDP but could be due to inhibitory neurons with decreased firing rates, (seen in the 1st minute of novel environmental exposure); which may facilitate plasticity in CA1. They also reported that 1-2 minutes of initial exposure to novel environment was not enough to develop stable representations on day1, but

only on day 2 after >3 minutes of exposure had happened and stabilized completely on the third day once the animal had at least 5–6 min of experience; indicating that time spent by the animal in novelty; and thus their length of experience, is critical for place field development and stability.

A modulatory role of dopamine in EC-CA1, but not CA3-CA1 circuitry has suggested the possibility of a fundamental role for EC-CA1synaptic transmission in terms of detection of spatial novelty, and intermediate-term, but not short-term spatial working memory or object-novelty detection (Vago & Kesner, 2008). CA1 is critical for correctly identifying novel relationships among objects within a particular spatial context, but not necessarily for identifying non-spatial object changes (Lee *et al.*, 2005; Hasselmo, 2008). Furthermore, it is proposed that CA1 is not necessary for short-term (< 5 min), but is required for intermediate-term (5 min – 24 hr) maintenance and retrieval of the familiar spatial context (Kesner *et al.*, 2002; Lee & Kesner, 2004a, 2004b; Rolls & Kesner, 2006). This could indicate the role of CA2, via the disynaptic pathway from EC to CA1 in novelty detection and immediate place field development.

1.6 Hippocampal circuitry:

1.6.1 Anatomical organization of the hippocampus:

The hippocampus contains four primary sub-regions/sub-fields: the dentate gyrus (DG) and cornu ammonis (CA) fields one through three (CA1, CA2, and CA3) (Lorente de Nò, 1934). He also established that the fields of the hippocampal

formation are linked by a sequence of unique, and largely unidirectional, connections when he first described one important relay of synaptic transmission through the hippocampus, the 'classical tri-synaptic loop'(Andersen *et al.,* 2007). Later work extended these early studies by proposing that the hippocampus is organized in parallel "lamellae," or small strips (Andersen *et al.*, 1971) and that excitatory activity travels from the entorhinal cortex, and through the hippocampus, within a series of parallel hippocampal "slices" or "lamellae". The temporal lobe interactions between the entorhinal cortex and the hippocampus were thought to be organized topographically, with different lamellae operating independently. This lamellar hypothesis of the hippocampus's anatomical organization has had tremendous influence on conceptualizing information processing in the hippocampus, with research today still aimed at understanding how layer-specific interactions among the various subfields contribute to memory formation and retrieval in the hippocampus. Although with the discovery that excitatory collaterals originating from CA3 dispersed over a much wider region than originally proposed, the lamellae hypothesis was found to be an incomplete description of hippocampal connectivity (Amaral & Witter, 1989; Ishizuka *et al.*, 1990), Of course, now, the hippocampus is known to have many independent and parallel circuitries within and outside its structure.

The hippocampus is characterized by three to five strata depending on the region, but all CA fields exhibit the four following layers: stratum lacunosum-moleculare, radiatum, pyramidal, and oriens, while CA3 and CA2 subfields also contain one additional layer known as the stratum lucidum. The somata of hippocampal pyramidal cells are located in the pyramidal cell layer and possess apical dendrites that extend into the stratum radiatum and lacunosume-moleculare, and basal dendrites that extend

into stratum oriens (Garcia-Lopez *et al.*, 2006). These dendrite containing layers serve as the main input zone for excitatory synapses from upstream pyramidal cells. The pyramidal cells, in turn, send their axons to a wide range of intra- and extra-hippocampal targets depending on the subfield. In addition to the pyramidal cells, the hippocampus is also populated by an extensive and diverse array of interneurons that primarily target local pyramidal cells, where they exert inhibition that contributes to the timing and organization of hippocampal activity (Klausberger & Somogyi, 2008).

Three major fiber systems are associated with input into and out of the hippocampus:

1. **The angular bundle**: this bundle carries fibers between the entorhinal cortex (EC) and the hippocampal fields (Andersen *et al.*, 2007). The EC is a specialized region of cortex that serves as the primary interface between the hippocampus and the associational cortices of the neocortex (van Groen *et al.*, 2003).

2. **The fimbria-fornix pathway**: this connects the hippocampus with the basal forebrain, hypothalamic, the locus coeruleus, and other brain stem regions (Andersen *et al.*, 2007).

3. **The dorsal and ventral commissures**: they connect the two hippocampi in each cerebral hemisphere.

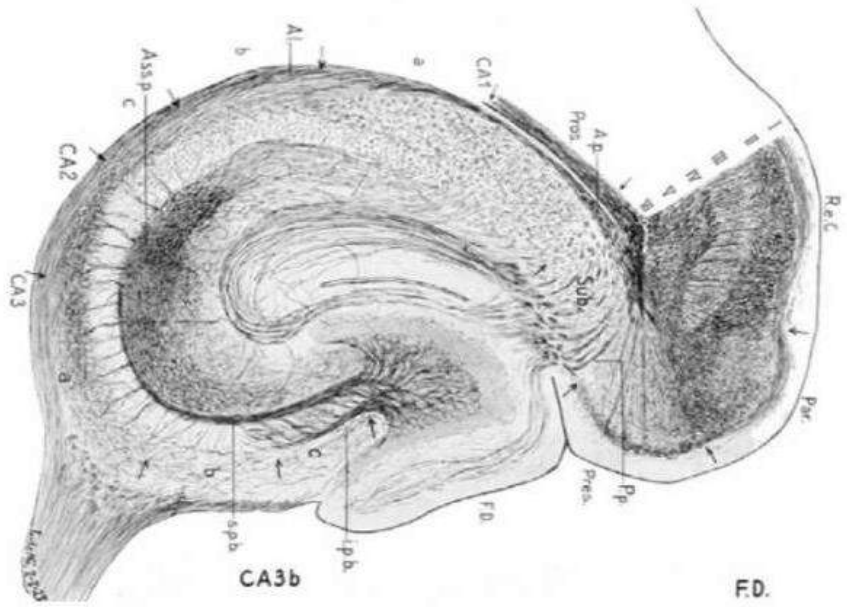


Figure 1.5: Dorsal hippocampus schema of the rat showing the different morphologies of pyramidal cells in all cornu ammonis (CA) subregions. (Lorente de Nò, 1934)

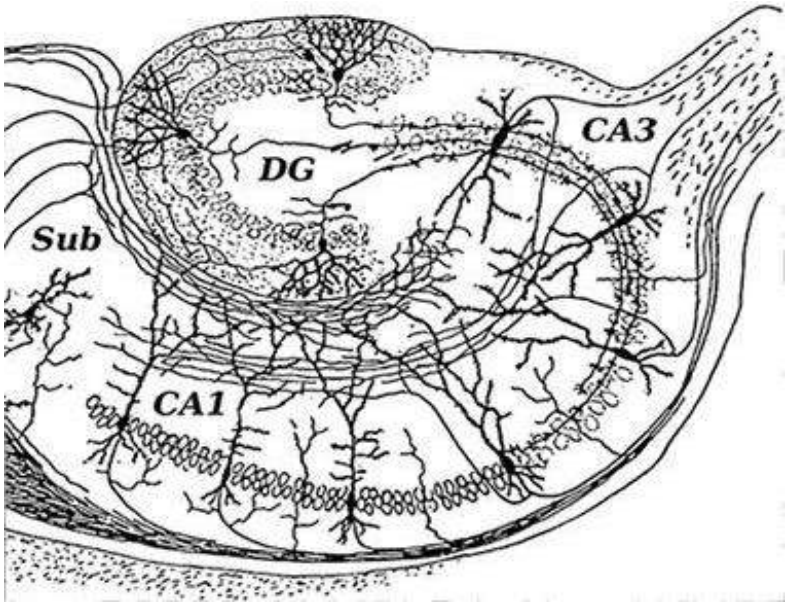


Figure 1.6: Hippocampal cross-section depicting various sub fields of the hippocampus (Ramón y Cajal, 1909)

1.6.2 Connections of the hippocampus:

The main input to the hippocampus comes from the entorhinal cortex, which projects highly processed sensory information (spatial and non-spatial) via its superficial layers (II and III) (Steward & Scoville, 1976). Both medial and lateral entorhinal cortex projections give different a kind of input to the hippocampus, modulating it's firing in different ways. While the MEC, is known to have grid cells (Hafting *et al.*, 2005) and computes the animal's spatial representation in space (McNaughton *et al.*, 2006; Sargolini, 2006; Solstad *et al.*, 2008); the LEC in contrast, responds to non-spatial stimuli modulation (e.g: object identity, context etc.) (Knierim *et al.*, 2006).

The connections within the hippocampus maybe broadly divided into 3 categories: (Amaral & Witter, 1989)

Perforant pathway: Layers II and IV of the EC project to the granule cells in the molecular layer of the dentate gyrus and the pyramidal cells of CA3.this is also known as the medial perforant pathway. Layers II and III of the entorhinal cortex project to the pyramidal cells of CA1 and the molecular layer of the subiculum. This pathway is known as the lateral perforant pathway. EC projections to both CA3 and CA1 end in stratum lacunosum moleculare of the hippocampus.

Mossy fibres pathway: the granule cells in the dentate gyrus project to the pyramidal cells of stratum lucidum of the CA3.

Schaffer collateral pathway: The CA3 projects to the CA1neurons. The CA3 also sends longitudinal association fibre projections back to itself, thereby forming an auto-associative pathway. Both these projections are terminated in the strata oriens and the strata radiate layers of CA1 and CA3 respectively. The CA1 projects to the subiculum in a columnar fashion, and a weaker projection goes back to the entorhinal cortex, thereby completing the circuit.

The MEC and the LEC have different termination patterns in the hippocampus (Witter & Amaral, 2004), in that in the DG and CA3, both areas converge on the same cells, thereby rendering these cells sensitive to spatial as well as non-spatial information (Leutgeb *et al.*, 2005); whereas in the CA1 and subiculum they terminate on different neurons. The MEC axons terminate on the proximal part of CA1 and distal part of subiculum, while the LEC neurons terminate on the distal part of CA1 and the proximal part of the subiculum (Tamamaki & Nojyo, 1995; Harris *et al.*, 2001). A reciprocal pattern is seen in the return projections form the CA1 and the subiculum, with the proximal CA1 and distal subiculum innervating the MEC and the distal CA1 and proximal subicular neurons going to LEC (Tamamaki & Nojyo, 1995; Naber *et al.*, 2001).

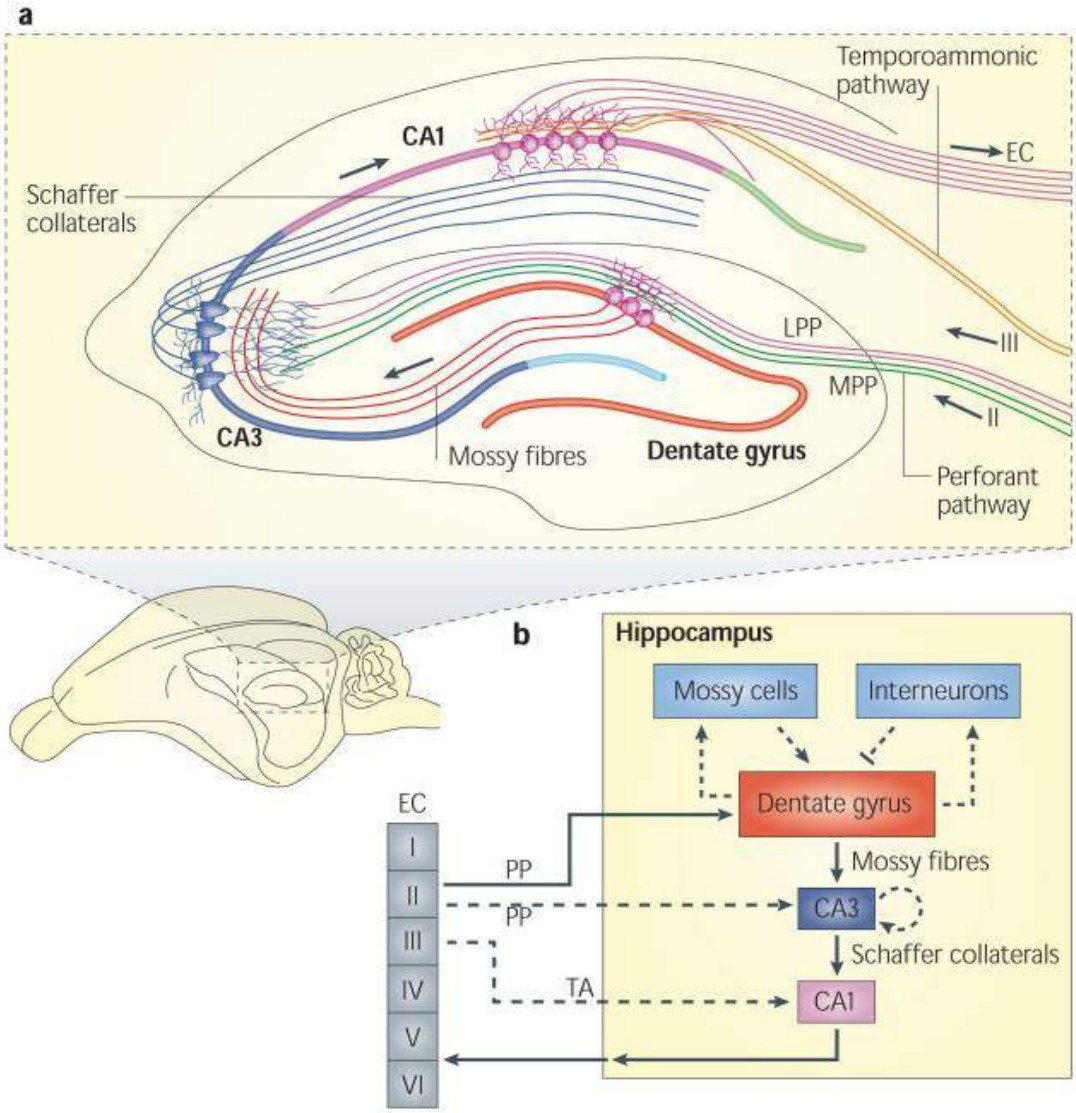


Figure 1.7:
Neural circuitry in the rat hippocampus, highlighting the classical trisynaptic pathway within the limbic structure (Deng et al., 2010).

The above-said indicates that spatial firing in CA1 is organized in a decreasing fashion along its proximo-distal axis, with strongest spatial tuning in the MEC innervated proximal part in comparison to the LEC efferent distal part (Henriksen *et al.*, 2010). It also gives direct evidence of the fact that the hippocampal place cells rely heavily on the input from the entorhinal cortex, which in itself is an important component of the head- direction system; emphasising the important interaction needed between the place cell system and the head-direction system for a cognitive map formation for navigation and accuracy. Entorhinal lesions result in the place cells losing their spatial sensitivity and their ability for normal acquisition of place memory (Hales *et al.*, 2014).

The CA1 efferents innervate the subiculum in a reciprocal topographic fashion, making it the main output region of the hippocampus. Information from proximal CA1 reaches distal subiculum through the alveus, the middle part of CA1 innervates the middle part of the subiculum in the deep part of stratum oriens while the distal part of CA1 sends out projections to proximal subiculum in all parts of stratum oriens layer (Amaral *et al.*, 1991). The subiculum in turn projects to the deep layers of the MEC and sends weaker projections to the superficial layers (Witter *et al.*, 1989). While proximal subiculum projects to LEC, distal subiculum projects to MEC (Tamamaki & Nojyo, 1995). Other than being a part of the entorhinal-hippocampal-subicular loop, the subiculum (distal part of dorsal subiculum) also projects to the retrosplenial cortex (Witter *et al.*, 1990), although no reciprocal connections from the RC exist to subiculum proper (van Groen & Wyss, 1992), but prominent projections from discrete RC regions do exist to the pre and para- subiculum. The subiculum also receives inputs from the AV region of the ADN of the thalamus; and different nuclei of this

region differentially innervate pre and para subiculum, which are also part of the subicular complex (O'Mara *et al.*, 2001).

HIPPOCAMPAL SUB-FIELD: CA2

1.7 CA2 as part of the hippocampal circuitry:

CA2 has recently been acknowledged as an important part of the hippocampal circuitry, rendering it much more than a mere 'transition zone' between CA3 and CA1. Its unique connections and striking differences in its biophysical and synaptic properties regarding synaptic plasticity (Zhao *et al.*, 2007) and resistance to LTP (Chevaleyre & Siegelbaum, 2010), along with different morphological characteristics and patterns of gene and protein expression. CA2 receives direct excitatory input from ECII and CA3 (Kohara *et al.*, 2014), and from several subcortical nuclei, including the supramammillary and paraventricular nuclei (PVN) of the hypothalamus, as well as the medial septum and diagonal band of broca (Cui *et al.*, 2013) making it an integral part of multiple important circuitries of the hippocampal- parahippocampal areas. Moreover, CA2 place cells appear to be the least spatially tuned pyramidal cells (Lu *et al.*, 2015; Mankin *et al.*, 2015), and remap upon exposure to novel or familiar conspecific animals while CA1 place fields do not (Alexander *et al.*, 2016). Many studies have also indicated that CA2 responds to environmental cues containing socially relevant information, which may be mediated in part by vasopressinergic signalling. Synaptic plasticity at the CA3-CA2 Schaffer collateral synapses is also uniquely regulated relative to the CA3-CA1 synapses, because CA2 synapses do not readily undergo high frequency stimulation-induced potentiation (Pagani *et al.*, 2015).

All the above studies highlight the uniquely distinct and strategic region CA2 is, poised to modulate both CA3 and CA1 firing as well as their synchrony with theta rhythm, thereby heavily influencing place cell firing and spatial navigation mechanisms within the hippocampus. CA2 is also been suggested as the intrinsic oscillator in hippocampal slices influencing theta oscillations, firing and synchrony, thereby impacting learning and memory capacity. In addition, CA2 neurons appear to

be electrically coupled via gap junctions (in slice cultures) and this coupling is enhanced by charbacol (muscarinic receptor agonist), commonly used to evoke theta oscillations (Fischer, 2004).

1.7.1 CA2 as a distinct sub-field of the hippocampus:

CA2 has long been regarded as an anomaly : sometimes as a separate region and sometimes as part of either CA1 or CA3, since Timm's staining of mossy fibres does not end at the CA3/CA2 boundary but tapers off towards the CA1 border and morphological features that some CA2 cells share with CA1 and/or proximal CA3 (Bartesaghi & Ravasi, 1999). But with CA2 being recognized as an important neuromodulatory region of the hippocampus, studies have attempted to re define CA2 as a distinct anatomical structure as well. The boundary of CA2 has been defined via in situ hybridization expression of pcp4/ purkinje cell protein 4 along the entire hippocampus. Also, the extent of CA2 defined on the basis of this gene expression is larger than that defined before, especially at the rostral pole of the hippocampus (Lein *et al.*, 2005). This provides a robust evidence for differential molecular expression and genetic boundaries between all sub-fields of the hippocampus. This study demonstrated that mouse CA2 is a thin sandwiched region with non-overlapping regions with CA1 and is of uniform width throughout the rostro-caudal extent of the hippocampus.

Anatomically, CA2 pyramidal neurons closely resemble CA3 neurons in size and dendritic branching patterns (Lorente de Nò, 1934; Woodhams *et al.*, 1993; Ishizuka *et al.*, 1995), but share with CA1 features that include the lack of large, thorny spines

characteristic of the mossy-fiber synapses (postsynaptic thorny excrescences) from the dentate gyrus (Lorente de Nò, 1934). CA2 cells have a larger somata than that found in CA1 and CA3, and also possess a unique branching pattern of apical dendrites (Mercer *et al.*, 2007).

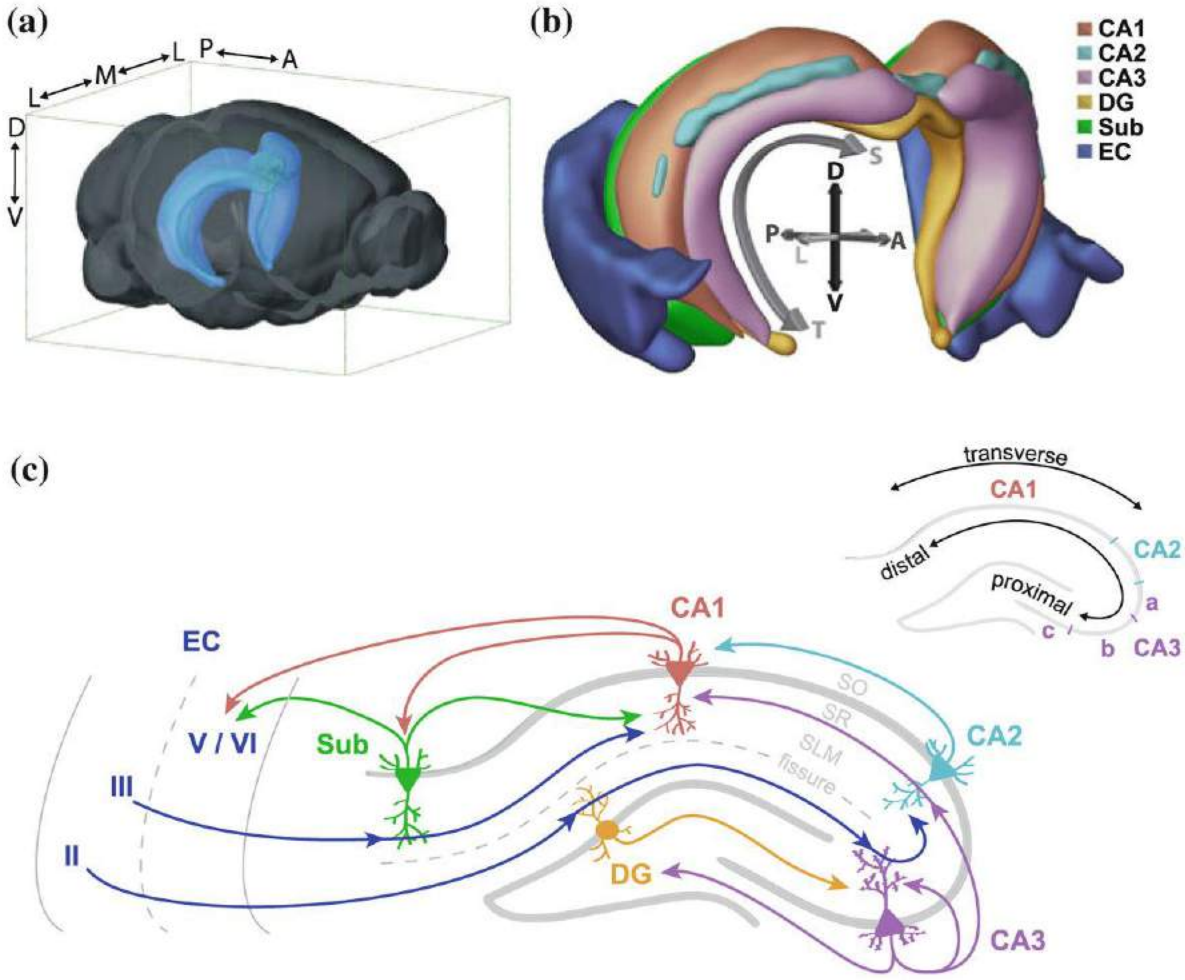


Figure 1.8: Anatomical organization in the hippocampus, including CA2

(Sosa *et al.*, 2016):

a. Relative location of the hippocampi within the mouse brain. Blue structures highlight the hippocampus proper (CA1, CA2, CA3, and DG) in each hemisphere. The geometry of the hippocampus is very similar in the rat brain. D dorsal, V ventral, A anterior, P posterior, M medial, L lateral.
b. Three-dimensional organization of the hippocampal formation and entorhinal cortex. The curved arrow delineates the septotemporal axis (S septal, T temporal). (Lein *et al.*, 2007)
c. The hippocampal circuit. Major projections into and within the hippocampal circuit are depicted here, following as closely as possibly the true trajectory of axons through the hippocampal layers (SO stratum oriens, SR stratum radiatum, SLM stratum lacunosum moleculare). For example, EC projections target distal apical dendrites of CA1, CA2, and CA3 neurons in SLM, while CA3 targets the proximal apical dendrites of CA2 and CA1 neurons in SR. Arrows represent synapses, but are not weighted by strength. Dotted grey lines represent a subset of layer boundaries, including the hippocampal fissure and the boundary between EC layers II/III and V/VI.

1.7.2 Distinct bilateral innervations of CA3 and CA2 on CA1 in Long Evans:

In Long Evans, tracer studies have revealed that CA1 is differentially innervated by CA3 and CA2, thereby providing strong evidence that CA2 has a strong and independent modulatory effect on CA1 firing. While CA3 and CA2 both strongly project to CA1, they have very distinct bilateral innervations: For CA3-CA1projection, while dorsal CA1 SR receives heavy ipsilateral projections, SO receives heavy contra lateral projections. The CA2-CA1 projection is heavily ipsilateral in both CA1 SO and SR, although it is much stronger in SO. These innervation projections may influence differential information processing in apical and basal dendrites of CA1. The strong CA2-CA1 SO innervations, that is close to SP may be a mechanism for strong synaptic inputs from CA2 (Shinohara *et al.*, 2012).

1.8 CA2 as part of an independent disynaptic circuitry in the hippocampus:

Till recent times, CA2 has been considered a mere 'transition zone' between CA3 and CA1 and an unimportant, non-independent part of the hippocampal-parahippocampal circuitry, with nothing new to offer on its own. But the recent barrage of studies show that CA2 is not only a structural, functional and genetically distinct part of the hippocampus, but plays a very important role in hippocampal dependant spatial memory (Brun, 2002; Nakashiba *et al.*, 2008), through a strong disynaptic circuitry from EC - CA2 - CA1 (Chevaleyre & Siegelbaum, 2010), which is independent and parallel to the DG-CA3-CA1 trisynaptic circuit; that helps preserve the firing properties of CA1, even in the absence of CA3 inputs.

It has been previously shown that dorsal psalterium volleys to EC layer II not only elicit activation of DG, CA3 and CA1, but parallelly stimulate CA2 neurons as well (Bartesaghi & Gessi, 2004), thereby mediating direct transfer of EC signals to hippocampus (CA1) without the involvement of CA3. In addition, CA2 neurons undergo a reverse synaptic strength rule: they are strongly activated by distal dendritic input from superficial layers of EC, but only weakly activated by proximal input from CA3. High frequency stimulation of EC layer III stimulation results in strong excitatory synapses on CA2, followed by a late EPSP and polysynaptic response in CA1. In contrast, moderate intensity stimulation of EC III only results in a small, direct EPSP in CA1 (SLM) (Temporoammonic pathway). Therefore, CA2 makes strong unitary, excitatory connections with CA1 (basal dendrites in SO) and is capable of eliciting CA1 firing. CA3 inputs to CA2 on the other hand, recruit strong feedforward inhibition, mediated by EC layer II input. (Chevaleyre & Siegelbaum, 2010). This inhibition is likely important for gating information flow in the hippocampus. Because this strong inhibition is initially engaged by the same input that directly activates CA2 (layer II input), it will preserve independence between the di- and the triynaptic loops. The strong inhibition might also terminate CA2 neuron firing after its initial activation by the disynaptic loop, and thus prevent excessive activation of CA1 neurons.

Therefore, the EC, CA3, CA2 and CA1 interact and cross talk amongst themselves such that independent loops within the hippocampus exist to control information flow during different behaviours and situations, without compromising on hippocampal mediated spatial learning and memory.

In addition, CA2 is the primary target (and the only hippocampal target) of glutamergic inputs from hypothalamic SuM (Maglóczky *et al.*, 1994), which is known to increase frequency and amplitude of hippocampal theta oscillations (Kirk & McNaughton, 1993), thereby making CA2 play a probable pivotal role in modulating CA3 and CA1 place cell firing with respect to theta synchrony via its feedforward and feedback circuitry within the hippocampus. In addition, Substance P – expressing SuM efferents target only CA2 in rats (Borhegyi & Leranth, 1997), and bath applied substance P has been shown to enhance CA1 responses (Langosch *et al.*, 2005). Substance P also enhances local inhibitory responses in hippocampus (Ogier & Raggenbass, 2003) and EC (Stacey *et al.*, 2002) by increasing GABA release.

Thus, CA2 receives convergent inputs form layer III (theta phase locked border, grid and head direction cells) and layer II (theta phase locked grid and border representation) to integrate spatial, directional motion and border information to influence CA1 place cell firing accordingly (Jones & McHugh, 2011).

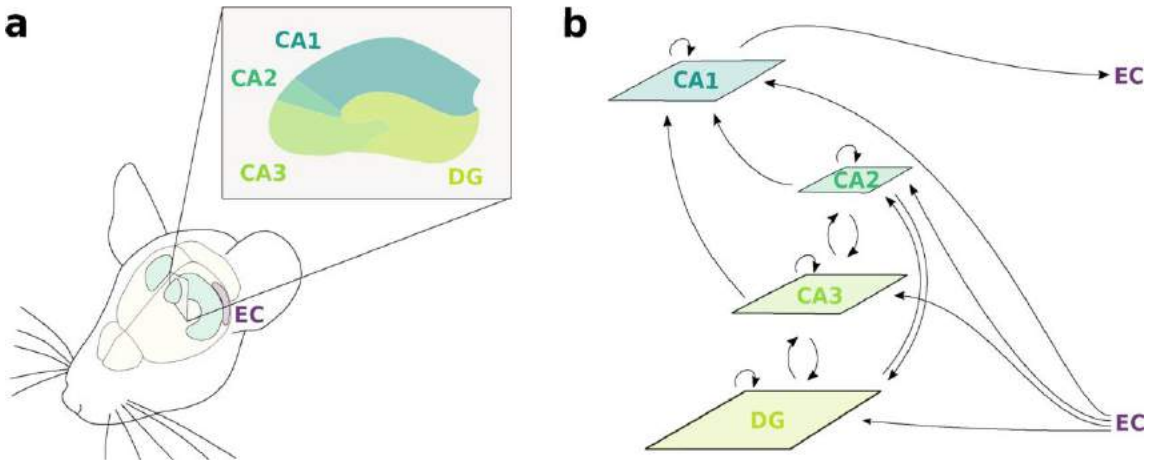


Figure 1.9: CA2connections
a. CA2 has a central role in the hierarchy of recurrently connected hippocampal subnetworks.
b. CA2 sends excitatory projections to all hippocampal regions and receives input from EC and all regions except CA1 (Lehr *et al.*, 2021)

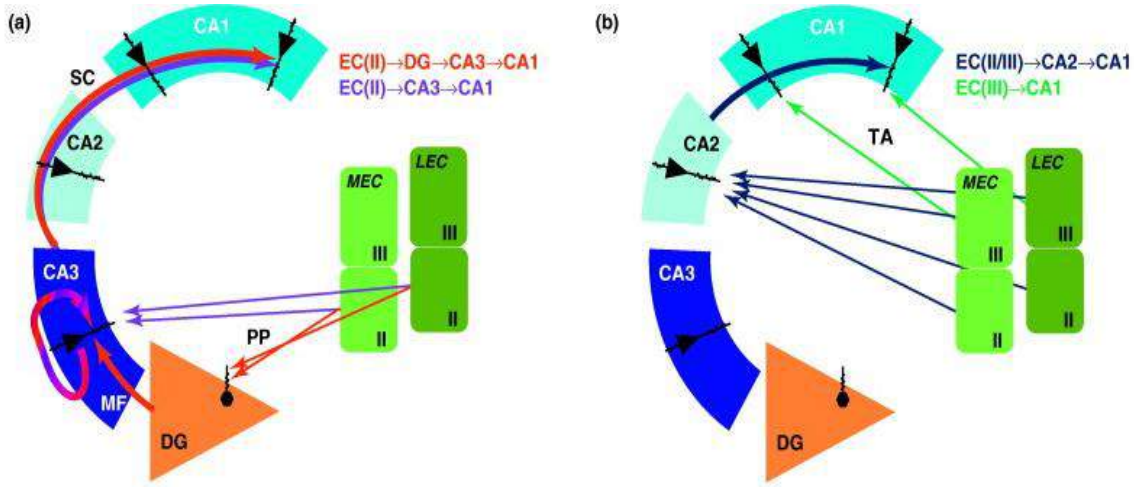


Figure 1.10: Multiple circuitries within the hippocampal sub-fields
a. the classical trisynaptic loop
b. the recently discovered disynaptic loop, involving CA2 (Jones & McHugh, 2011)

1.9 CA2 morphology:

1.9.1 CA2 and CA1 connectivity:

CA2 and CA1 pyramidal neurons share very distinct morphological features, branching and connectivity, leading to marked differences in synaptic integration and outputs. Even though CA2 and CA1 pyramidal neurons are targeted by common inputs at similar dendritic locations, the dendritic integration is very different in these 2 classes (Piskorowski & Chevaleyre, 2012).

CA1 pyramidal neurons have a single apical dendrite that usually does not bifurcate, or bifurcates relatively far from the soma. Numerous secondary oblique dendrites arise from the apical dendrites in the SR but only a few branches are present in the SLM. In comparison, the apical dendrite of CA2 neurons bifurcates close to the soma into two or three apical dendrites that extend to the SLM. Also, in contrast to CA1, very few secondary oblique dendrites are observed in the SR, but many branches are present in the SLM. While the basal dendrite length of both neurons is 35% of total dendritic length, apical branching is very different for the two. 47% of total dendritic length is present in SR for CA1 neurons, while CA2 neurons have only 31% present in this region. Conversely, 31% for total dendritic length is present in SLM for CA2 but only 19% for CA1.

This difference of predominant branching in different layers between these neurons seems to suggest preferential connections for different inputs and influence on pyramidal firing patterns. The abundant branching of apical dendrites of CA2 neurons close to cell body may contribute to larger EPSPs recorded at CA2 soma while

eliciting distal inputs. Since back-propagating somatic spikes are necessary for spike timing-dependant plasticity, the more proximal bifurcation of the CA2 apical dendrite may lead to a dampening of the spike and thus may restrict the extent of the plasticity evoked along the dendrite.

1.9.1.1 Compartmentalization of Excitatory inputs:

The proximal dendrites of both CA1 and CA2 receive local inputs while the more distal dendrites contain synapses with axons originating from the cortex.CA1 basal dendrites share connections with axons from neighbouring CA2 neurons and with axons that project from contra lateral CA3 pyramidal neurons. In the SR, Schaffer collaterals from CA3 form synapses onto the proximal region of the apical dendrites of both CA1 and CA2 neurons. CA2 also sends axons to the same region onto CA1 (in addition to targeting the basal dendrite of CA1). In addition, both basal and apical dendrites of CA2 neurons are likely targeted by ipsilateral CA2 axons. Finally, the distal tuft of the apical dendrite of both CA1 and CA2 is connected by cortical inputs. However, CA1 neurons only receive inputs from layer III of the entorhinal cortex, while both layer III and layer II connect CA2 neurons. They are the first to generate responses (highest EPSP responses) to layer II stimulation in comparison to both CA1 and CA3; and to layer III in comparison to CA1. Also, CA2 receives inputs on its distal dendrites from thalamic nucleus reunions and from medial hypothalamic supramammillary nucleus on its soma (glutamergic inputs).

The difference in dendritic branching of both classes of neurons may be responsible for larger EPSPs in CA2 v/s CA1 neurons. The CA1 dendrites attenuate distal EPSPs

by a factor of 50-100 at the soma, and could be due to oblique branching from CA1 apical dendrites in SR, that exhibit a shunting effect. Also, HCN1 and 2 and A-type potassium channels are expressed in an increasing gradient from proximal to distal dendrites along CA, these conductances are known to dampen EPSP amplitude/duration. On the other hand, due to more distal apical branching as it approaches the tuft, in CA2 neurons, attenuation is not more than 7 fold or 8 fold. Higher branching in SLM also ensures that a cortical axon synapses more on CA2 than CA1 neuron. This allows for different synaptic signals to summate onto the single common branch of CA2 as signal approaches the soma, resulting in a larger EPSP. Also since oblique branching in SR is very less, and expression of HCN channels low, there is minimal shunting effect.

1.9.1.2 Compartmentalization of Inhibitory inputs:

Numerous interneurons innervate hippocampal pyramidal cells and influence their firing patterns. OLM cells target the apical tuft of pyramidal neurons and most likely affect distal excitatory synapses onto the neurons. Conversely, bistratified cells target basal and proximal apical dendrites and influence proximal inputs more. Basket cells influence the probability of the pyramidal cell firing because they target the perisomatic region of the cell.

In humans, CA2 has the highest density of interneurons, as well as the highest number expressing parvalbumin, in comparison to rest of the hippocampus. In rodents, interneurons expressing somatostatin are highest in CA2. CA2 also has a stronger inhibitory drive, because the density of connected interneurons to pyramidal cells is more in CA2.

CA3 excitatory inputs to CA1 increase in amplitude after blocking inhibition, whereas they are smaller in amplitude in CA2. But under intact inhibition, EPSPs are completely blocked by IPSPs in CA2. Inhibition even prevails over excitation in proximal CA2 dendrites. The very large feed forward inhibition in CA2 prevents any excitatory inputs from CA3 to affect CA2 firing. On the other hand, a large feed forward inhibition in CA1 (SLM) is seen following cortical input excitation, which completely blocks EPSPs. In CA2 however, even though the amplitude of inhibitory input is equal to that in CA1, because of larger cortical drive to CA2, this inhibition is masked and excitation prevails over inhibition over fourfold. Thus, when inhibition is blocked, EPSPs evoked by distal cortical inputs are as large as those by proximal SC inputs to CA2. But in intact inhibition, while cortical inputs are affected only mildly, SC inputs are completely masked and nullified.

Thus, despite having similar inputs in their proximal and distal compartments, their processing in very different due to different branching patterns in SR and SLM, and due to exclusive inputs to both CA1 and CA2, indicating that CA1 and CA2 participate in different hippocampal circuitries and modes of information processing and memory mechanisms.

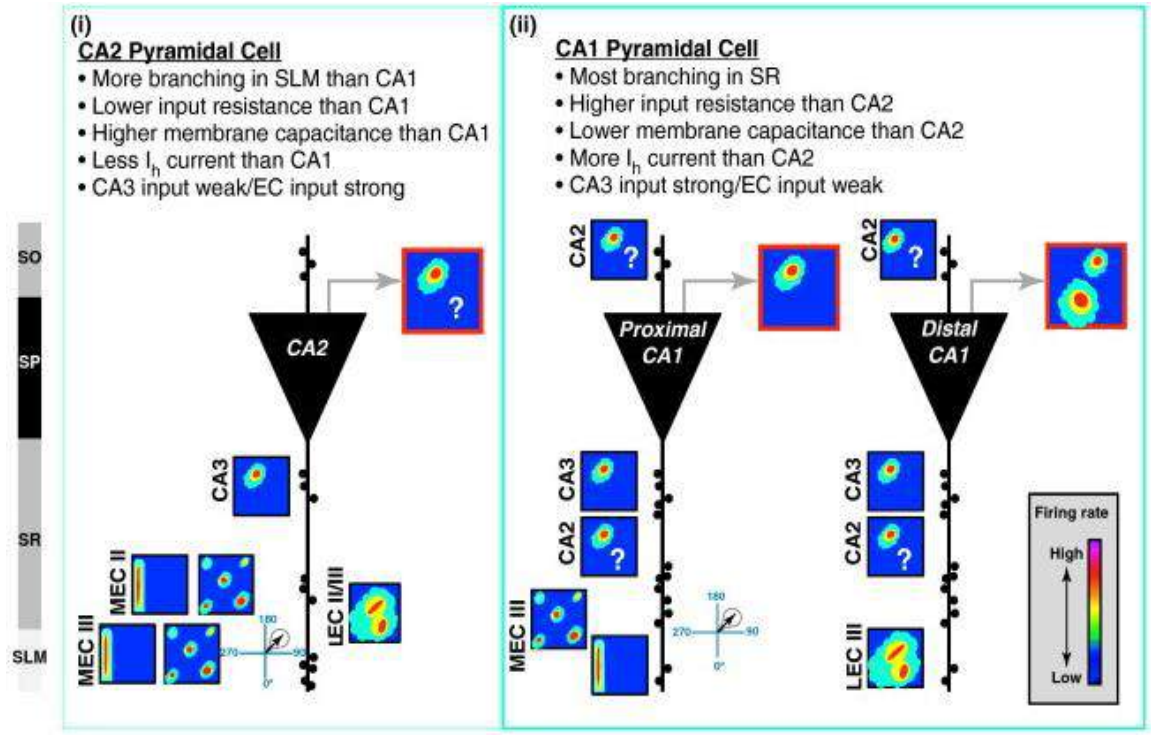


Figure 1.11: Various differences between CA1 and CA2 pyramidal neuron properties, making the two sub-fields structurally and functionally distinct (Jones & McHugh, 2011)

1.10 Plasticity mechanisms in CA2:

One of the most distinctive features of hippocampal CA2 is that the pyramidal neurons have an unprecedented synaptic stability, making it one of the few "plasticity resistant" areas of the brain, being resistant to normal LTP and LTD induction protocols, which is the very trademark of hippocampal memory mechanisms.

While LTP cannot be generated in CA2 entirely in rat and cannot be stabilized in later time points in mouse, LTD induction is possible in some but not all neurons, indicating that CA2 pyramidal cells are a heterogeneous population. CA2 neurons also have larger leak currents and more negative resting membrane potential than CA1 neurons, thereby making it more difficult to generate action potentials.

The lack of LTP in this area may be influenced by the following molecules, known to be present abundantly in CA2, but not CA3 or CA1: A1 adenosine receptors, extracellular matrix proteins, distinct oligodendrocytes and astrocytes, calbindin, TREK1 and TREK2 channels, STEP and lack of β integrin subunit. On the other hand, induction of LTD in some neurons may be a result of differential expression of chromogranin A and vasopressin 1b receptor in CA2 neurons (Zhao *et al.*, 2007). The presence of many molecules known for downregulating plasticity are enriched in this region e.g. – chondroitin sulphate proteoglycan, (Brückner *et al.*, 2003) which is an extracellular matrix component that increases during development and is regulated by visual experience. Destruction of this matrix reactivates plasticity at ages beyond the 'critical period' and increase dendritic spine motility (Oray *et al.*, 2004).

However, not all forms of synaptic plasticity mechanisms are absent or inhibited in CA2. Short term facilitatory mechanisms are intact in CA2 and can be induced by both paired-pulse facilitation and short term post tetanic potentiation of SC inputs (Zhao *et al.*, 2007). LTD is also occasionally induced in CA2, as opposed to complete lack of LTP, and may be due to low levels of calcium intracellularly but with a wide range, sufficient for LTD (Feldman, 2000). Additionally, inputs from layers II and III of EC that form synapses on distal apical dendrites of CA2 cells are highly plastic (Chevaleyre & Siegelbaum, 2010), indicating that strong regulation of CA2 output by EC may influence CA1 firing accordingly.

More importantly, the apparent lack of plasticity of SC inputs to CA2 can be reversed by application of certain potent molecules such as adenosine A1 receptors (Ochiishi *et al.*, 1999), vasopressin 1b receptor (Avpr1b gene) (Pagani *et al.*, 2015), and even caffeine (Simons *et al.*, 2012).

Calcium buffering and extrusion in CA2:

It has been shown that evoked transients of calcium are significantly smaller in CA2, with the average change in free calcium being only approximately 227nM in is spines. This indicates that CA2 pyramidal neurons have a significantly higher endogenous rate of calcium buffering as well as calcium extrusion. This tight regulation of endogenous calcium can account for lack of LTP in these neurons under basal conditions. Increasing calcium extracellularly permits LTP induction to a similar magnitude as that of SC inputs to CA1. Therefore, while NMDA dependant LTP

induction machinery is present in CA2, tight calcium regulation prevents this synaptic plasticity mechanism. (Simons *et al.*, 2009).

Role of RGS – 14:

CA2 is also rich in another protein: RGS-14, which is a scaffolding protein, that integrates G protein and H-ras/ERK/MAP kinase pathway, making it a key molecule in regulating synaptic plasticity in CA2 neurons. It is known to play a role in both suppression of plasticity and hippocampal learning and memory. RGS14 KO mice show enhanced spatial learning and novel object recognition. RGS 14 is found in CA2 soma and neurites that project to fasciola cinerea (FC) through SLM and SR layers, and in CA2 dendritic spines. It blocks synaptic plasticity by suppressing the MAP kinase pathway in CA2 neurons, leading to loss of LTP and limited induction and expression of synaptic potentiation (Lee *et al.*, 2010). Thus, the presence of high rates of calcium buffering and extrusion, along with presence of RGS14 in CA2 pyramids, leads to LTP blockage.

Role of Caffeine:

Caffeine, known to be adenosine receptor antagonist, is known for its role in attention and cognition. It improves mental acuity by blocking the normal inhibitory functions of adenosine. A1Rs are richly found in CA2, and a brief 5-min application of a physiologically relevant concentration of caffeine or other selective A1R antagonist induces a lasting potentiation of synaptic transmission in CA2, but not in areas CA1 or CA3. Even small doses applied on hippocampal slices induce a long lasting and potent effect. It is also accompanied by an increase in volume of CA2 pyramidal cell dendritic spines (secondary and tertiary), indicating that the effect is post synaptic.

Moreover, even when physiologically relevant doses of caffeine were administered orally to rats, it led to potentiation of basal synaptic responses in CA2. This was achieved as a result of modifications at synapses because neuronal excitability remains unaffected under high doses of caffeine (Simons *et al.*, 2012). This potentiation is not dependant on calcium levels, but is sensitive to adenyl cyclase 5 and 6 which are high in CA2, cAMP activation of PKA levels during acquisition and Ras/MAP pathway for stabilization (Lein *et al.*, 2007).

Role of vasopressin:

Vasopressin is known to play a role in aggression and social memory. While V1a receptor is abundant in DG, CA3 , CA2 and CA1, Vib is exclusively found in dorsal CA2 (Young *et al.*, 2006), and is known to play a role in potentiating synapses at CA2, while enhancing social recognition memory and social novelty tasks. Avpr1b KO mice also show memory impairment for temporal order of tasks involving 'what-when-where' tasks. They are unable to make temporal associations in tasks as well (Dere *et al.*, 2005b; DeVito *et al.*, 2009). Recently it has been reported that vasopressin selectively inhibits the EC – CA2 – CA1 disynaptic pathway by suppressing LTP potentiated EPSPs, while unaffecting the SC- CA1 pathway (Chafai *et al.*, 2012). AVP decreased on EPSPs and not IPSPs in CA2 neurons. Also, AVP could attenuate both SC and EC layer III evoked EPSPs in CA2 but not CA1.

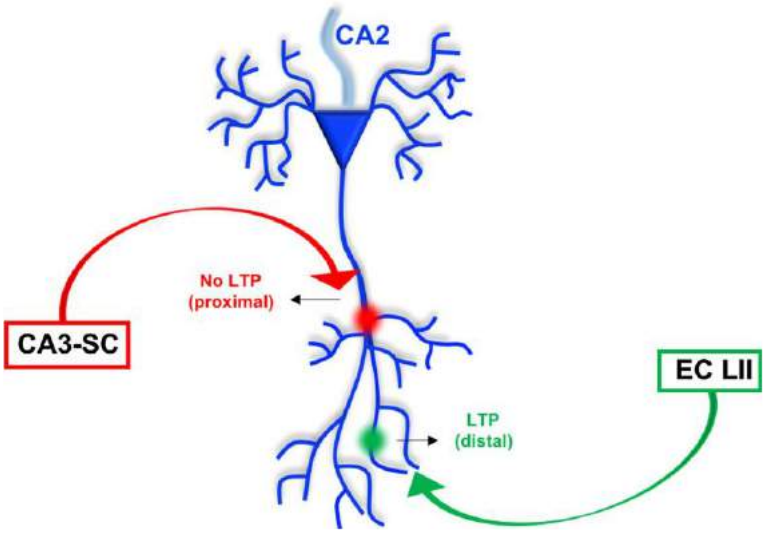


Figure 1.12: LTP mechanism in CA2
A unique LTP gradient along the proximal–distal axis of CA2, that is resistant at SC junction proximally but not at EC input distally (Benoy *et al.*, 2018)

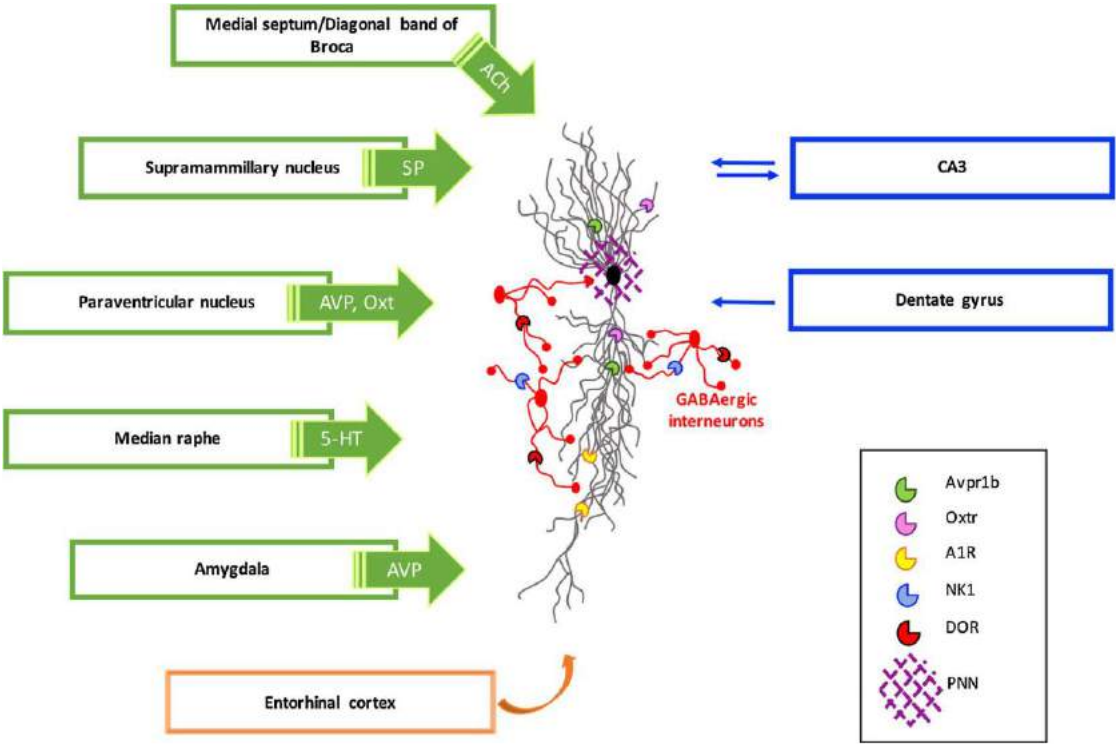


Figure 1.13: Various neuromodulatory inputs to CA2 (Benoy *et al.*, 2018)

1.11 Probable bio markers for CA2 staining:

A large number of molecules are also found to be selectively found only in CA2, but not CA3 and CA1, further cementing evidence for CA2 as a functional distinct region. Eg: Fgf2 (Williams *et al.*, 1996), adenosine A1 receptors (Ochiishi *et al.*, 1999), Egfr (Tucker *et al.*, 1993) are exclusively found in CA2 while Ntf3 (Vigers *et al.*, 2000) and Pcp4 (Zhao *et al.*, 2001) are expressed in CA2 and DG but not CA3 and CA1. Therefore, owing to their differential expression in hippocampal CA2, the following molecules may be used as a probable biomarker for the region:

Pcp4- purkinje cell protein 4

Rgs14- regulator of G-protein signalling 14

Avpr1B – arginine vasopressin receptor 1B

Timm's staining- CA2 does not stain due to lack of innervations from DG

α actinin 2 protein – immunoperoxidase technique

CACNG5

TARP – γ

Adenosine A1 / vasopressin 1b receptor antagonists

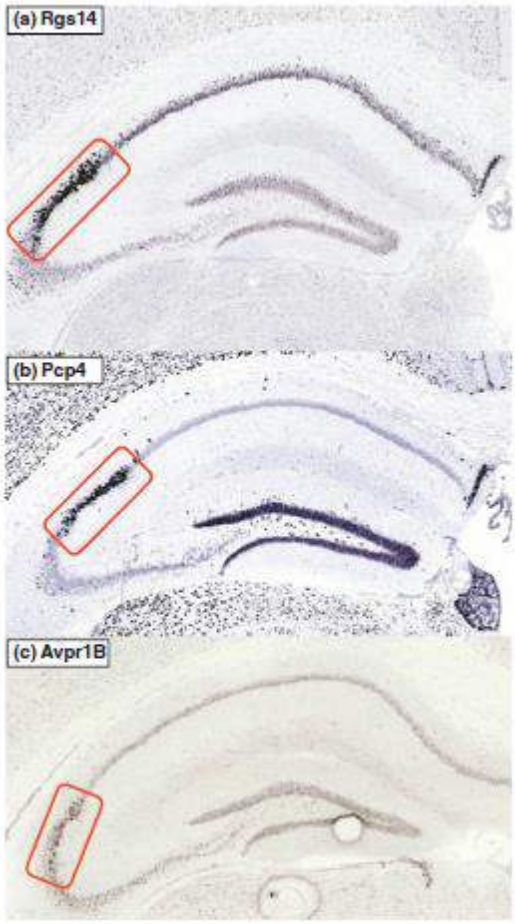


Figure 1.14: CA2 bio-markers
In situ hybridization of various genes expressed only in the CA2 sub field of mouse hippocampus. (Lein *et al.*, 2007; Jones & McHugh, 2011)

1.12 CA2 in disease:

Owing to morphological, anatomical and molecular differences in CA2 from its neighbouring regions, various changes become apparent immediately after a pathological event and support the notion that the CA2 neurons fundamentally differ from CA1 and CA3 neurons in cell excitability, calcium buffering, and/or phosphatase activity (Zhao *et al.*, 2007). It is resistant to temporal epilepsy and may be involved in the onset of schizophrenia (Benes *et al.*, 1998).

Epilepsy:

CA2 is spared in several models of hypoxia/ischemia (Kirino, 1982; Sadowski *et al.*, 1999) and is resistant to a variety of ischemia related insults such as cell loss, relative to other hippocampal subfields. This may be due to the abundance of adenosine A1 receptors in CA2 and their anti-convulsion properties. Also, cell loss in CA2 is de-correlated to cell loss in DG, indicating they are part of separate independent loops (Jones & McHugh, 2011). A study of human epileptic CA2 region has shown that it generates a spontaneous interictial like activity, that is similar but independent to that generated in subiculum. There is preservation of somatic inhibitory input to CA2 cells and loss of PV from interneurons rather than their death (Wittner *et al.*, 2009).

Schizophrenia:

CA2 possesses a unique pathology in schizophrenic patients (Benes *et al.*, 1998; Benes & Todtenkopf, 1999). The number of non-pyramidal (inhibitory) neurons decreases in CA2 individuals with schizophrenia, and caffeine worsens symptoms of psychosis. A profound loss of PV +ve immunoreactivity from CA2 interneurons is seen here. Schizophrenia as well as bipolar disorder is associated with a selective

decrease in density and number of non-pyramidal cells of CA2 (Benes *et al.*, 1998). These patients show both lack of social behavior and episodic memory. Selective decreases of CA2 inhibition, leading to CA1 hyper excitability is also seen in this disease (Chevaleyre & Siegelbaum, 2010).

Alzheimer's Disease:

CA2 neurons of normal aged humans without Alzheimer's disease often contain large, lipofuscin-containing deposits not seen in other CA areas (Braak H, 1980), suggestive of distinct protein or lipid metabolism in this area. CA2 volume reduction (CA1/2 border) maybe the distinguishing factor between AD and MCI, as a selective reduction has been seen in MCI, and indicated involvement of CA2 in cognitive processing (Jones & McHugh, 2011).

1.13 Importance of studying CA2:

1.13.1 Contribution of CA2 in hippocampal circuitry dynamics in spatial learning and memory:

Recent literature, has pointed out that the DG-CA3-CA1may not be the only circuit responsible for place cell firing and spatial memory, since CA1 activity still persists even when their CA3 inputs have been disrupted (Brun, 2002; Nakashiba *et al.*, 2008).With the additional understanding of the disyanptic circuit as a separate functioning hippocampal loop, CA1 output can be viewed as result of differential modulation via CA3 and EC inputs (and CA2). In-vivo stimulation of the inputs to

EC can trigger the initial firing of spikes in the CA2 region, followed by CA1 and then CA3 (Bartesaghi & Gessi, 2004), suggesting that CA2 neurons may mediate a disynaptic link from EC to CA1 (Bartesaghi *et al.*, 2006).

With the advent of CA2 as part of a distinct, independent and important sub field of the hippocampus, the role of various hippocampal circuitries and their contribution to spatial memory needs to be re visited. The establishment of CA2 as a separate region comprising distinct genetic, molecular, structural and functional profile from both CA3 and CA1, makes it imperative to study the physiological properties of CA2 in vivo and to distinguish its unique contribution to hippocampal dependent learning and memory mechanisms with respect to spatial memory. Consequently, the classic 'trisynaptic loop', comprising of DG, CA3 and CA1, known to be responsible for main pathway for the information processing in the hippocampus (Amaral & Witter, 1989) needs to be updated with the inclusion of CA2.

To summarize, CA2 is a truly unique region because:

1. It is the only region of the hippocampus that receives a direct, glutamergic input from medial hypothalamic supramammillary nucleus, which is responsible for hippocampal theta activity (Maglóczky *et al.*, 1994), which has a significant role to play in spatial learning an navigation.
2. It gets convergent excitatory inputs from both layers II and III of EC, thus coalescing spatial and non-spatial information from EC (Chevaleyre & Siegelbaum, 2010).

3. It makes strong unitary excitatory connections with CA1, independent of the trisynaptic circuit.

4. It follows a 'reverse synaptic rule' and is excited by EC inputs on its distal dendrites as opposed to SC inputs from CA3 on its proximal dendrites.

5. It defies the very hallmark of hippocampal plasticity and is one of the very few 'plasticity resistant' areas of the brain that fails to undergo LTP under normal conditions. (pts 2-5: Chevaleyre & Siegelbaum, 2010).

6. It is the most apt area to control and influence flow of information within the hippocampus via feed forward inhibition of CA1 and feedback inhibition of CA3.

1.13.2 Possible roles of CA2 in learning and memory:

1. Novelty detection/ novel recognition memory

When the rat enters a new environment, CA1 firing is initially driven by EC (through CA2), but a rapid shift from EC to CA3 occurs, such that eventually CA3 spatially tunes CA1 cells for stable spatial representation (Frank *et al.*, 2000). Thus, there are indications that the temporoammonic / disynaptic pathway is important for encoding of novel information, and functions as an independent circuit for mnemonic processes, which is capable of spatial recognition memory (Brun, 2002). Although CA3 NMDARs are crucial for rapid acquisition of one-trial memory of novel environment (Nakazawa *et al.*, 2003), their ablation does not block encoding of novel environment. Therefore, while encountering a novel environment, CA2 may play a bigger role in influencing CA1 place cell firing as opposed to CA3 at initial time points.

2. Memory for temporal order

Knock out studies in mice have revealed that vasopressin 1b receptor plays a role in aggression and social memory. Mice lacking this vasopressin receptor show impairments in temporal components of object recognition and paired association tasks. They cannot distinguish the order in which they encounter objects, although their object recognition memory is intact. They cannot compare events that occurred at different times, nor can they make association between two different events occurring at different time points. Thus on a 'what-when-where' task, they do not score well on the 'when' part of the task. CA2 is the only hippocampal region that is rich in this receptor and hence may influence temporal processing in episodic memory. CA2 may consequently play an important role in modulating the input of delayed information (slower time signals) to CA1, as displayed by the inability to perform memory tasks involving a memory signal over a delay period (DeVito *et al.*, 2009).

3. Social recognition memory

Knockouts for vasopressin receptor 1b also show impairment in social memory and preference for social novelty. Mice vasopressin 1b KO cannot discriminate between their own littermates versus novel mice and are thus less attracted to social stimuli. Hence they do not recognize their own littermates and are not differentially attracted to novel social stimuli (DeVito *et al.*, 2009).

4. Spatial learning

While vasopressin affects social recognition and temporal memory, vasopressin KO do not show any deficits in spatial learning and navigation and are equally exploratory in behaviour in comparison to their normal counterparts. Interestingly, CA2 is rich in another gene: RGS14, the deletion/blocking of which leads to enhanced spatial learning as well as object recognition memory. Both the absence of RGS 14 and stimulation of Avpr 1b permits induction of plasticity at SC-CA2 connection, and while one shows no deficit in spatial learning, the other enhances spatial learning (Simons *et al.*, 2012; Pagani *et al.*, 2015)

Why study spatial novelty in CA1 and CA2:

Hippocampal place cells are the neural correlates of the internal GPS of the brain, and constitute an integral part of the cognitive map (O'Keefe & Nadel, 1978). The different subfields of the hippocampus (CA1, CA2, CA3 and DG) contribute differentially to update, represent and stabilize a continuously evolving spatial map of any given environment. A key role of spatial exploration is successful detection and encoding of novel stimuli (contextual or social) and integrating it with familiar stable spatial maps of an environment, leading to continual upgradation of stored memories.

Recent studies have shown that CA2 plays a role in social and novel contextual information processing (Hitti & Siegelbaum, 2014; Mankin *et al.*, 2015; Alexander *et al.*, 2016) and has a relatively flexible spatial code when compared to that of CA1 and CA3. In previous studies, CA1 has also been recognized a broadcaster of a novelty signal in the hippocampus and is essential for spatial refocused re-explorations (Larkin *et al.*, 2014) and a disruption of EC-CA1 pathway leads to impairment in spatial novelty detection (Vago & Kesner, 2008). The discovery of the novel disynaptic pathway (EC(II) to CA2 to CA1) (Chevaleyre & Siegelbaum, 2010; Kohara *et al.*, 2014) which is parallel and independent to the classic trisynaptic pathway (EC to DG to CA3 to CA1), makes CA1 uniquely poised to be neurmodulated to receive and assimilate spatial information through diverse circuitries within and beyond the hippocampus by both CA3 and CA2. Additionally, the mutual inhibitory relationship between CA3-CA2 that is controlled by feed forward circuitry (Chevaleyre & Siegelbaum, 2010; Kohara *et al.*, 2014) and limited plasticity (Zhao *et al.*, 2007) indicates a competition for active control of the

hippocampal circuitry. It also makes it clear that multiple independent circuitries co-exist within the hippocampus that are activated parallelly or alternatively for different functional optimization; with maximum influence being exerted on the most downstream subfield of the hippocampus i.e. CA1.

Each environment is represented by a unique ensemble of active place cells (O'Keefe & Dostrovsky, 1971). It is well documented that CA3 hippocampal ensembles orthogonalize neural representations, so as to maximize the number of experiences that can be stored within the same network (Leutgeb, 2004; Alme *et al.*, 2014) while CA1 ensembles are non-orthogonal and are overlapping in nature (Skaggs & McNaughton, 1998). How CA2 represents any environment, familiar or novel is yet unknown and remains to be seen.

While CA3-CA1 pathway has been extensively studied with respect to spatial navigation and memory, with some even looking at CA3-CA2 place cell topography (Lee *et al.*, 2015; Lu *et al.*, 2015); in vivo data demonstrating how CA1 and CA2 interact and contribute to spatial novelty detection, encoding and consolidation are sparse and few (Mankin *et al.*, 2015; Alexander *et al.*, 2016). Most of the studies have focused on either social or novel contextual changes in an environment (Wintzer *et al.*, 2014; Alexander *et al.*, 2016) or non spatial aspects of memory (Mankin *et al.*, 2015). Other studies have primarily focused on novelty detection and encoding in CA1 only, in an object-place recognition task (Larkin *et al.*, 2014), place field plasticity (Frank, 2004) or temporal coding and episodic memory (Mankin *et al.*, 2012).

With both CA1 and CA2 contributing to a spatial novelty detection signal in the hippocampus, it is imperative to tease out their specific contributions and functions

towards spatial mnemonic processes such as detection, encoding and consolidation for a better understanding of spatial navigation and memory mechanisms. For the same, a completely novel 3 day long paradigm was designed, whereby the animal was introduced to a completely novel (track + spatial cues) environment on the first day and to varying degrees of familiarity and novelty of the track in the same environment on the subsequent days, as the track from day1 was elongated further on both days 2 and 3. This allowed me to observe how a dynamically modifying environment would be encoded and consolidated in the hippocampus (CA1 and CA2), not only with respect to varying degrees of familiarity and novelty across days, but also within the same day (days 2 and 3), where the animal would experience relative novelty in the same lap itself.

CHAPTER 2

MATERIALS AND METHODS

2.1.1 Animal handling and surgical procedures

5-6 month old *Long evans* rats (n = 4, male) were housed individually on reversed light-dark (12:12 h) cycle and the experiments were carried out during the dark phase of the cycle. All surgical procedures were performed under aseptic conditions. All the procedures (animal care, surgical procedures and euthanasia) were performed in accordance with NIH guidelines and were approved by the Institutional Animal Ethics Committee (IAEC) of National Brain Research Centre at Manesar, Haryana, constituted by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

The rats were initially anaesthetized using ketamine (administered at 60 mg/kg body weight) and xylazine (administered at 8mg/kg body weight) and subsequently shifted to gaseous anaesthesia using isoflurane for the rest of the surgery. A custom-built recording device (Microdrive) contained inside a dual bundle, was made entirely from scratch in the laboratory, containing 20 independently movable tetrodes (each bundle consisting of 9 recording tetrodes +1 reference tetrode). This drive was then surgically implanted over the right hemisphere at 3.5-3.7 mm posterior to bregma and 1.7-1.8 mm lateral to midline; to simultaneously access different regions of the hippocampus (CA1 and CA2). (Figure2.1).

2.1.2 Post-surgical procedures and animal training

The rats were given a post-surgery recovery period of 7 days, where post-surgical care was provided by the experimenter. Following post-surgical recovery, the tetrodes were slowly advanced, targeting CA1 and CA2 regions of the hippocampus over a

period of 10-15 days, by keeping the rat on a pedestal next to the recording system. During this period, the rats were also trained in the adjacent behavioural room to run clockwise, seeking chocolate sprinkles placed at random locations on a centrally placed black circular track (10 cms wide, elevated 90 cms from floor level) for 30 min/day for 8-10 days. The behaviour room was covered with circular plain black curtains at its perimeter and had no other cues (proximal or distal) of any kind. During training and subsequent experimental recordings, the rats were maintained at 85% of their free feeding weights.

2.1.3 Electrophysiology and recording

17 μm platinum-iridium wire from California Fine Wire, USA was used to make the tetrodes and the tips of individual wires of these tetrodes were electroplated with platinum black solution (Neuralynx Inc., USA) to 100-150 kΩ with 0.2 μA current. Multi tetrode electrophysiological recordings were carried out using 96-channel data acquisition system (Digital Lynx 10S, Neuralynx Inc., USA) by amplifying the signals through a headstage preamplifier (Neuralynx Inc., USA). The microdrive was fitted with an EIB-27 board at its centre, which then connected to the headstage preamplifier HS-27, which was in turn connected to the commutator using HS-27 tethers. The commutator was connected with the recording cables to the 96-channel Digital 145 Lynx 10S data acquisition system (Neuralynx Inc., USA). The units were recorded against a reference electrode from that particular bundle of the dual – bundle microdrive, which was present in a cell-free zone in the brain (the 'silent zone' above the hippocampal pyramidal layer) by filtering the signal between 600 Hz and 6 kHz. Spike waveforms above the threshold were sampled at 32 kHz for 1 ms. Local field

potentials (LFPs) were recorded against a ground screw anchored to the skull above the frontal cortex, filtered between 0.1 Hz and 1 kHz, and continuously sampled at 4 kHz. The position and the head direction of the animal were tracked with the red and green LEDs attached to the headstages, which was captured through a color CCD camera that was mounted at the ceiling, central in position to the room (CV-S3200, JAI Inc, San Jose, USA) at 25 Hz.

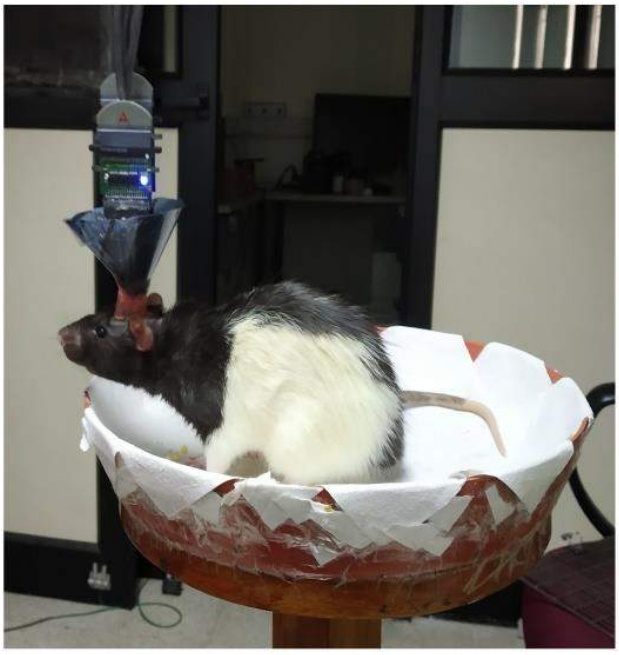


Figure 2.1: 18 + 2 dual bundle microdrive
18 channels plus 2 reference channels, each bundle containing 9+1 channels, made from scratch in the laboratory, implanted in the right hemisphere of *Long evans* rat.

2.1.4 Histological procedures and identification of recording sites

After successful completion of the electrophysiological experiments, marker lesions were performed on few selected tetrodes by passing current at 10 μA for 10 seconds. Rats were transcardially perfused the next day with 4% formalin solution, the brain was extracted and stored in 30% sucrose-formalin until it sank in the solution. These brains were then sectioned in the coronal plane (40 μm thick), mounted, and stained with Nissl's staining using 0.1% cresyl violet. Images of serial sections were captured on Leica DFC265 digital camera attached to Leica M165-C stereo microscope and saved as TIFF files. Sections showing clear tetrode traces in CA1 and CA2 were photographed again on Olympus microscope (BX51) for better clarity and resolution at a higher magnification (4X). Images were acquired using the virtual slide scan module from StereoInvestigator software. The distance from midline to the tetrode track markings were measured from these serial sections, plotted in excel spreadsheet to visualize the configuration of tetrode tracks. The tetrodes were identified by comparing this configuration with the actual arrangement of tetrodes in the microdrive, and cross verifying with the marker lesions. Depth reconstruction of the tetrode tracks was performed to identify the brain region at which the cells were recorded on each day, based on the distance from the bottom tip of the tetrode by taking into account 15% shrinkage of tissue due to histological processing.

2.2 Experimental procedures

Once the tetrodes reached the desired recording sites, and the animal was sufficiently trained to run clockwise, the experiment began. During the entire length of the experiment, the tetrode positions were not adjusted or moved by the experimenter. The animal was only introduced to the experimental environment on the day of the experiment, hence the paradigm was completely novel for the animal on day 1.

At the beginning of the experiment, 4 distinct shaped visual cues were hung over the black curtains at 90 degrees to each other. A large 1X1 foot black square platform was kept in the centre of the room, elevated 90cms from the ground, over which all the differently shaped, closed loop plain black tracks (10cms wide) were subsequently introduced each day, all elevated at 15 cms from the square platform. On day 1, a small square black track (45X45 cms) was kept on the lower left quadrant of the square platform. This track was divided into 4 equal length arms: arm1, arm2, arm3, arm4. The corner junction of arm3 and 4 was covered with yellow sandpaper (10cmsX10cms), which served as the location of reward for the rat (a single chocolate sprinkle) each time it completed a lap. The position of this corner was at the centre of the behaviour room, and remained constant throughout the length of the experimental paradigm (Figure 2.2-day1). On day2, the square track from day1 was elongated into a rectangle shaped black track (45X80cms), such that the track now occupied the lower and upper left side of the square platform (2 quadrants). The track was further divided into 6 arms: arms1,2a and 4b were the same arms from day1 track, while arms 2b, 3 and 4a were the additional/novel arms on day2. The reward corner, between arms 4a and 4b, was at the same physical location i.e. centre of the room (Figure2.2-day2). On the 3rd day, the rectangular track from day 2 was elongated to extend to the top right quadrant of the platform and formed a L-shaped track (3 quadrants). This

track was divided into 8 arms: arms 3b,4 and 5 were the additional/novel arms, arms 1,2a and 6 were the same arms from day1 track and arms 2b and 3a were the 2 remaining arms from the novel arms of day2. The position of reward area, now at the junction of arms 5 and 6 was again at the same location as previously mentioned (Figure 2.2-day3).

All days had 4 sessions of run, consisting of 20 clockwise laps each (except day 1 of 1 rat, which had 3 sessions). This novel paradigm was designed such that at each elongation of the track, from day 1 to day 2 to day 3, the rat took a longer route to complete each lap. This was done so that the rat experiences a completely novel environment on day 1 (0%familiarity, 100% novelty) and varying degrees of familiarity and novelty on day 2 (50% familiarity, 50% novelty) and day 3 (67% familiarity and 33% novelty) of track environment. Except this change in the length and thus, the shape of the environment, the relative position of distal visual cues, the position of reward corner, the entry point of rat on track (arm1) and the direction of running (clockwise) all remained constant throughout the experiment. Once the tetrodes reached the hippocampal layer, their position were not disturbed or moved by the experimenter on any day of the experiment. Each session was interleaved with 10-15 seconds of break where the rat was removed from track and put in a box to dispense its sense of direction and orientation. Then it was released on the same starting position on the track for the next session. On completion of all the experimental sessions, the track was wiped clean with 70% ethanol to clear off any traces that could act as potential cues for next day of recording.

A schematic of the 3-day novel experimental paradigm is given below (figure 2.2)

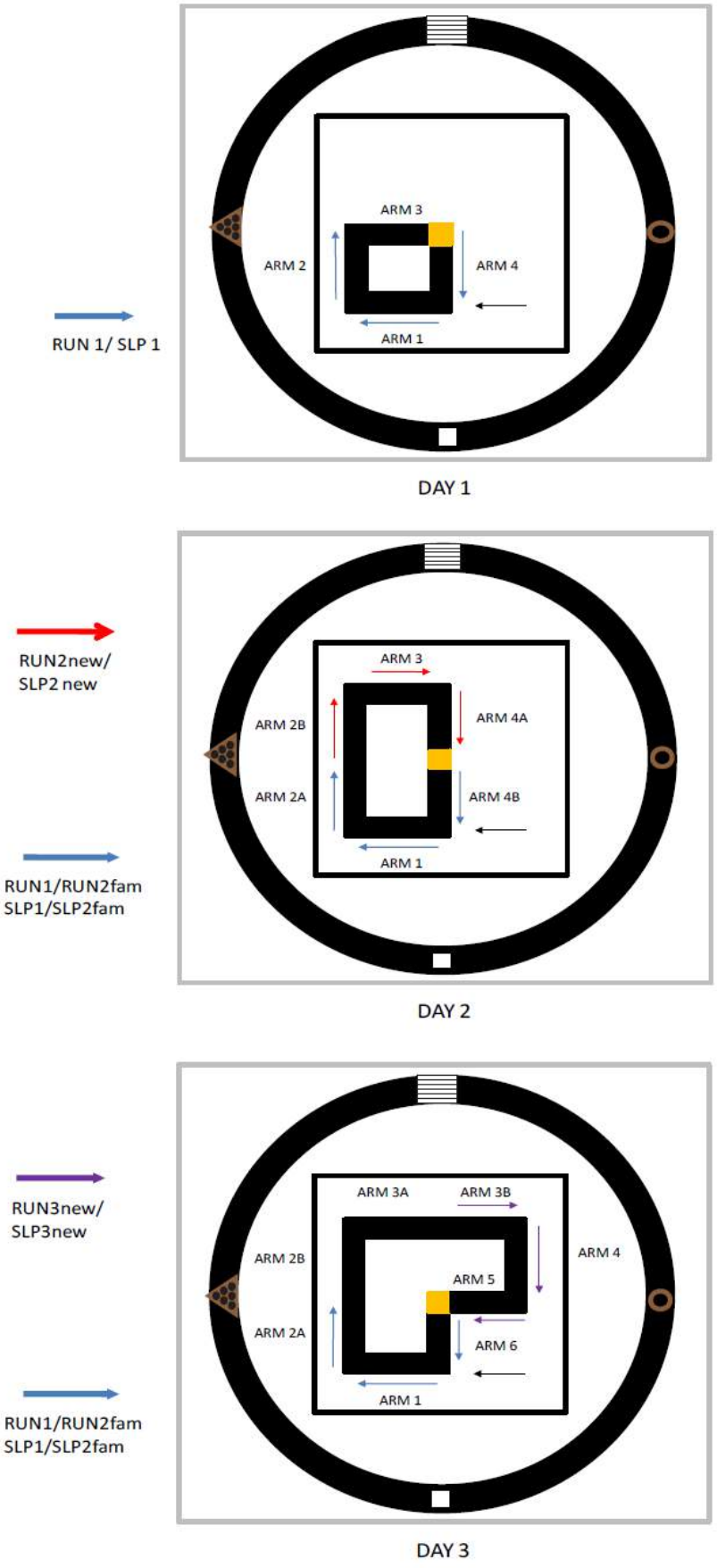


Figure 2.2: 3-day novel paradigm

The experiment was further divided into 2 stages: RUN session, where the animal ran 4X20 laps, followed by post run SLEEP session for 3-4 hours.

2.2.1 RUN SESSION

On day 1, although the rat ran in a clockwise fashion on a square track, only place cells from arm1, 2 and 4 were considered for appropriate comparison and analysis. This is termed as RUN1. Since these arms are present on both day 2 (arms1, 2a and 4b) and 3 (arms1, 2a and 6) as well, though numbered differently to maintain arm count continuity each day, they are the only common arms between each track, across all days. They are termed RUN2fam and RUN3fam respectively on day 2 and day 3. Correspondingly, the added/novel arms on day 2 (arms 2b, 3 and 4a) and day 3 (arms 3b, 4 and 5) are termed RUN2new and RUN3new, respectively. All the above mentioned C-shaped, 3-arm tracks: RUN1, RUN2fam, RUN2new, RUN3fam and RUN3new are similar in shape and size and are thus used for comparative analysis with each other. The middle arms (arms 2b and 3a) on day 3 are excluded from the same to maintain track length accuracy for analysis (Figure 2.2).

Therefore, not only can RUN1 be compared across days with RUN2fam and RUN3fam, as this area becomes more and more familiar from being completely novel across days; it can also be compared with RUN2new and RUN3new, since all 3 are novel areas of the track when first introduced in the environment on their respective days. Furthermore, comparison within the same day between familiarity and novelty can also be done on day 2 (RUN2fam v/s RUN2new) and day 3 (RUN3fam v/s RUN3new). The same comparisons have been applied to sleep analysis as well.

2.2.2 SLEEP SESSION

Each day's recording was followed by the rat being placed on a pedestal in a room adjacent to the behaviour room, where he slept afterwards and 3-4 hours of sleep was recorded. The experimenter was present throughout this sleep recording and only recorded when they visually inspected that the rat had fallen asleep, with his eyes closed and there was no movement made by him for at least 5 minutes. During any intermittent waking of the rat (if any), the recording was switched off and resumed only after he fell asleep again. It was observed that on all days, usually the rat slept within 15-20 minutes of being brought outside the behaviour room and kept on the pedestal after completion of the experimental task.

2.3 Data analysis:

All quantitative analysis of data was performed with custom-written software on MATLAB (R2013a, 2018), (www.mathworks.com) and Microsoft Excel 2016 as described below. The significance value (alpha) of all statistical tests performed is set at 0.05, unless specified otherwise.

2.3.1 Isolation of single-units

Isolation of single-units was performed manually with custom-written spike-sorting software Winclust (Savelli *et al.*, 2017). Cells were isolated based on the peak amplitude and energy of the waveforms recorded on four wires of the tetrode. Offline spike sorting of multiple clusters recorded from the same tetrode was done by principle component analysis (PCA) and isolating each cluster in various projections (six projections for four wires of each tetrode: 1/2,1/3,1/4,2/3,2/4,3/4. Based on their isolation quality (distance from the background and separation from other clusters), the units were rated on a scale ranging from 1 to 5 (1-very good; 2-good; 3-fair; 4-marginal; 5-poor) and the units rated 'fair -marginal' and above were used for further analysis (Figure 2.3). The same was done for clusters recorded in sleep as well. RUN clusters and SLEEP clusters recorded on the same tetrode were then compared and each isolated cluster's corresponding boundaries were overlapped with each other across all six projections and only those sleep clusters that could be successfully identified with their corresponding run cluster (50% overlap or more in most projections) were chosen for further analysis.

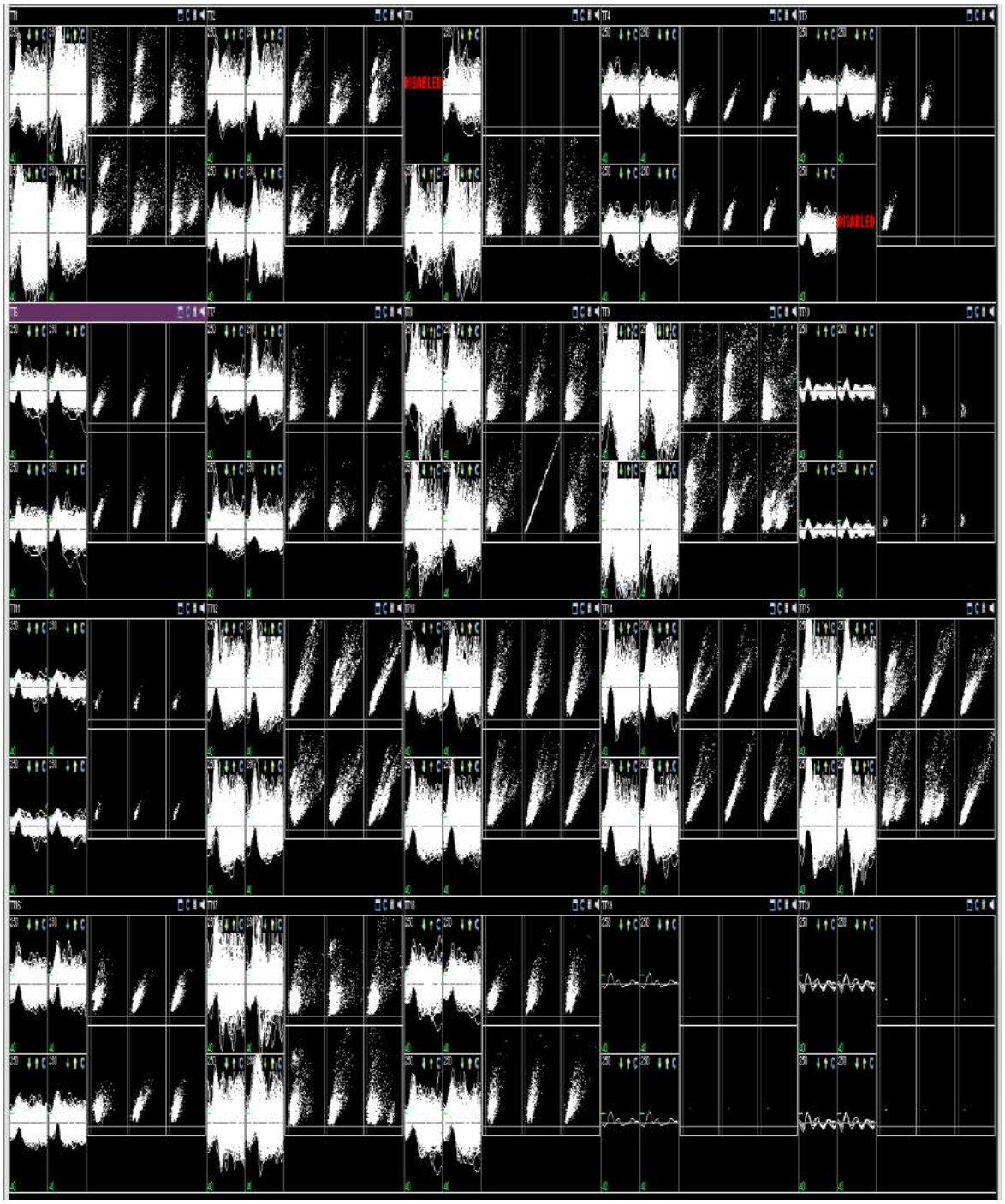


Figure 2.3: Multi tetrode in-vivo spike recordings from rat hippocampus
Recordings are from the right hemisphere (Rat 2). The 1st 18 channels are hippocampal pyramidal neuronal recordings while the last 2 are reference channels, hence quiet (no spike activity). Each tetrode shows spike recordings on all 4 channels, translated into 6 projections.

2.3.2 Linearization of tracks

Position data for all 4 sessions of a particular day for each rat was loaded in MATLAB, and outer and inner boundary was defined, in alignment with the track by removing outliers. The position where the rat was left on track at the beginning of each run session was defined as the starting point of arm1 and subsequent arms of each track were defined clockwise. All tracks were linearized and converted to 1 dimension tracks and divided into 2 cm spatial bins. Track 1 had 72 bins, track 2 had 106 bins and track 3 had 142 bins. Finally, a speed filter of 2cms/sec was applied to the same.

2.3.3 Defining place fields

Spatial information score: The spatial information score was calculated for all previously chosen clusters defined as place cells. The spatial information value indicates the amount of information about the rat' position, conveyed by the firing of a single spike from a cell (William E. Skaggs & Bruce L. McNaughton, 1992), and was calculated as:

$$I = \sum_x \lambda(x) \log_2 \left(\frac{\lambda(x)}{\lambda} \right) p(x)$$

where x is spatial bin, λ(x) is the firing rate of the cell at location, λ is the mean firing rate and p(x) is the probability of occupancy at bin x. A cell was classified as a Place cell if its spatial information score was found to be significant ($p < 0.05$) based on cell shuffling procedure (defined below) performed on each cell individually in any of the experiment sessions recorded in a particular day.

Data shuffling procedure: An individual cell's entire spike sequence recorded in a particular session was time shifted by a random interval between 20th second and 20

seconds before termination of that session. Spikes exceeding the total time of the session were wrapped around to be assigned to the beginning of session to generate new spike time sequence for that cell. This procedure was repeated 100 times for each cell separately. The 99th percentile value of the shuffled distribution of each score was taken as the threshold value for that particular cell (Langston *et al.*, 2010).

The chosen clusters that fit the criteria for being defined as a place cell based on its spatial information score were subsequently loaded in MATLAB and only those clusters that fired more than 50 spikes in each session were eventually chosen for analysis. The firing rate was calculated as the ratio between the number of spikes and time spent in each bin. Linearized rate maps of 2 cm spatial bins were smoothed with a Gaussian smoothing function of 4 cm standard deviation and characterized as having a firing rate greater than 1Hz over a minimum of 5 continuous spatial bins and an occupancy rate greater than 0.1Hz. Place fields were further defined as having a mean firing rate between 0.1Hz and 5 Hz and a peak firing rate of minimum 2 Hz. A cell's peak rate was defined as the firing rate in the bin with the highest rate on the linear track. Place field borders were defined as where the firing rate fell to less than 10% of peak firing rate of the cell or less than 1Hz, whichever was higher. Firing rates for place cells firing in multiple sessions were averaged for the day, across all sessions. Place field centres were calculated for all place cells based on the spatial bin that had the highest firing rate for that particular cell (Dragoi & Tonegawa, 2013; Pfeiffer & Foster, 2013). Using place field centres, cells were arranged on each track for a particular day from RUN start till end in the direction the rat traversed the tracks (clockwise). Place cells firing for reward area each day were eliminated in both run and sleep and were not used for further analysis (Figure 2.4, 2.5a, b, c).

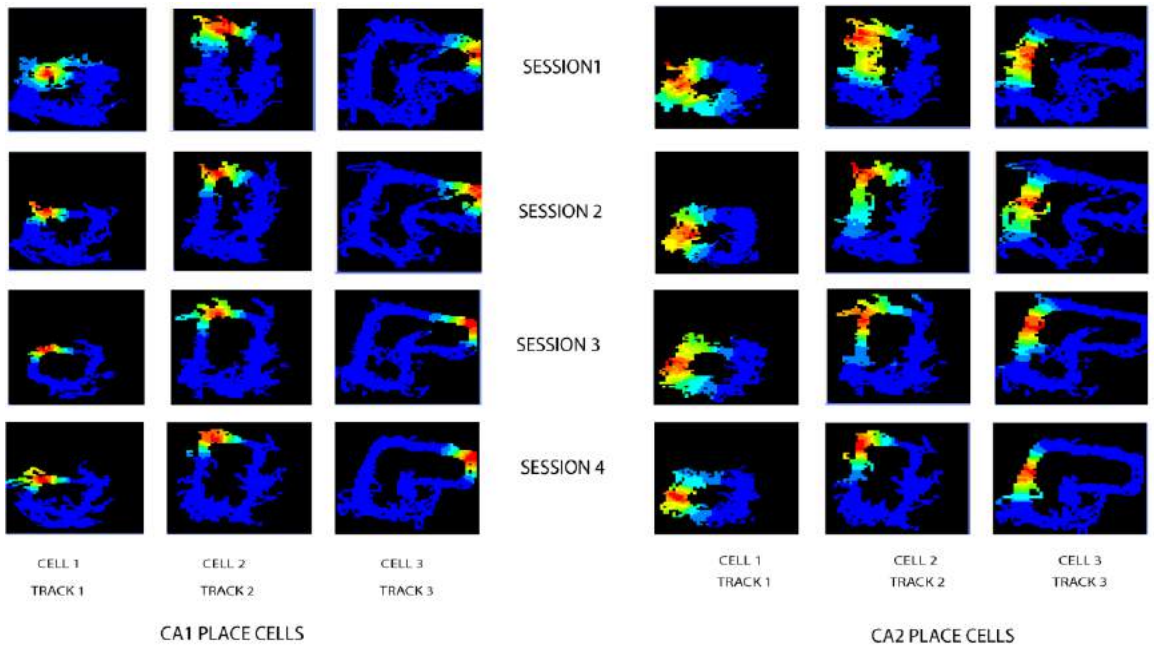
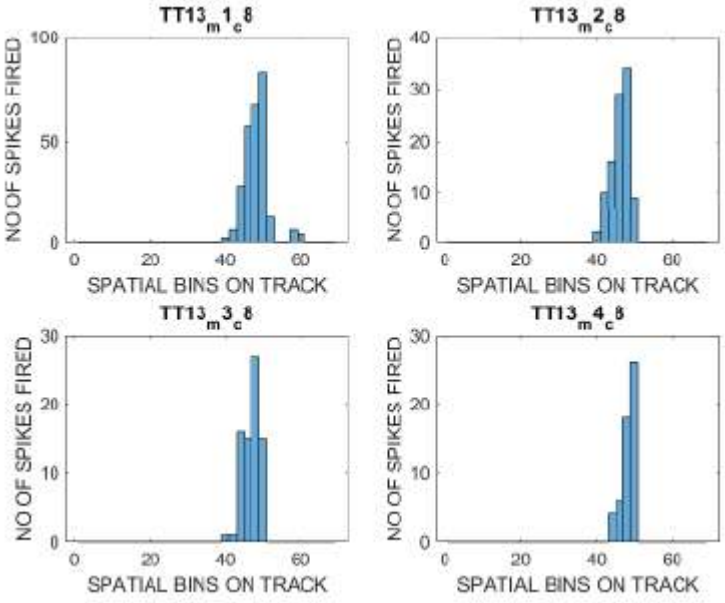
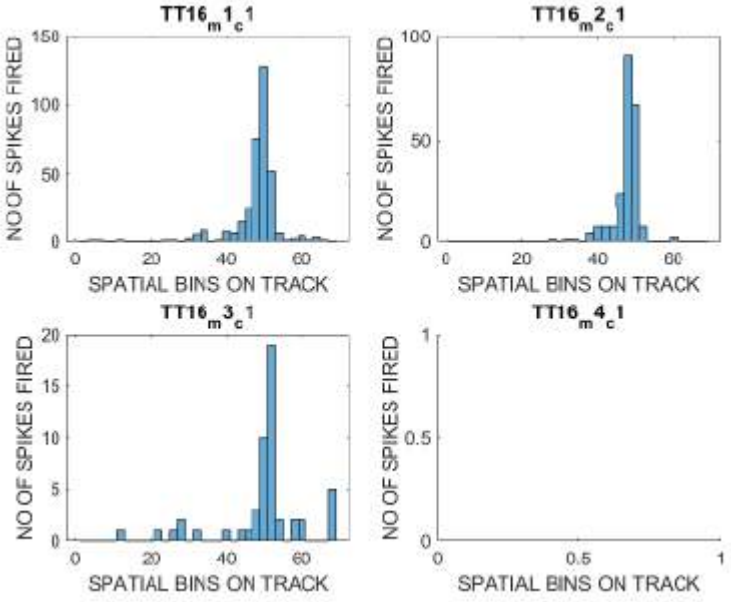


Figure 2.4: RUN session
Color coded firing rate maps of CA1 and CA2 hippocampal place cells recorded on all 3 days (and all 3 tracks).



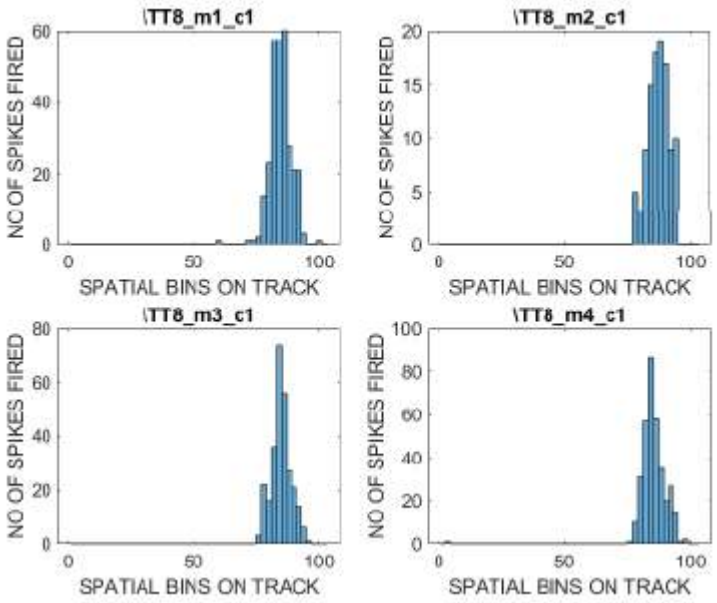
Linearized place field firing of a CA1 place cell on day 1



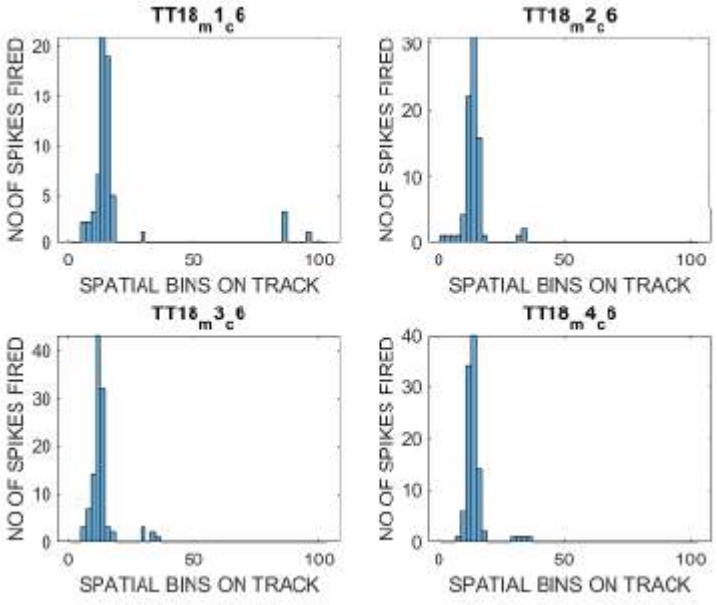
Linearized place field firing of a CA2 place cell on day 1

(did not fire in the last session)

Figure 2.5 a

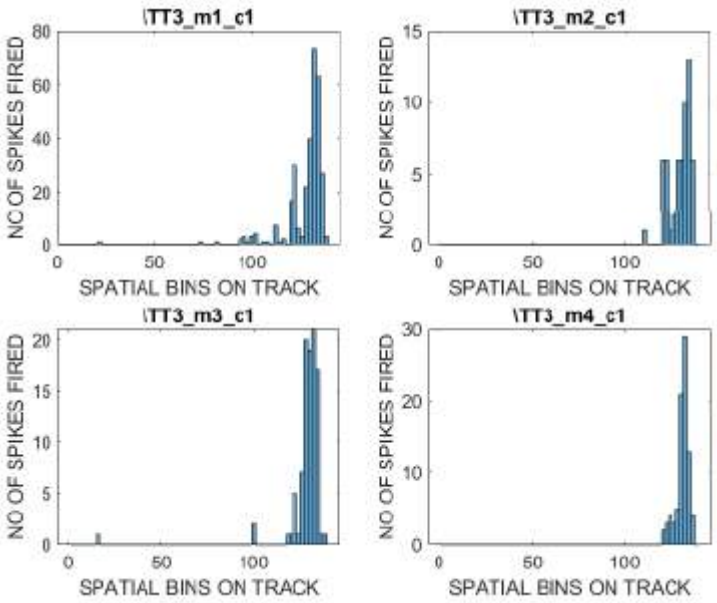


Linearized place field firing of a CA1 place cell on day 2

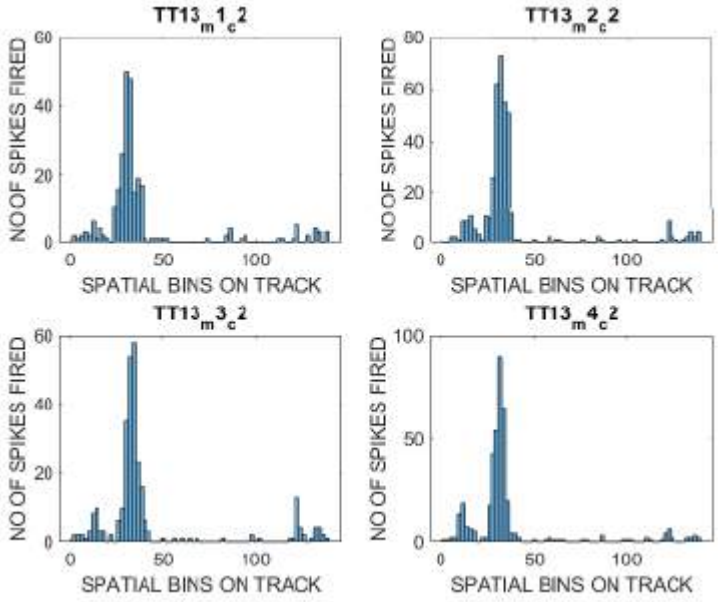


Linearized place field firing of a CA2 place cell on day 2

Figure 2.5 b



Linearized place field firing of a CA1 place cell on day 3



Linearized place field firing of a CA2 place cell on day 3

Figure 2.5 c

2.3.4 Offline detection of SWRs and sleep analysis:

The tetrode that had a clean LFP pattern by spectrogram (plotted in MATLAB) and showed clear SWRs (viewed in NeuraView software, by Neuralynx) were chosen each day. LFP envelope was computed by first band pass filtering and subsequently taking the hilbert transforms between 140-250Hz. REM and nREM phases were distinguished by computing delta/theta ratio of 2 -3 (theta band: 6-12 Hz, delta band : 1-4Hz) using Thompsons multi taper estimate (Thomson, 1982). Within the nREM epochs, each SWR was identified when the signal exceeded 3SD (standard deviation) for 15 ms or more, and its beginning and end was marked by 1SD (standard deviation). Candidate replay events lasting >500ms were discarded. Only spikes firing within the interval of each sharp wave ripple event were considered for further analysis (Csicsvari *et al.*, 2007; Karlsson & Frank, 2009).

Replay sequences were identified using an algorithm, previously described in detail in (Gupta *et al.*, 2010), that detects sequence structure in the pattern of place cell activity by comparing the times and place cell centres of spike pairs occurring in a flexible time window within each identified SWR. At least 4 spikes firing from 3 different place cells within a SWR was termed as a candidate replay event. For cells with multiple place fields, spikes were assigned the place field centre that maximized the forward or backward score. This algorithm resulted in a series of time windows, place field centre-labelled spikes, and scores for each forward and backward sequence.

These sequences were then analysed to identify significant sequence replays using two independent bootstrapping procedures: spike time shuffle and centre peak shuffle. The first method involved shuffling of spike times, while preserving the identity of

spikes of each cell while the second involved shuffling of peak firing position of each cell, keeping the cell identity intact. Each event was shuffled 300 times, and its sequence score was computed again. If the unshuffled replay score was greater than 85% of both independent sets of shuffled replay scores, they were deemed to be significant and were chosen for further analysis.

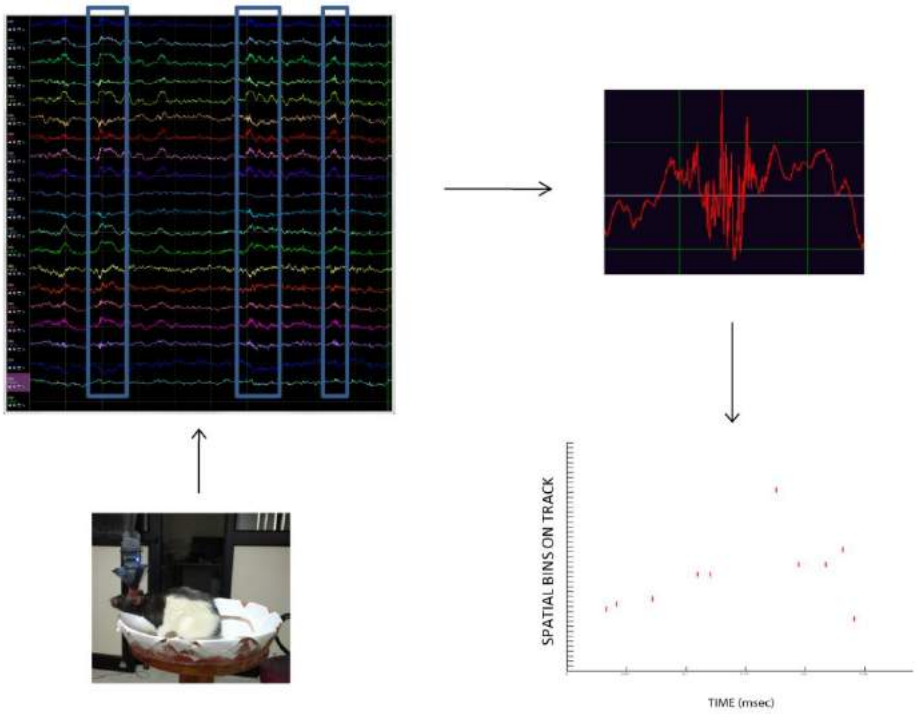


Figure 2.6: SLEEP SESSION:
LFP (local field potential) recordings from the rat hippocampus on each of the 18 tetrode recording channels of the microdrive, highlighting sharp wave ripples(SWRs) (in blue), a signature of hippocampal LFP (left). A candidate replay event recorded (bottom right) during a SWR (top right).

CHAPTER 3

SPATIAL NOVELTY DETECTION

IN CA1 AND CA2

The first part of the experimental paradigm involved the animal running 20 clockwise laps in 4 sessions each, on a novel track that was subsequently modified in shape, size and length each day from day 1 to 3. The track was elongated from a square track on day 1, to a rectangle on day 2 and to an L-shaped track on day 3. Consequently, the length of the track increased from 72 bins (linearized 2 cm spatial bins, as described in materials and methods chapter previously) on day1, 106 bins on day 3 and 142 bins on day 3.

In addition, as the original track from day 1 became more and more familiar on one hand, subsequent additions to the length of the track each day resulted in novelty being introduced into the existing environment each day. Thus, the animal experienced complete novelty on day1, 50% novelty on day 2 and only 33% novelty on day 3. Conversely, familiarity of the environment increased from 0% on day1, 50% on day 2 and 67% on day 3 (including middle arms of the track as well).

Therefore, the animal experienced varying degrees of familiarity and novelty not just across days but also in the same lap itself on days 2 and 3. Under such circumstances, it was important to observe how CA1 and CA2 place cell distribution on each track would occur, and how they would be spatially represented by the two place cell populations; as well as if there would be any discernible differences in other firing properties of these place cells, with respect to familiarity and novelty of the environment.

Thus, this part of the study focused on place cell distributions, average (mean) firing rates and pair wise cross correlations between place cell pairs from CA1 and CA2 neuronal populations on all tracks, across all days. The aim was not only to compare the above place cell characteristics across days, but also within days (on days 2 and

3) with respect to novelty. Further, CA1 and CA2 population responses were observed separately to tease out any differences between the two in spatial representation of the same (if any).

RESULTS

Results:

3.1.1 Number of place cells recorded:

RUN session: A total of 427 CA1 and CA2 place cells were recorded on all 3 days of the experiment: 121 cells on the 1st day, 155 cells on the 2nd day and 151 cells on the 3rd day. After excluding cells that fired for the reward area, a total of 105 place cells were recorded on day1, 136 place cells on day 2 and 141 on day 3 (total number: 382 cells). Out of these 382 place cells, 288 were recorded from CA1 and 94 from CA2.

SLEEP session: Following the RUN session each day, a total of 275 place cells were recorded in SLEEP: 69 place cells on day1, 103 on day 2 and 102 on day 3. After excluding cells that fired for the reward area in sleep, a total of 269 place cells remained: 192 from CA1 and 77 from CA2.

REWARD AREA: A total of 43 place cells were recorded on all 3 days that fired for the reward area: 16 cells on day1, 17 on day 2 and 10 on day 3. It was observed that, with respect to the proportion of cells firing for the track each day, the percentage of cells that fired for reward decreased each day. (16 cells out of 121, 17 out of 155 and 10 out of 151 place cells. Thus, on day1, while 13% of place cells fired for reward area, 11.6% fired on day 2 and only 6.7% on day3.

A detailed break up of place cell counts from various parts of the track each day is provided in Table 3.1 as well as Figure 3.2.

		DAY 1			
S.NO	RAT NO	TOTAL CELLS	ON TRACK	REWARD	IN SLEEP
1	33	19	14	5	8
2	47	59	54	5	27
3	58	15	11	4	11
4	A-16	28	26	2	23
TOTAL		121	105	16	69

			DAY 2			
S.NO	RAT NO	TOTAL CELLS	FAM	NEW	REWARD	IN SLEEP
1	33	28	10	15	3	19
2	47	73	20	43	10	45
3	58	22	9	11	2	18
4	A-16	32	10	20	2	21
TOTAL		155	49	89	17	103

			DAY 3				
S.NO	RAT NO	TOTAL CELLS	FAM	NEW	MIDDLE	REWARD	IN SLEEP
1	33	25	15	7	1	2	22
2	47	59	29	16	10	4	34
3	58	31	13	7	8	3	21
4	A-16	36	20	10	5	1	25
TOTAL		151	77	40	24	10	102

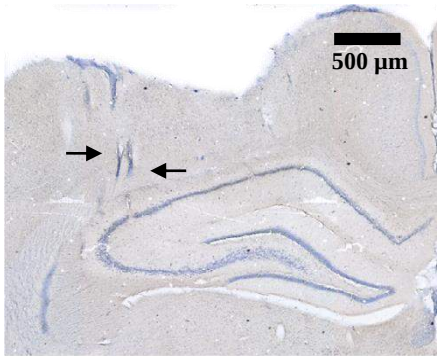
Table 3.1: Total number of CA1 and CA2 place cells recorded on all days in RUN and SLEEP sessions.

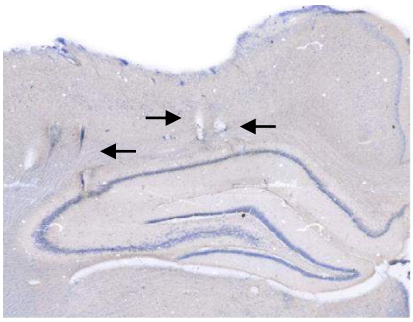
3.1.2 Histology and Tetrode track identification:

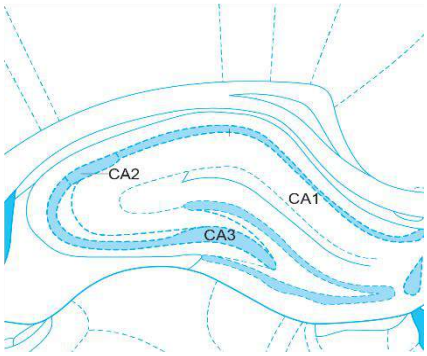
Four adult Long-Evans rats aged between 5-6 months were implanted with the dual-bundle micro-drive and histology was performed once all experiments were completed and the brain was perfused. Each rat was implanted to target CA1 and CA2 simultaneously, with one bundle aimed at each of these hippocampal sub-fields. The dual tetrode bundle (containing two bundles of 10 tetrodes each: 9 recording tetrodes and 1 reference channel) were made using 16 G stainless steel tubing of 1.2 mm inner diameter and 1.6 mm outer diameter. The total length of the 2 bundles was 3.6mm and the breadth was 1.7mm. The bundles, centre to centre were 1.75 mm apart. These bundle configurations enabled maximum coverage of the hippocampus medio-laterally to enable simultaneous recordings from both CA1 and CA2. All implants were done with respect to the lateral-most tetrode in the entire bundle configuration, aimed at CA2, such that the medial ones could aim CA1. The final depth of the tetrode tips were marked by performing electrolytic lesions on a few selected tetrodes. This enabled identifying the location of each tetrode in the rest of the bundle in comparison to the lesion marks. This configuration was then cross referenced with actual configuration of the bundle that was determined while loading the tetrodes in the microdrive. Finally, depth reconstruction of the tetrode configuration was done to identify accurate anatomical locations for the cells (place cells) recorded; i.e. to observe which tetrodes recorded from CA1 and which recorded from CA2 (Figure 3.1). Post perfusion and staining procedures of brain slices have been elaborated in materials and method section.

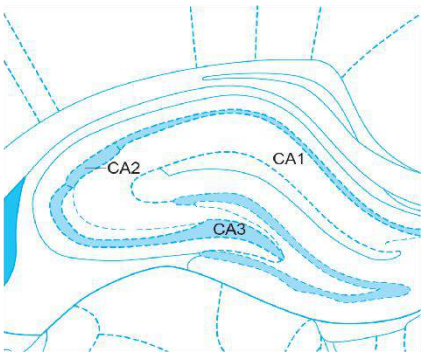
S.no	Rat no	ML(mm)	AP(mm)
1	33	1.8	3.5
2	47	1.7	3.7
3	58	1.7	3.5
4	A-16	1.8	3.5

Table 3.2: Dual- bundle microdrive implant co-ordinates for each rat.

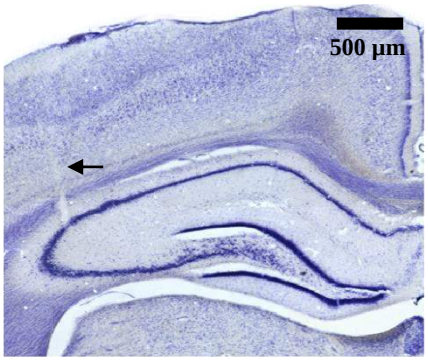


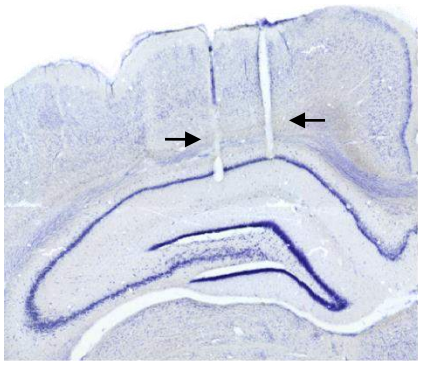


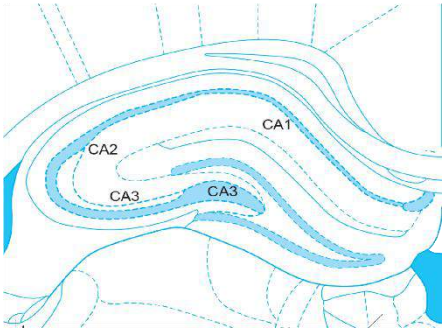


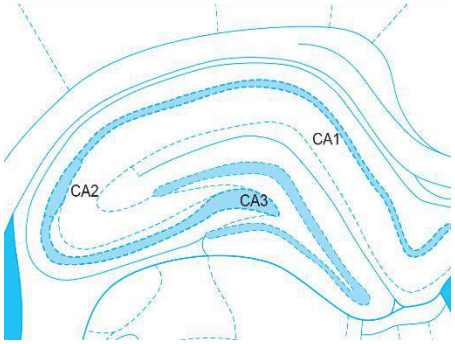


Rat 1

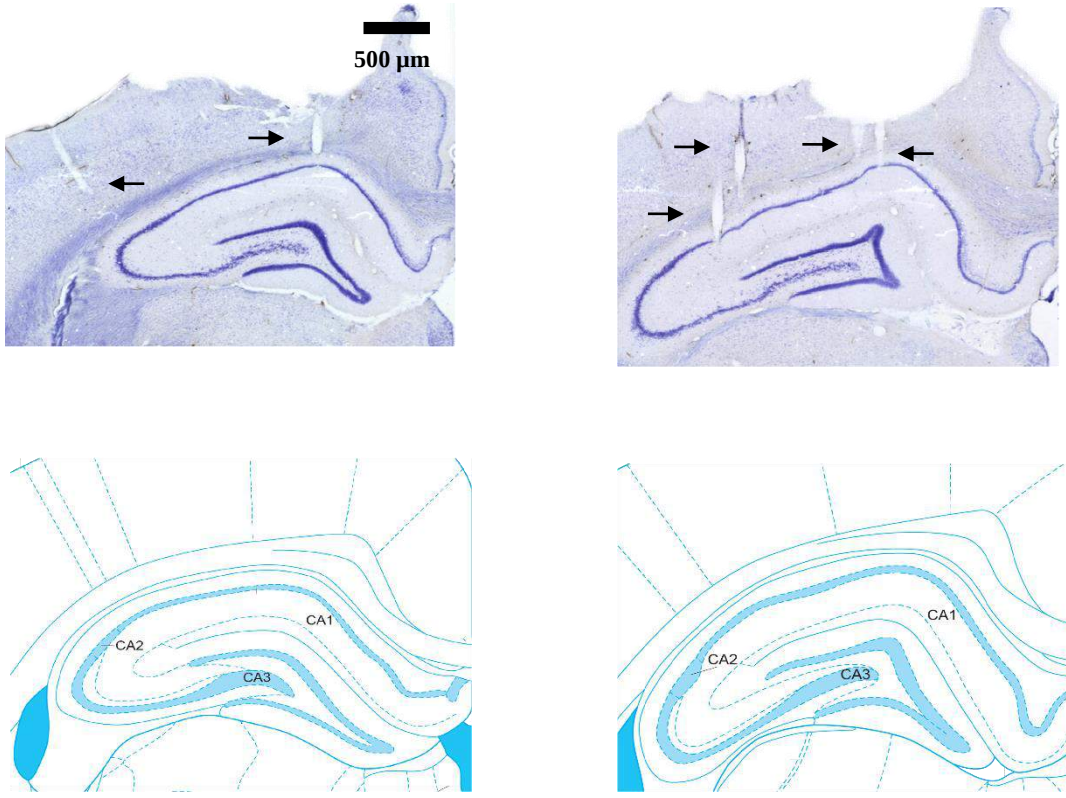




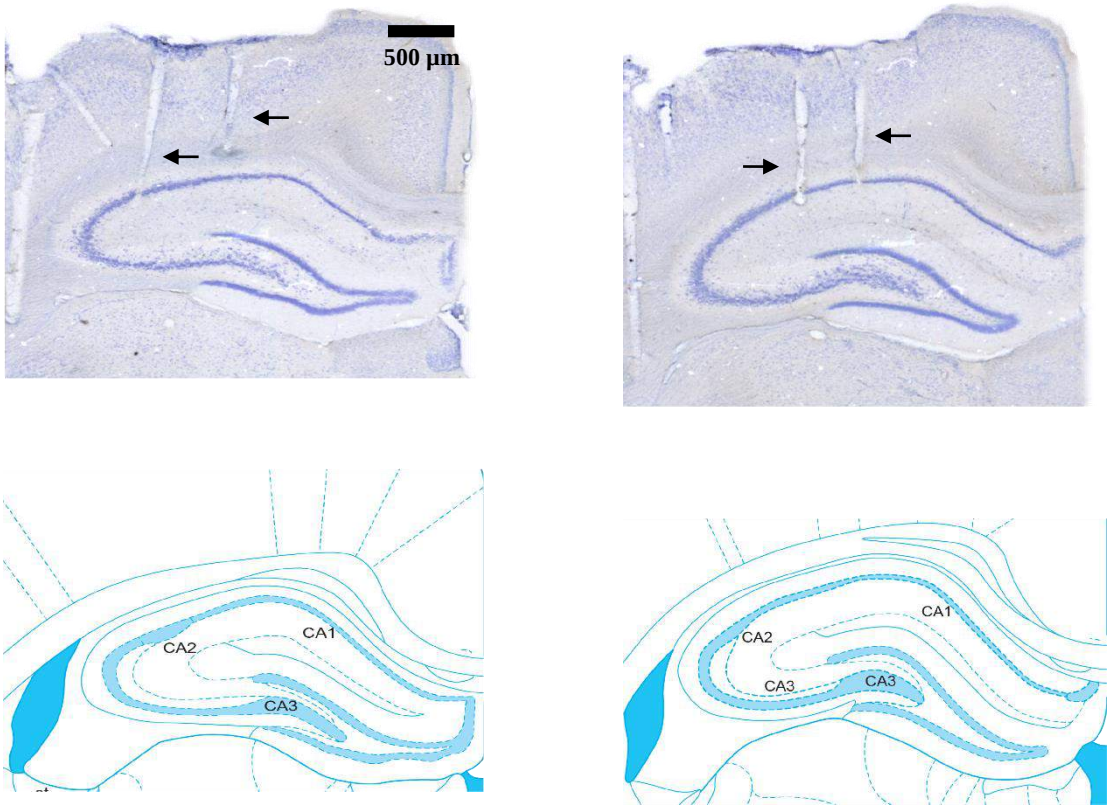




Rat 2



Rat 3



Rat 4

Figure 3.1: Tetrode tracks identification in CA1 and CA2 rat hippocampal coronal sections

3.1.3 Place cell counts

When the track was elongated each day, from day 1 to 2 to 3, it was seen that there was an overall increase in total place cell population counts of CA1 (day1: 81 cells, day 2: 99 cells, day 3: 108 cells) and CA2 from day 1 to day 2 but a slight decrease on day 3 (day1: 24cells, day 2: 37 cells, day3: 33 cells) (Figure 3.2-top left panel). But when this comparison was done for each RUN session, it was seen that while from RUN1 to RUN2 (RUN2fam + RUN2new) cell populations increased in number, it decreased from RUN2 to RUN3 (RUN3fam + RUN3new). This was observed in both CA1 (64 cells on day1, 99 cells on day2 and 92 cells on day3) and CA2 (19 cells on day1, 37 cells on day2 and 26 on day3). This was despite the fact that RUN2 and RUN3 track lengths are the same (Figure 3.2-top right panel).

Further, when the above RUN distribution of both CA1 and CA2 place cells combined were redistributed according to novel and familiar areas on track on day 2 and 3, it was observed that on day 2 most place cells fired for novel part of the track (RUN2new) and that this count was similar to RUN1 place cell number (RUN1: 83 cells and RUN2new: 88 cells). On the other hand, from day 1 to day2, place cell number on RUN2fam decreased to almost half the original place cell number from RUN1 (RUN1:83 cells, RUN2fam:48 cells). For day3, it was expected that as seen on day2 (RUN2fam: 48 cells, RUN2new: 88 cells), maximum number of place cells would continue to fire for novel portion of the track, but the reverse was actually seen (RUN3fam:76 cells, RUN3new:42 cells). Most cells fired for familiar part of the track on day3 and not for the novel part, for which the cell count dropped to almost half from that of the previous day (RUN2new: 88 cells, RUN3new:42 cells). Also, the

number of place cells firing for familiar portion of track returned to similar numbers as that on day 1, as opposed to day 2 where they had dropped to half their original count (RUN1:83 cells, RUN2fam: 48 cells, RUN3fam:76 cells) (Figure 3.2-middle panel). Further, when the above said RUN distribution was divided into CA1 and CA2 ensemble populations separately, it was observed that both ensembles showed a very similar trend in their cell counts across RUN1, RUN2fam, RUN2new, RUN3fam and Run3new. Both populations had higher cell counts for RUN2new as compared to RUN2fam, which had comparable counts to RUN1 (CA1: RUN1:64 cells, RUN2new: 65 cells, RUN2fam: 34 cells; CA2: RUN1: 19 cells, RUN2new: 23 cells, RUN2fam: 16 cells). On day3, however, the reverse trend was observed and RUN3fam had a higher cell count than RUN3new and had a comparable count to RUN1 (CA1: RUN1: 64 cells, RUN3fam:60 cells, RUN3new:32; CA2:RUN1:19 cells, RUN3fam:16 cells, RUN3new: 10 cells).

Thus, when compared across RUN2 and RUN3, the place cell distribution for familiar and novel track parts seem to flip and be the opposite of one another (CA1:RUN2fam: 34 cells, RUN2new: 65 cells; RUN3fam:60 cells, RUN3new:32 cells; CA2: RUN2fam:14 cells, RUN2new:23cells; RUN3fam:16 cells, RUN3new:10 cells) (Figure 3.2 middle and bottom panel, refer to Figure 3.14 and Figure 4.5-left column).

Chi square test-

All place cells: across days comparison: RUN1/RUN2fam/RUN3fam: p=.0559, RUN1/RUN2new/RUN3new: p=.0022, within day comparison: RUN2: RUN2fam/RUN2new: p=.0142 and RUN3:RUN3fam/RUN3new: p=.0253.

CA1 place cells: across days comparison: RUN1/RUN2fam/RUN3fam: p=.0403, RUN1/RUN2new/RUN3new: p=.0106, within day comparison: RUN2: RUN2fam/RUN2new: p=.0253 and RUN3:RUN3fam/RUN3new: p=.0367.

The test was not significant for CA2 place cells separately, probably due to less number of place cell counts, despite it showing the same trend as CA1 place cells. (Figure 3.2 middle and bottom panel and refer Figure 4.5-left column).

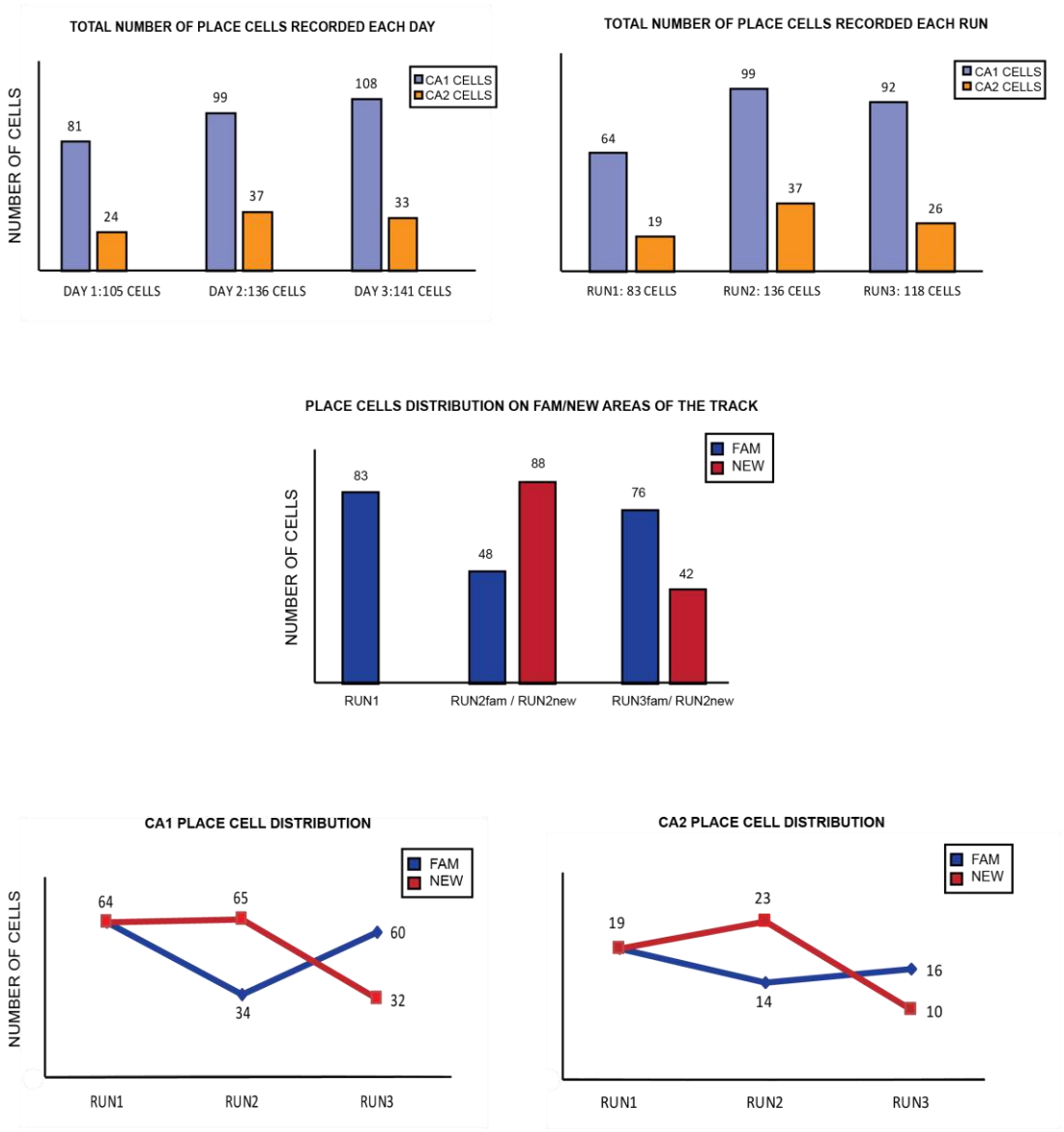


Figure 3.2:

a. Total number of CA1 and CA2 place cells recorded each day (eliminating place cells that fired for reward area) (top panel-left)
b. Total number of CA1 and CA2 place cells recorded in each RUN session (RUN1: arms1,2,4;RUN2:arms1,2a,4b(RUN2fam)arms2b,3,4a(RUN2new);RUN3: arms1,2a,6(RUN3fam) arms3b,4,5(RUN3new)). (top panel-right)
c. Total number of CA1 and CA2 place cells recorded from different areas of the track (familiar and novel areas) in each RUN session (RUN2 divided into RUN2fam+RUN2new and RUN3 into RUN3fam+RUN3new). (middle panel)
d. Comparison of CA1 and CA2 place cell distribution across RUN1, RUN2fam, RUN2new, RUN3fam, RUN3new. Both ensembles show the same trend in place cell allocation as new tracks are added on days 2 and 3. In RUN2, for both ensembles RUN2new>RUN2fam and in RUN3, RUN3fam>RUN3new for place cell distribution on tracks. (bottom panels)

3.1.3.1 Representation of track by hippocampal place cells:

The above myriad of comparisons gives a clear picture of spatial area representation by place cell counts of CA1 and CA2 ensembles, both across and within days, as the environment is modified each day. As familiarity of the track increases from 0% on day1 to 50% on day2 and 67% on day3 (with 2/3rds of the track being relatively familiar now), it was observed that 0% of place cells fired for familiarity on day 1 (RUN1), 35% of place cells fired for familiar track on day 2 (RUN2fam) and 70% on day 3 (combining RUN3fam, arm 2b, and arm 3a). Similarly, as novelty of the track decreased from 100% on day1, 50% on day2 and 33% on day3, the % of place cells firing for novel track portion also decreased from 100% on day 1 (RUN1), to 65% on day 2 (RUN2new) to 30% on day 3 (RUN3new). While for day 1 and day3, the correlation between the distribution of familiarity and novelty on track closely reflected the place cell distributions of both CA1 and CA2, the same was not observed for day 2. There was a skewed representation of place cell distribution on day2, with respect to both familiarity and novelty. While the track on day 2 comprises of 50% familiar (arms 1, 2a,4b) and 50% novel track (arms 2b,3,4a), the place cell ensemble distribution for both cell populations was 35% for familiar track and 65% for novel track. This skewed representation was not seen on day 3, where 30% of total place cell population fired for 33% of novel track and 70% cells fired for rest of the track, although the majority fired for RUN3fam (54%) and while only a minority fired for arms2b and 3a (16%). This difference in place cell distribution between day 2 and 3 might indicate that when novelty is introduced in an environment for the first time (early detection), the neuronal ensemble response of both CA1 and CA2 is an increase in place cell number firing for that particular novel region, but not on subsequent detections (whenever novelty is introduced next) where both ensembles choose to

redistribute its place cells in accordance with relative familiarity and novelty in the environment. (Figure 3.3, 3.4).

Chi square test for place cell distribution (all cells) on day2: p=.0142, on day3: p=.792. Thus, the skewed distribution is only observed on day 2 during early spatial novelty detection but not day 3 during subsequent novelty detection.

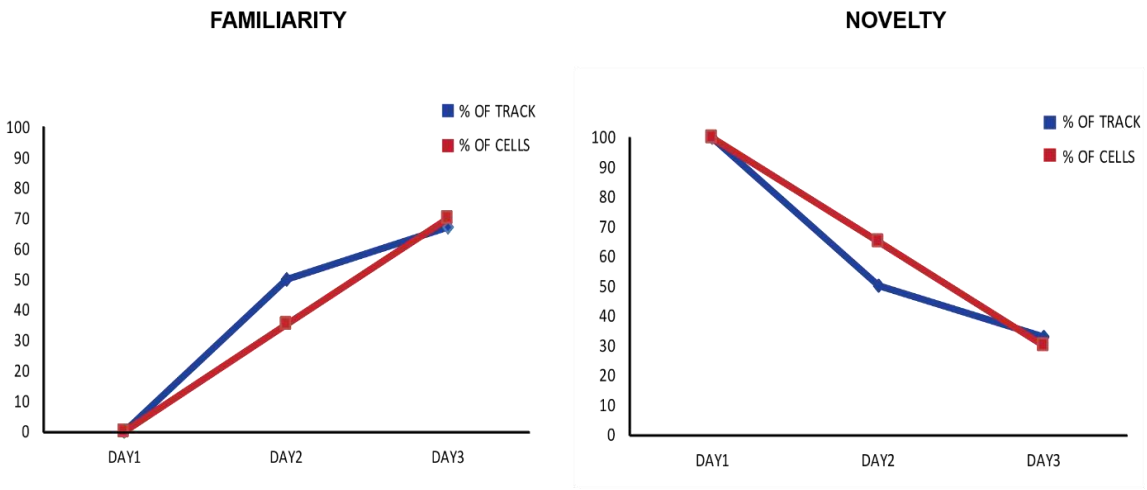


Figure 3.3: Percentage of place cells coding for familiarity and novelty on each track across days
As familiarity increases and novelty decreases across days (and across each RUN) on the track, place cell distribution for both CA1 and CA2 closely mirrors this trend on day 1 (100% place cells for 100% novel track) and day 3 (70% place cells for 67% familiar track and 30% place cells for 33% novel track). The same is not observed on day2 where a skewed distribution of place cells (65% place cells for 50% novel area, 35% place cells for 50% familiar area) occurs, when novelty is introduced in a relatively familiar environment for the first time (termed early novelty detection).

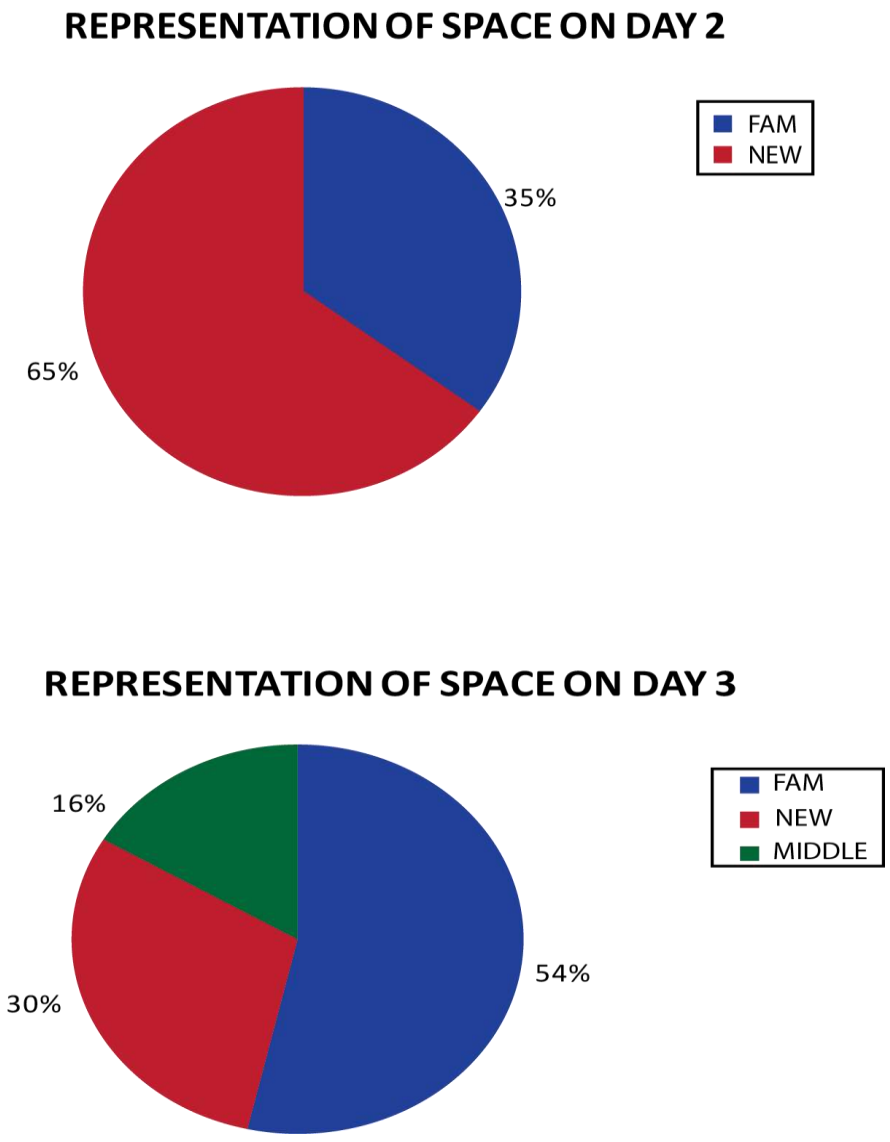


Figure 3.4: Track representation by place cells on day 2 and day 3
Representation of track area by CA1 and CA2 place cells reveals that on day 2, more place cells code for novel space, even though familiar and novel space is equal in size, shape and other dimensions (65% place cells code for 50% novel area while only 35% code for 50% familiar area of the track). This skewness is not observed for day 3, where 70% cells code for relatively familiar 67% of the track and 30% cells code for the novel 33% track area. Thus a 'skewed distribution' of place cells is seen on day 2 but not day 3.

3.1.3.2 Place cell distribution across tracks

Place cell distributions were compared across all arms of all 3 tracks on all days, i.e. 3 arms on day1, 6 arms on day2 and 8 arms on day3 (Figure 2.2). This was done to eliminate the possibility of attributing any of the above results to a preferential arm of any track by chance or for the reward area. It was observed that this was not on the case on either day 2 or 3. On day2, all 3 arms of the novel track showed an increase in number of place cells firing for it when compared to 3 arms of the familiar track (RUN2fam v/s RUN2new). Similarly, the result seen on day 3 was due to an increase in place cells firing for all 3arms of familiar track when compared with all 3 arms of novel track (RUN3fam v/s RUN3new). The place cell distribution across the particular arms connected to the reward area on any of the days also did not show a preferential increase/decrease compared to any other arm of the tracks. Thus, the place cells were pretty evenly distributed across all arms of novel tracks and familiar tracks, and were not influenced by either a particular arm of any track or the position of reward area on any day.

Kruskal Wallis test: RUN1 arms place cell distribution (arm1/arm2/arm4): p=.9, RUN2fam (arm1/arm2a/arm4b): p=.06, RUN2new (arm2b/arm3/arm4a): p=.67; RUN3fam (arm1/arm2a/arm6): p=.058, RUN3new (arm3b/arm4/arm5): p=.25 (Table 3.3)

	PLACE CELL DISTRIBUTION ACROSS ALL DAYS ON DIFFERENT ARMS OF EACH TRACK							
DAY 1	**RUN 1**	ARM 1	ARM 2	ARM 4				
		30	23	30				
DAY 2	**RUN2fam**	ARM 1	ARM2a	ARM 4b	**RUN2new**	ARM 2b	ARM 3	ARM 4a
		20	21	7		31	24	33
DAY 3	**RUN3fam**	ARM 1	ARM 2a	ARM 6	**RUN3new**	ARM 3b	ARM 4	ARM 5
		15	22	40		16	17	8

Table 3.3: Place cell distribution of both CA1 and CA2 place cells across all arms of tracks being used in comparison: RUN1, RUN2fam, RUN2new, RUN3fam and RUN3new.

Place cells were found to be pretty evenly distributed across individual arms of the above tracks on all days, and no bias of place cell firing was found for proximity to reward area or towards any particular track arms on any of the days.

3.1.3.3 Place cell distribution across sessions within each day

On each day of the experiment, the rat ran 4 sessions of 80 clockwise laps, with every session consisting of 20 laps each. As the rat traversed through these sessions, it got more and more familiar with the environment and got more accustomed to each track and the associated paradigm (for e.g.: it would get a chocolate sprinkle only on the patch of sandpaper on the track after each lap). Thus, on day1, where the environment was completely novel for the rat in the 1st session, it got relatively familiar by the 80th lap in the last session. The same was experienced on day 2 and day 3 as well. This was also evident in its navigation of each track by the last session (running speed etc.) and its expectancy of reward at the same position after each lap.

Therefore, to check if increase in relative familiarity of a track/ environment within a day would have an effect on number of place cells firing each session, the total number of active place cells in each session were plotted for each day (Figure 3.5a). The same was done for CA1 and CA2 place cells separately as well (Figure3.5b and c). Although a general trend was observed that place cell counts increased after the 1st session, it was not conclusive.

Finally, place cell counts across each of the 4 sessions were pooled across days (e.g.: place cell counts for session1 of each of the days 1, 2 and 3 were summed across) and it was observed that within each session, place cell counts increased from day1 to day 2 to day3 (Figure 3.5d). This is attributed to increase in track length across days as well, but the increase in cell counts was more pronounced from day1 to day 2 than from day2 to day3, even though the increase in track length was the same on both days. The same comparison was done for CA1 and CA2 place cells separately as well. While for CA1, place cell counts increased each day within a session, for CA2 the

place cell count decreased from day 2 to day 3 in all sessions except session 1. However, this trend was again not found to be conclusive.

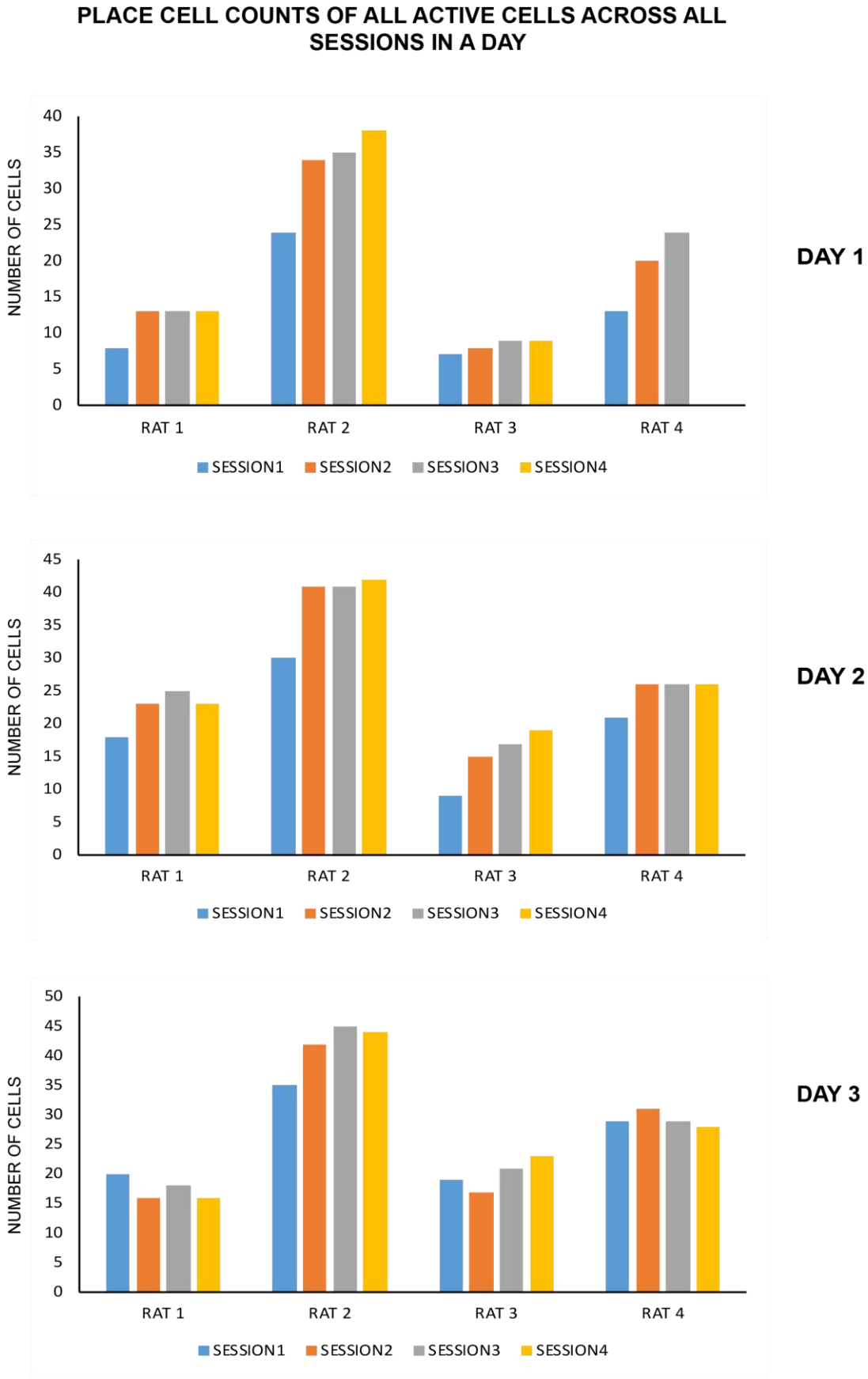

PLACE CELL COUNTS OF ALL ACTIVE CELLS ACROSS ALL SESSIONS IN A DAY
NUMBER OF CELLS
40
35
30
25
20
15
10
5
0
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 1
NUMBER OF CELLS
45
40
35
30
25
20
15
10
5
0
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 2
NUMBER OF CELLS
50
45
40
35
30
25
20
15
10
5
0
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 3

Figure 3.5 a

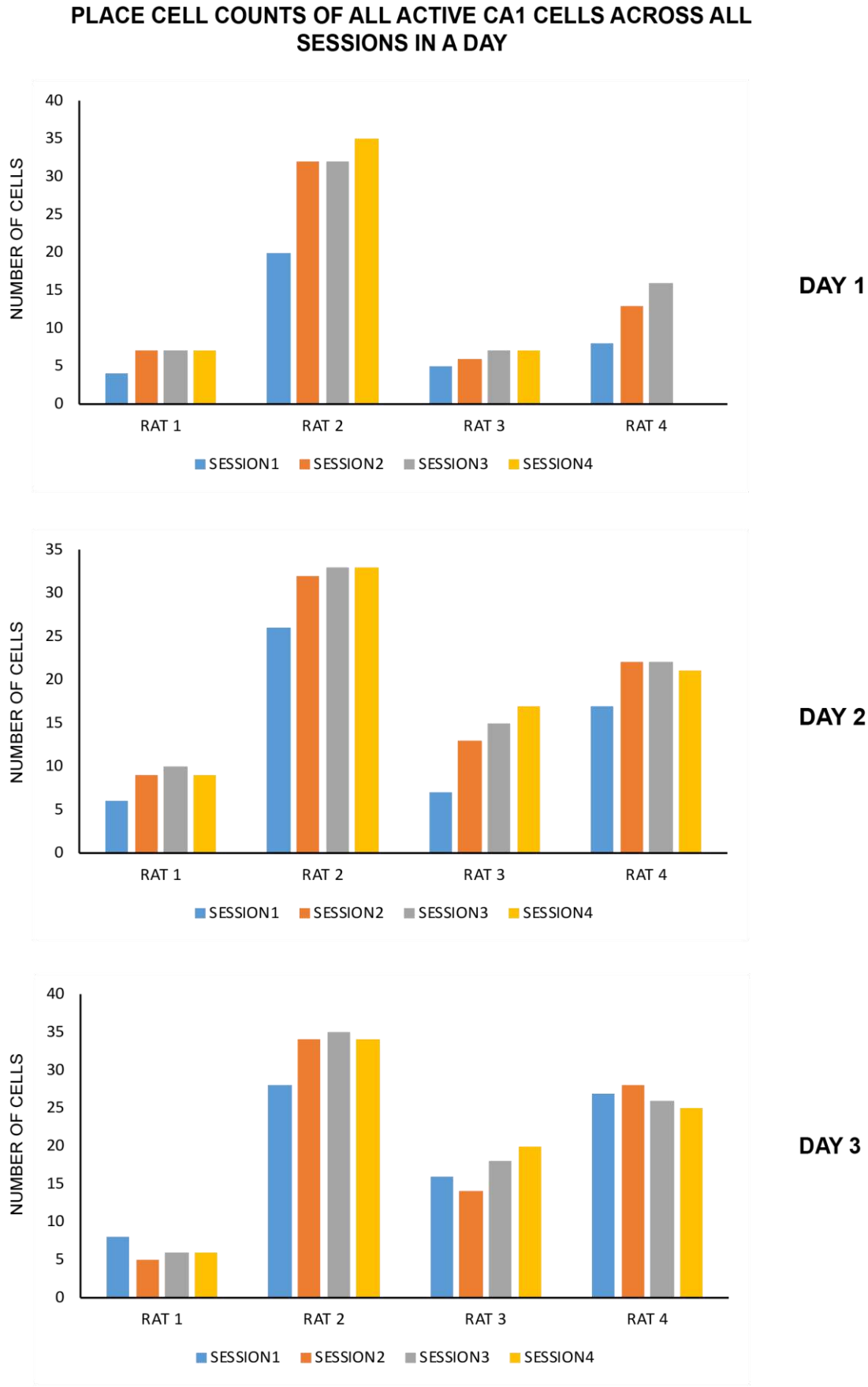

PLACE CELL COUNTS OF ALL ACTIVE CA1 CELLS ACROSS ALL SESSIONS IN A DAY
NUMBER OF CELLS
40
35
30
25
20
15
10
5
0
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 1
NUMBER OF CELLS
35
30
25
20
15
10
5
0
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 2
NUMBER OF CELLS
40
35
30
25
20
15
10
5
0
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 3

Figure 3.5 b

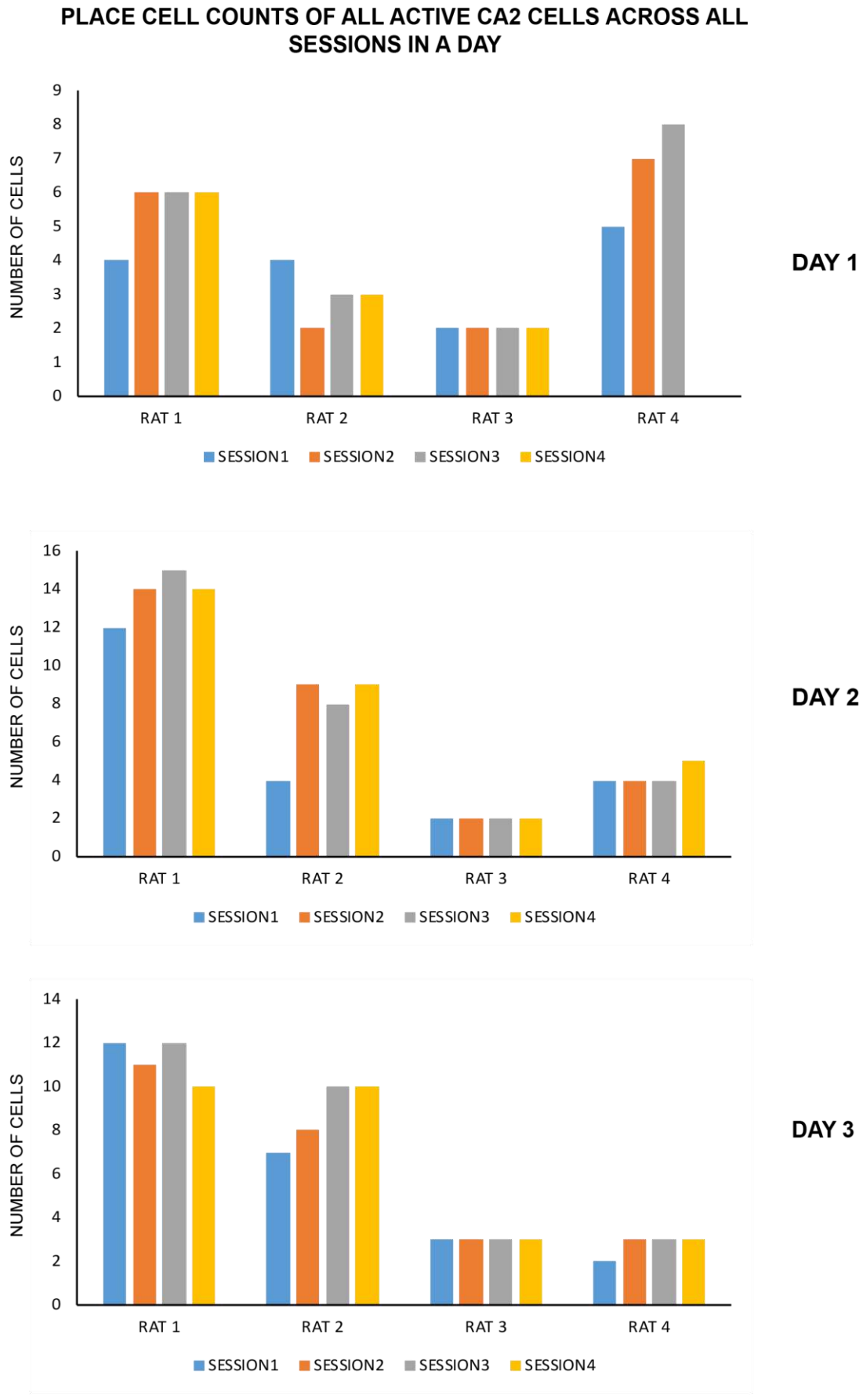

PLACE CELL COUNTS OF ALL ACTIVE CA2 CELLS ACROSS ALL SESSIONS IN A DAY
NUMBER OF CELLS
0
1
2
3
4
5
6
7
8
9
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 1
NUMBER OF CELLS
0
2
4
6
8
10
12
14
16
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 2
NUMBER OF CELLS
0
2
4
6
8
10
12
14
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 3

Figure 3.5 c

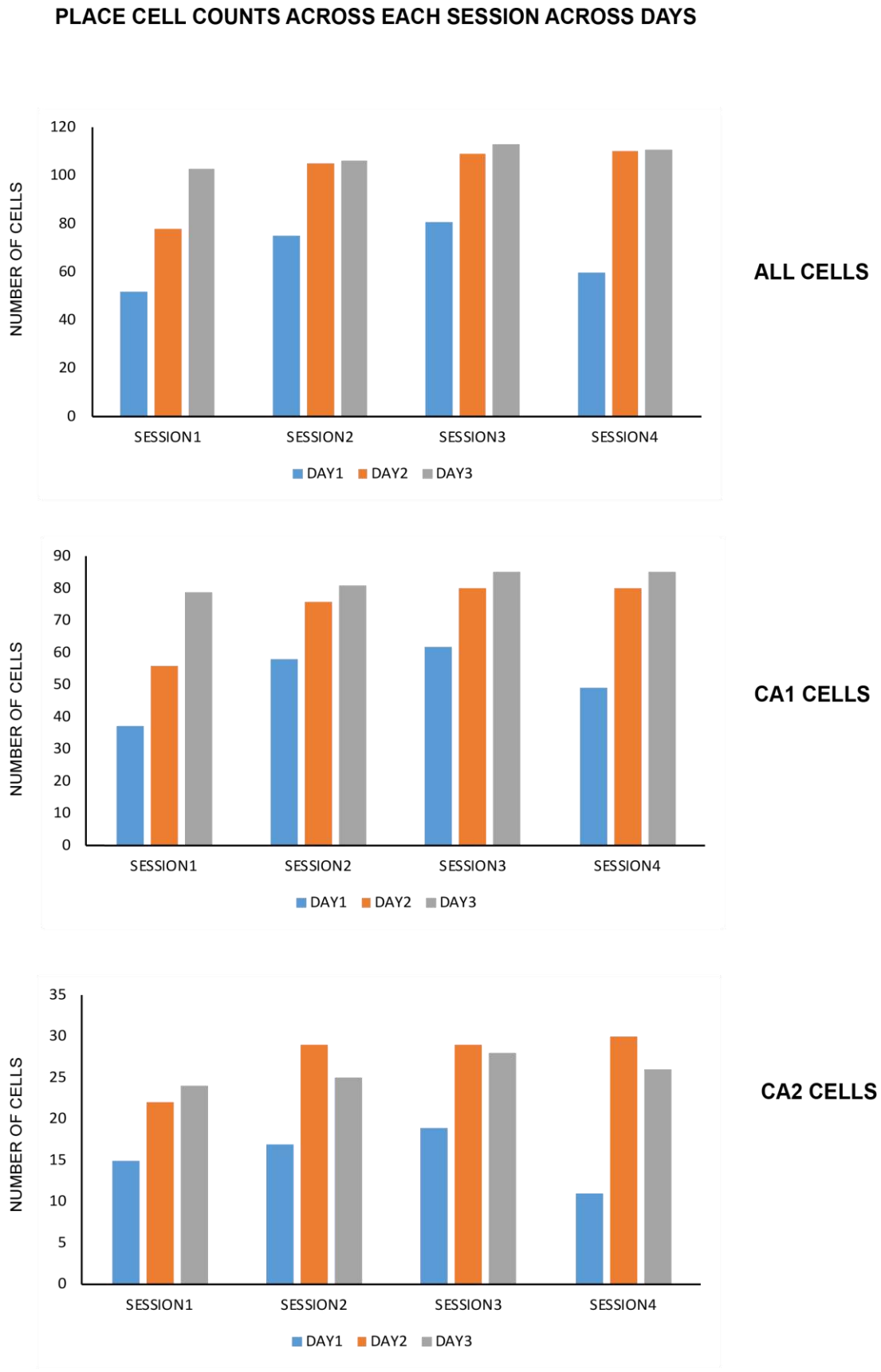

PLACE CELL COUNTS ACROSS EACH SESSION ACROSS DAYS
NUMBER OF CELLS
120
100
80
60
40
20
0
SESSION1
SESSION2
SESSION3
SESSION4
DAY1
DAY2
DAY3
ALL CELLS
NUMBER OF CELLS
90
80
70
60
50
40
30
20
10
0
SESSION1
SESSION2
SESSION3
SESSION4
DAY1
DAY2
DAY3
CA1 CELLS
NUMBER OF CELLS
35
30
25
20
15
10
5
0
SESSION1
SESSION2
SESSION3
SESSION4
DAY1
DAY2
DAY3
CA2 CELLS

Figure 3.5 d

3.1.4 Average firing rate of place cells

Introduction of novel /added arms to an already pre-existing environment does result in higher number of place cells firing for that space, (Frank *et al*,2004), but as observed in the results for place cell distribution, this phenomenon occurs only during early detection (first novelty introduction) and not during subsequent detections of novelty. Nonetheless, it is imperative that no matter how many times novelty is introduced in an environment, the animal recognizes this addition/modification and behaves accordingly. Thus, to explore what the neuronal ensemble response might be during subsequent novelty detections (if not an increase in place cell count), differences (if any) in other firing properties of these hippocampal pyramidal neurons were studied.

3.1.4.1 Average firing rates across days

The average firing rate of all place cells from both cell populations were calculated for each arm separately from all tracks, across all days (Table 3.4). It was observed that on day1, the arm with place cells having the highest average firing rate was arm2; while on day2, the highest firing rate was from place cells belonging to arm2b (the 1st arm of Run2new) and on day3, were from arm4 (middle arm of Run3new). (Figure 3.6-top panel). The same was observed for CA1 and CA2 populations separately, (Figure 3.6-middle and bottom panel). This seems to suggest the possibility that place cells firing for novel part of the track have a higher firing rate than those firing for the familiar part. Note: Arms shown in blue are common arms of the track (3 arms of RUN1, RUN2fam and RUN3fam), arms in red correspond to added track arms on day2 and 3 (3 individual arms of RUN2new and RUN3new), while orange arms are

not included in any analysis to maintain corresponding comparisons between remaining track areas, as explained in materials and methods section.

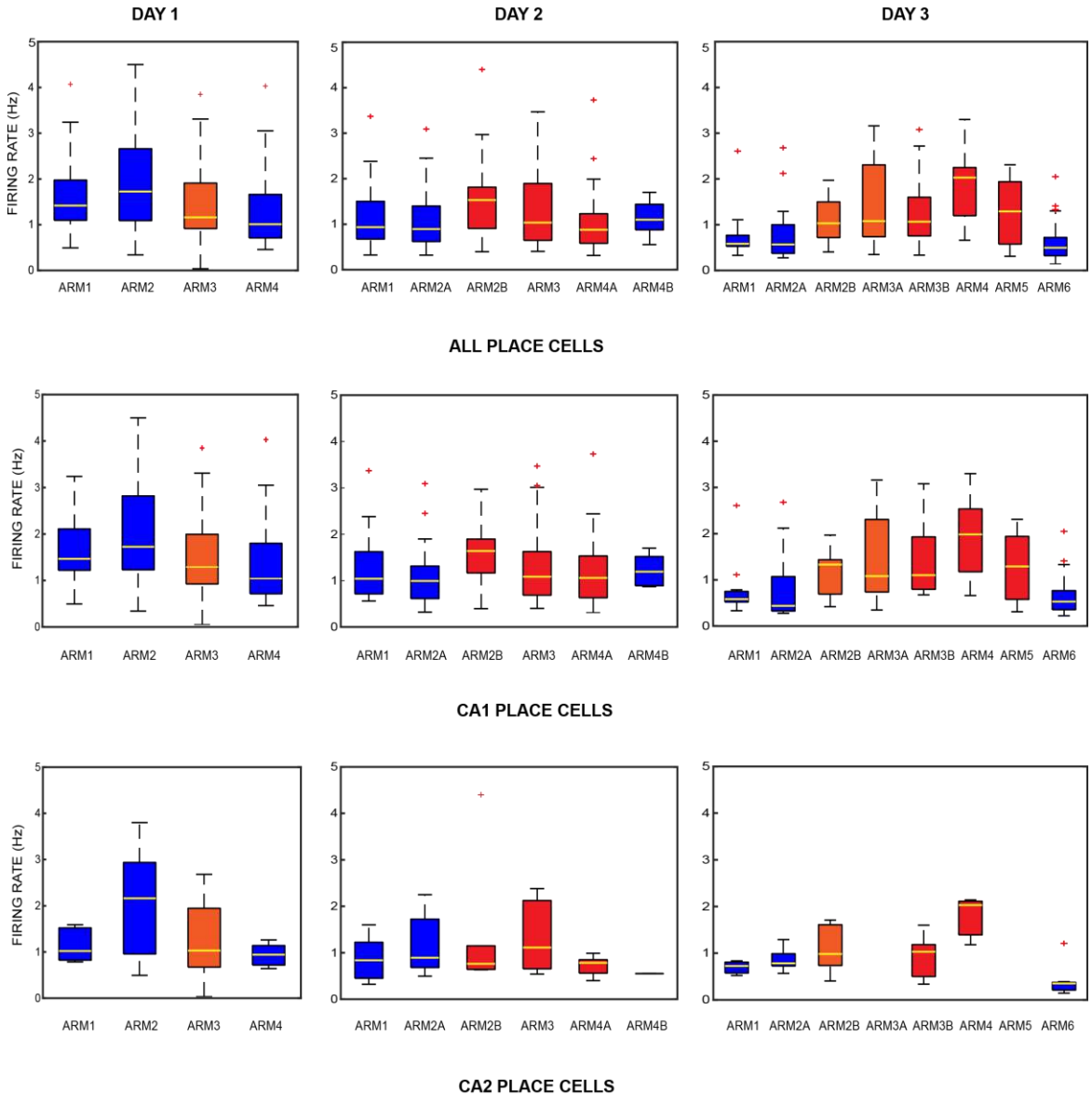


Figure 3.6:
Average firing rate of all place cells (CA1+CA2) across all arms of all tracks each day (Top panel). The arm with highest average firing rate on day 1 was arm2; on day 2 was arm2b (the 1st arm of novel track RUN2new) and on day 3 was arm 4(2nd arm of novel track RUN3new).

The same trend was seen when the average firing rates of CA1 place cells and CA2 place cells were compared separately (Middle and bottom panel).

3.1.4.2 Average firing rates across RUN

Within day comparisons of firing rates were made on days 2 and 3, in each RUN session, and higher firing rates were observed for place cells firing on RUN2new/Run3new arms, compared to RUN2fam/RUN3fam arms (Figure 3.7). Despite this observation, the increased firing rates for RUN2new were not statistically significantly higher than RUN2fam (Figure 3.7-middle column). In contrast, when the same comparison was extended to RUN3, the difference between firing rates of place cells on RUN3fam and RUN3new were more pronounced and significant (Mann Whitney test-all place cells: RUN3fam/RUN3new: $p<.0001$) (Figure3.7-right column, Figure 3.14). The same was observed for CA1 and CA2 separately as well (Mann Whitney test-RUN3fam/RUN3new: CA1: $p=.0001$; CA2: $p=.0219$) (Figure 3.7-middle and bottom panel, Figure 3.14). Gaps indicate lack of place cell firing in that arm. Color coding remains the same.

Next, average firing rates for CA1 and CA2 were calculated across RUN1, RUN2 (combining RUN2fam and RUN2new arms) and RUN3 (combining RUN3fam and RUN3new arms) sessions, and it was observed that they decreased across days (Figure 3.8-left column), further cementing the previous observation that as a completely novel area gets more and more familiar (0% familiar on day1, 50% familiar on day2 and 67% familiar on day3 as stated before), the firing rates of place cells coding for that area decrease, despite introduction of varying degrees of novelty in that environment. (Kruskal Wallis test: RUN1/RUN2/RUN3: CA1: $p<.0001$; CA2: $p=.0442$). This distinction was also observed within day comparison for RUN2 and RUN3, where both cell populations showed a higher average firing rate on RUN2new and RUN3new, compared to RUN2fam and RUN3fam respectively (Figures 3.7).

This indicates that the animal was able to distinguish between relative novelty and familiarity even when acquiring spatial episodic memories of a given environment in real time.

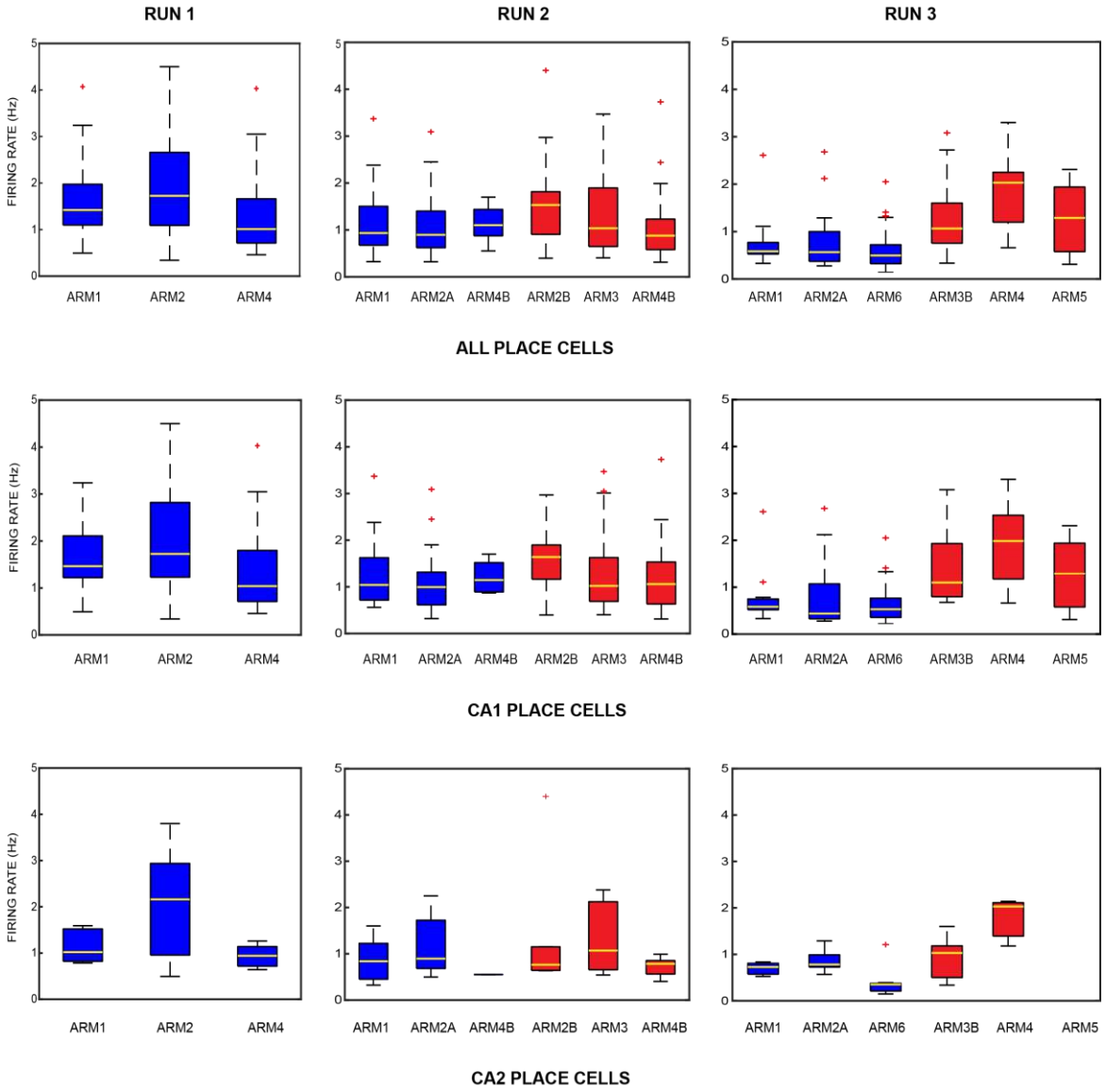


Figure 3.7: Average firing rates across each RUN session for all place cells (top panel) and for CA1 and CA2 cells separately (middle and bottom panels).

a. Within day comparison for RUN2 (middle column): average firing rates for both CA1 and CA2 place cells firing in 3 arms of RUN2new (red) were higher than the 3 arms of RUN2fam (blue), but not statistically significant.
b. Within day comparison for RUN3 (right column): average firing rates for both CA1 and CA2 place cells firing in 3 arms of RUN3new (red) were significantly higher than the 3 arms of RUN3fam (blue).

3.1.4.3 Average firing rates across common arms of the track

Further, the firing rate of place cells on common arms from RUN1 (arm1,2,4) were compared with RUN2fam (arm1,2a,4b) and RUN3fam (arm1,2a,6) correspondingly and it was seen that across all 3 arms, the firing rate decreased from day 1 to 2 to 3 (Kruskal Wallis test: RUN1/RUN2fam/RUN3fam $p<.0001$). The same was observed for CA1 and CA2 populations separately as well (Kruskal Wallis test: RUN1/RUN2fam/RUN3fam- CA1: $p<.0001$; CA2: $p=.0003$) (Figure 3.8-middle column).

Furthermore, the average firing rate on each of the 3 arms of the common tracks of RUN1, RUN2fam and RUN3fam were compared individually across days and it was observed that the firing rate decreased each day across each of the individual arms as well (Kruskal Wallis test: RUN1/RUN2fam/RUN3fam: arm1: $p=.0002$, arm2: $p=.001$, arm4: $p<.001$). The same was observed for CA1 place cells separately as well (Kruskal Wallis test: CA1: RUN1/RUN2fam/RUN3fam: arm1: $p=.0025$, arm2: $p=.0008$, arm4: $p=.0001$), but not for CA2. Although the same trend in each of the arms was observed in CA2 as well, it wasn't statistically significant, probably due to less number of cells. Therefore, as a novel spatial area becomes more and more familiar, the average firing rate of place cells firing for that space decreases (Figure 3.8-right column).

Finally, since RUN1 was completely novel on day1, the average firing rates were compared for RUN1/RUN2new/RUN3new for all cells together as well as CA1 and CA2 place cells separately, and it was observed that all 3 had similar average firing rates, thereby not showing a statistically significant test; indicating that average firing

rates of novel place cells are similar to one another. (RUN1/RUN2new/RUN3new: Kruskal Wallis test: all place cells: p=.107; CA1 cells: p=.259; CA2 cells: p=.250)

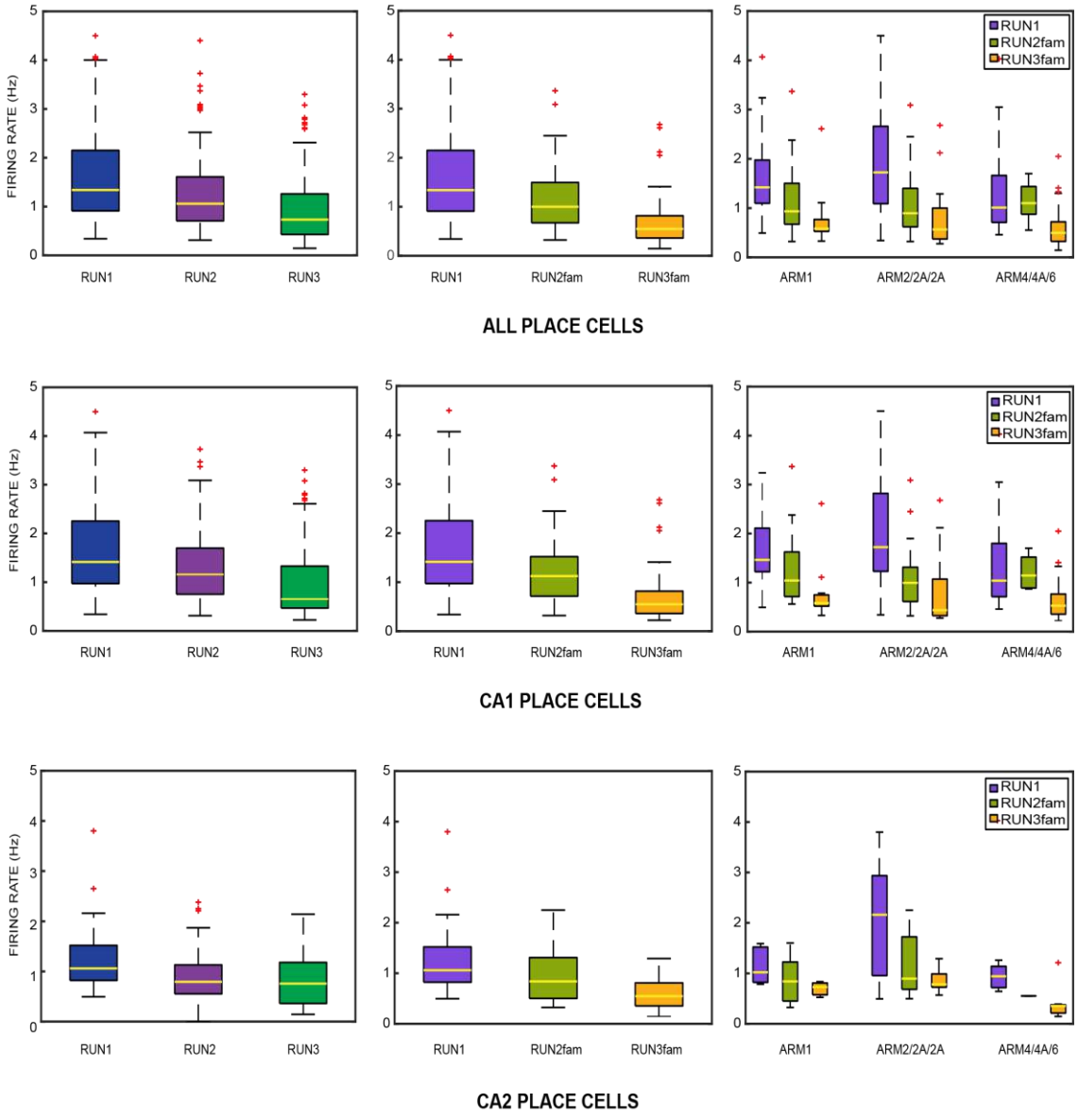


Figure 3.8:

a. Average firing rates cross RUN1, RUN2 and RUN3 (left column). For both cell populations, the average firing rates decreased as the overall familiarity of the environment increased (0% familiarity on day1, 50% familiarity on day 2 and 67% familiarity on day 3).
b. Average firing rates across common arms of all tracks (middle column): RUN1/RUN2fam/RUN3fam comparison for individual arms across days: as the novel area of RUN1 became more and more familiar on day2 (RUN2fam) and day3 (RUN3fam), the average firing rates of place cells decreased each day.
c. This trend was observed further within each individual arm of RUN1, RUN2fam and RUN3fam (right column) where the firing rate of cells decreased with increasing familiarity

3.1.4.4 Average firing rates across each session in a day:

Since increasing familiarity of the environment across days led to decrease in firing rates of CA1 and CA2 ensembles for not just common arms of the track but also across overall RUN sessions, average firing rates for place cells were observed across all sessions within each day, to check if increasing familiarity would also lead to decrease in firing rates of place cells within the same day from session1 to session4. In contrast to the influence of increasing familiarity across days, the influence of increasing familiarity within a day did not lead to the same observation. Firing rates varied across sessions within a day for all days, and no conclusive trend of increasing or decreasing firing rates was observed (Figure 3.9a). It wasn't observed for CA1 or CA2 cells separately as well (Figure 3.9 b and c).

As was done for place cell counts, the firing rates for one session were pooled across days and this time, it was observed that firing rates significantly decreased within each session across days. The same was observed for both ensembles separately as well, though not statistically significant. (figure 3.9d). Kruskal Wallis test: all cells: p=.0434.

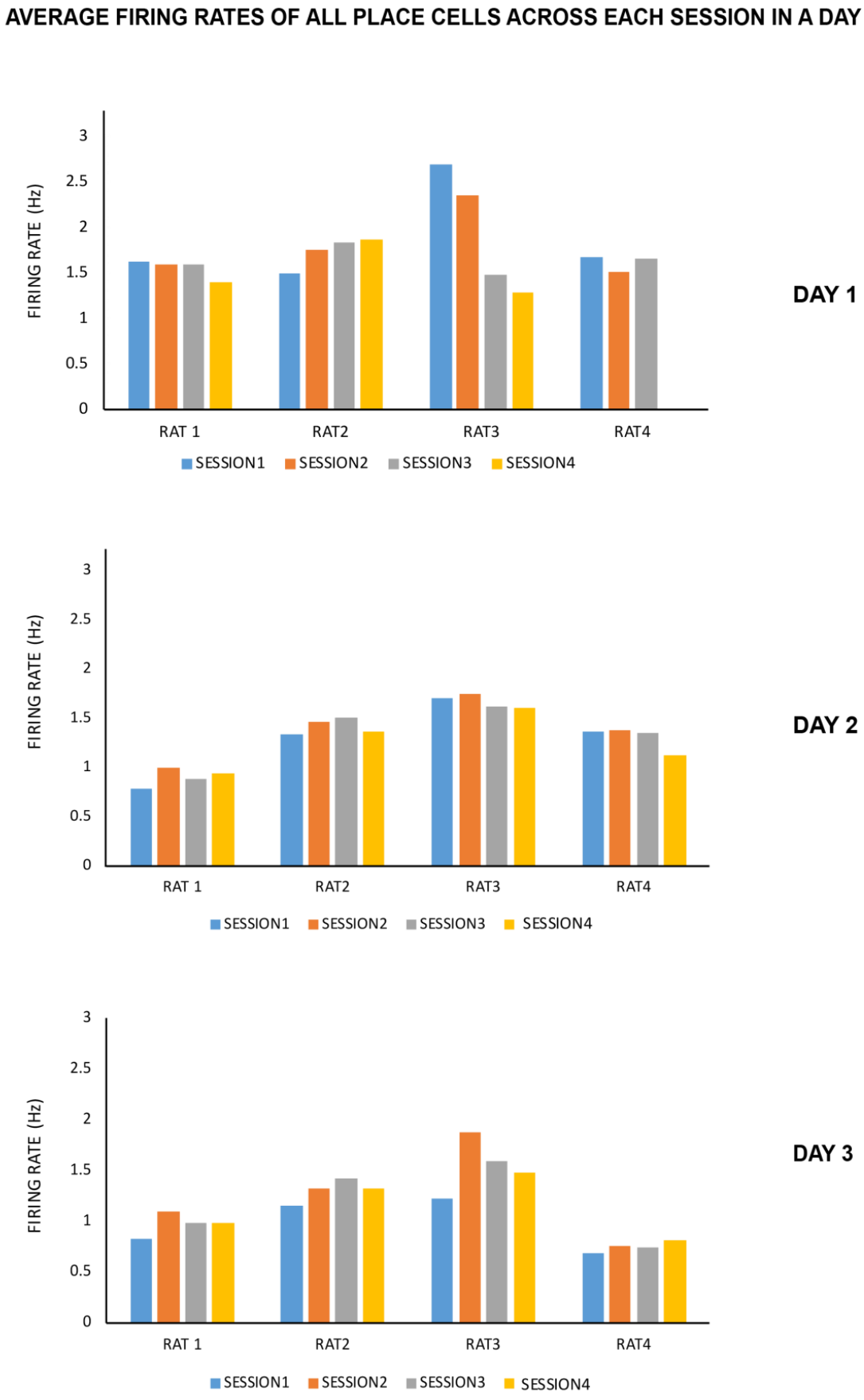

AVERAGE FIRING RATES OF ALL PLACE CELLS ACROSS EACH SESSION IN A DAY
FIRING RATE (Hz)
3
2.5
2
1.5
1
0.5
0
RAT 1
RAT2
RAT3
RAT4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 1
DAY 2
DAY 3

Figure 3.9 a

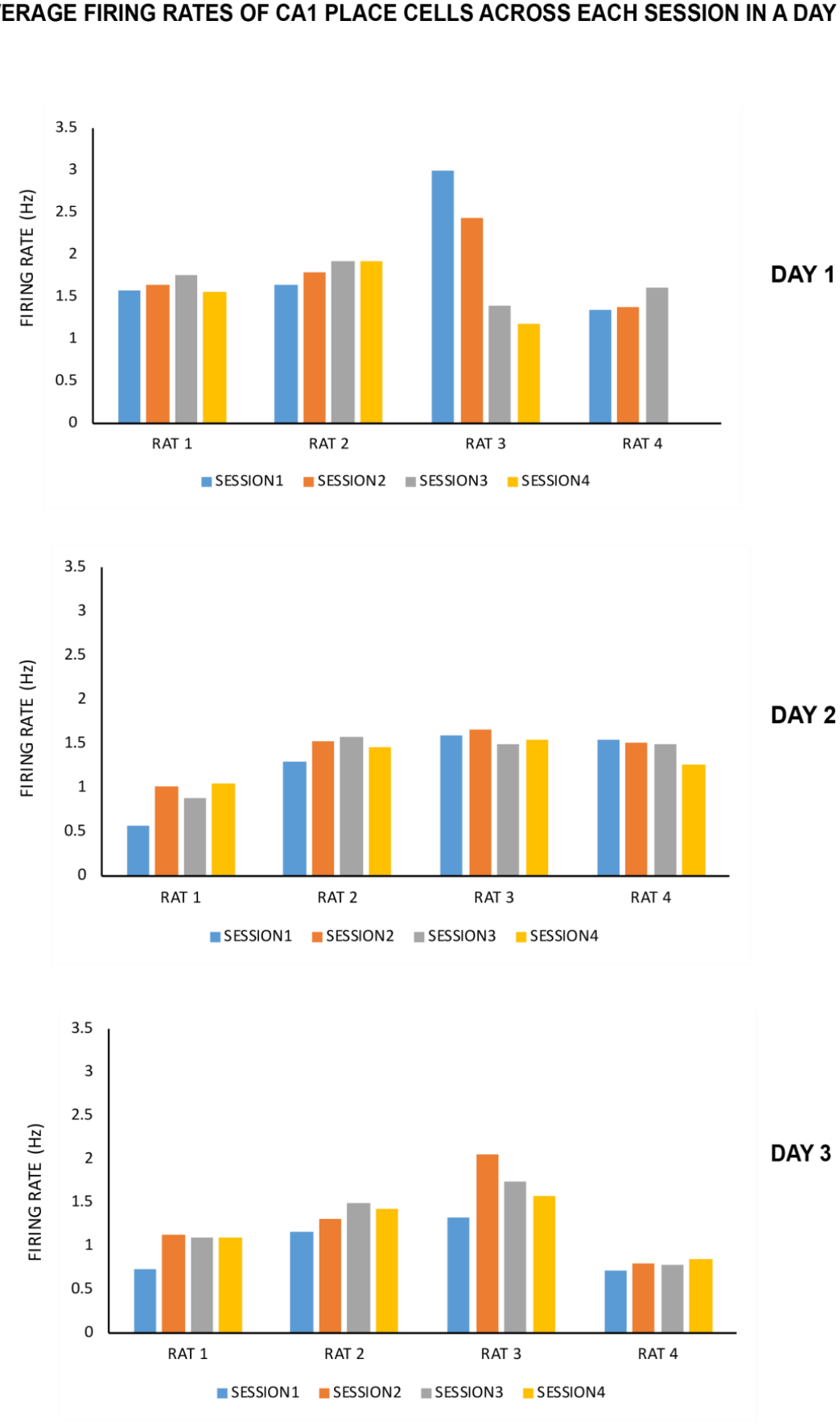

AVERAGE FIRING RATES OF CA1 PLACE CELLS ACROSS EACH SESSION IN A DAY
FIRING RATE (Hz)
3.5
3
2.5
2
1.5
1
0.5
0
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 1
DAY 2
DAY 3

Figure 3.9 b

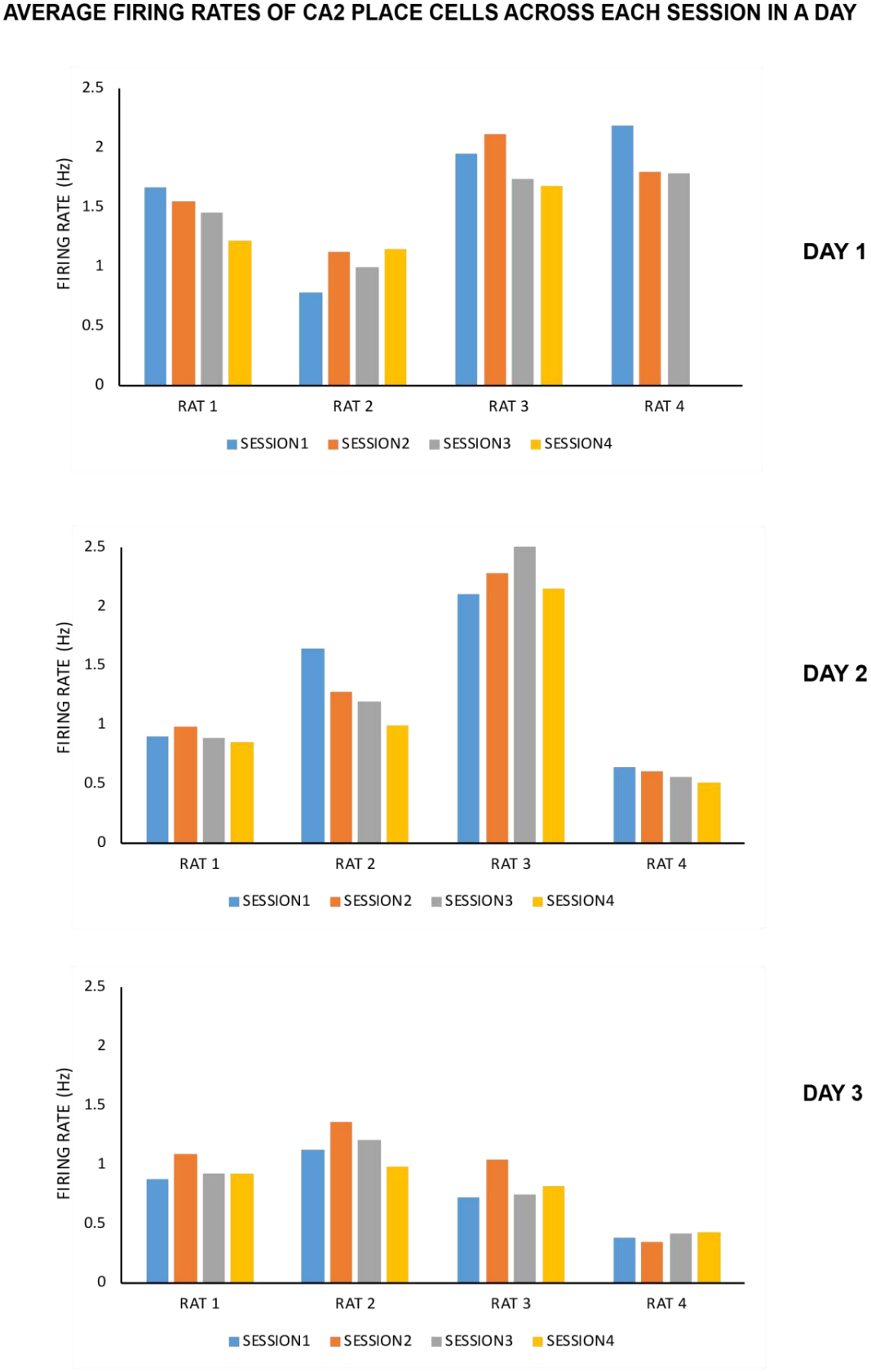

AVERAGE FIRING RATES OF CA2 PLACE CELLS ACROSS EACH SESSION IN A DAY
FIRING RATE (Hz)
2.5
2
1.5
1
0.5
0
RAT 1
RAT 2
RAT 3
RAT 4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 1
DAY 2
DAY 3

Figure 3.9 c

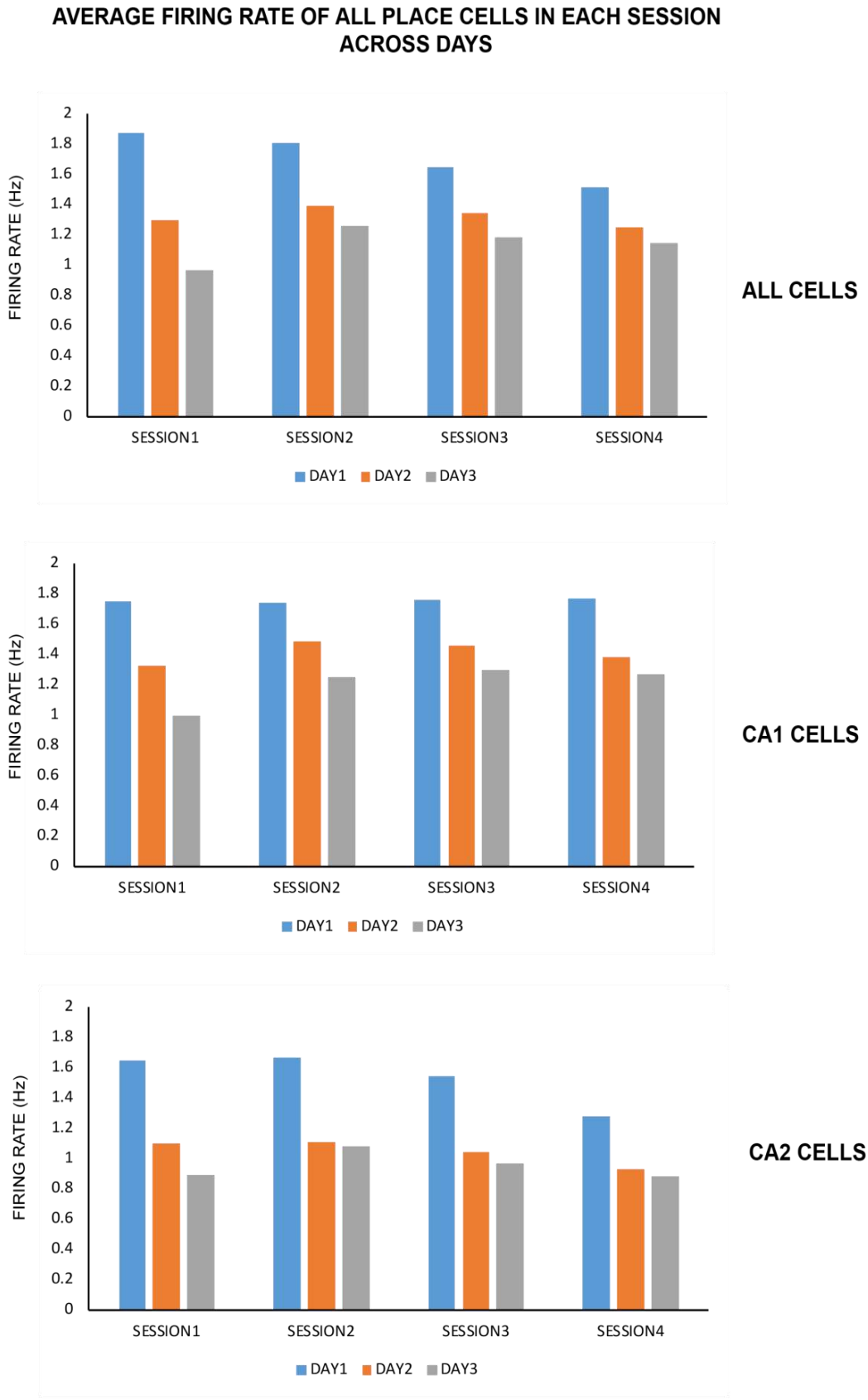

AVERAGE FIRING RATE OF ALL PLACE CELLS IN EACH SESSION ACROSS DAYS
FIRING RATE (Hz)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SESSION1
SESSION2
SESSION3
SESSION4
DAY1
DAY2
DAY3
ALL CELLS
CA1 CELLS
CA2 CELLS

Figure 3.9 d

AVERAGE FIRING RATE FOR CA1 AND CA2 PLACE CELLS ACROSS ALL DAYS

DAY1	ARM1	ARM2	ARM3	ARM4	TOTAL/AVERAGE
CA1 CELLS	24	18	13	22	77
AVERAGE FIRING RATE	1.653	2.039	1.572	1.418	1.671
CA2 CELLS	6	5	7	7	25
AVERAGE FIRING RATE	1.126	2.043	1.272	0.936	1.344

DAY2	ARM1	ARM2A	ARM2B	ARM3	ARM4A	ARM4B	TOTAL/AVERAGE
CA1 CELLS	12	16	23	21	21	6	99
AVERAGE FIRING RATE	1.331	1.151	1.583	1.346	1.201	1.212	1.304
CA2 CELLS	7	5	6	7	9	1	35
AVERAGE FIRING RATE	0.876	1.187	1.392	1.368	0.732	0.551	1.018

DAY3	ARM1	ARM 2A	ARM 2B	ARM 3A	ARM 3B	ARM 4	ARM5	ARM6	TOTAL/AVERAGE
CA1 CELLS	12	16	7	11	13	12	8	29	108
AVERAGE FIRING RATE	0.775	0.797	1.139	1.517	1.435	1.896	1.279	0.653	1.186
CA2 CELLS	3	5	6	0	7	3	0	8	32
AVERAGE FIRING RATE	0.695	0.861	1.071	0.000	0.925	1.783	0.000	0.404	0.956

Table 3.4: Total number of CA1 and CA2 place cells in each arm of all tracks across days and their corresponding average firing rates.

3.1.5 Pairwise cross correlations between novel and familiar place cell pairs

Previous studies have reported that CA1 cell pairs with overlapping place fields show a higher correlation and co-ordinated activity during high frequency events (HFE) in novel environments and decrease with increasing familiarity (Cheng & Frank, 2008). Therefore, pairwise cross correlation differences between novel cell pairs and familiar cell pairs on all tracks, across and within days were compared. Those cell pairs were chosen that had overlapping place fields (peak distance<15cms) and were recorded on different tetrodes (Wilson & McNaughton, 1994; Skaggs & McNaughton, 1996). These cell pairs included CA1-CA1 pairs, CA1-CA2 pairs and CA2-CA2 pairs. Since CA2-CA2 pairs were relatively fewer in number, comparisons were limited to all cell pairs taken together, and CA1-CA1 and CA1-CA2 pairs separately (Table 3.5).

3.1.5.1 Pairwise cross correlations between novel and familiar place cell pairs across days

Pairwise cross correlations from all cell pairs from each arm of all 3 tracks were compared, as was previously done for average firing rates. It was observed that on day2, the highest correlation was between cell pairs belonging to arm 2b (the first arm of Run2new) and on arm 5, followed by arm 4 (2^{nd} and 3^{rd} arms of RUN3new) on day3(Figure 3.10-top panel). This is the same trend observed while comparing average firing rates previously. The same comparison was done for CA1-CA1 pairs and CA1-CA2 pairs separately as well: peak cross correlations were seen for cell pairs belonging to arms 4a (CA1-CA1 pairs) and arm2b (CA1-CA2 pairs) on day 2 (both arms belonging to RUN2new); and on arms 5 (CA1-CA1 pairs) and arm 3b (CA1-CA2 pairs) on day 3 (both arms belonging to RUN3new) (Figure 3.10-middle and bottom panel). Color coding of all arms remains the same. Gaps indicate lack of place cell firing in that arm from either CA1 or CA2.

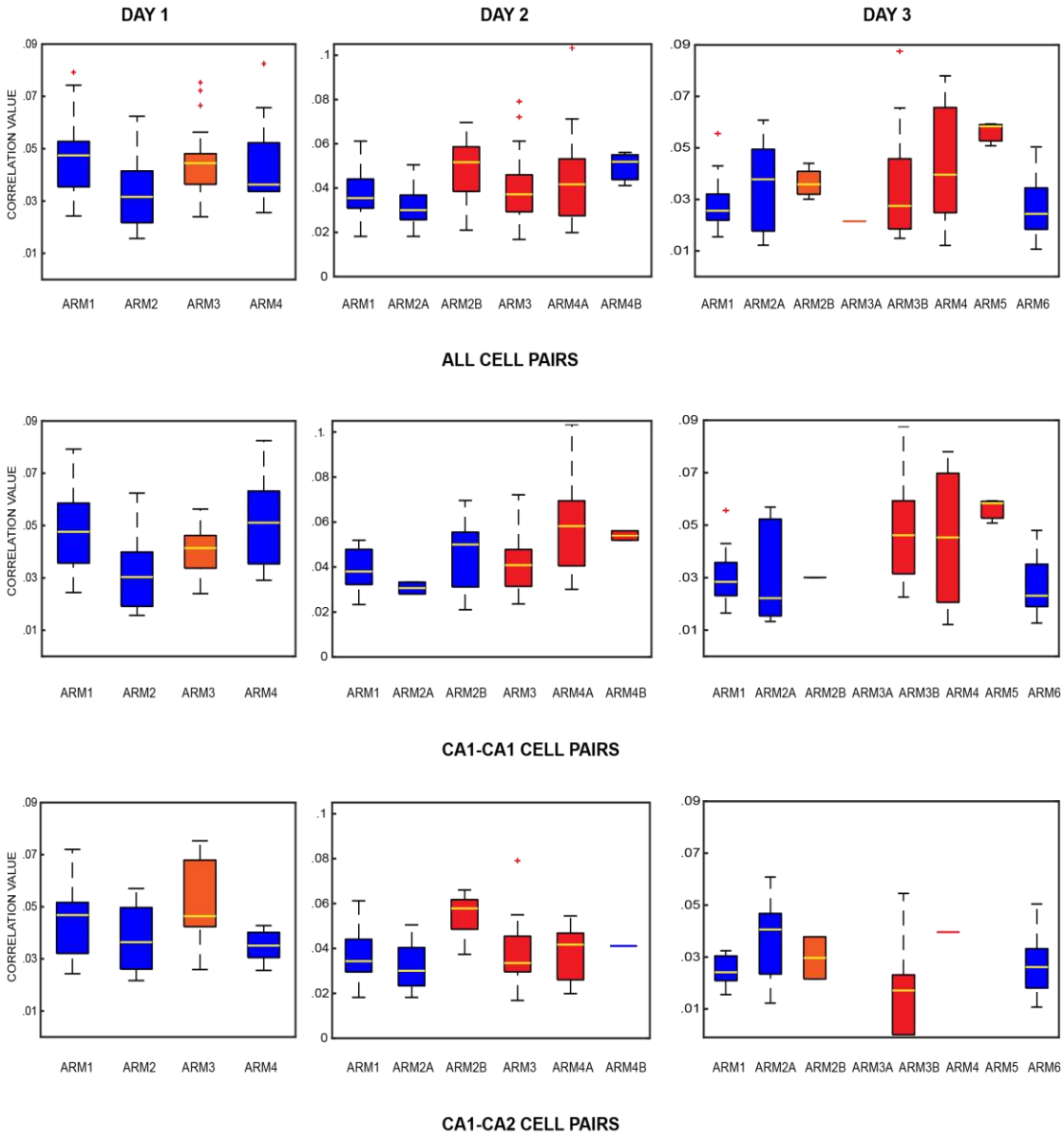


Figure 3.10:

a. Pairwise cross correlations of CA1 and CA2 cell pairs (CA1-CA1, CA1-CA2, CA2-CA2) across all arms of all tracks each day. The arm with highest average firing rate on day 2 was arm2b (the 1st arm of novel track RUN2new) and on day 3 was arm5 followed by arm 4(1st 2 arms of novel track RUN3new). (top panel)

b. Similar trend was seen when Pairwise cross correlations were seen independently for CA1-CA1 place cells pairs and CA1-CA2 place cell pairs. (middle and bottom panels)

3.1.5.2 Pairwise cross correlations between novel and familiar place cell pairs across RUN

Cross correlations between cell pairs were plotted for each RUN session across days. Correlations were compared across RUN1, RUN2 and RUN3 but no significant trend was observed due to comparable correlation values between RUN1 and RUN2 although a decrease was seen in RUN3 (Figure3.12-left column). For within day comparisons on day 2 and 3, it was observed that correlations between pairs on RUN2new were higher than RUN2fam and for RUN3new than RUN3fam. The same was observed for CA1-CA1 and CA1-CA2 pairs separately as well. As observed for average firing rates, the differences were more pronounced on day 3 than day2. (Mann Whitney test: RUN3fam/RUN3new-all pairs: p.0312; CA1-CA1 pairs: p<.001) (Figure 3.11, 3.14).

3.1.5.3 Pairwise cross correlations between novel and familiar place pairs across common arms of the track

Next, pairwise cross correlations between all cell pairs were compared across RUN1, RUN2fam and RUN3fam, and it was observed that they decreased across days (Kruskal Wallis test: RUN1/RUN2fam/RUN3fam: p<.0001). There was no correlation found for within individual arm comparison (arms1,2,4) for the same, as was found for average firing rates; therefore, all arms were taken together and compared across days. The same was observed for CA1-CA1 and CA1-CA2 cell pairs separately as well (Kruskal Wallis test: RUN1/RUN2fam/RUN3fam: CA1-CA1 pairs: p<.001; CA1-CA2 pairs: p=.0009) (Figure 3.12-right column).

Furthermore, the cross correlations between cell pairs on novel areas from all 3 days were compared as well (RUN1/RUN2new/RUN3new) and there wasn't any significant difference found, similar to the trend observed previously for average firing rates as well; indicating that pairwise cross correlations between novel cell pairs are also similar in values. (RUN1/RUN2new/RUN3new: Kruskal Wallis test: all pairs: p=.088; CA1-CA1 pairs: p=.485).

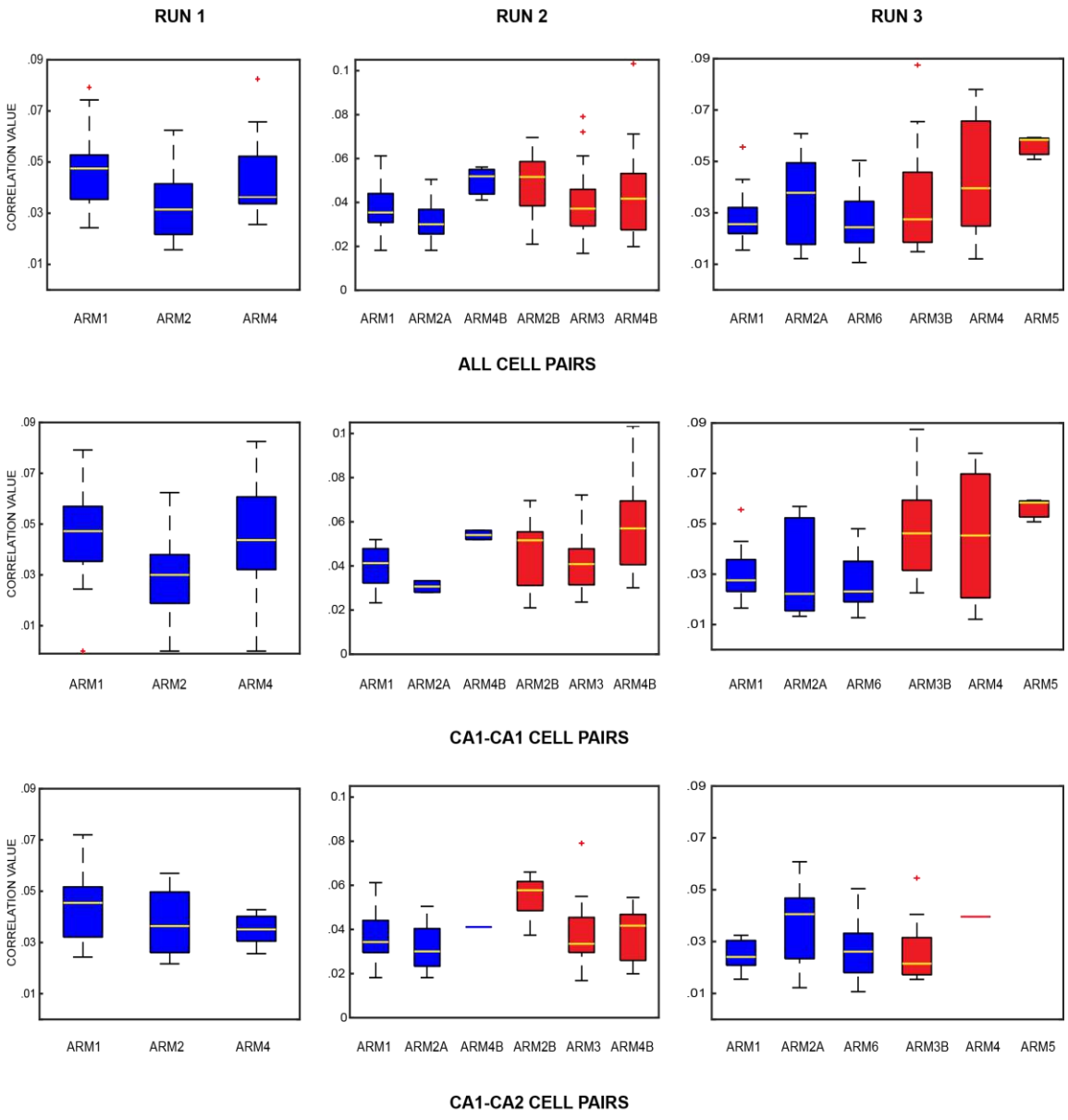


Figure 3.11: Pairwise cross correlation values comparison across all RUN sessions.

a. Within day comparison for RUN2 (middle column): pairwise cross correlations for CA1-CA1 and CA1-CA2 cell pairs, firing in 3 arms of RUN2new (red) were higher than 3 arms of RUN2fam (blue), but not significantly higher.

b. Within day comparison for RUN3 (right column): pairwise cross correlations for CA1-CA1 and CA1-CA2 cell pairs firing in 3 arms of RUN3new (red) were significantly higher than 3 arms of RUN3fam (blue).

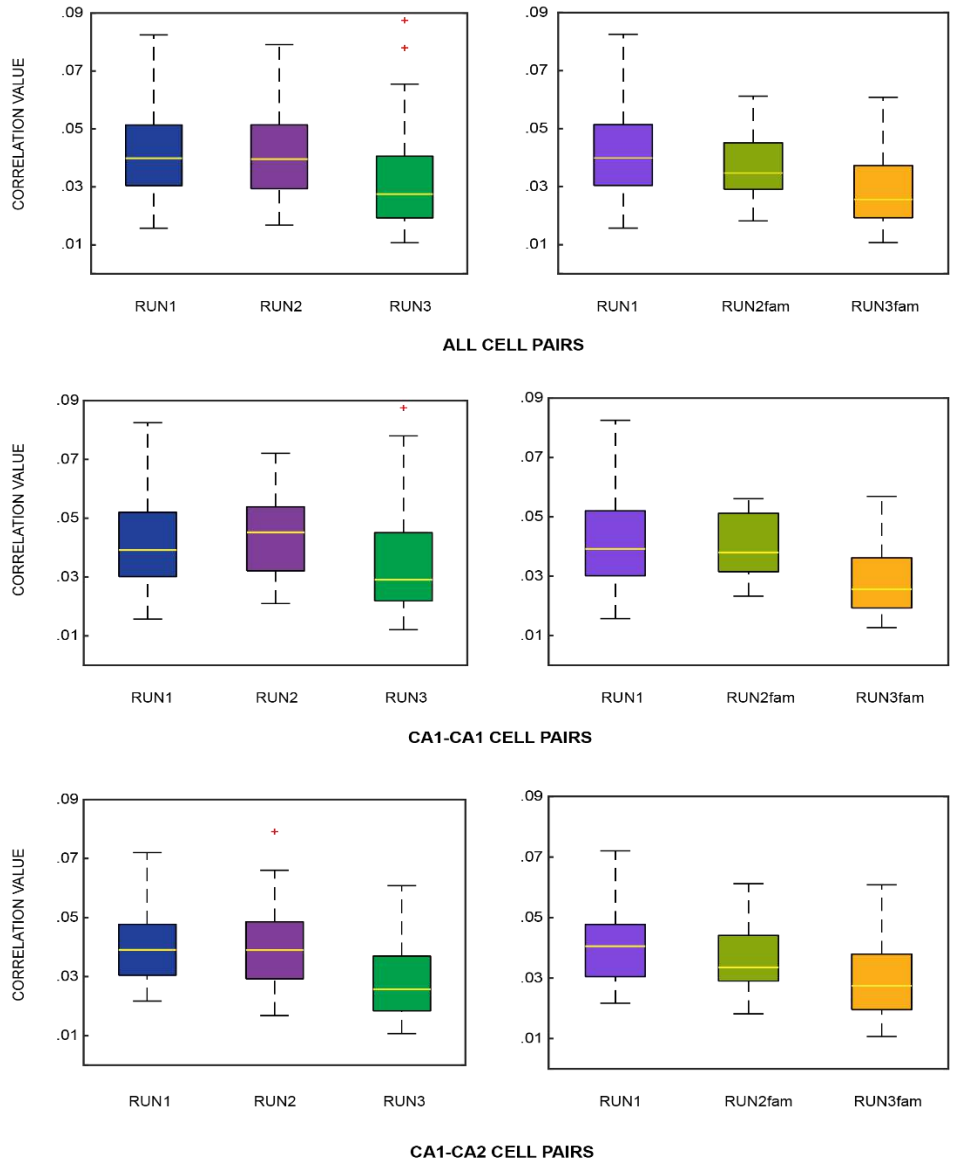


Figure 3.12

a. Pairwise cross correlation across RUN1, RUN2 and RUN3 (left column).

b. Pairwise cross correlation across common arms of all tracks (right column): RUN1/RUN2fam/RUN3fam comparison for individual arms across days: as the novel area of RUN1 became more and more familiar on day2 (RUN2fam) and day3 (RUN3fam), the cross correlation values of cell pairs decreased each day.

3.1.5.4 Pairwise cross correlation across sessions in a day:

As was previously done for place cell counts and average firing rates, pairwise cross correlation values were compared across sessions in each day. Similar to previous comparisons, no conclusive trend was observed for corellation values of all cell pairs across each session on any of the days (Figure 3.13a), or for CA1-CA1 and CA1-CA2 cell pairs separately (Figure3.13b and c).

When correlation values were pooled across days for individual sessions, a trend similar to the one observed for average firing rates emerged. Correlation values decreased across days within each session (Figure 3.13d) and was observed separately for CA1-CA1 and CA1-CA2 cell pairs as well, but was not deemed to be significant.

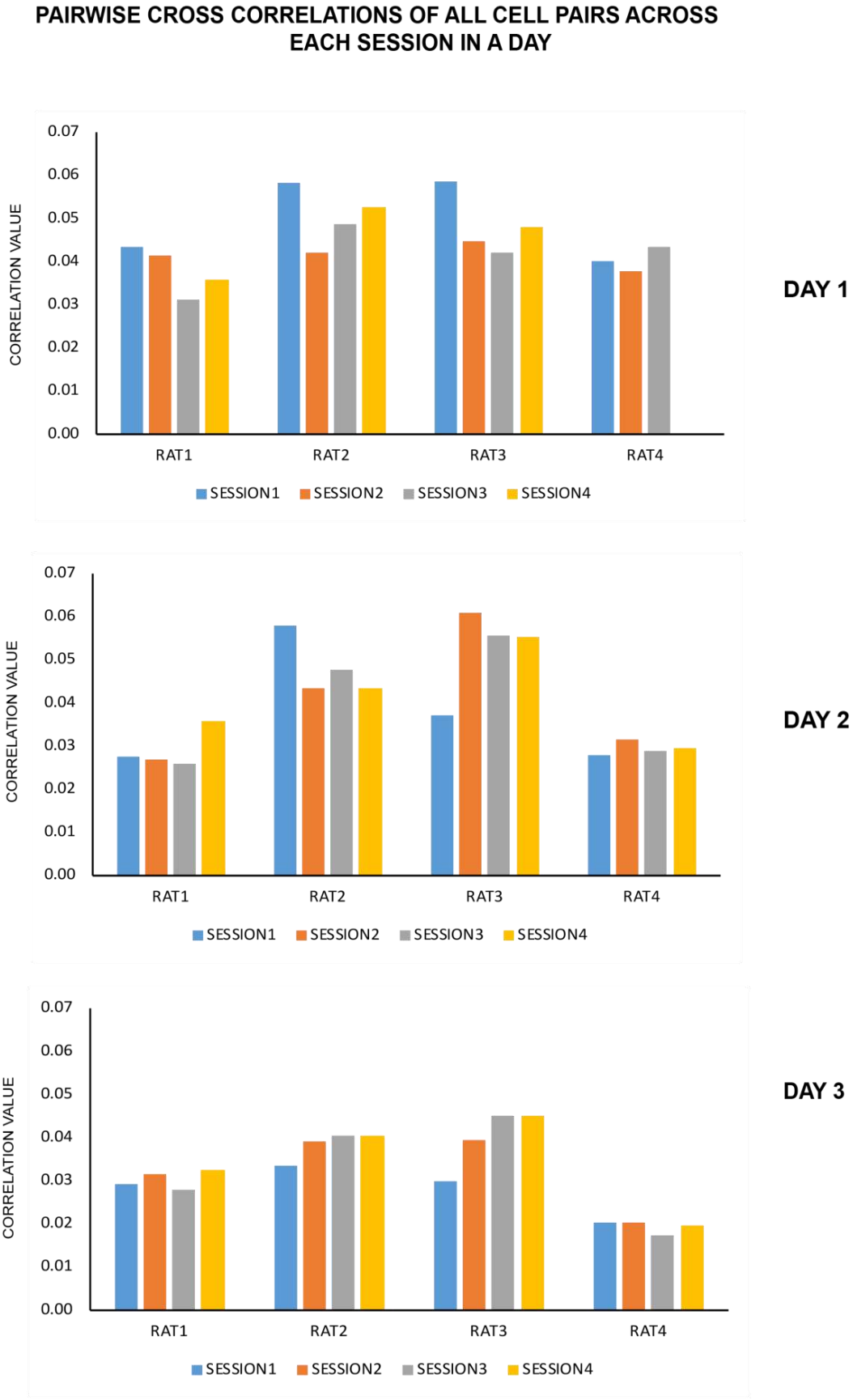

PAIRWISE CROSS CORRELATIONS OF ALL CELL PAIRS ACROSS EACH SESSION IN A DAY
CORRELATION VALUE
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
RAT1
RAT2
RAT3
RAT4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 1
DAY 2
DAY 3

Figure 3.13 a

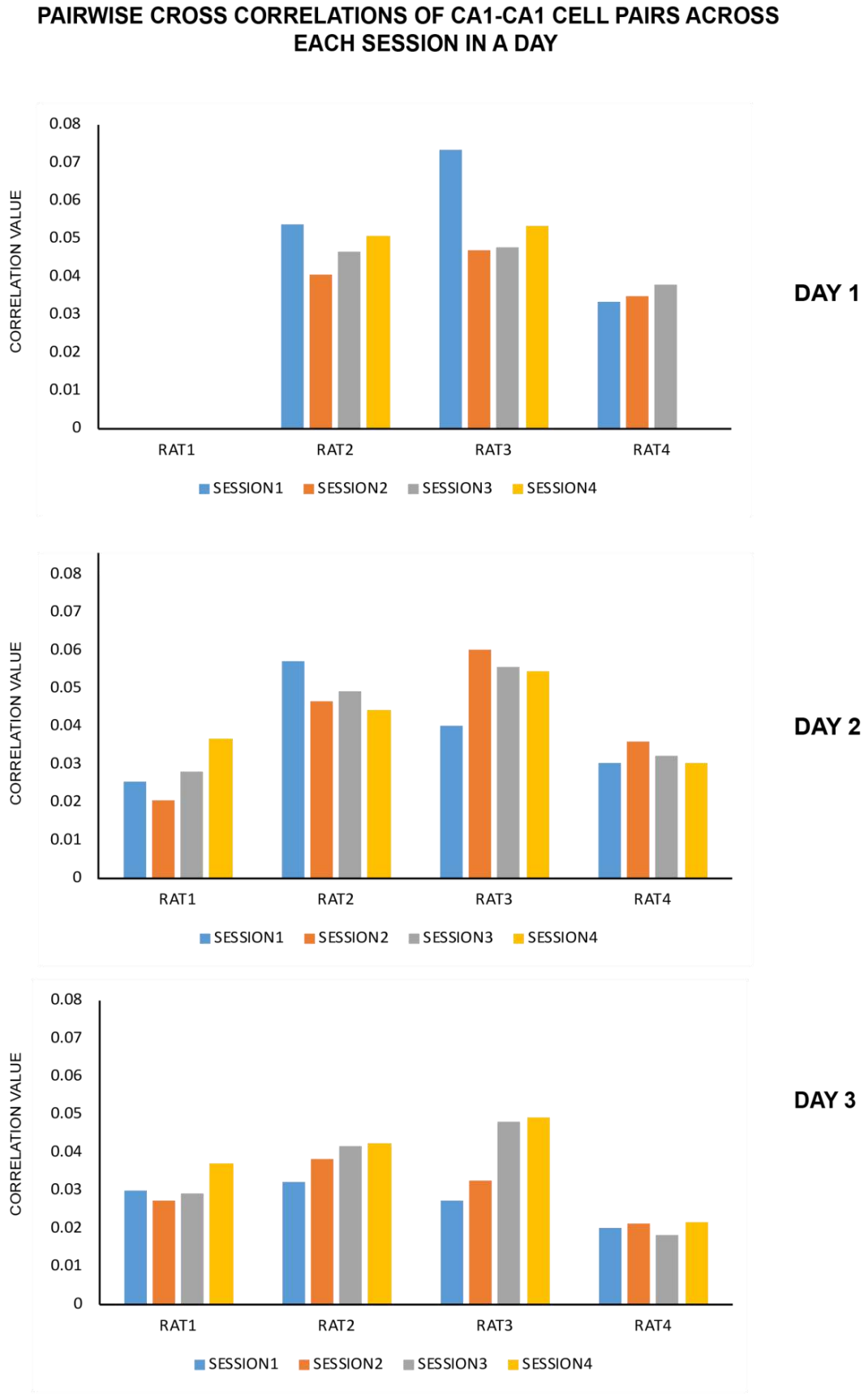

PAIRWISE CROSS CORRELATIONS OF CA1-CA1 CELL PAIRS ACROSS EACH SESSION IN A DAY
CORRELATION VALUE
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
RAT1
RAT2
RAT3
RAT4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 1
DAY 2
DAY 3

Figure 3.13 b

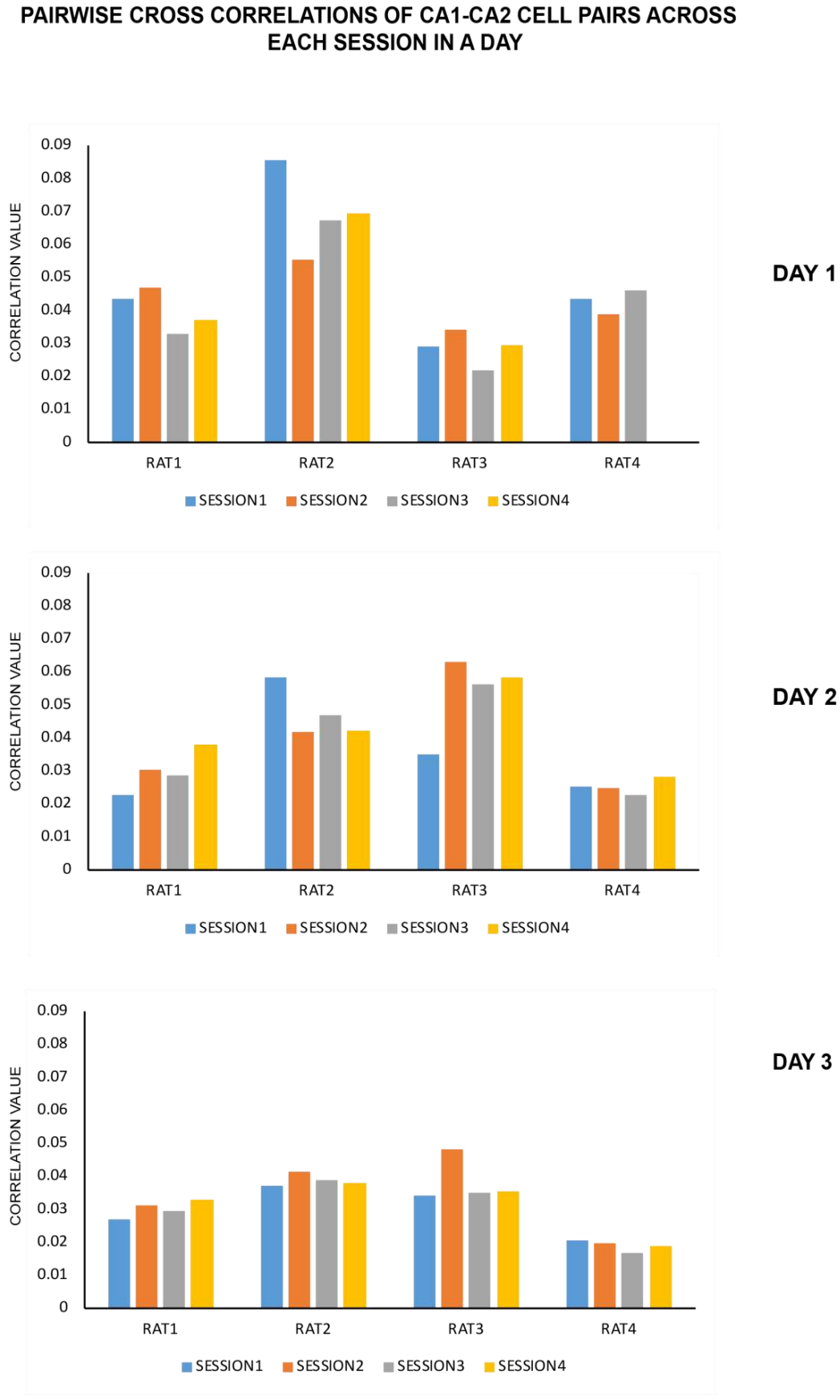

PAIRWISE CROSS CORRELATIONS OF CA1-CA2 CELL PAIRS ACROSS EACH SESSION IN A DAY
CORRELATION VALUE
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
RAT1
RAT2
RAT3
RAT4
SESSION1
SESSION2
SESSION3
SESSION4
DAY 1
DAY 2
DAY 3

Figure 3.13 c

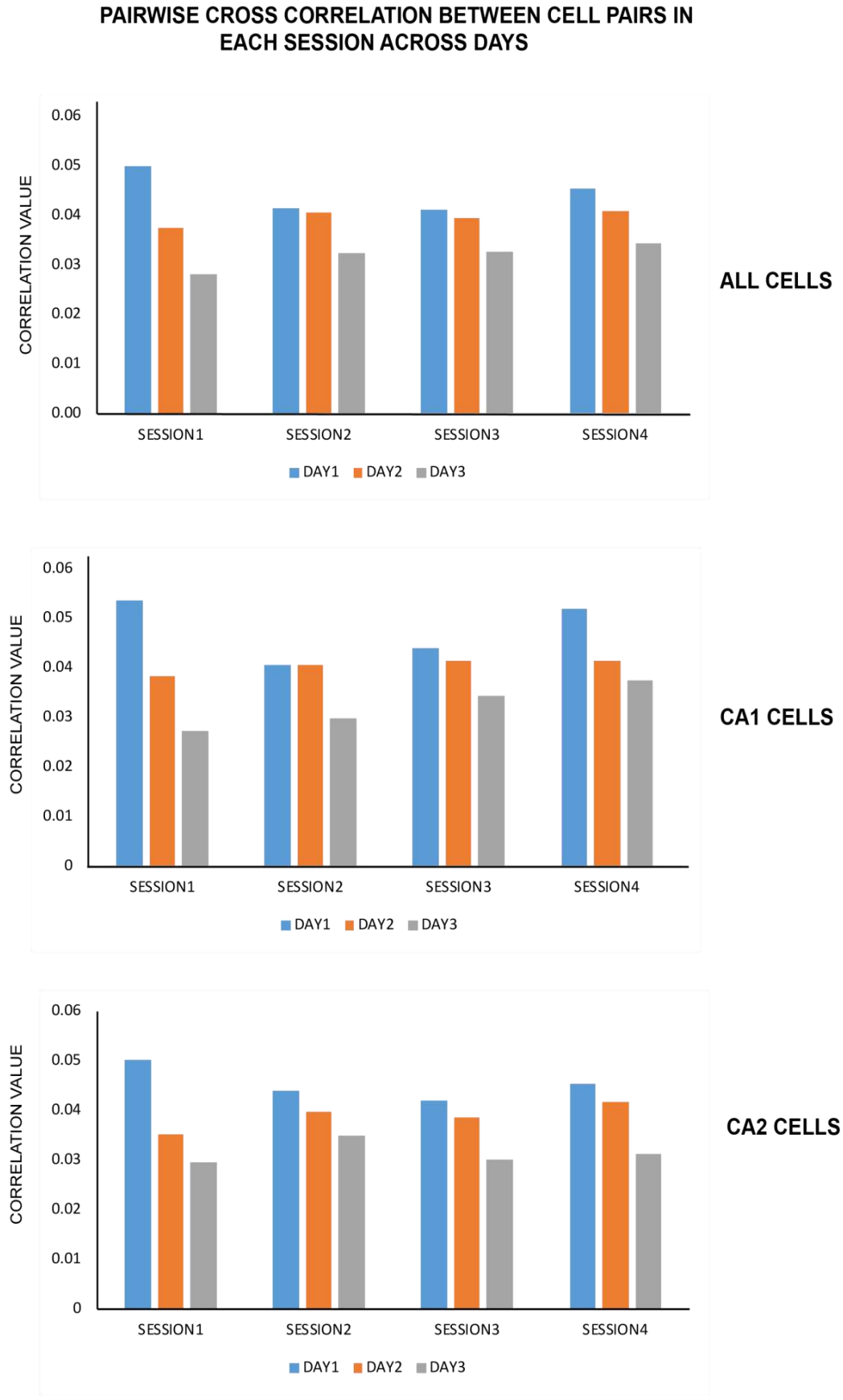

PAIRWISE CROSS CORRELATION BETWEEN CELL PAIRS IN EACH SESSION ACROSS DAYS
CORRELATION VALUE
0.06
0.05
0.04
0.03
0.02
0.01
0.00
SESSION1
SESSION2
SESSION3
SESSION4
DAY1
DAY2
DAY3
ALL CELLS
CORRELATION VALUE
0.06
0.05
0.04
0.03
0.02
0.01
0
SESSION1
SESSION2
SESSION3
SESSION4
DAY1
DAY2
DAY3
CA1 CELLS
CORRELATION VALUE
0.06
0.05
0.04
0.03
0.02
0.01
0
SESSION1
SESSION2
SESSION3
SESSION4
DAY1
DAY2
DAY3
CA2 CELLS

Figure 3.13 d

AVERAGE PAIRWISE CROSS CORELLATION OF CA1 AND CA2 CELL PAIRS ACROSS ALL DAYS

DAY1	ARM1	ARM2	ARM3	ARM4	TOTAL/AVERAGE
CA1-CA1 PAIRS	18	14	13	7	**52**
CROSS CORELLATION	0.048	0.031	0.041	0.051	**0.043**
CA1-CA2 PAIRS	10	4	9	6	**29**
CROSS CORELLATION	0.044	0.038	0.051	0.035	**0.042**
ALL PAIRS	28	18	22	13	**81**
CROSS CORELLATION	0.047	0.033	0.045	0.043	**0.042**

DAY2	ARM1	ARM2A	ARM2B	ARM3	ARM4A	ARM4B	TOTAL/AVERAGE
CA1-CA1 PAIRS	7	2	19	19	7	2	**56**
CROSS CORELLATION	0.039	0.031	0.046	0.041	0.059	0.052	**0.045**
CA1-CA2 PAIRS	15	6	9	19	9	1	**59**
CROSS CORELLATION	0.037	0.032	0.054	0.038	0.037	0.041	**0.040**
ALL PAIRS	22	8	28	41	19	3	**121**
CROSS CORELLATION	0.038	0.032	0.048	0.038	0.044	0.047	**0.041**

DAY3	ARM1	ARM2A	ARM2B	ARM3A	ARM3B	ARM4	ARM5	ARM6	TOTAL/AVERAGE
CA1-CA1 PAIRS	15	8	1	0	9	4	3	24	**64**
CROSS CORELLATION	0.031	0.031	0.030	0.000	0.050	0.045	0.056	0.026	**0.034**
CA1-CA2 PAIRS	11	13	2	0	12	1	0	19	**58**
CROSS CORELLATION	0.025	0.036	0.030	0.000	0.040	0.040	0.000	0.027	**0.025**
ALL PAIRS	26	22	4	1	26	5	3	43	**130**
CROSS CORELLATION	0.028	0.035	0.034	0.022	0.036	0.044	0.056	0.027	**0.035**

Table 3.5: Total number of CA1-CA1, CA1-CA2 and all (CA1-CA1, CA1-CA2, CA2-CA2) place cell pairs in each arm of all tracks across days and their corresponding pairwise cross correlation values.

DISCUSSION

Discussion:

The aim of this study was to elucidate how hippocampal ensembles, particularly CA1 and CA2, dynamically evolve, modify and code for spatial familiarity and novelty in a given environment. When novelty is first introduced in a given environment, both ensembles devoted more number of place cells to encode for the novel area, as compared to the relatively familiar one, even though both spaces were similar in length, spatial structure and form, i.e. C-shaped tracks (RUN2fam/RUN2new). Additionally, place cells from both ensembles coding for the novel space had a relatively higher average firing rate and pairwise cross correlation compared to place cells firing for familiar locations as well, though not achieving statistical significance. Thus, the very first introduction of novelty resulted in a disproportionate allocation of place cell distribution (65% place cells coding for 50% novel area, 35% place cells for 50% familiar area). Conversely, when novelty was introduced on the subsequent day (day 3), no such disparity in place cell distribution was seen in any ensemble (30% place cells coding for 33% novel space, 70% place cells for 67% familiar space). Nevertheless, the place cells coding for novel space did have a significantly higher average firing rate and pairwise cross correlation than their familiar counterparts (RUN3fam/RUN3new). In addition, as a novel space became more and more familiar over successive days, the average firing rate and pairwise cross correlation for cells coding for this area decreased with increasing familiarity (RUN1>RUN2fam>RUN3fam). These observations indicate how hippocampal ensembles continually evolve, update and code for novelty and familiarity in the same space, in real-time, as the rat traverses through the given environment, and how robustly it does so, even as relative familiarity and novelty is experienced in the same

lap itself (days 2 and3). Thus it seems that even within laps, the brain is able to distinguish between the relative novelty and familiarity pretty well, and this 'novelty coding' is rooted in specific characteristics of the novel place cells that develop as the rat traverses that area for the first time.

3.1.6 Early and subsequent spatial novelty detection is not the same

In vivo data demonstrating how CA1 and CA2 interact and contribute to continual spatial novelty detection and its subsequent consolidation are sparse, with most studies focusing on either social or novel contextual changes in an environment (Wintzer *et al.*, 2014; Alexander *et al.*, 2016) or non-spatial aspects of memory (Mankin *et al.*, 2015). Other studies have primarily focused on novelty detection and encoding in CA1 only, in an object-place recognition task (Larkin *et al.*, 2014), place field plasticity (Frank, 2004) or temporal coding and episodic memory (Mankin *et al.*, 2012). To my knowledge, this is the first time where the roles of both CA1 and CA2 have been simultaneously examined with respect to spatial novelty detection and its subsequent consolidation. It was imperative to observe how an existing hippocampal place cell ensemble for a completely novel environment (day1) would reorganize to incorporate the coding for increasing spatial RUN area across days (days 2 and3). Apart from the above, how would place cell redistribution occur, as many new place cells would come up for the extended part of the track each day, and many would continue to fire for the familiar portion of the track. This would include activation of previously 'silent' place cells as well as some active place cells remapping to fire for another part of the track. Given the fact that the same hippocampal ensemble is highly malleable and flexible in encoding various different environments, it is imperative to

see how the same ensemble would modify/reorganize to parametric variation of geometry, i.e. length of track. Given the 'nontopographical mapping' of any environment by hippocampal place cells (O'Keefe, 1976; Wilson & McNaughton, 1993), this question becomes even more pertinent and of greater importance.

For both CA1 and CA2 ensembles, on day 2, more place cells fired for the added portion of the track compared to the existing part of the track, while the reverse was seen on day 3. This makes sense as it isn't energy efficient or physically possible for a given hippocampal place cell ensemble to keep modifying its distribution of place cells every time a spatial area is added to an existing environment, nor can it afford to dedicate more and more place cells that fire for the novel area each time it is introduced in the environment, as it would result in a highly uneven and inadequately sparse distribution of place cells for the entire spatial area. But it is essential that each time modifications such as elongation of a track etc. are implemented in an otherwise stable environment (with respect to other spatial cues, reward area, beginning/end of lap etc.), the animal acknowledges this change and recognizes the novel/added spatial area. This is probably the reason why even though on the 3rd day, number of place cells that came up for the added/novel track were lower in number, they still had a significantly higher average firing rate and a higher pairwise cross correlation between them when compared to their familiar counterparts.

Therefore, spatial novelty detection seems to be a complex, dynamic mnemonic process, characterized by different and distinct hippocampal pyramidal neuronal properties. Early/first time response to spatial novelty detection is characterized by not only higher number of place cells firing for the novel area, but also higher values of spatial firing properties and characteristics of novel place cells such as average firing rate and pairwise cross correlations. On the other hand, subsequent novelty

detections are expressed through higher average firing rate and pairwise cross correlations but not higher place cell count. Thus, the biggest difference between the hippocampal neuronal responses to novelty on day 2 and day 3 is higher number of place cells firing for the novel area when compared to the familiar one on day 2 but not day 3 (Figure 3.14 a, b, c – note: pairwise cross correlation graph for CA1-CA2 cell pairs is not shown as there weren't enough cell pairs in all 3 arms of RUN3new. Refer to Figure 3.11. for the same). Additionally, average firing rates and pairwise cross correlations between place cells firing for novel areas on all days (RUN1/RUN2new/RUN3new) were comparable to one another, indicating that novelty detection is expressed through these specific attributes and characteristics of novel place cells. Other characteristics of such novel place cells, not examined in this study may also contribute to subsequent spatial novelty detection as well, and is a bright future prospect to explore further.

It is reported here for the first time that not only CA1 but also CA2, a small area, 1-step upstream and very much capable of influencing CA1 also shows the same response for both early and subsequent spatial novelty detections.

Previous studies have shown that both CA3 and CA1 place cells are less stable in novel v/s familiar environments (Leutgeb, 2004), whereby CA1 input is largely dominated by the powerful disyanptic pathway of EC-CA2-CA1, and is only later on taken over by the classic trisynaptic pathway, over days (post 24 hours) to stabilize CA1 place fields (Karlsson & Frank, 2008). Lesion studies of EC-CA1 pathway have impaired spatial coding (Brun *et al.*, 2008), indicating that CA2 directly influences spatial acquisition and learning driven processes in CA1. CA2 is uniquely poised within the hippocampal circuitry, with strong unidirectional connections to CA1 (Chevaleyre & Siegelbaum, 2010) and bi-directional feed forward connections to CA3

(Kohara *et al.*, 2014; Boehringer *et al.*, 2017). While dorsal CA2 projections to ventral hippocampus are required for social recognition memory (Meira *et al.*, 2018), the same projections (excitatory) to dorsal CA1 seem to be involved in maintaining sequential firing patterns and working memory in CA1 (MacDonald & Tonegawa, 2021). Further, changes in an environment such as shape (Mankin *et al.*, 2015) affect CA2 place fields the least when compared to CA1 or CA3 place fields. On the other hand, their sensitivity to smaller contextual changes and local cues, suggests that CA2 activity is an indicator of novelty signal to its downstream areas, i.e. CA1. Additionally, CA2 also receives projections from novelty signalling areas such as supra-mammillary nucleus (Chen *et al.*, 2020) and non-dopaminergic inputs from ventral tegmental area (VTA) (Lisman & Grace, 2005). Thus, during the development of novel place fields, the input to CA1 is primarily dominated by CA2, which in itself codes for a novelty signal, and hence may explain why both ensembles show the same characteristic response to both types of novelty detection (early and subsequent).

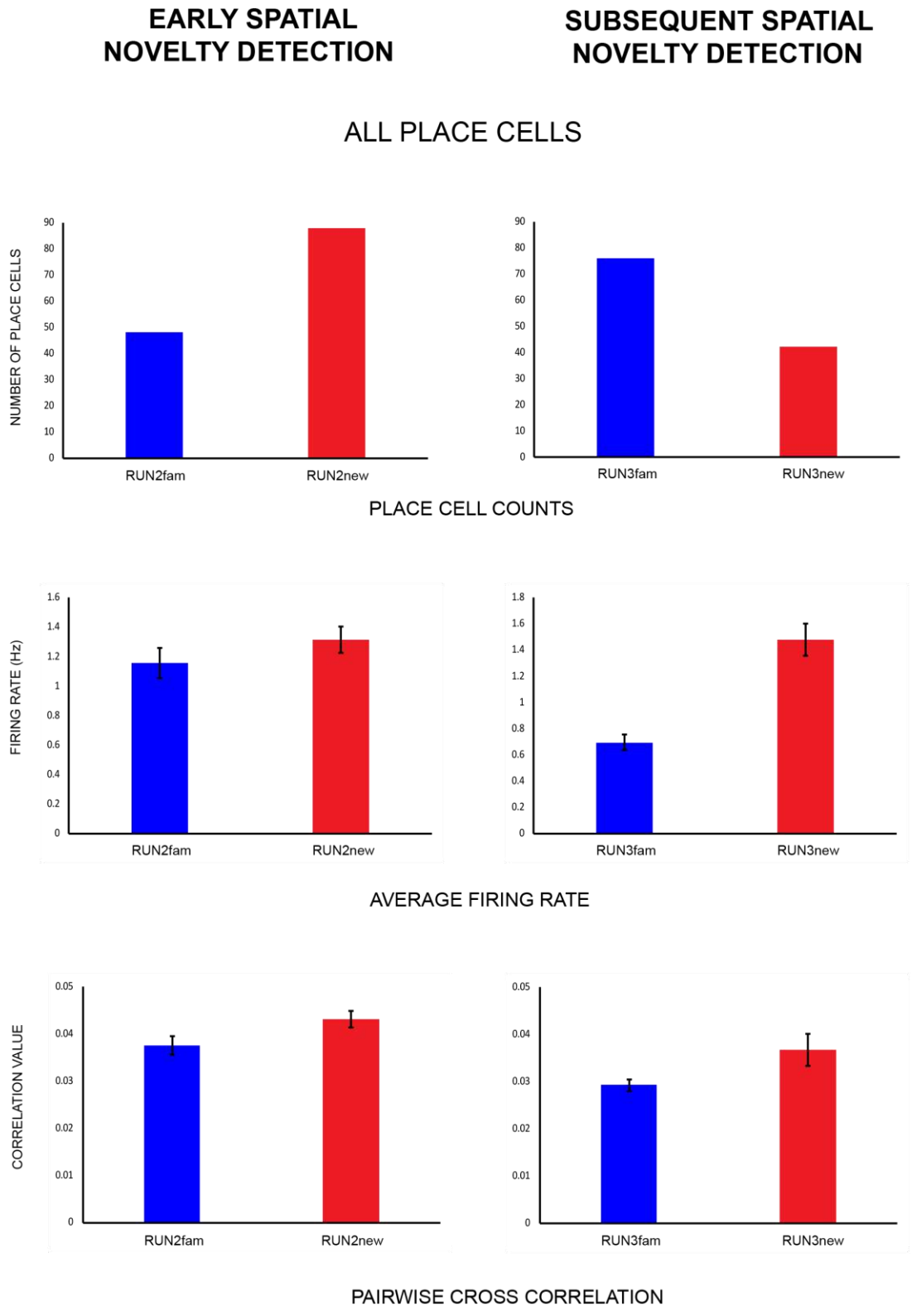


Figure 3.14 a

Early novelty detection is attributed by a higher place cell count, higher average firing rate and pairwise cross correlation values for place cells firing on novel arms, in comparison to cells firing on familiar arms (observed on day 2) while subsequent novelty detection is attributed by higher average firing rate and cross correlation values but not a higher place cell count for place cells firing for novel arms in comparison to cells on familiar arms (observed on day 3). Bars represent standard error of mean.

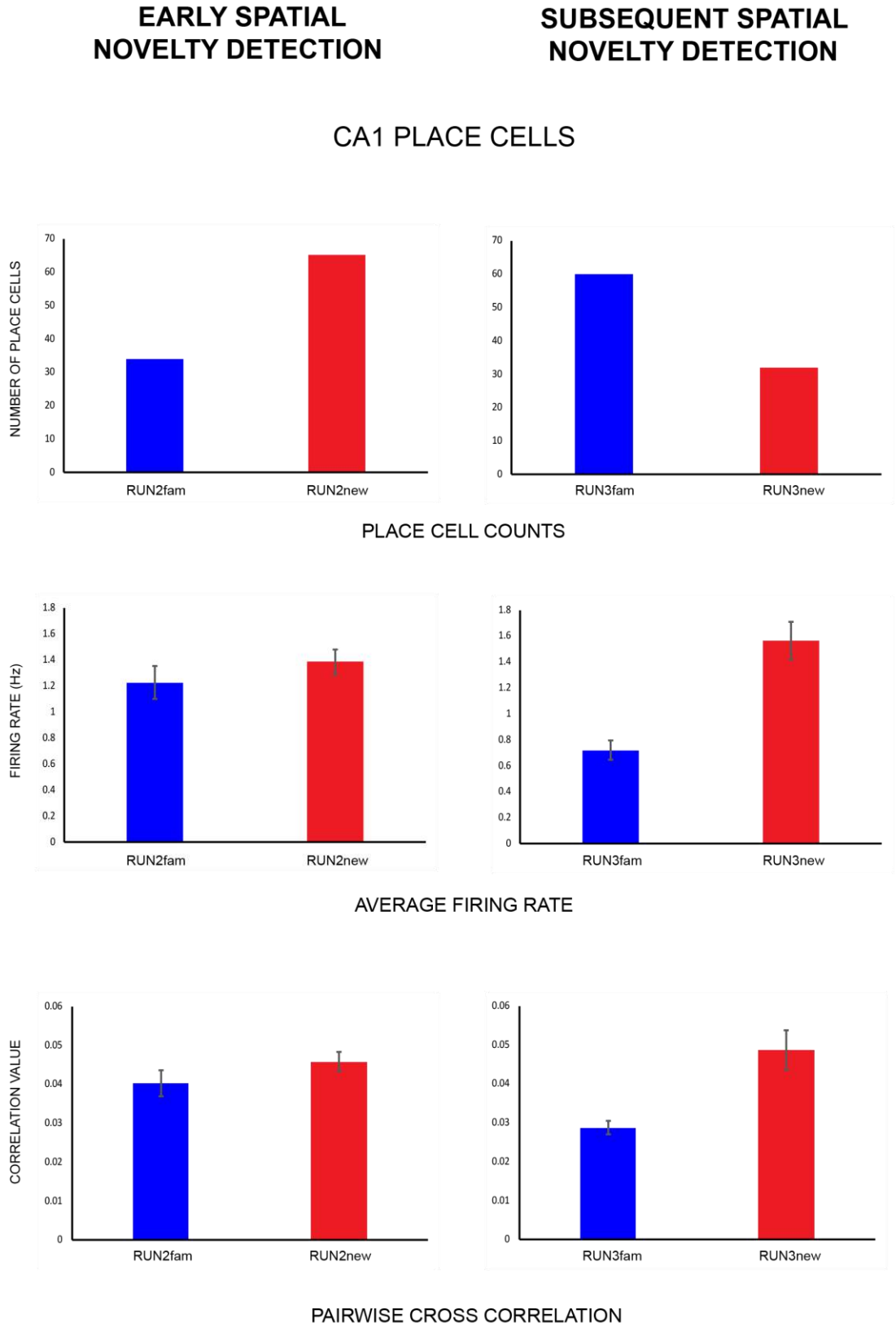

EARLY SPATIAL NOVELTY DETECTION
SUBSEQUENT SPATIAL NOVELTY DETECTION
CA1 PLACE CELLS
NUMBER OF PLACE CELLS
70
60
50
40
30
20
10
0
RUN2fam
RUN2new
RUN3fam
RUN3new
PLACE CELL COUNTS
FIRING RATE (Hz)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AVERAGE FIRING RATE
CORRELATION VALUE
0.06
0.05
0.04
0.03
0.02
0.01
PAIRWISE CROSS CORRELATION

Figure 3.14 b

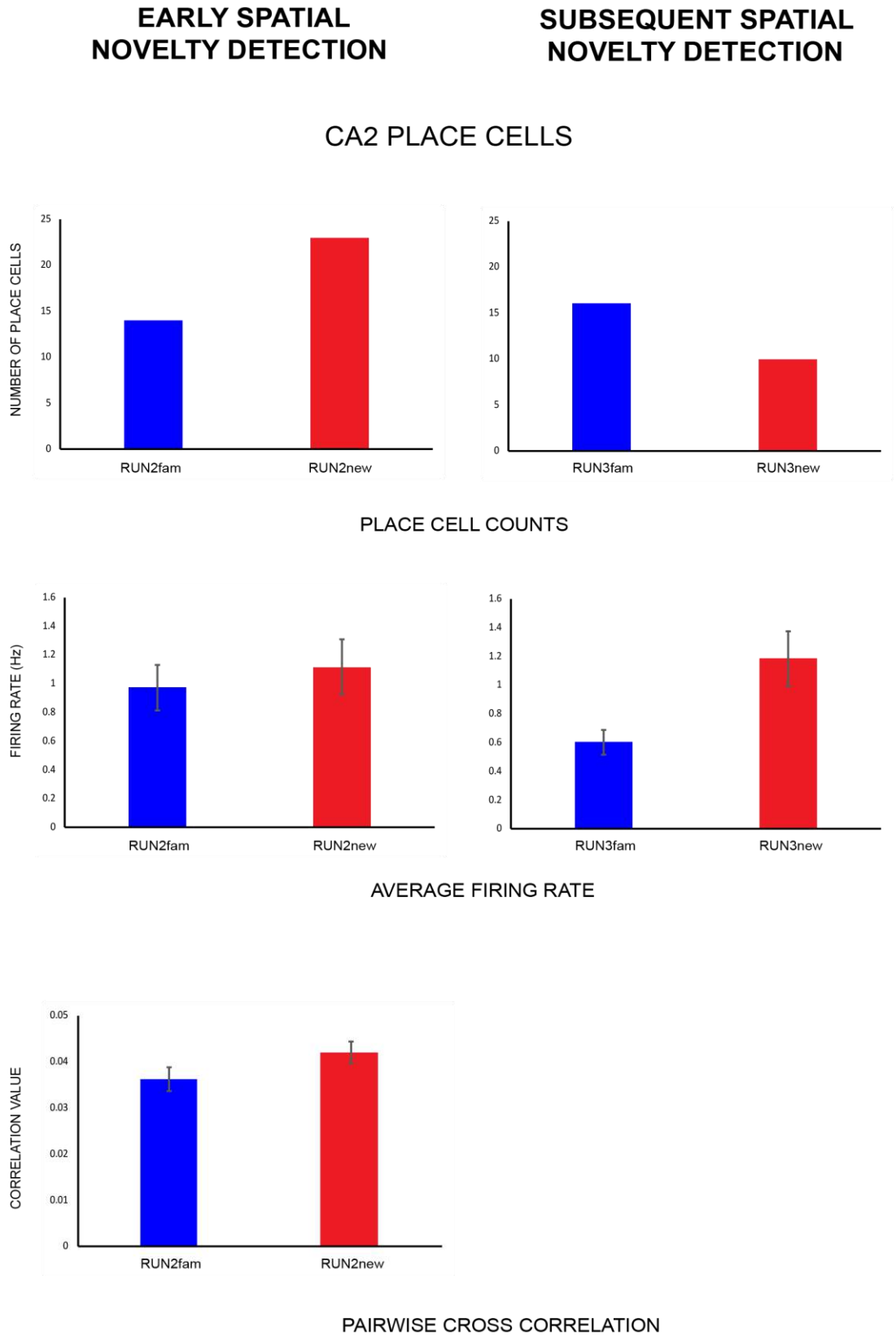

EARLY SPATIAL NOVELTY DETECTION
SUBSEQUENT SPATIAL NOVELTY DETECTION
CA2 PLACE CELLS
NUMBER OF PLACE CELLS
25
20
15
10
5
0
RUN2fam
RUN2new
RUN3fam
RUN3new
PLACE CELL COUNTS
FIRING RATE (Hz)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AVERAGE FIRING RATE
CORRELATION VALUE
0.05
0.04
0.03
0.02
0.01
PAIRWISE CROSS CORRELATION

Figure 3.14 c

CHAPTER 4

SPATIAL NOVELTY CONSOLIDATION

IN CA1 AND CA2

The second part of the study focused on better understanding the assimilation and consolidation of such an ever-changing environment across days. If 'replay' of place cells occurring during SWRs in nREM sleep is how spatial memory consolidation takes place, then it is important to study the preferential activation of place cells (if any) that takes place during these SWRS and to which part of the track/area they belong to. Particularly, with respect to this paradigm, given that relative familiarity and novelty existed in the same closed loop track itself, it is essential to observe how different parts of the track are represented in sleep, not just across days as novelty varies in the environment, but also within the same day, how that particular track is represented/consolidated in sleep.

For this aim, the focus of this part of the study was to observe track representation in sleep of all 3 tracks post behaviour; via spikes fired from place cells representing different parts of the track (familiar and novel) in SWRs during nREM sleep. This comparison was done not just across all 3 days of the experiment but also within the same day (day2 and 3) as done for spatial novelty detection in the previous chapter. Moreover, spatial representation of the environment in sleep was evaluated separately for CA1 and CA2 place cells, to observe any differences in spatial consolidation (if any) within the 2 populations, as no discernible differences were observed between the two in spatial novelty detection (as reported in the previous chapter).

Further, this study also focused on how the given environment is represented within each individual replay sleep sequence, by classifying each sequence as either same arm replay (if replay consisted of spikes from place cells firing in the same continuous location on track), mixed replay (spikes consisted of place cells from 2/3 more arms of the track) or full track replay (spikes firing from place cells scattered all across the

track). This was done to better understand what ‘mode of consolidation’ the hippocampus prefers to assimilate any previous experienced environment.

Finally, the top 3-5 place cells (from either CA1 or CA2) that were part of maximum number of replay sequences fired in sleep for each day were correspondingly mapped on track according to their firing field location. The field location of these cells were found to be overlapping/belonging to same arm of the track across all rats on days 2 and 3; indicating that as a spatial area becomes more and more familiar, certain places in that environment might serve as anchors/cues/preferred locations on track for the animal to consolidate or remember that environment better.

RESULTS

Results:

4.1.1 Spatial novelty consolidation during SWRs replays in sleep

Using cluster tracking mechanisms described in materials and method section previously, those sleep clusters that overlapped with their corresponding run clusters more than 50% on most of the projections (in cluster cutting software) were chosen for this analysis. A total of 69 sleep clusters on day 1, 104 clusters on day 2 and 102 clusters on day 3 were obtained this way (Figure 4.1-top left panel).

Since both CA1 and CA2 showed the same firing responses for both early spatial novelty detection and subsequent novelty detection, I further wanted to investigate for differences (if any) in spatial consolidation mechanisms between the two neuronal ensembles. Therefore, spiking activity within the confines of SWRs, isolated from nREM sleep, from each day's post run was analysed. A total of 203 such significant sequences were detected on day1, 461 on day 2 and 314 on day 3. These sequences were further divided into 2 categories: CA1 sequences and CA1+CA2 sequences (since sequences that only had spikes from CA2 cells in any rat on any day could not be found, and the probability of doing so is anyway extremely low owing to the diminutive physical area of CA2 as compared to CA1). The total number of sequences generated by both CA1 and CA2 place cells were highest for day2, and not day 3, despite the fact that the biggest spatial area traversed by the rat was on that day (Figure 4.1 –top right panel)

4.1.2 Sleep replay scores:

Next, the sequences scores calculated for CA1 sequences and CA1+CA2 sequences were looked at and the proportion of positive scores sequences (forward replay) and negative sequence scores (reverse replay) was compared. A total of 238 forward replays were found for CA1 sequences and 238 for CA1+CA2 sequences (cumulative number across all days). In contrast, 314 reverse replays for CA1 sequences and 188 for CA1+CA2 were found (Figure 4.2).

Although this preference for reverse replay in CA1 sequences and for forward replay in CA1+CA2 sequences was hinted at, it wasn't uniform enough across animals to emerge as a strong and conclusive trend. Nonetheless, both reverse replay and forward replay have different functional contributions to memory consolidation mechanisms (Diba & Buzsáki, 2007; Davidson *et al.*, 2009; Ambrose *et al.*, 2016; Bhattarai *et al.*, 2020) and this preference for one type of replay order by CA1 (reverse replay) and another for the CA2 (forward replay) may indicate the differential contributions and processing mechanisms in both these neuronal cell populations that influence memory consolidation and other mnemonic processes in the hippocampus.

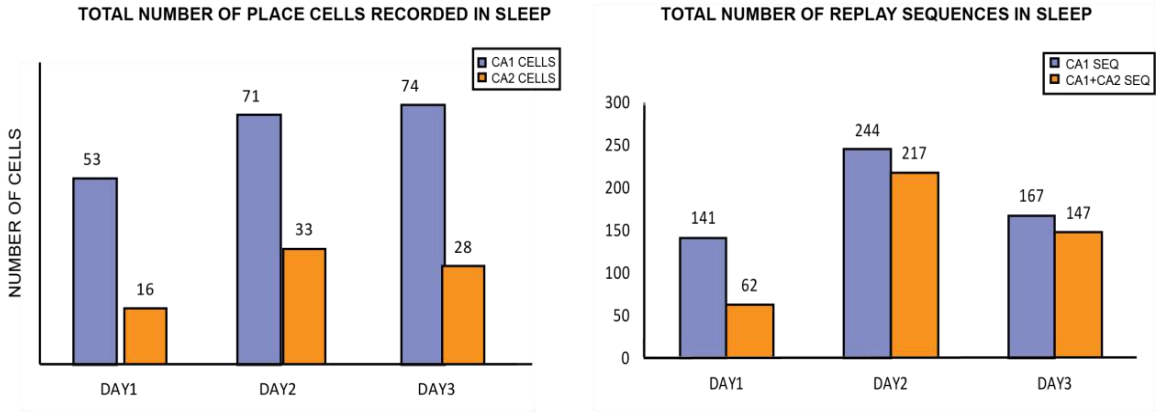


Figure 4.1:

a. Total number of place cells recorded in sleep for CA1 and CA2 place cells.
b. Total number of CA1 and CA1+CA2 replay sequences recorded in sleep each day. The highest number of sleep sequences were recorded on day2, despite a longer track length on day3.

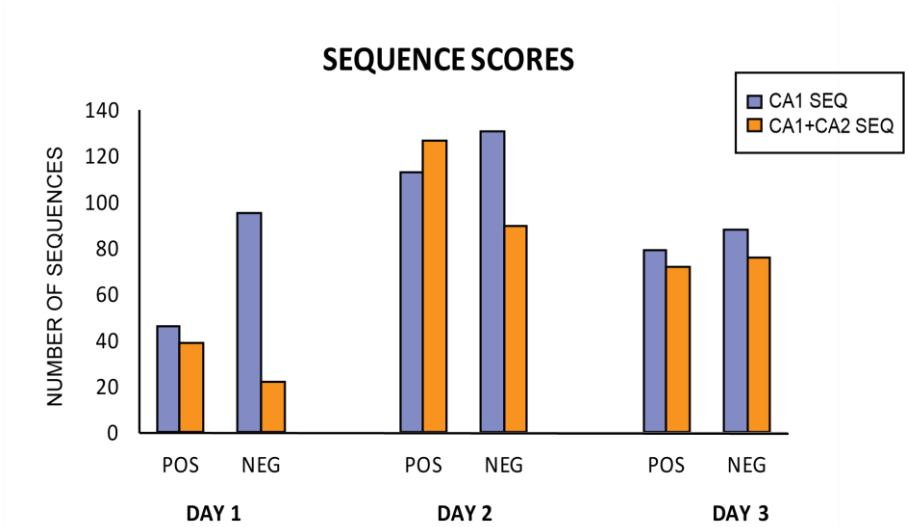


Figure 4.2: Forward and reverse replay scores for CA1 and CA1+CA2 sequences recorded in sleep.

4.1.3 Average length of sleep replays:

Since the increase in the length of the track is the only change in the environment each day of the experiment, the next step was to check if this influences the length of replay sequences during SWRs in any way. As we go from day 1 to day 3, the track length almost doubles in size (72 spatial bins to 142 bins), therefore it was imperative to see its effect on how an ever-changing (length increase) environment would be consolidated in sleep. It was found that the average length of replays during all 3 days remained constant at ~5 spikes/replay sequence. (Kruskal Wallis test: p=.943). When differentiated into CA1 and CA1+CA2 sequences, however, the average length of CA1+CA2 sequences did increase to 6 spikes/sequences while that for CA1 sequences remained the same at 5 spikes. But even then, there was no change or increase in replay length across days for either of the 2 groups (Figure 4.3).

4.1.4 Average number of sleep replays:

Next, the total number of significant sequences in sleep replay for each day were plotted to check if their number varied as a function of increasing track length. Again, no correlation as found on any of the days, as the total number of sequences recorded varied each day and showed no trend of increase/decrease across days for any of the rats. Even when the total number of CA1 sequences and CA1+CA2 sequences were looked at separately, there was no correlation found with track length (Figure 4.4).

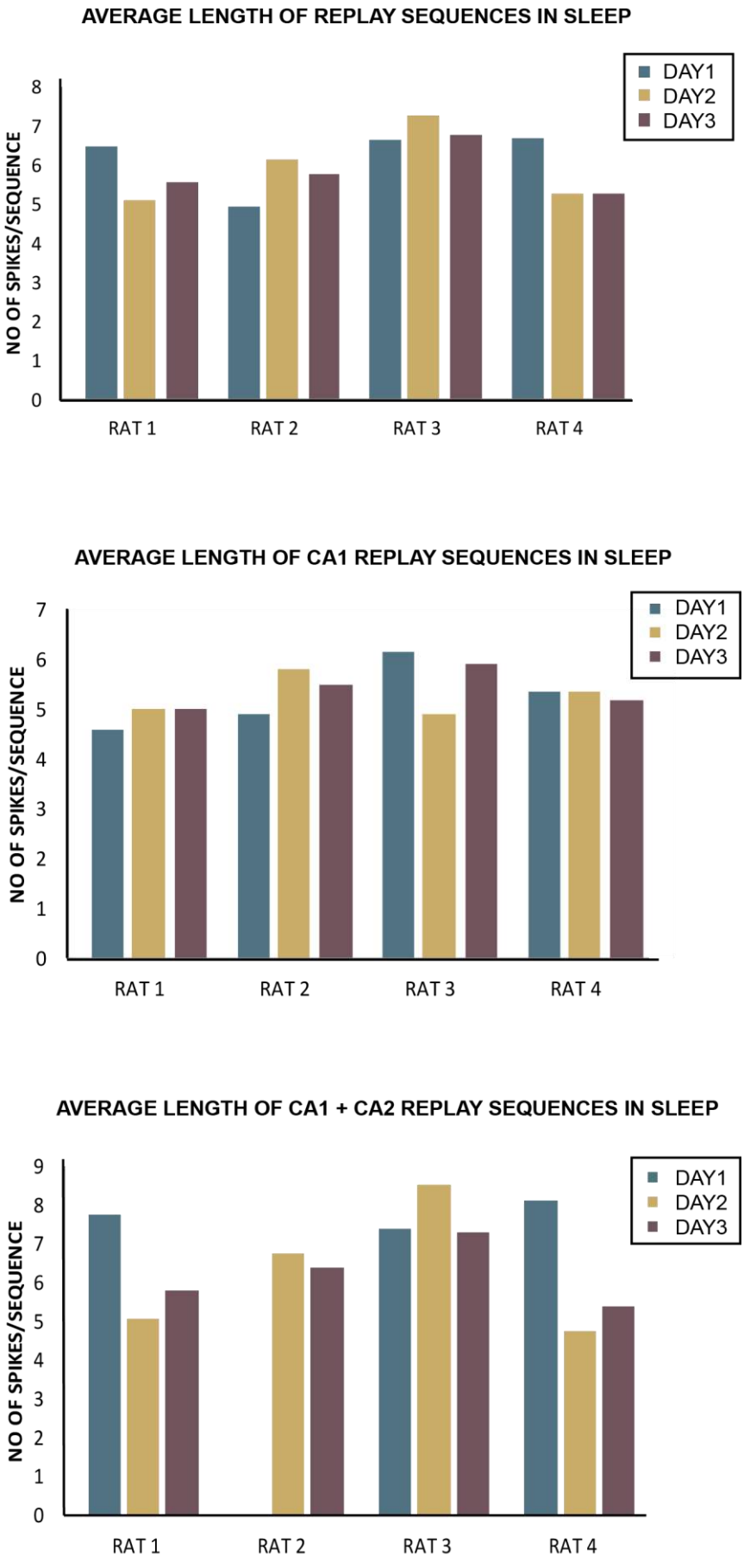
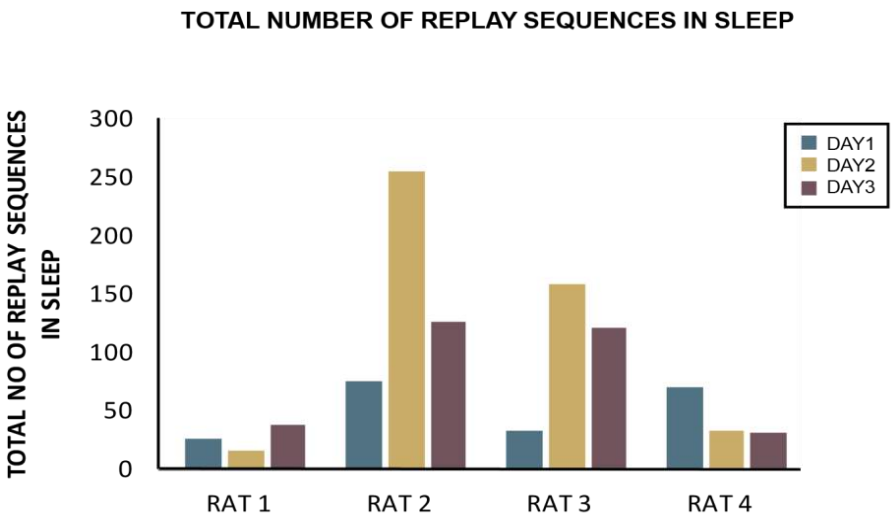
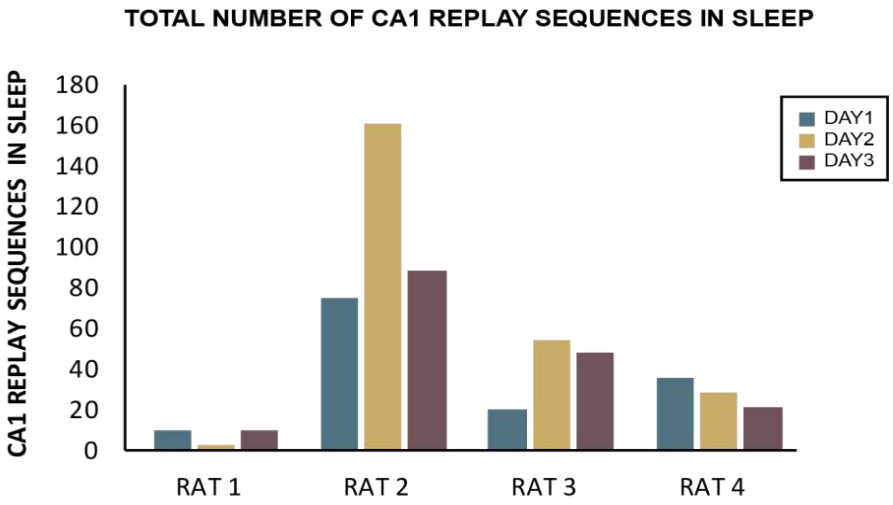

AVERAGE LENGTH OF REPLAY SEQUENCES IN SLEEP
NO OF SPIKES/SEQUENCE
0
1
2
3
4
5
6
7
8
RAT 1
RAT 2
RAT 3
RAT 4
DAY1
DAY2
DAY3
AVERAGE LENGTH OF CA1 REPLAY SEQUENCES IN SLEEP
NO OF SPIKES/SEQUENCE
0
1
2
3
4
5
6
7
RAT 1
RAT 2
RAT 3
RAT 4
DAY1
DAY2
DAY3
AVERAGE LENGTH OF CA1 + CA2 REPLAY SEQUENCES IN SLEEP
NO OF SPIKES/SEQUENCE
0
1
2
3
4
5
6
7
8
9
RAT 1
RAT 2
RAT 3
RAT 4
DAY1
DAY2
DAY3

Figure 4.3


TOTAL NUMBER OF REPLAY SEQUENCES IN SLEEP
TOTAL NO OF REPLAY SEQUENCES IN SLEEP
300
250
200
150
100
50
0
RAT 1
RAT 2
RAT 3
RAT 4
DAY1
DAY2
DAY3


TOTAL NUMBER OF CA1 REPLAY SEQUENCES IN SLEEP
CA1 REPLAY SEQUENCES IN SLEEP
180
160
140
120
100
80
60
40
20
0
RAT 1
RAT 2
RAT 3
RAT 4
DAY1
DAY2
DAY3

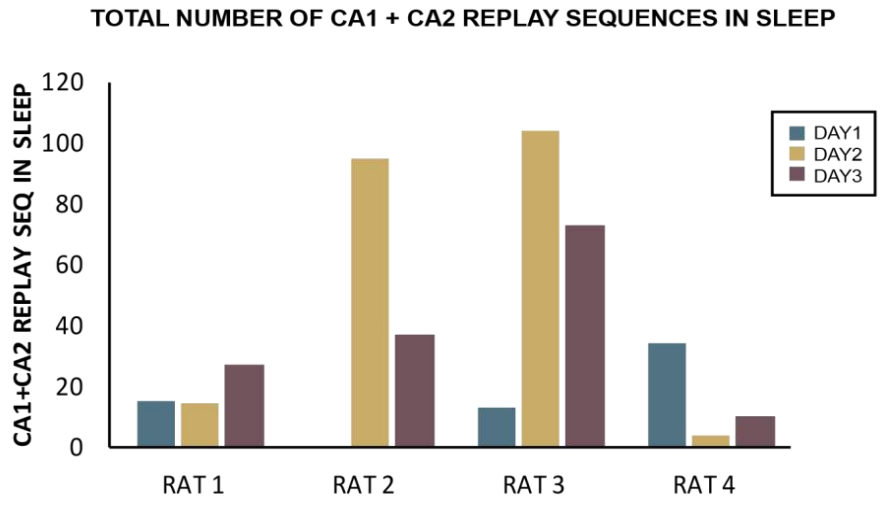

TOTAL NUMBER OF CA1 + CA2 REPLAY SEQUENCES IN SLEEP
CA1+CA2 REPLAY SEQ IN SLEEP
120
100
80
60
40
20
0
RAT 1
RAT 2
RAT 3
RAT 4
DAY1
DAY2
DAY3

Figure 4.4

4.1.5 Track representation in sleep:

Next, the individual spikes firing in each of the significant replay sequences were classified further based on their peak firing positions on RUN for that particular day, and divided further into SLP1 (corresponding to RUN1), SLP2fam, SLP2new (corresponding to RUN2fam and RUN2new from day2) and SLP3fam and SLP3new (corresponding to RUN3fam and RUN3new from day3). Cumulative spikes fired in sleep sequences from all place cells belonging to either familiar or novel arms of the track on both day 2 and 3 were compared within the corresponding days. Each of the spikes firing within each sleep SWR, were classified as 1.CA1fam 2.CA1new 3.CA2fam and 4.CA2new for days 2 and 3. For day1, spikes were identified on basis of whether they belonged to CA1 or CA2. All the spikes belonging to one category were summed across and their cumulative totals were compared.

For within day comparison on day2, when CA1fam and CA1new were compared across all rats and it was observed that more spikes from place cells belonging to the novel track fired in SWR during sleep compared to their familiar counterparts. (Figure 4.5-right column) (Chi square test: SLP2fam/SLP2new: $p<.0001$). This was the same observation in RUN2 (RUN2new>Run2fam). Similarly, for day 3, it was observed that a higher number of spikes fired in sleep from familiar part of the track (SLP3fam) across all rats when compared to novel part of the track (SLP3new) (Figure 4.5-right column) (Chi square test: SLP3fam/SLP3new: $p<.0001$). This was again in sync with RUN3 (RUN3fam>RUN3new). Thus, for CA1, on both day 2 and 3, whichever part of the track in RUN had higher number of place cells firing for it, had a higher number of spikes firing in sleep from those place cells belonging to that part of the track.

Therefore, a ‘coherent response’ was observed from CA1 place cells in RUN and SLEEP firing responses for a particular day (Figures 4.5, 4.6b and c).

Conversely, the same was not observed for CA2 spikes in sleep. For both day 2 and 3 when the cumulative total for SLP2fam v/s SLP2new and SLP3fam v/s SLP3new were compared, for some rats, more spikes from novel track fired more in sleep while for others, spikes from place cells belonging to familiar track fired more (Figures 4.5-right column, 4.6b and c). Thus, there was no clear, conclusive trend seen for CA2 cells firing in sleep replays, and they showed no preference for any particular part of the track: familiar or novel, irrespective of how many place cells fired for each of those parts in RUN. This is not only in contrast to CA1’s spiking response in SLEEP but, more importantly, to CA2’s own firing response in RUN on both day2 and 3 (which was exactly similar to CA1’s RUN on both days).

Therefore, even though CA1 and CA2 showed the same response and preference to a particular track part in RUN on both days, the ‘coherent response’ observed between SLEEP and RUN in CA1 was not observed in CA2. Thus, a stark difference in spatial memory consolidation mechanisms between CA1 and CA2 was observed.

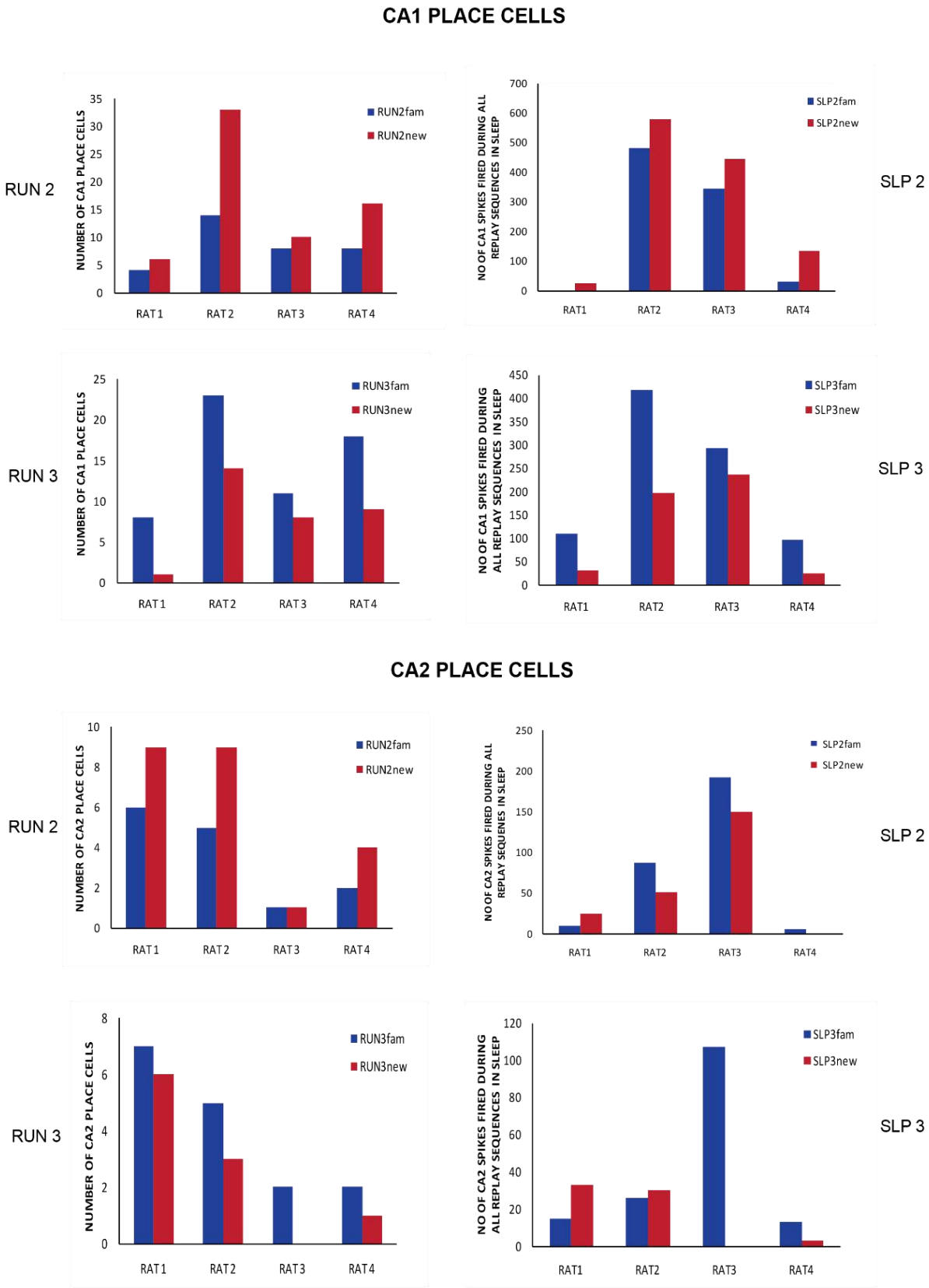


Figure 4.5: Hippocampal ensemble response in RUN and SLEEP for each rat

a. **CA1 place cells**: Day2: RUN2 and SLP2 show the same response as higher number of place cells fire for RUN2new than RUN2fam and correspondingly in SLP, spikes from place cells belonging to SLP2new fire more than spikes from SLP2fam during SWR-replays. Day3: similar to day2's response, RUN3 and SLP3 show the same response. Higher number of place cells fire for RUN3fam than RUN3new and correspondingly in sleep spikes from place cells from SLP3fam fire more than SLP3new during SWR-replays. Thus, on both days, CA1 shows a 'coherent response' between a particular day's RUN and SLP session.

b. **CA2 place cells**: conversely, CA2 cells do not show the 'coherent response' on either day 2 or day3. While RUN2 and RUN3 responses mirror that of CA1's RUN2 and RUN3 response (RUN2new>RUN2fam; RUN3fam>RUN3new), SLP responses (SLP2 and SLP3) do not show any conclusive trend. Thus, CA2 does not seem to show any preference for a particular part of the track as it did in its RUN responses (novel in RUN2 or familiar in RUN3).

4.1.5.1 Track representation in sleep- all days:

Finally, spatial (track) coverage in RUN and SLEEP sessions across all days was plotted from CA1 and CA2 place cells combined for each track. If SWRs in sleep are a form of memory consolidation (Wilson & McNaughton, 1994; Kudrimoti *et al.*, 1999; Nádasdy *et al.*, 1999; Lee & Wilson, 2002), it is imperative to see how a completely novel environment (day1) is represented by CA1 and CA2 in sleep and on subsequent days, when it becomes increasingly familiar (days 2 and 3), while the track also simultaneously changes form, with respect to shape and length. All place cells (CA1+CA2) that fired on track in RUN sessions (RUN1, RUN2fam, RUN2new, Run3fam, RUN3new) for that particular day were plotted for that particular track, in accordance with their peak firing position. Since the place cells corresponding to reward area and any spatial area outside of RUN were eliminated for analysis, some gaps may be observed in the plotting. (Figure 4.6 - left panel: RUNl, RUN2, RUN3). Out of those place cells, cells that fired in replays corresponding to SLP1, SLP2fam, SLP2new, SLP3fam and SLP3new were plotted for all days, respectively (figure 4.6 - right panel: SLP1, SLP2, SLP3). For plotting purposes, only the 1st spike from each cell that fired multiple times in a replay sequence was taken per sequence.

When all cumulative replay sequences were plotted together, it was observed that on day 2, place cells corresponding to SLP2new fired more than SLP2fam, while the reverse was seen on day 3 (SLP3fam>SLP3new). This is in congruence with more place cells (CA1 and CA2) firing in RUN2new when compared to RUN2fam and RUN3fam when compared to RUN3new. This is also in accordance with CA1 cell firing responses in sleep, which in itself was congruent with its own RUN responses on both day 2 and 3.

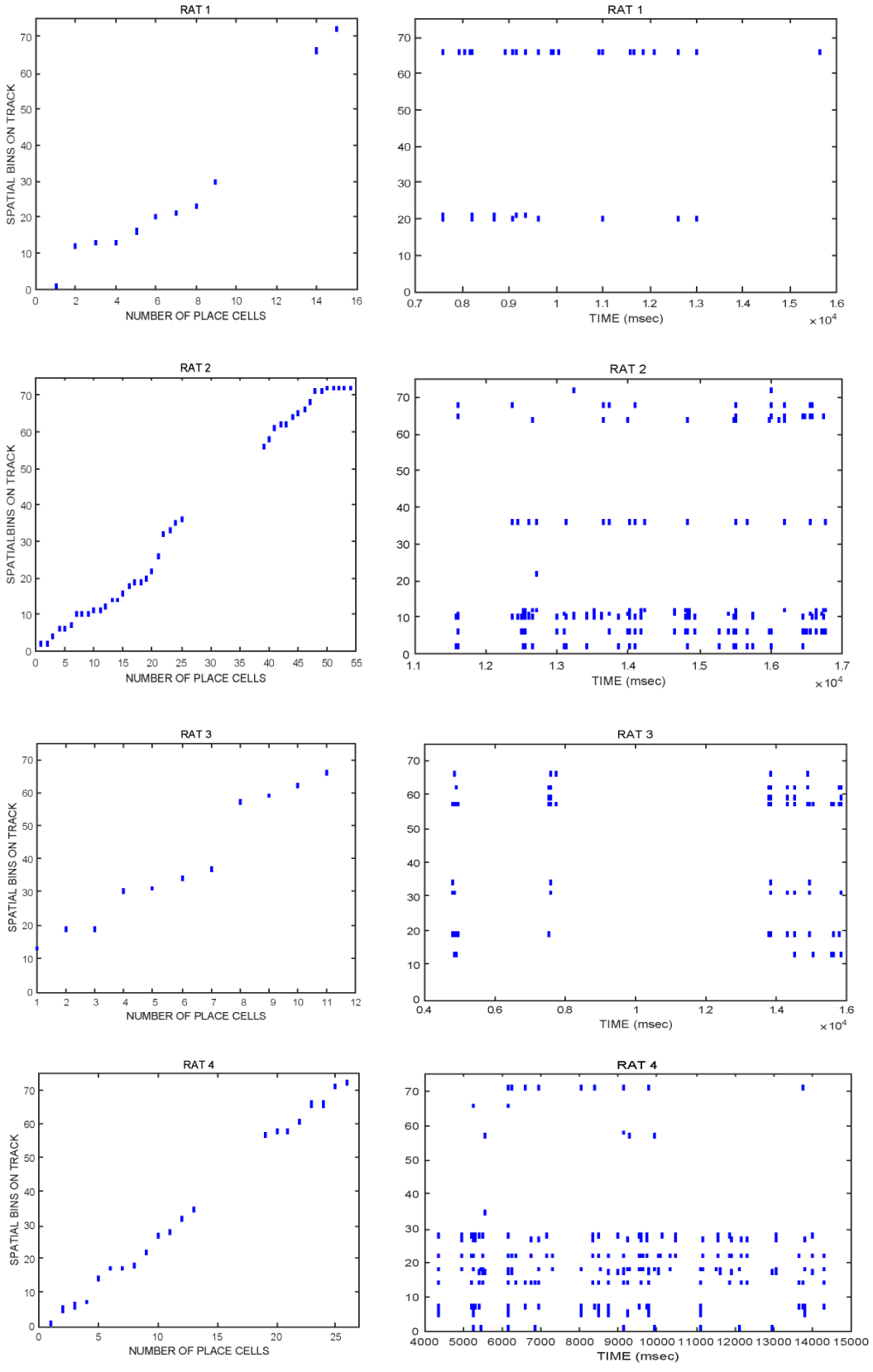

RAT 1
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 1
TIME (msec)
RAT 2
SPATIALBINS ON TRACK
NUMBER OF PLACE CELLS
RAT 2
TIME (msec)
RAT 3
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 3
TIME (msec)
RAT 4
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 4
TIME (msec)
RUN 1
SLP 1

Figure 4.6 a

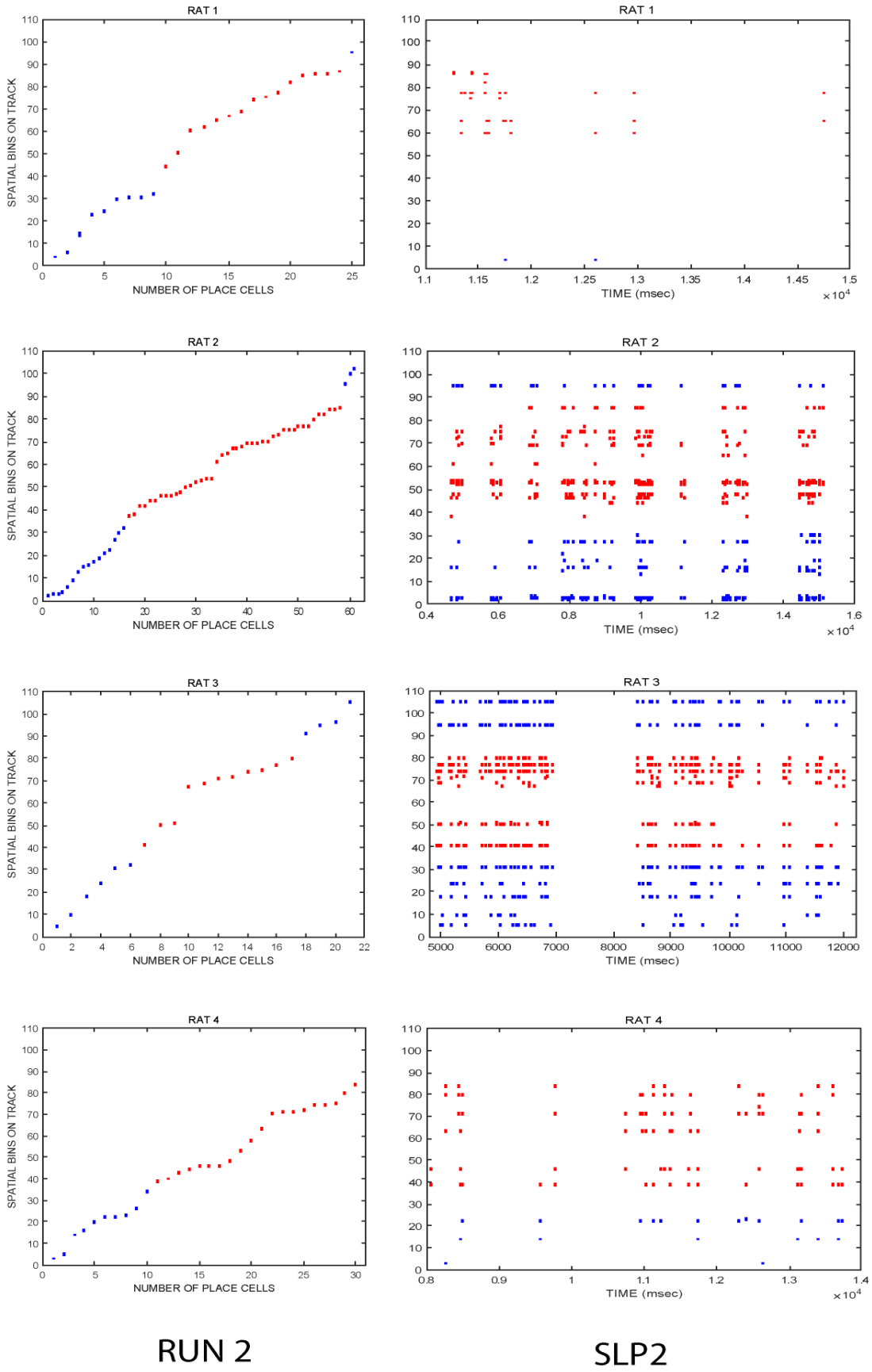

RAT 1
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 1
TIME (msec)
×10^4
RAT 2
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 2
TIME (msec)
×10^4
RAT 3
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 3
TIME (msec)
RAT 4
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 4
TIME (msec)
×10^4
RUN 2
SLP2

Figure 4.6 b

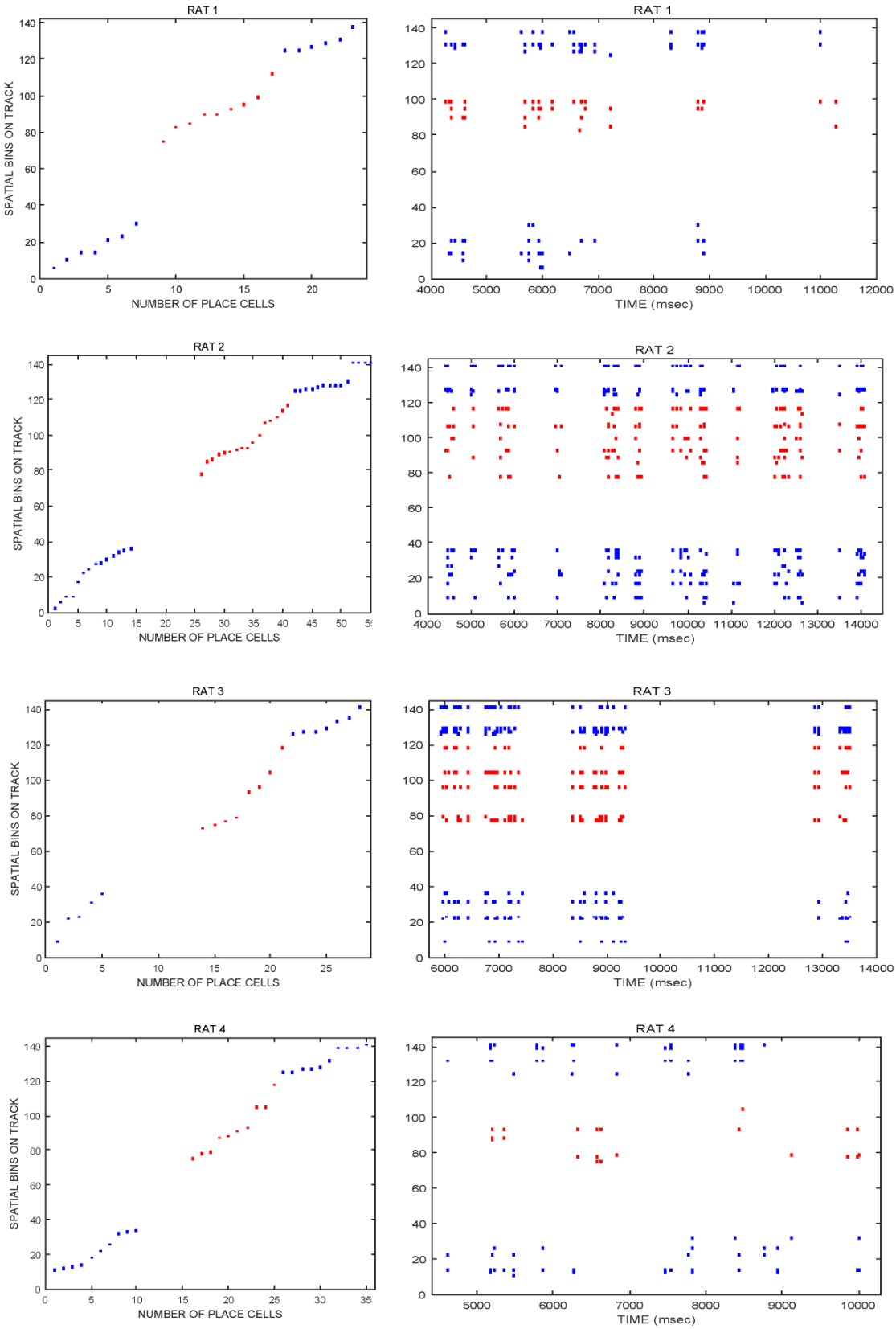

RAT 1
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 1
TIME (msec)
RAT 2
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 2
TIME (msec)
RAT 3
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 3
TIME (msec)
RAT 4
SPATIAL BINS ON TRACK
NUMBER OF PLACE CELLS
RAT 4
TIME (msec)
RUN 3
SLP 3

Figure 4.6 c

4.1.6 Spatial consolidation in 1st hour of sleep:

The content of hippocampal replay is known to most strongly resemble the preceding behavioural experience in the 1st hour of sleep than the hours after that (Ji & Wilson, 2007) . Consolidation of declarative memory, as well as improved hippocampal dependent learning using task related cues also relies on early periods of sleep, presumably because of prominence of nREM sleep during the 1st hour (Plihal & Born, 1999; Rudoy *et al.*, 2009). Therefore, the constituents of sleep replays that occurred during the 1st hour of nREM sleep in each of the rats were observed and compared against replays during the entire length of nREM sleep for each particular day. This was done to observe whether the trend observed for track representation (via spikes firing from place cells belonging to different regions of the track in replays) during the entire sleep was also reflected in the 1st hour of sleep, or if there was any difference. Any difference would indicate that different modes of consolidation are employed by the hippocampus as a function of sleep time, resulting in variable track representation as robustness of spatial consolidation changes/decreases with increasing sleep time.

Thus, all the spikes that fired during all sleep replays during the 1st hour of nREM sleep were coalesced and differentiated into the same 4 categories: CA1fam, CA1new, CA2fam and CA2new, as done before for the entire sleep duration. No discernible differences were observed in replay constituents in the 1st hour of sleep v/s entire sleep, and the trend observed was the same. This could indicate that consolidation mechanism employed by the hippocampus does not vary as a function of sleep time.

The trend was observed separately for CA1 and CA2 ensembles. For CA1 on day2, in the 1st hour as well as for full sleep, number of CA1new spikes fired more during

replays than CA1fam; while for day 3 the reverse was observed. For CA2 on the other hand, on both days 2 and 3, number of spikes fired from CA2fam exceeded CA2new for not only the entire sleep duration but also the 1st hour of sleep. This was again because of the previous trend observed that CA2 spike firing during sleep replays varied with each rat, showing no conclusive trend, and hence no preference for a particular part of track representation in sleep on any of the days (Figure 4.7).

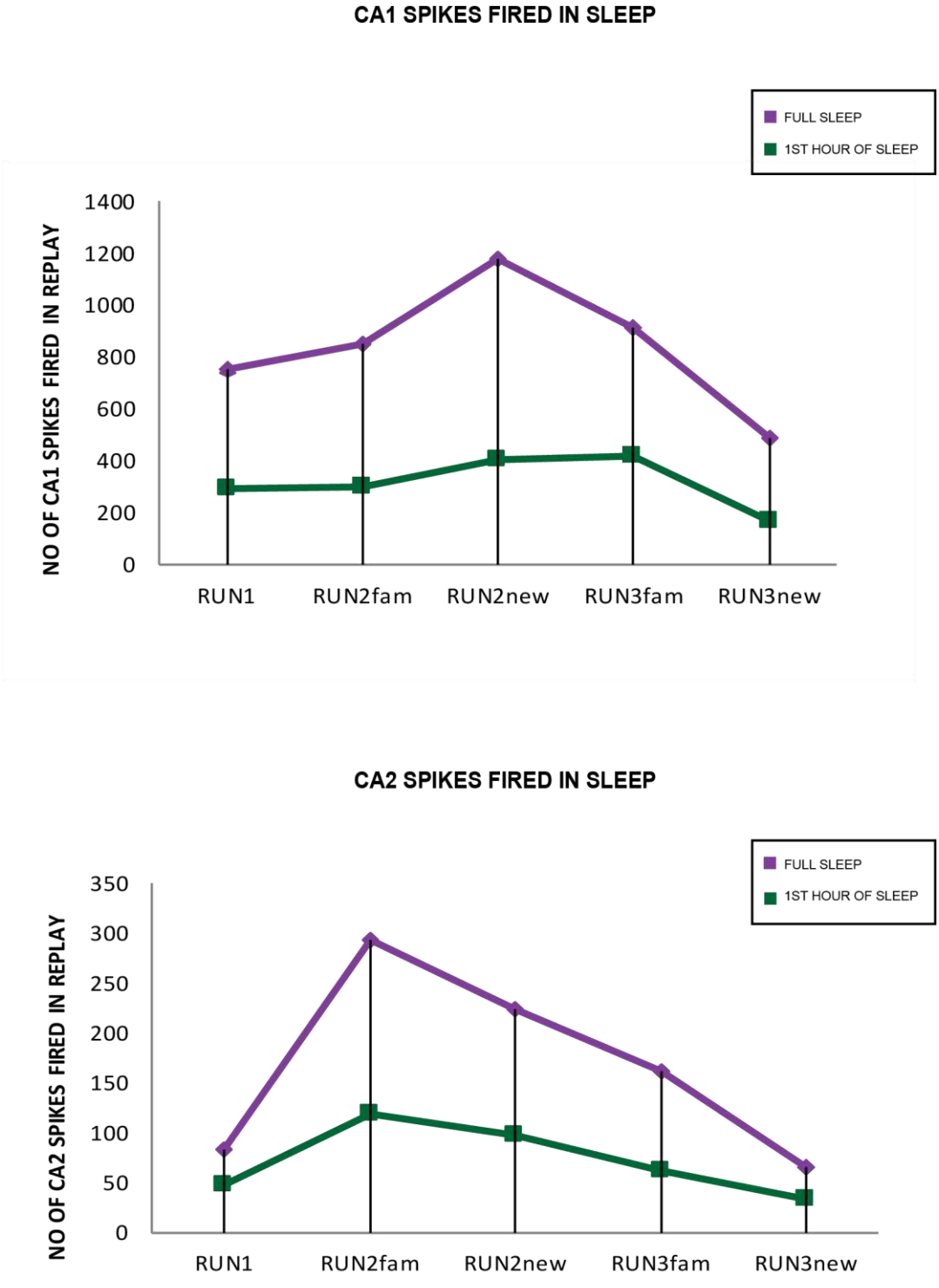


Figure 4.7: Comparison of number of spikes fired from different parts of the track in 1st hour of nREM sleep v/s the entire nREM sleep duration

4.1.7 Different types of sleep replays:

It is imperative to understand how a dynamically changing environment is consolidated by the hippocampus, with not only varying degrees of familiarity and novelty being introduced each day, but also the change in length, shape and form of the track. For the same, it is essential to observe what the sleep replays themselves constitute i.e. place cells from which part of the track are being replayed the most (if any). Thus, all the replay sequences obtained in each day's sleep were mapped correspondingly on to the track to better understand what portion of the track was being represented in each of the individual sequences. These individual replay sequences were then differentiated by whether they constituted of spikes firing from place cells firing from same arm of the track (replay for a small, specific and continuous portion of track) termed 'continuous replay', from 2 or more arms of the track but not all arms, termed as 'mixed replay' or 'full track replay', that constituted cells from more or less all arms of the track in a single sequence.

For all days and in all rats it was seen that most replays were of the mixed kind, whereby 2 or more arms of each track were represented, followed by full track replays and the least number of replays were from a small specific portion of the track. On day1, 35 replays were from same arm of track, 120 were mixed replays and 48 all track replays, on day2: 95 replays were from same arm of the track, 253 mixed replays and 111 all track replays, while on day3: 57 were replays from same arm, 195 mixed replays and 62 from all track. However, only replays from day 1 were found to be statistically significant, even though on all 3 days, more than 50% replays constituted 'mixed replay' kind. (Figure 4.8). (Kruskal Wallis test: day1: p=.049, day2: p=.429 and day3: p=.12)

This could indicate that the hippocampus prefers to consolidate a given spatial area by small non-continuous chunks being represented of that area in each individual sleep replay. Different areas of the given environment are thus represented, and all sleep replays taken together may end up representing the entire area eventually.

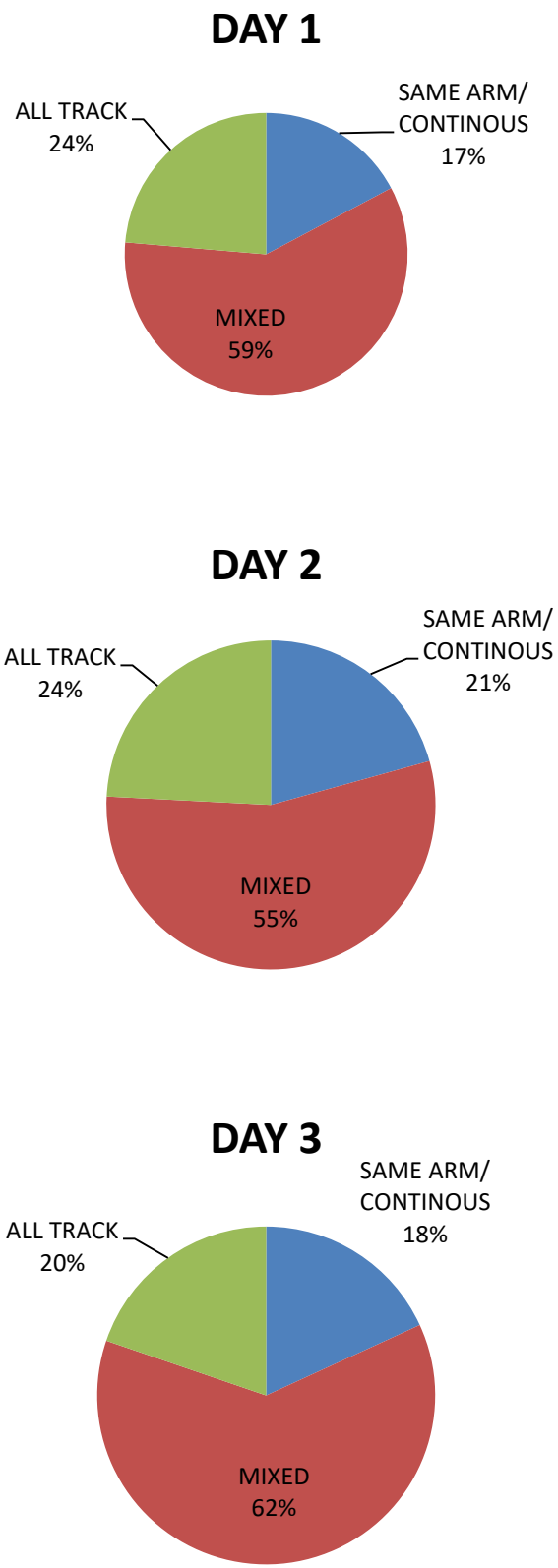


Figure 4.8: Different types of replays observed in sleep

4.1.8 Individual place cell firing frequency in sleep replays:

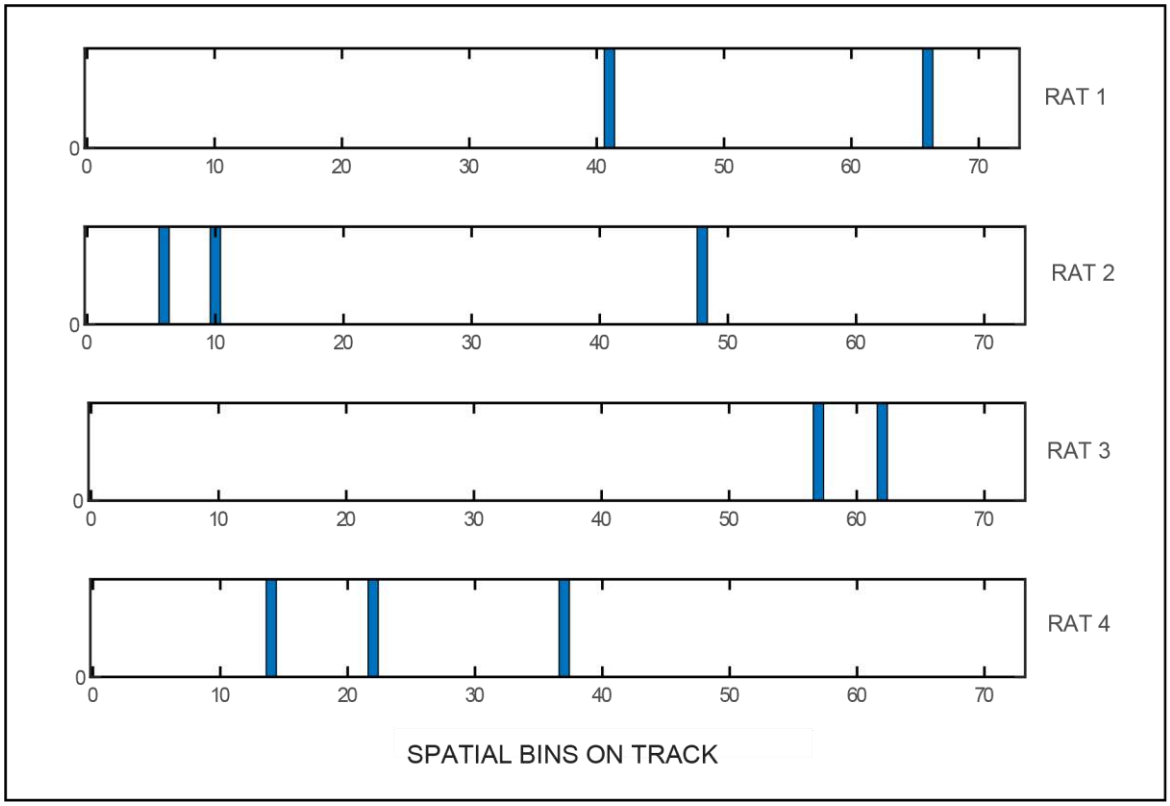
After checking for a preference for a specific part of the track (if any) in sleep sequences (as observed above), the next step was to check if there are certain place cells (either from CA1 or CA2), that are being replayed more than the others, i.e. if any particular place cells were repeatedly a part of different replay sequences in sleep. That could indicate the innate preference by the hippocampus of certain specific areas of the track/environment during sleep consolidation.

Hence, place cells from both CA1 and CA2 were compared for which of them fired the most in sleep and were consequently, a part of maximum number of replay sequences for that day. Based on the above criteria, the top 3-5 replayed cells from each rat individually were plotted on track in accordance with their spatial firing field. This was done for all days and for each particular day, and their corresponding firing positions on track were compared across all rats. It was seen that for day 1, different rats had different cells, corresponding to different parts of the track as their top firing cells in replay (Figure 4.9-top panel). Conversely, for day 2, most cells that fired the most in sleep replay corresponded to SLP2new (particularly ARM4a) across all rats (Figure 4.9-middle panel). Similarly, for days 3, it was seen that cells corresponding to last arm of the track (ARM6) fired the most for all rats, particularly bin 140 (Figure 4.9-bottom panel).

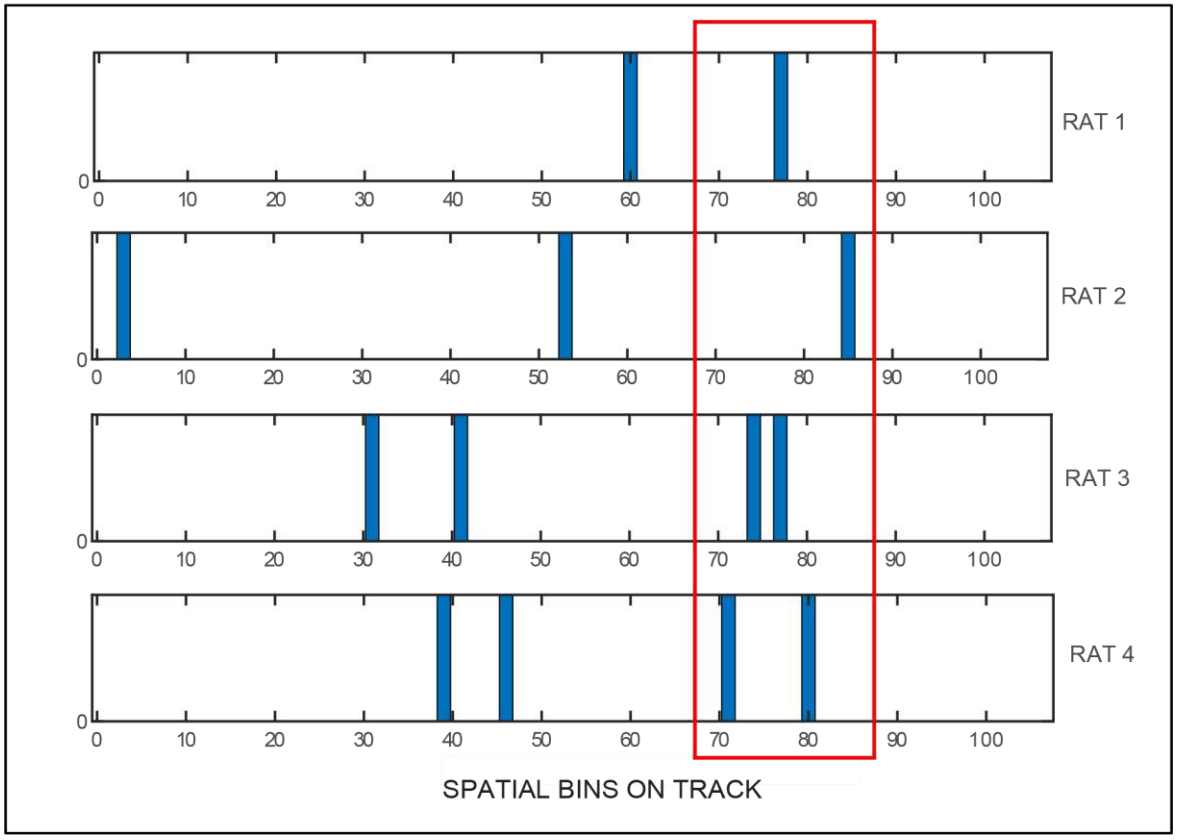
It is pretty surprising and interesting that on both day 2 and 3, cells from a very particular portion of the track fired the most in sleep replays and that 'preferred' arm was consistent across rats as well. This preference was comparatively missing on day 1 where the environment and the track were completely novel for the rat. Thus, as a novel environment becomes more and more familiar, certain places in that

environment seem to serve as anchors/reference points/spatial cues for mapping out and coding the rest of the environment. This preference also seems to be ‘innate’ as this conservation was seen across animals; indicating that the hippocampus may intrinsically prefer certain locations for a specific reason; and hence they were part of maximum number of replay sequences, as that area was being consolidated in sleep.

DAY 1


RAT 1
RAT 2
RAT 3
RAT 4
0
0 10 20 30 40 50 60 70
SPATIAL BINS ON TRACK

DAY 2


RAT 1
RAT 2
RAT 3
RAT 4
0
0 10 20 30 40 50 60 70 80 90 100
SPATIAL BINS ON TRACK

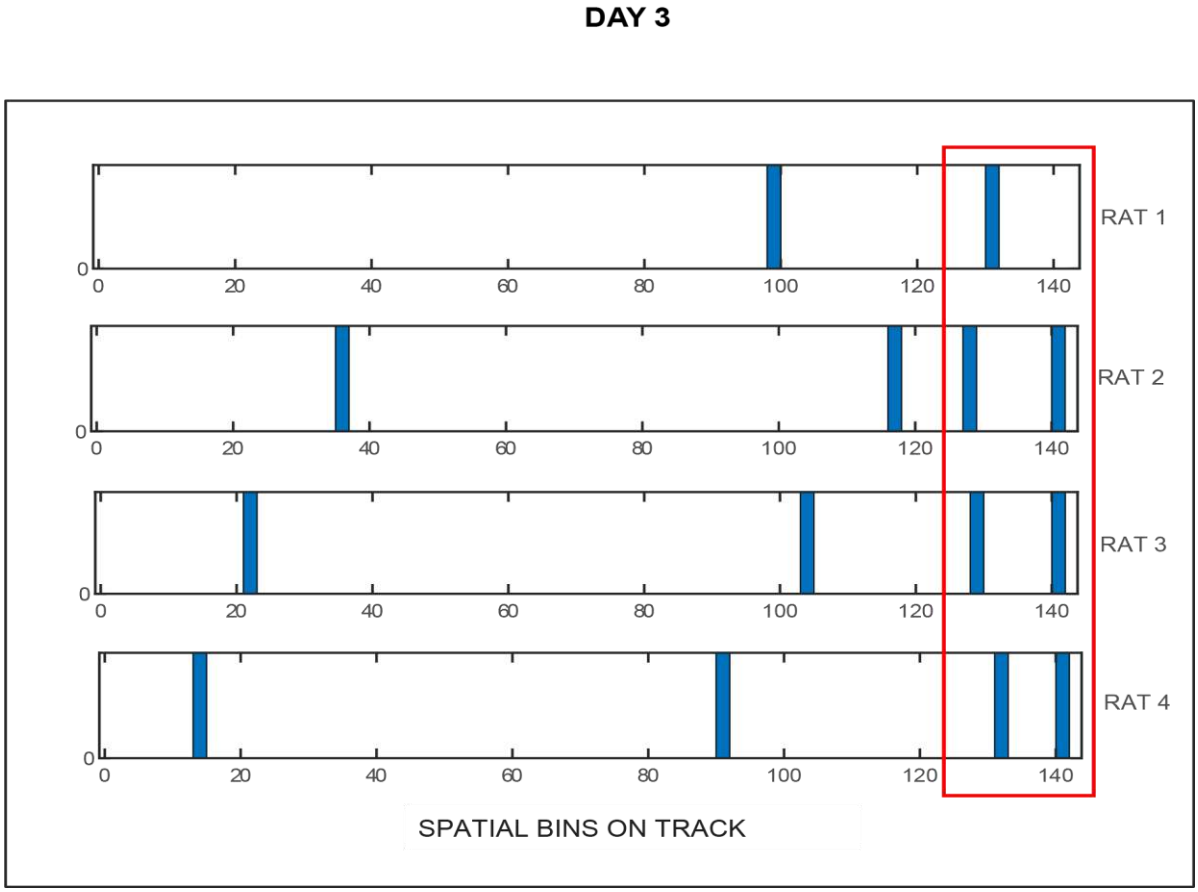


Figure 4.9: Place cell representation on track for top 3-5 place cells replayed in sleep for each rat across all days.
Some place cells that fired in maximum number of replays for all rats were coded for a similar/same place on a common arm of the track, highlighted in red. This was observed on both days 2 and 3.

DISCUSSION

Discussion:

For a successful navigation strategy, mere mapping of novel geometry and its encoding is not enough. Assimilation of novel stimulus with existing memory traces of the environment and remembrance of various paths and spatial cues (proximal and distant) is equally essential. Consolidation of spatial memory is dependent on the re-activation of place cells that fired during recent behaviour and this replay is the cornerstone of higher order representations, which are spatial as well as non-spatial in nature. Patterns of network activity that occur within sharp wave ripples during sleep/awake resting pauses are on a time compressed scale that reflect recent tasks and episodic behaviour (Kudrimoti *et al.*, 1999; Lee & Wilson, 2002; Davidson *et al.*, 2009). This study would not be complete without focusing on learning and memory consolidation mechanisms in CA1 and CA2 and understanding their contribution to these mnemonic processes. Therefore, this research question focused on spatial memory consolidation, particularly during sharp wave ripples during nREM sleep, which has been well established to be playing a crucial role in these processes (Buzsáki, 1986; Maquet, 2001; Marshall & Born, 2007).

4.1.9 Place cell representation of a dynamic environment in sleep replays differs in CA1 and CA2

As the track changed geometry and introduced familiar and novel spatial areas across days, next step was to focus on each track's representation in that corresponding day's sleep by CA1 and CA2 place cell firing during SWR-replays and how it would be consolidated each day. To the best of my knowledge, this is the first study to focus on

spatial representation of a changing environment in sleep by the CA2 neural network. While a couple of studies have recently come up on studying activation of CA2 neurons in sleep, they have focused on either social memory (Oliva *et al.*, 2020), its role during immobility (Kay *et al.*, 2016) or its role in triggering SWRs (Oliva *et al.*, 2016).

It was found that contrary to the hypothesis that CA1 and CA2 place cells from novel/added area of the track would be represented more during SWRs in sleep, i.e. more spikes firing from novel place cells than place cells from familiar parts of the track, neither CA1 nor CA2 showed this 'novelty preference.' This may indicate that other areas of the hippocampal- parahippocampal network are actually involved in spatial novelty consolidation or that novelty consolidation mechanisms are not just limited to/reflected in replay activity during nREM sleep but rather are a result of much higher and complex network mechanism in the brain.

Nonetheless, differences in CA1 and CA2 firing patterns in sleep were observed; which was surprising, given that in RUN, both ensembles behaved the exact same way on all days, with respect to both early and subsequent spatial detection. While CA1 showed a 'coherent response' in SLEEP with that corresponding day's RUN, CA2 did not. Thus, for CA1, whichever part of the track for a particular day had a higher number of place cells firing for it in behaviour, had a higher number of spikes firing in that day's sleep replay as well (for day2: RUN2new and SLEEP2new; for day3: RUN3fam and SLEEP3fam). In contrast, for CA2, irrespective of whichever part of the track was represented more in behaviour (for day2: RUN2new; for day3: RUN3fam), there was no such clear preference in sleep. While in some animals, spikes from place cells belonging to familiar part of track fired more in sleep replays, in others, spikes from place cells belonging to novel part of track fired more. This

inconsistency was observed on both days 2 and 3, and is in direct contrast to the clear preference for a particular portion of track observed in RUN. This seems to indicate that CA2 place cells fired from all parts of the track with similar/same probability in sleep replays, irrespective of its coding for familiarity or novelty in RUN/behaviour. This also seems to indicate a dichotomy for CA2 neural network ensemble, whereby a clear preference is shown by it for a particular track region during spatial novelty detection but is lost during consolidation (subsequent sleep).

Relatively minimal work on CA2 network dynamics and mechanisms with respect to various aspects of spatial learning and memory leaves a lot to desire when making interpretations or hypothesis of such results. The inconclusive trend observed for CA2 in sleep could also be attributed to less number of CA2 cells recorded, in comparison to CA1, and consequentially less number of spikes fired in sleep by those CA2 cells. Although, this did not hinder the consistent trend that was observed in CA2 RUN, with respect to place cell number, average firing rate or pairwise correlations. Therefore, while in RUN sessions across days, CA1 and CA2 show the same neuronal responses (which could be attributed to the independent influence of CA2 over CA1 via the disyanptic loop in place field development), in SLEEP sessions the case is not so. The differing spiking responses between the two heavily interconnected regions in sleep replays seems to indicate that the role of CA2 in sleep goes much beyond just directly influencing CA1 firing and instead has a much larger role to play in influencing and co-ordinating the overall hippocampal circuitry. CA2 receives a direct input from the supramammillary nucleus (Haglund *et al.*, 1984; Berger *et al.*, 2001) and has been shown to be the initiator of SWRs in awake immobile and sleep, acting as a trigger and initiator for sleep replays (Oliva *et al.*, 2020). It also plays a role in coding for current location during immobility and sleep through brief periods of

desynchronization in slow wave sleep (SWS) (Kay *et al.*, 2016). Chemo genetic inactivation of CA2 during a novel spatial task resulted in poor replay quality and fidelity during subsequent sleep and a lower ripple power in CA1 (He *et al.*, 2020). Further, the N units/ramping cells in CA2 may help in choosing and selecting which particular experience will be re activated during a subsequent sharp wave ripple in sleep (Kay *et al.*, 2016; Stöber *et al.*, 2020). More studies still have indicated that the competitive, alternate and independent circuitries via CA3 (trisynaptic pathway) and CA2 (disynaptic pathway) to CA1 as well as excitatory projections from CA2 to CA3 (Kohara *et al.*, 2014) modulate the flow of spatial information in the hippocampus depending on different behavioural states (sleep v/s awake). It has been proposed that CA2 drives sensory based representations in awake state while CA3 drives memory based representation in sleep (Middleton & McHugh, 2020) via adenosine; which allows CA3 to control sleep replay content. Therefore, CA3 takes over (via the classical trisynaptic loop) and influences CA1 firing in sleep replays more for memory consolidation of prior experience. CA3 itself has been recently shown to preferentially replay novel trajectories with higher fidelity in nREM sleep (Hwaun & Colgin, 2019).

The influence of CA3 over CA1 could explain the coherent response seen in CA1 for both day2 and 3's SLEEP, whereby CA3 specifically focuses on novel trajectories irrespective of RUN, whereas CA1 focuses on reflecting trajectories that had a higher number of place cells coding for it on that particular RUN. This 'switch' between dominance of influence over CA1 from CA2 in RUN/behaviour and CA3 in sleep could explain why CA1 and CA2 show the same response to spatial novelty detection but not in subsequent consolidation. This could be the first step towards better understanding what each of these hippocampal subfields independently contributes to during memory consolidation of space.

Most replay studies have focused primarily on CA3 or CA1 (Buzsáki, 1986; Lee & Wilson, 2002; Csicsvari *et al.*, 2007; Davidson *et al.*, 2009), and more still have looked at fidelity of replays, whether in sleep or awake state, (Foster & Wilson, 2006; Karlsson & Frank, 2009) or on direction of replays (forward/reverse) (Diba & Buzsáki, 2007). In this study, it was chosen to focus on what the replays themselves constitute/ are comprised of, and what the representation from each place cell that fired from a particularly specific spatial area in prior behaviour might reflect as a spike fired in a temporally compressed manner during sleep instead. The relative preferences of firing of different trajectories during sleep replays with respect to familiarity and novelty on track by these subfields (CA3, CA2, CA1) can help in understanding memory consolidation of a dynamic environment better in the hippocampus.

Furthermore, the preference/dominance of 'mixed replays' observed in all rats and on all days indicate that the hippocampus consolidates a spatial area in 'non continuous chunks', with different such 'chunks' being replayed in individual sequences throughout nREM sleep. This also indicated that relative novelty and familiarity in the same given environment has no effect on the mode of replay as mixed replays were observed the most on all days of the experiment.

Finally, it was found that as a novel environment gets more and more familiar, certain places may act as anchors/reference points for the mapping out the rest of the environment during memory consolidation in sleep replays. This may help the animal in remembering spatial cues, contexts and paths better for the future.

Taken together, the above findings may be a good starting point to begin to understand how assimilation of a given spatial area takes place in the hippocampus during sleep via individual replay sequences.

CONCLUSION AND FUTURE DIRECTIONS

In conclusion, the questions that have been asked in this research study is through a completely novel paradigm specially designed to answer and shed light on some important characteristics of hippocampal neuronal ensembles that have been unexamined or overlooked. The paradigm is especially suited to better understand how place fields themselves develop in a novel environment (day 1) and how these ensembles redistribute their place cell 'allocation' subsequently when varying degrees of familiarity and novelty is introduced (days 2 and 3). This relative familiarity and novelty is experienced by the animal not only in varying degrees across days, but also within the same laps on days 2 and 3, making it a powerful tool to study the influence of spatial novelty and familiarity on hippocampal place cell development, coding and distribution, and subsequently for higher network mnemonic processes. To the best of my knowledge, this is the first study that has aimed to tease apart differences in spatial early detection and subsequent detection with respect to novelty. It is also the first to shed some light on spatial memory consolidation mechanisms in sleep replay by looking at what the replay events actually constitute, in terms of spatial area representation, and not just the fidelity of those replays. Finally, this study opens avenues where detection, encoding and consolidation mechanisms in CA1 and CA2 and be understood better and bigger, bolder questions may be asked through this paradigm to understand various higher-order mnemonic processes and hippocampal network interplay better.

PUBLICATION:

Dynamic representation of space in the hippocampus: spatial novelty detection and consolidation in CA1 and CA2

Guncha Bhasin

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Life is not always a matter of holding good cards, but sometimes, playing a poor hand well

-Jack London